WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 1742-1759. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF EFINACONAZOLE MICROEMULSION WITH EUCALYPTUS CITRODORA OIL

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Article Received on
13 August 2022,

Revised on 02 Sept. 2022,
Accepted on 23 Sept. 2022

DOI: 10.20959/wjpr202213-25709

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ABSTRCT

The aim of present study is preparation and evaluation Efinaconazole microemulsion along with eucalyptus citrodora oil to achieve the synergistic antifungal effect. Efinaconazole is a new triazole antifungal, developed for topical treatment of mild to moderate onychomycosis. higher concentration required to achieve the therapeutic effect, essential oil is used in the formulation to reduce the concentration of drug and achieve the better therapeutic effect. We developed a Pseudoternary phase diagram to find out the microemulsion existence regions by using eucalyptus citrodora oil (oil), tween 20 (surfactant), propylene glycol (co-surfactant) by water titration method (ternaryplot.com software). We prepared 3 formulations (ECM1-ECM3) by changing the oil and Smix ratios. Developed microemulsions was characterized for various parameters like %transmittance, viscosity, pH, drug content, surface morphology, zetapotential, *in vitro* drug release study, release kinetics study. The optimum formulation ECM3 showed higher %transmittance 99.54±0.26, lesser viscosity 12.15±0.24cps, comfortable pH 6.61±0.016, high % drug content 98.80±0.12. 287nm of globule size, zetapotential found to be -6.03mV, and high *in vitro* drug release 91.66% after 6 hrs and fallows the zero-order kinetics. The optimized formulation further converted into Microemulgel by dispersing the microemulsion to 1% Carbopol gel base and evaluated for various parameters. The antifungal efficacy was carried out for optimized microemulsion and its Microemulgel by agar well diffusion method against *Trichophyton rubrum* and compared with standard drug which showed ECM3 and ECM3-G have a better antifungal effect than standard drug, it proved that the synergistic effect could

be achieved by both eucalyptus citrodora oil and Efinaconazole drug by microemulsion formulation.

KEYWORDS: Microemulsion, Efinaconazole, eucalyptus citrodora oil, onychomycosis.

INTRODUCTION

Onychomycosis is a very common nail infective disorder, it is caused mainly by dermatophytes, in particular by Trichophyton rubrum and T. mentagrophytes Yeasts, like Candida albicans and C. parapsilosis. Toenails are more commonly affected than fingernails, Onychomycosis in childhood is rare and affects approximately 0.5% to 2.6% of all children and it is a gender- and age-related disease, being more common in males and increasing with age in both genders. In the aged, onychomycosis might have an incidence >40%. Predisposing elements are diabetes mellitus, peripheral arterial disease, immunosuppression due to HIV or immunosuppressive agents. Clinical diagnosis of onychomycosis always needed laboratory confirmation, and treatment depends on many factors, like the fungus species and the number of affected nails. The disadvantages of therapies are that oral treatments are often restricted by drug interactions and potential hepatotoxicity, while topical antifungals have a limited success if used without nail plate debridement. Efinaconazole 10% nail solution is a promising drug, approved by the FDA in June 2014, for toenail onychomycosis. It is a new triazole antifungal developed for topical treatment of mild to moderate onychomycosis, applied once daily without nail debridement. Cure rates are similar to those seen with oral itraconazole.[1] Efinaconazole inhibited ergosterol biosynthesis in both Trichophyton mentagrophytes and Candida albicans, and was more active than itraconazole and clotrimazole. Apply once daily for 48 weeks, utilizing a flow-through brush applicator with application to the infected toenail and its undersurface, nail folds, nail bed, and hyponychium. Two drops are sufficient for the toenails.[2] due to lower nail permeability from the topical solution, it has disadvantages such as the greater concentration of the drug needed to produce the therapeutic effect. Also, the drug solution can simply wipe out from the nail surface after application Hence, there is a requirement for a drug delivery system that overcomes the complications associated with the existing conventional topical solution. In the recent era, colloidal based drug delivery has enormous importance in onychomycosis treatment due to its higher efficacy with less side effects. The colloidal-based drug delivery systems include nanoparticles, microemulsions, Nano emulsions, nano capsules, nanovesicles, transferosomes, liposomes, and hydrogel systems. Among all these drug

delivery systems, the microemulsions based drug delivery has proven to be a thermodynamically stable and clinically beneficial system because of its versatility, biocompatibility, capability to penetrate a drug molecule to deep layers of nail unit due to unique hydration properties of microemulsion ingredients as well as the longer shelf life of formulation, The microemulsion based formulation may eventually improve antifungal activity and enhance the patient compliance over a prolonged duration in comparison to other drug delivery system. the microemulsion system possesses very low viscosity, consequently, the formulation residence time is very short when applied to the affected nail parts. In onychomycosis disease, it is required to retain formulation on nail part for a required period of time to exert drug effect. Hence, to enhance the viscosity of the formulation and to provide release of the drug for a longer period of time, the gelling agent has been added into the microemulsion to form microemulsion based gel.[3] Essential oils are mixtures of chemical compounds that present aromatic structures of natural origin, where their constitution differs widely among plant species and is generally classified as terpenes, terpenoids and phenolic compound. Eucalyptus citriodora belongs to the Myrtaceae family, essential oil has a wide spectrum of biological activities, including herbicidal, antifungal, insecticide, antioxidant, antimicrobial properties. but they subjected to hydrolysis, oxidation, dehydration and isomerization reactions. So, essential oils needed a nanotechnology to enable the use of such constituents, improving their physical-chemical stability and promoting protection against external factors.[4] Hence, in the present work an attempt to study the antifungal effect of Efinaconazole along with eucalyptus citrodora oil (essential oil) to be formulated as microemulsion and microemulsion based gel to explore the synergetic antifungal effect.

MATERIALS AND METHODS

Materials: Efinaconazole was gifted by (MSN Laboratories, Hyderabad India). Tween20, Tween80 (Thomas baker PVT LTD, Mumbai), propylene glycol and methanol (S D Fine chemicals Mumbai), Carbopol 934(Central drug house, new Delhi), Eucalyptus citrodora oil purchased from (Swastik eucalyptus oil. Co., Ooty), tea tree oil purchased from (Heilen bio pharm, Gujarat).

Methods

Solubility study of Efinaconazole

Solubility determination in the different oils, surfactants and co-surfactants for formulating micro emulsion drug delivery system. The solubility of the drug in various oils is an essential

step for the micro emulsion formulation. So before starting the phase diagram one must have to choose the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is required for the formulation of micro emulsion drug delivery system. Drug powder of Efinaconazole was added in excess to each of the oils, surfactants (S), cosurfactants (CoS) and then vortexed for mixing. After vertexing the samples were kept for 72 hours at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 5000 rpm for 30 minutes to separate the undissolved drug. the supernatant was taken and diluted with methanol and observed by UV spectrophotometric method at 262nm.[5]

Construction of Pseudoternary phase diagram

To determine the existence range of microemulsions, pseudo ternary phase diagrams were constructed utilizing water titration method at ambient temperature (25 ºC). Based upon on the available solubility profile of the drug, Eucalyptus citrodora oil was selected as an oil phase, Tween20 and propylene glycol were used as surfactant and co-surfactant respectively. The Smix (surfactant+Co-surfactant) ratios were selected to be 1:1, 2:1 and 3:1 w/w and used. For each phase diagram at specific Smix concentration the Eucalyptus citrodora oil was added from the range of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1(%w/w) and the mixture were diluted with distilled water by sequential addition of 0.1ml of water using a micropipette. Water was added drop by drop while blending on a magnetic stirrer at room temperature, and the samples were marked as being visually clear or turbid. The microemulsion regions were identified as transparent and isotropic mixtures. The percentage of three different phases, that is oil, water, and the mixture of surfactant and co-surfactant were calculated (Table 04). From the endpoint compositions of titrated samples, the mass percent composition of the components like oil, Smix and water was calculated and then plotted on ternaryplot.com to construct the pseudo ternary phase diagram.[6]

Preformulation studies: Various pre formulation parameters were studied like identification of drug, determination of λ max, standard calibration curve, compatibility studies, melting point determination by DSc.

Preparation of Efinaconazole loaded microemulsion: Dissolve the drug in oil and then add surfactant and co-surfactant in fixed ratios and vertex the mixture for 15minutes continuously. Then add require quantity of demineralized water dropwise drop to the above

mixture while stirring and allow to forming a clear transparent liquid, it shows formation of a microemulsion.

Finally, the optimized microemulsion formulation incorporated to 1%w/w Carbopol 934 gel base.[7]

Evaluation of Efinaconazole microemulsion[8-12]

1. Percent transmittance

The transparency of the microemulsion was determined by measuring the percentage transmittance at 650nm against distilled water as blank by using UV spectrophotometer (UV 1800, Shimadzu, Japan).

%T= Antilog (2-Absorbance)

2. pH and Viscosity measurements

The Rheological behaviour of the microemulsion formulation was evaluated using an Ostwald viscometer at a room temperature**.** The pH of Efinaconazole microemulsion formulations was determined by using digital pH meter. The measurement of pH of each formulation was done in triplicate and standard values were calculated.

3. % Drug content

For the determination of drug content about one ml of each microemulsion formulation was transfer to a 10 ml volumetric flask and dissolved in methanol. It was diluted appropriately and analyzed spectrophotometrically at 262 nm.

4. Measurement of globule size and zeta potential

The average globule size and zeta potential of the optimized microemulsions were measured using a Malvern Zeta seizer instrument at a temperature 25 °C.

5. Surface morphology

Surface morphology of the optimized microemulsion formulations ECM3 will be determined by using a scanning electron microscope (SEM).

6. Centrifugation test

The optimized microemulsion formulation ECM3 was centrifuged at 3500 rpm for 30 min to ensure physical stability.

7. *In vitro* drug release study

In *in vitro* diffusion study, the diffusion medium used was phosphate buffer pH 7.4. Assembly of diffusion cell for in-vitro diffusion studies the diffusion cell was designed as per the dimension given. Diffusion cell with an effective diffusion area of 3.14 cm2 was used for in-vitro permeation studies. The egg membrane was mounted on the cell carefully so as to avoid the entrapment of air bubble under the egg membrane. Intimate contact of egg membrane was ensured with receptor fluid by placing it tightly with clamp. The diffusion cells were placed on the receptor compartment with magnetic stirrer. Then add 1gm of microemulsion to the donor compartment and 200ml of phosphate buffer pH 7.4 to receptor compartment. The speed of the stirrer and temperature was kept constant throughout the experiment. With the help of 1ml pipette 1 ml of sample was withdrawn at a time interval of 60 min (0 to 6hrs) from receptor compartment and same volume was replaced with receptor medium in order to maintain sink condition. The samples were appropriately diluted and the absorbance was measured at 262 nm using UV spectrophotometer.

Evaluation of prepared microemulsion gel[13-16]

1. Spreadability

Spreadability was carried out by using two glass slides of length 7.5 cm. 350 mg of Microemulgel was weighed correctly and it was taken on one glass slide. One more glass slide was placed above it from a height of 5 cm. A weight of 5 gm was kept on the upper slide and after 1 min, diameter of circle that was spread was noted in cm. The observed diameter specifies the type of gel.

2. Viscosity and rheological studies

Brookfield digital viscometer (Model LVDV–E, USA) was used for the determination of viscosity and rheological properties of microemulsion based gel using spindle no 6. 10gm of sample was taken into a small sample holder and the viscosity of gel was measured at a temperature of 25 °C.

3. Determination of pH

The apparent pH of the gel was determined by pH meter in triplicate at 25±1ºC.

4. Determination of % drug content

For the determination of drug content 1 gm of gel formulation as weighed in 10 ml volumetric flask and dissolved in methanol. It was diluted appropriately and analyzed

spectrophotometrically at 262 nm.

5. *In vitro* drug release studies

An *in vitro* drug release study was performed using diffusion cell. Egg membrane was lay down between receptor and donor compartments. Microemulsion gel equivalent to 0.2gm was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4. The diffusion cells were maintained at 37 ± 0.5℃ with stirring at 100 rpm all over the experiment. At fixed time interval, 5ml of sample was withdrawn for every 1,2,3,4,5 and 6 hrs. and same volume was replaced with receptor fluid solution in order to continue sink condition. The collected samples were analyzed by UV spectrophotometer at λ max 262nm.

6. Stability studies: The prepared gel (ECM3-G) was subjected to stability study for a period of three months at room temperature.

7. *In vitro* antifungal activity studies

Sterile Sabourd Dextrose Agar plates were prepared, by pouring the sterile agar into sterile Petri dishes under aseptic conditions. 0.1 ml of the test organism *(Trichophyton rubrum)* was spread on agar plates. 5 mm diameter holes were made in the agar plates using a sterile bore. 500µg/ml drug, 10µl of formulation ECM3, 10µl of essential oils (ECO) and 20mg of gels (ECM3-G) were added into each hole separately. The plates were maintained at +4˚C for 4 hrs to allow the diffusion of solution into the agar medium. All the plate-containing *Trichophyton rubrum* were incubated at 28˚C for 48 hrs. zones of inhibition of microbial growth around the well were measured and recorded after the incubation time.

RESULTS AND DISCUSSIONS

Based on the Efinaconazole solubility study data, the oil, surfactant, and co-surfactant components were selected in the present research work. Efinaconazole has shown the highest solubility in eucalyptus citrodora oil (154.56±6.32 mg/mL), methanol (136.21±5.06 mg/mL) and Tea tree oil (143.49±1.91 mg/mL) components among the various oils, surfactants and co-surfactants. Table 1 summarizes the solubility data for Efinaconazole by UV method.

The pseudo ternary stage charts of different proportions of surfactants (Tween 20) Co-surfactant (Propylene glycol) were utilized to develop. The Smix weight proportions [1:1, 2:1, 3:1] are addressed in Fig.1 and Table 04, in pseudo-ternary stage graph where

microemulsion regions are noticed by using Ternary plot.com software.

Table 1: Solubility profile of Efinaconazole.

Phase type	Excipient	Solubility mg/ml
Aqueous	Water	0.259±0.015
Organic solvent	Methanol	136.21±5.06
Oils	Tea tree oil	143.49±1.91
	Eucalyptus citrodora oil	154.56±6.32
	Thyme oil	134.34±1.16
Surfactants	Tween20	21.37±0.333
	Tween80	4.24±0.190
Co-Surfactants	Propylene glycol	8.96±0.271
	Poly ethylene glycol 400	7.89±0.063
Phosphate buffers	pH 6.8	0.133±0.019
	pH 7.4	0.370±0.036

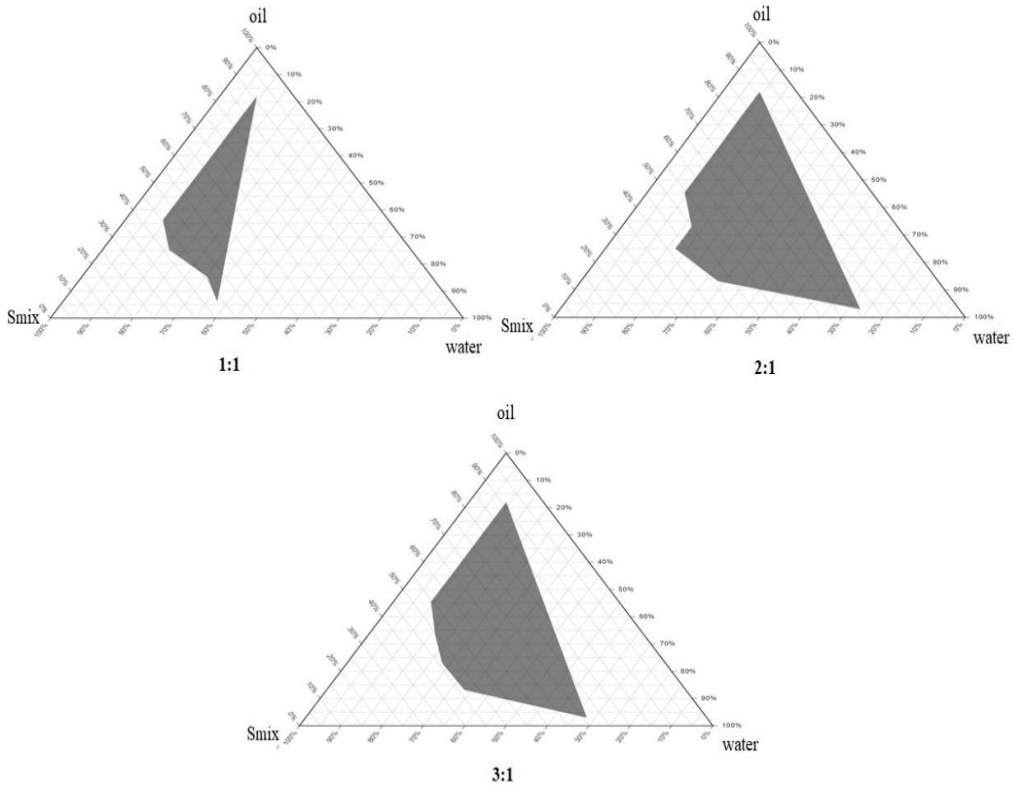

Fig 01: Pseudoternary phase diagram of Eucalyptus citrodora oil, Tween 20, propylene glycol contains different Smix ratio (1:1, 2:1 and 3:1).

The Efinaconazole melting point was found to be 89℃ by Thiel's method and 104.98 ℃ by DSC (Fig 02) method which complied with researcher's reports, thus indicating the purity of the drug.

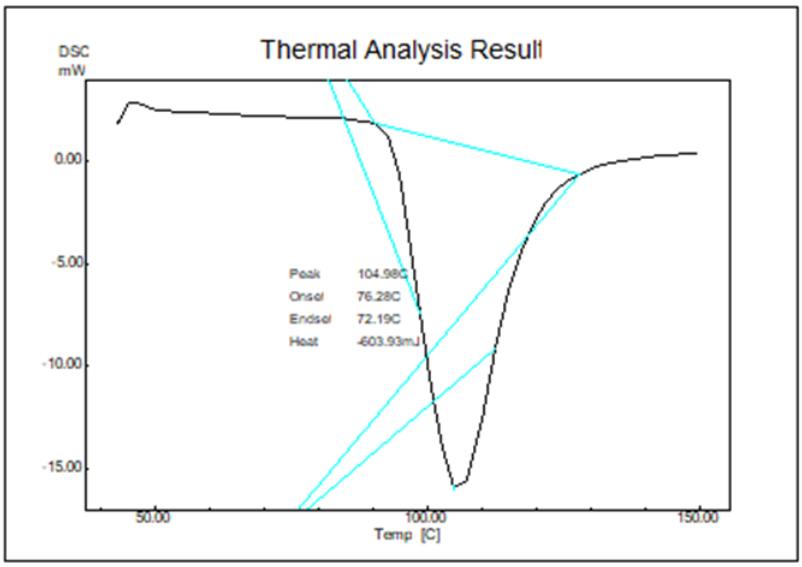

Fig 02: DSc thermograph of Efinaconazole.

The λ max of the Efinaconazole in methanol with phosphate buffer pH 7.4 was found to be 262 nm and UV spectrawere shown in Fig 03.

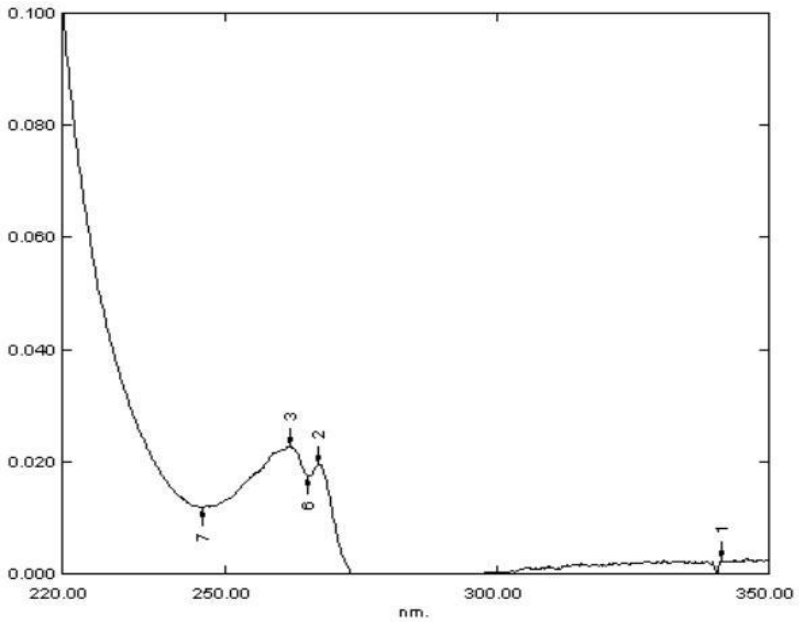

Fig 03: UV spectrum of Efinaconazole at 10µg/ml concentration.

Efinaconazole obeys Beer's law in the concentration range of 0 - 250 µg/ml in methanol with phosphate buffer pH 7.4. The regression coefficient (r2) of 0.9983 and slope (m) of 0.0024 and the standard calibration curve data (Table 02) and a calibration curve was constructed (Fig 04) and confirmed the linearity.

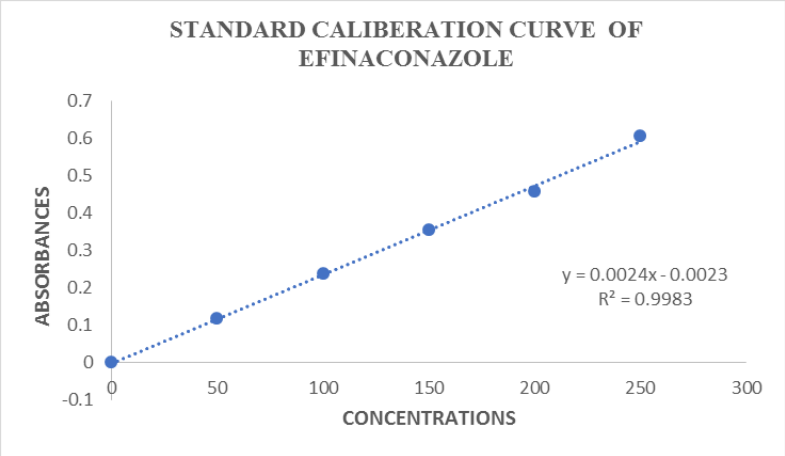

Fig 04: Standard calibration curve of Efinaconazole.

Table 02: Data for Standard calibration curve of Efinaconazole.

Sl. no	Concentration in µg/ml	Absorbance at 262nm			Standard deviation (SD)
		Trail-I	Trail-II	Trail-III	
1.	0	0	0	0	0
2.	50	0.116	0.118	0.121	0.118±0.0025
3.	100	0.238	0.234	0.233	0.233±0.0026
4.	150	0.353	0.374	0.376	0.376±0.012
5.	200	0.457	0.467	0.463	0.463±0.005
6.	250	0.604	0.606	0.605	0.605±0.001

Efinaconazole FTIR spectra were showed sharp characteristic peaks 3676.45 cm^{-1}, 2937.68cm^{-1}, 1653.05cm^{-1}, 1381.08cm^{-1}, and 1022.31 cm^{-1}, (Fig 05) and formulation ECM3 showed the entire characteristic peaks of pure drug, confirmed no interaction between the drug and excipients. Comparative studies of FTIR graphs are shown in (Fig 06) and Table 03.

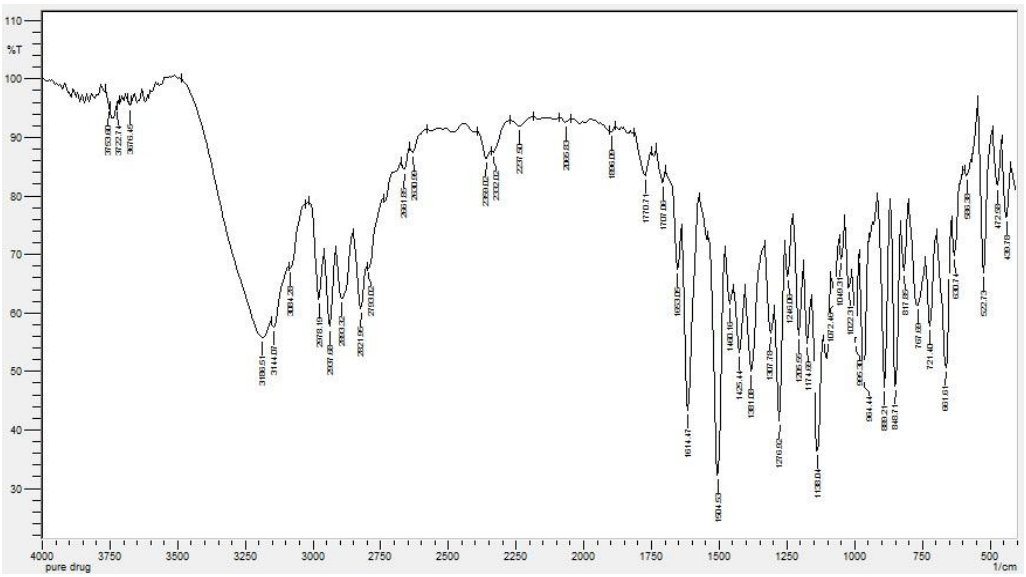

Fig 05: FTIR spectra of Efinaconazole.

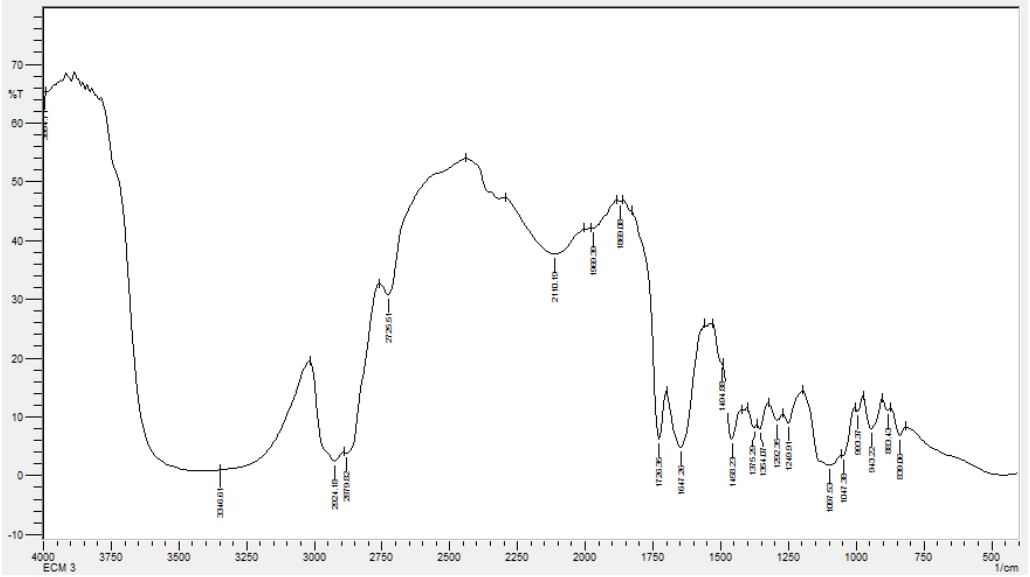

Fig 06: FTIR spectra of ECM3 Formulation.

Table 03: FTIR comparison of characteristic peak of pure drug and formulation.

Functional group	Wave number (cm^{-1}) of pure drug	Wave number (cm^{-1}) of ECM3formulation
-OH (Stretch)	3676.45	3346.61
C-F(stretch)	1381.08	1375.29
C=C(stretch)	1653.05	1647.26
C-N(Stretch)	1022.31	1047.38
C-H (Stretch)	2937.68	2924.18

Table 04: formulation development of Efinaconazole microemulsion with selected oil, Smix, water from the Pseudoternary phase.

Formulation code	Smix ratio	Surfactant	Oils	Percent w/w component in formulation			
				Oil %	Smix %	Water%	Drug %
ECM1	1:1	Tween20	Eucalyptus citrodora oil	26	55	17	5
ECM2	2:1			25	54	22	5
ECM3	3:1			32	50	18	5

Evaluation of microemulsion

- **% Transmittance:** Clarity of microemulsion was checked by % transmittance. The transmittance values of all formulations are above 90% as shown in Table 5. The ECM3 formulation showed 99.54±0.265 compare to other formulations. which indicates that the microemulsions were clear and transparent in nature also indicates the globules in the formulation is in the nanometer range.
- **pH and viscosity measurements:** The pH of all the formulations is found in the range of 5.98 to 6.61 as shown in Table 5. This is well between the range for topical

administered formulation. Formulation of ECM3 has shown pH 6.61±0.016. Therefore, there is no need for adjusting the pH of the formulation. The viscosity of microemulsion formulation was determined as shown in Table 5, all samples exhibited Newtonian flow behaviour and formulation ECM3 showed 12.15±0.249cps shows less viscous compared to other microemulsion formulations.

- **Drug content:** The drug content of all the formulations of Efinaconazole microemulsion is shown in Table 05. ECM3 was exhibited 98.80±0.12% higher drug content than other formulations. The microemulsion drug content of all formulations was found to be within the range of 85-99% which was within the limits of USP specifications. it indicates uniformity in drug content without any degradation.

Table 05: Evaluation of Efinaconazole microemulsion formulation ECM1-ECM3.

Formulation code	Transmittance	Viscosity cps	pH	% Drug content
ECM1	92.11±0.121	18.22±0.62	5.98±0.029	92.86±0.12
ECM2	97.18±0.467	13.67±0.72	6.22±0.095	96.38±0.81
ECM3	99.54±0.265	12.15±0.249	6.61±0.016	98.80±0.12

Measurement of globule size and zeta potential: The globule size and zeta potential were measured by a Malvern zeta analyzer and it was Found to be 287.1nm for ECM3(Fig 07). Established that microemulsions are within the required size ranges. The Zeta potential of microemulsion ECM3 was found to be -6.03 Mv (Fig 08) which indicates that the globules aggregation is not expected to take place so, they are sufficient to be stable.

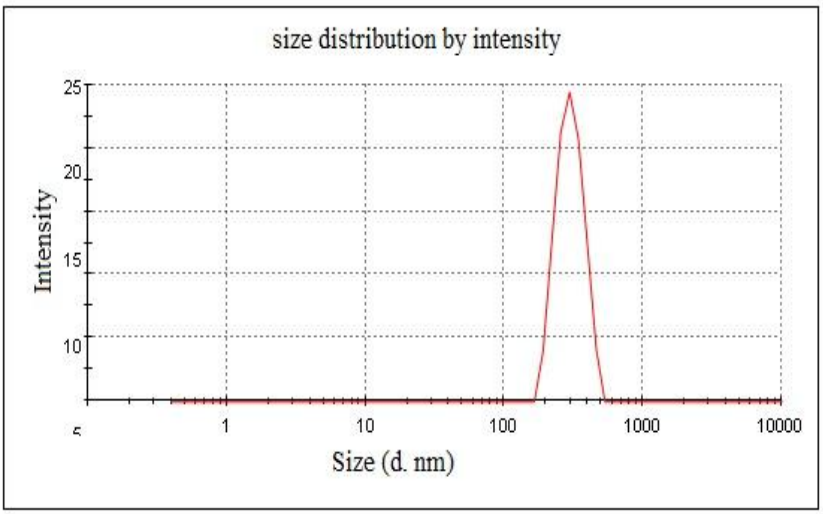

Fig 07: Globule size of ECM-3.

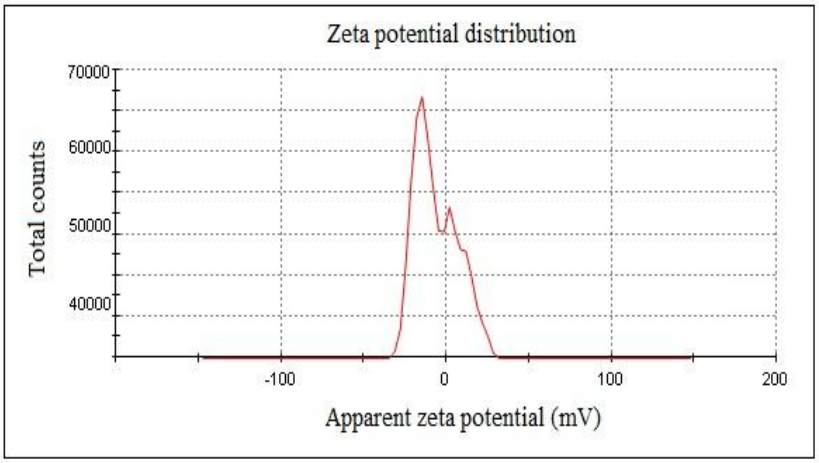

Fig. 08: Zeta potential of ECM-3.

- **Surface morphology:** The surface morphology was studied by SEM for the optimized formulations which were confirmed that the drug is completely dissolved. This can have the ability to form a microemulsion. And the particles are globular with globule size in the nano meter scale with a smooth surface as shown in Figure 09, for ECM3.

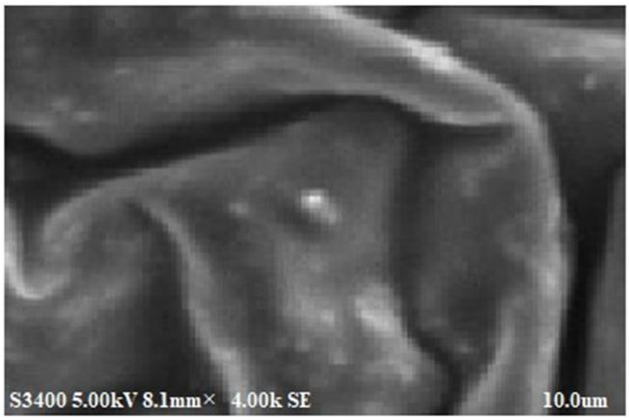

Fig. 09: SEM image of ECM3.

- **Centrifugation test:** There is no phase separation of optimized microemulsions formulations. Consequently, ECM3 will be the monophasic in nature.
- ***In vitro* diffusion study:** From the *in vitro* release studies, we observed that 0 - 20% of the drug was delivered in 1hrs and over half drug released in 3 hrs, and more than 80% of the drug released in 6 hrs. The formulation of ECM3 showed 91.66% (Figure 10). And it has shown a higher % of drug release when compared with other formulation. (Table 6).

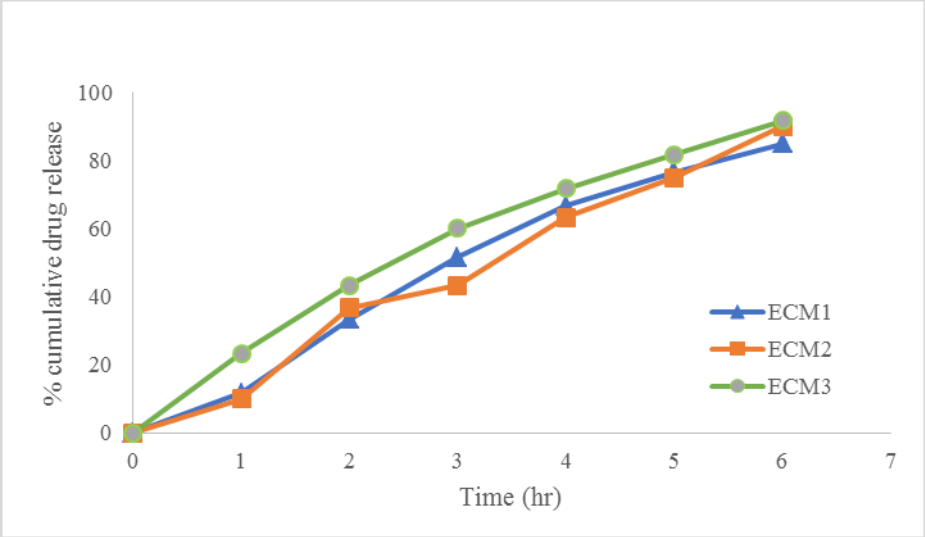

Fig. 10: Comparison of % cumulative drug release of ECM1-ECM3.

Table 06: *In vitro* diffusion study of Eucalyptus citrodora oil microemulsion.

Time in hrs	%CDR		
	ECM1	ECM2	ECM3
0	0	0	0
1	11.6	10.0	23.33
2	33.3	36.6	43.3
3	51.6	43.3	60.0
4	66.6	63.3	71.66
5	76.6	75.0	81.66
6	85.0	90.0	91.66

Evaluation of microemulsion based gel

- **Spreadability:** The spreadability is an important property of topical formulation from a patient compliance point of view. The increase in the diameter due to spreading of the formulation gel ECM3-G1 was found to be 2.73±0.169cm.
- **Viscosity determination:** The microemulsion gel formulation ECM3-G1 showed 7186.6±49.88cps. this value indicates probable retention of drug formulation on nail affected surface area without any drainage.
- **pH measurement:** The pH of microemulsion gel ECM3-G1 was found to be 6.71 ± 0.04 (Table 7) and is suitable for topical application with minimum discomfort**.**
- **% Drug content:** The prepared Efinaconazole microemulsion gel ECM3-G1 subjected to drug content uniformity. The microemulsion gel was in the permissible range from 97.41% respectively which indicated the drug uniformly dispersed throughout the formulation.

Table 07: Spreadability, Viscosity, pH and % drug content of microemulsion gel.

Formulation code	Spreadability	Viscosity	pH	% Drug content
ECM3-G2	2.73±0.169	7186.6±49.88	6.71 ± 0.04	97.41± 0.18

- ***In vitro* Drug release:** The result of the *in vitro* release of Efinaconazole from the gel formulation. However, the results clearly show that the gels can retain the drug for prolonged periods. The % CDR of microemulsion gel formulation ECM3-G1 was found to be 88.33% as shown in Figures 11.

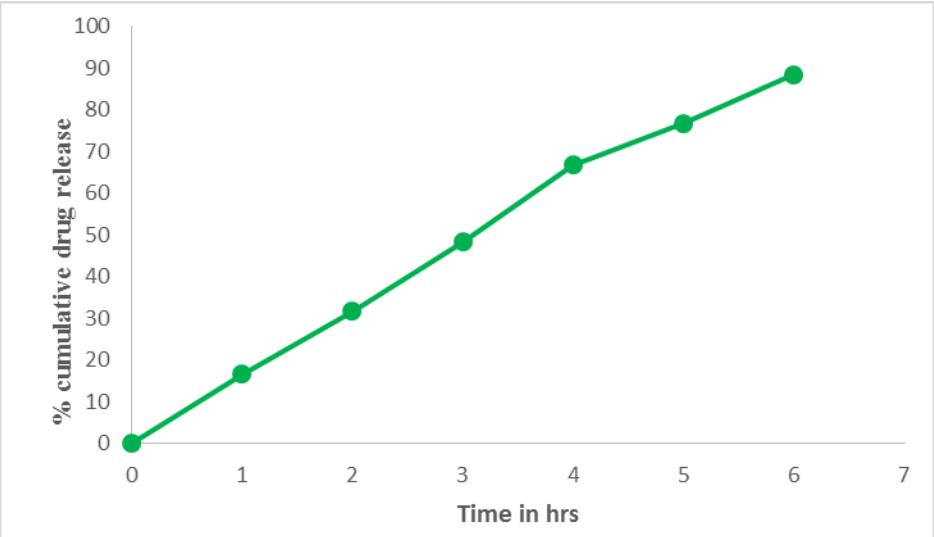

Figure 11: *In vitro* drug release of ECM3-gel.

- **Release kinetics:** the cumulative amount of drug released from optimized Efinaconazole microemulsion and microemulsion based gel formulations at different time interval was fitted to discrete models to find out the mechanism of drug release. The correlation coefficients table 8 showed that the kinetic of drug release from microemulsion (ECM3) and (ECM3-G) microemulsion based gel stick to zero order model of kinetics. 'n' values were found to be more than 0.5 this shows that the release approximates non-Fickian diffusion mechanism.

Table 08: Release kinetics of optimized microemulsion and microemulsion based gel.

Formulation code	Zero order	First order	Higuchi model	Peppas model	n values
	R^2	R^2	R^2	R^2	
ECM3	0.975	0.965	0.970	0.607	1.69
ECM3-G	0.9935	0.9514	0.9315	0.6859	1.77

- ***In vitro* antifungal activity studies:** The *in vitro* antifungal effect of optimized microemulsion formulations, Eucalyptus citrodora oil, and the drug has done against using fungal strain *Trichophyton rubrum.* The zone of inhibition was measured in terms of millimetres. The zone of inhibitions was measured 19mm for 500ug/ml of drug and 16mm for 10 µl of Eucalyptus citrodora oil, 27mm for ECM3, and 23mm for 20mg of ECM-3 gel. (Table 9) These microemulsion formulations have more effective in inhibiting the growth of *Trichophyton rubrum* fungal strain used in this study. (fig:12) Optimized microemulsion and microemulsion gel (ECM3 and ECM3-G) have a better antifungal effect than standard Drug, this proved that the synergistic effect could be achieved by Eucalyptus citrodora oil with Efinaconazole drug by microemulsion formulations.

Table 09: Report of antifungal activity against *T. rubrum*.

Sl no	Samples	Quantity Used	Zone of inhibition in mm	Sensitivity
1.	Efinaconazole	500µg/ml	19	sensitive
3.	ECM-3	10µl	27	sensitive
5.	ECM-3 GEL	20mg	23	sensitive
7.	Eucalyptus citrodora oil	10µl	16	sensitive

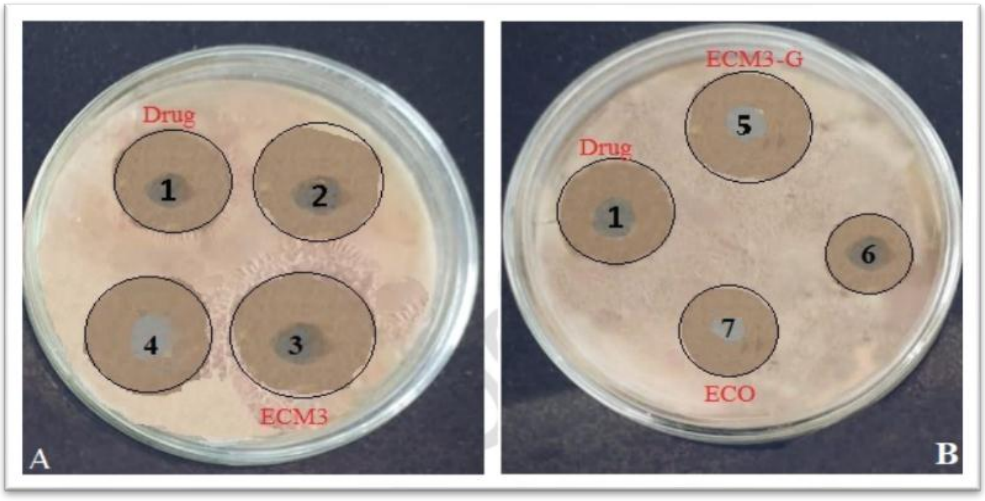

Figure 5.12: The antifungal activity against *T. rubrum* using agar well diffusion method.

A- Drug, ECM3

B- Drug, ECM3-G, Eucalyptus citrodora oil

- **Stability studies:** Stability studies of microemulsion gel formulation ECM3-G1 Shows that negligible change in drug content and % CDR revealed that the formulations are stable on storage.

CONCLUSION

Efinaconazole microemulsions with eucalyptus citrodora oil were prepared and evaluated successfully. The optimized formulation (ECM3) shows %transmittance (99.54±0.26), pH (6.61±0.016), drug content (98.80±0.12), less viscous (12.15±0.24cps), *in vitro* release (91.66%). and 287.1nm globule size and -6.03mV zetapotential obtained it indicates formulations are stable. the optimized formulation converted into gel and it shows good spreadability, pH (6.71 ±0.04), drug content, *in vitro* release and high viscosity it indicates that drug retain for a prolong period of time on surface of nail or nail bed. The antifungal activity of optimized microemulsion and its gel shows more antifungal activity compare to drug and eucalyptus citrodora oil individually. So, successfully we achieved the synergistic effect of drug and eucalyptus citrodora oil in the form microemulsions.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to management, principal and Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India for providing necessary facilities to carry out this research work.

REFERENCES

1. Piraccini BM, Alessandrini A. Onychomycosis: a review. Fungi, 2015; 1(1): 30-43.
2. Lipner SR, Scher RK. Onychomycosis: diagnosis and therapy. Medical Mycology: Current Trends and Future Prospects, 2015; 28.
3. Agrawal V, Patel R, Patel M, Thanki K, Mishra S. Design and evaluation of microemulsion-based Efinaconazole formulations for targeted treatment of onychomycosis through trans ungual route: Ex vivo and nail clipping studies. Colloids Surf Bio., 2021; 201: 111652.
4. Abreu FO, Costa EF, Cardial MR, André WP. Polymeric nanoemulsions enriched with Eucalyptus citriodora essential oil. Polímeros, Oct 5, 2020; 30.
5. Bharade S, Gupta N, Jain S, Kapoor V, Rajpoot DS. Development of Micro-Emulsion Gel Based Topical Delivery of Salicylic Acid and Neem Oil for the Management of Psoriasis. J Drug Deliv Ther., 2019; 9(4): 186-91.
6. Sunitha S, Jitendra W, Sujatha D, Santhosh Kumar M. Design and evaluation of hydrogel-thickened microemulsion for topical delivery of minoxidil. Iran J Pharm Sci., 2013; 9(4): 1-4.

7. Sadan P gowda, parthiban s, Senthilkumar G p, Preparation and evaluation of topical salicylic acid microemulsion formulated with garlic oil. Inventi Rapid: pharm Tech, 2020; 3: 1-5.
8. Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of clopidogrel. Iran J Pharm Res., 2010; 9(4): 327.
9. Pavankumar K M & Parthiban S. Design and characterization of fluconazole microemulsion formulated with lemongrass oil. J Pharm Sci Innov, 2021; 10(3): 80-86.
10. Evelyn D, Wooi CC, Kumar JR, Muralidharan S, Dhanaraj SA. Development and evaluation of microemulsion based gel (MBGs) containing econazole nitrate for nail fungal infection. J Pharm Res., 2012; 5(4): 2385-90.
11. Biswal B, Karna N, Nayak J, Joshi V. Formulation and evaluation of microemulsion based topical hydrogel containing lornoxicam. J Appl Pharm Sci., 2014; 4(12): 077-84.
12. Trivedi K, Shaikh N, Ravikumar P. Development of novel microemulsion based topical formulation of clobetasol propionate and salicylic acid for the treatment of psoriasis. Int Res J Pharm., 2018; 9: 1-7.
13. Dandagi PM, Pandey P, Gadad AP, Mastiholimath VS. Formulation and evaluation of microemulsion based luliconazole gel for topical delivery. Indian J Pharm Educ Res., 2020; 54(2): 293-301.
14. Nautiyal U, Mohammed J, Kazmi I. Preparation and Evaluation of Antifungal Micro-Emulsion/Gel using reduce Dose of Silver, Supported by Ciprofloxacin. Int Pharm Sci., 2012; 2: 72-87.
15. Kumar KJ, Muralidharan S, Dhanaraj SA. Anti-fungal activity of microemulsion based fluconazole gel for onychomycosis against Aspergillus Niger. Int J Pharm Pharm Sci., 2012; 5(1): 96-102.
16. Pavankumar K M & Parthiban S. Design and characterization of fluconazole microemulsion formulated with lemongrass oil. J Pharm Sci Innov, 2021; 10(3): 80-86.