RESEARCH PAPER

HERBAL ESSENTIAL OIL-ENRICHED MICROEMULSION GEL AS A NOVEL TOPICAL CARRIER FOR ANTIFUNGAL AGENT

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ABSTRACT

The current investigation aimed to study the antifungal effect of Miconazole Nitrate, along with herbal essential oils such as Cinnamon oil and Nigella seed oil, when prepared as a microemulsion, and to explore the synergistic antifungal effect. Microemulsions were prepared by titrating different oil-to-Smix (Surfactant + co–surfactant) ratios with water, and the region was determined using a pseudoternary phase diagram. Formulations were characterised for viscosity, pH, Drug content, Globule size, Zeta potential, and stability. Optimised formulations (COM3 and NOM3) were incorporated into 1% w/w Carbopol gel to form COM3-G1 and NOM3-G2, which were evaluated for physicochemical properties, Drug release, and antifungal activity. An in vitro antifungal study confirmed that when Miconazole Nitrate is combined with essential oils in a microemulsion, it exhibits a synergistic antifungal effect compared with the drug and the oils used separately. Miconazole nitrate microemulsions based on Cinnamon oil and Nigella seed oil were prepared for topical application. COM3 and NOM3 formulations were considered optimised because of high transmittance, low viscosity, high drug content, topical pH, greater in vitro drug release, and good stability. In vitro antifungal effects were studied using the Candida Albicans strain. The prepared microemulsion drug delivery system can improve the therapeutic effect of topical drugs, and a combination of a drug and essential oils in microemulsion form showed synergistic activity, yielding a better therapeutic effect.

Keywords: Microemulsion, Pseudoternary diagram, Miconazole Nitrate, Antifungal agent, Cinnamon oil, Nigella seed oil.

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INTRODUCTION

Topical preparations are applied to specific body parts. Formulations that solely impact a specific area of the body and are designed to minimise systemic absorption of the drug are referred to by this term. The conventional topical drug delivery techniques basically involve either breaking down the horny layer at the molecular level or assisting or manipulating the skin's barrier function (topical antibiotics, antibacterials, emollients, and sunscreen agents) to deliver drugs to the viable epidermal and dermal tissues without the use of oral, systemic, or other therapies. [1]

A microemulsion is a good candidate for the oral delivery of poorly water-soluble drugs because it can improve drug solubilisation. Absorption rate of a drug increases as its thermodynamic activity in the vehicle increases.[2]

Microemulsions are widely used for topical drug delivery. These optically isotropic, thermodynamically stable systems of water, oil, surfactant, and/or co-surfactant have been investigated as drug delivery systems due to their ability to solubilise poorly water-soluble drugs and to improve topical and systemic availability. It provides rapid, effective skin penetration and aids solubilisation of the lipophilic drug moiety.[3]

As a result, it is beneficial when administering topical medications. Transparent, isotropic, and thermodynamically stable mixtures of two immiscible liquids, microemulsions are made possible by the presence of a suitable surfactant, usually in conjunction with a co-surfactant.4 In addition to the usual advantages of improved drug stability and availability as a result of surfactant solubilisation, the microemulsion method significantly affects transdermal dispersion. Additionally, because microemulsion sizes are usually quite small, they are a great way to deliver drugs. Microemulsion, hence, has great potential for drug delivery through the skin.[5]

Miconazole nitrate, a synthetic imidazole derivative, has a broad-spectrum antibacterial action and can be used to treat fungal infections both locally and systemically. In particular, it is effective against species of *Microsporum, Trichophyton, Epidermophyton*, and *Candida*, and has some activity against gram-positive bacteria. It indicates that the cell membrane is the main site of action. According to research on *Candida albicans*, Miconazole selectively inhibits the uptake of mucopolysaccharides (glutamine) and RNA and DNA precursors (purines) at low concentrations by acting mainly on the yeast cell membrane.[6, 7]

Therefore, the goal of the current study was to investigate the antifungal effect of Miconazole Nitrate in combination with essential oils such as Cinnamon oil and Nigella seed oil to create a microemulsion gel and examine the advantages of a stronger antifungal effect.

MATERIALS AND METHODS

Materials

The following chemicals and reagents were used in the study. Miconazole Nitrate (Pharma grade, Mahrshee Laboratories, Gujarat) served as the active antifungal drug**.** Carica papaya seed oil and Cinnamon oil (LR grade, RV Essential, New Delhi) were used as natural antifungal agents. Tween 20 and Tween 80 (LR grade, Thomas Baker Pvt. Ltd., Mumbai) act as non-ionic surfactants for microemulsion stabilisation**.** Propylene glycol and PEG 400 (LR grade, S D Fine Chem. Ltd., Mumbai) were used as co-surfactants and penetration enhancers**.** Carbopol 934 (LR grade, CDH Pvt. Ltd., New Delhi) served as a gelling agent for microemulsion gel formation. Methanol (LR grade, S D Fine Chem. Ltd., Mumbai) was used as a solvent. Potassium dihydrogen orthophosphate (Thermo Fisher Scientific, Mumbai) and Sodium hydroxide(S D Fine Chem. Ltd., Mumbai) were used for buffer preparation and pH adjustment.8 All chemicals used were of analytical or laboratory reagent grade.

Method of Preparation of Microemulsion Gel

The drug was first dissolved in the selected oil, followed by the addition of a fixed ratio of surfactant and co-surfactant. The resulting mixture was vortexed continuously for about 15 minutes to ensure proper mixing. Subsequently, the required quantity of demineralised water was added dropwise to the mixture with constant stirring. The process was continued until a clear and transparent liquid was obtained, indicating the formation of a microemulsion. The prepared microemulsion was then incorporated into a 1% w/w Carbopol 934 gel base to obtain the final microemulsion gel formulation suitable for topical application.[9]

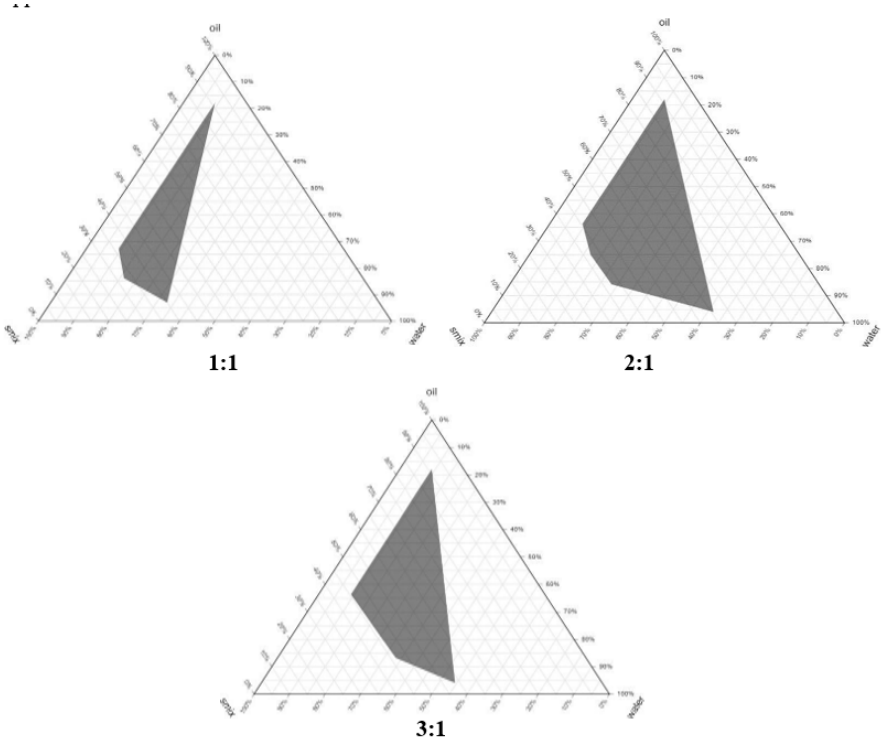

Fig. 1: Pseudoternary phase diagram of Cinnamon oil, Tween 80, and Propylene glycol with different Smix ratios (1:1, 2:1, and 3:1).

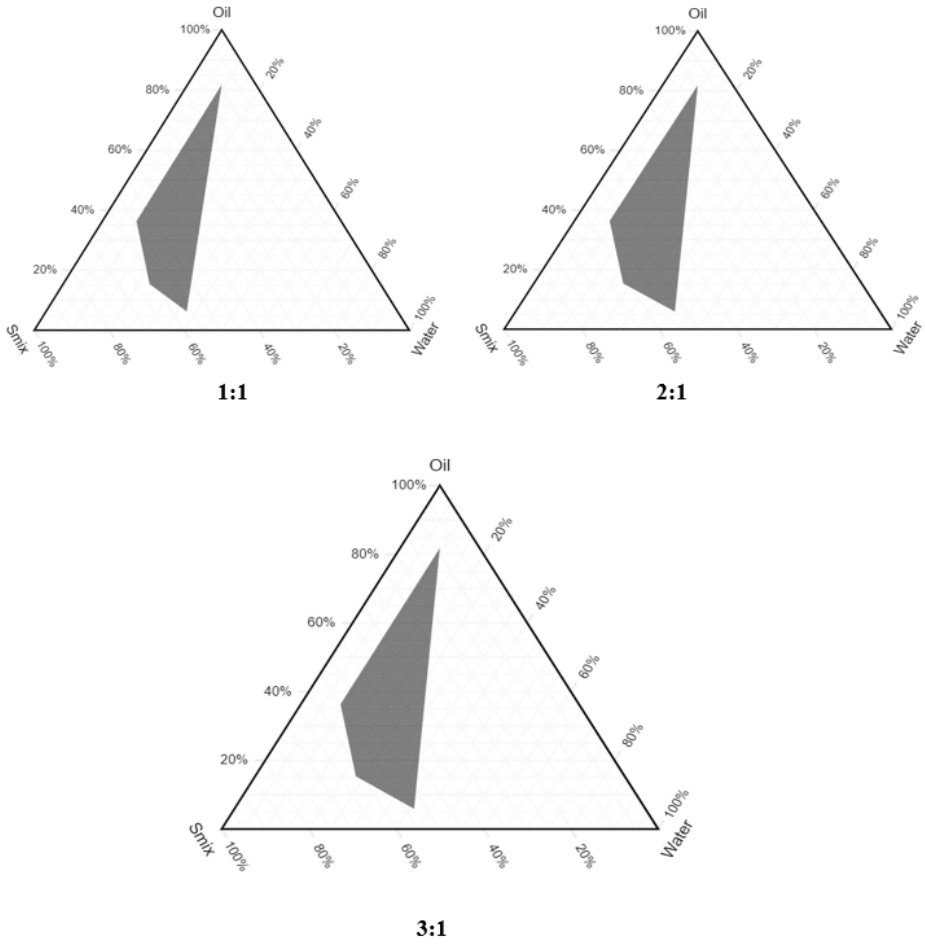

Fig.2: Pseudoternary phase diagram of Nigella seed oil, Tween 80, and Propylene glycol containing different Smix ratios (1:1, 2:1 and 3:1)

Table 1: Formulation development based on Pseudo-ternary phase

Formulation Code	Smix ratios	Co-surfactant	Surfactant	Oils	Percentage w/w component in formulation			
					Oil %	Smix %	Water %	Drug %
COM1	1:1	Propylene glycol	Tween80	Cinnamon oil	26	55	17	1
COM2	2:1				25	54	22	1
COM3	3:1				32	50	18	1
NOM1	1:1			Nigella seed oil	15	55	30	1
NOM2	2:1				20	50	30	1
NOM3	3:1				35	50	15	1

EVALUATION OF MICROEMULSION

Percentage transmittance: The transparency of the microemulsion was determined by measuring the percentage transmittance at 272 nm against distilled water as a blank by using a UV spectrophotometer.[10]

$$Percent\ Transmittance = -Log_{10}(2 - Absorbance)$$

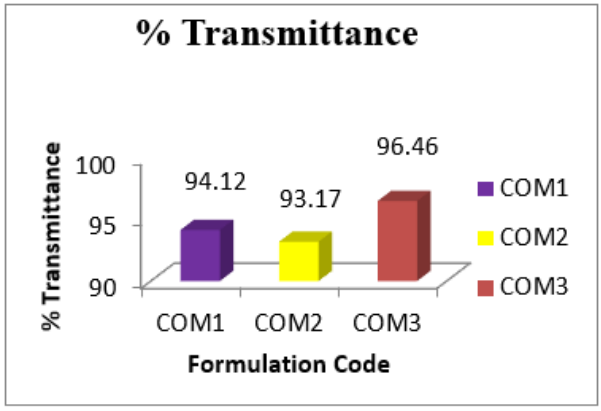

Fig.3: % Transmittance of COM1-COM3

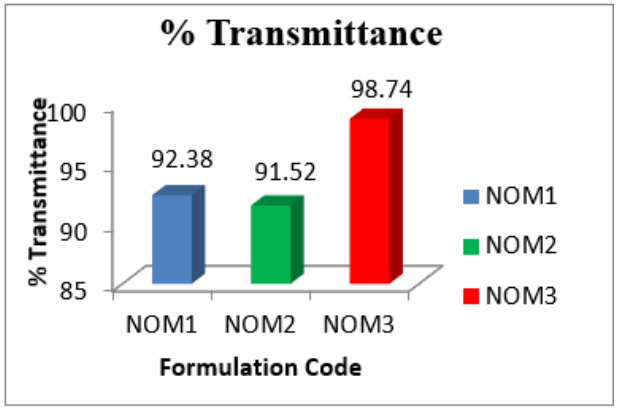

Fig.4: % Transmittance of NOM1-NOM3

Viscosity measurements: The Rheological behaviour of the microemulsion formulation was evaluated using an Ostwald viscometer at room temperature**.**

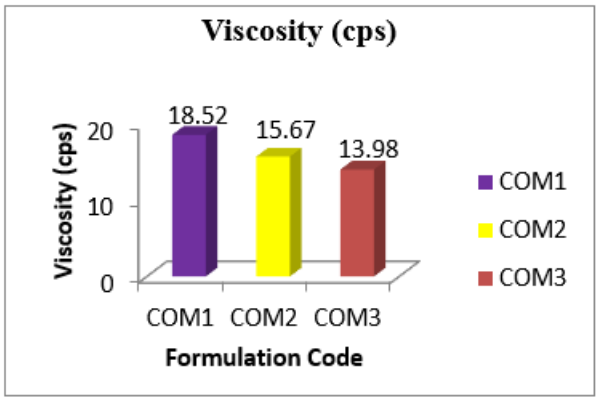

Fig.5: Viscosity of COM1-COM3

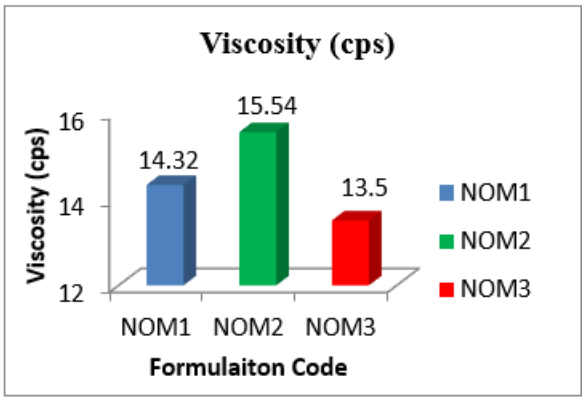

Fig.6: Viscosity of NOM1-NOM3

Measurement of pH: The pH of Miconazole Nitrate microemulsion formulations was determined by using a digital pH meter. The pH of each formulation was measured in triplicate, and the average values were calculated.[11]

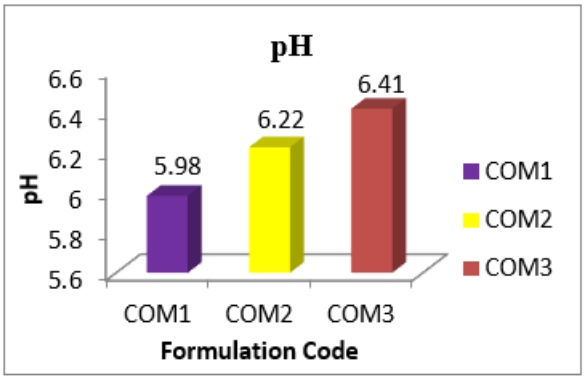

Fig.7: pH of COM1- COM3

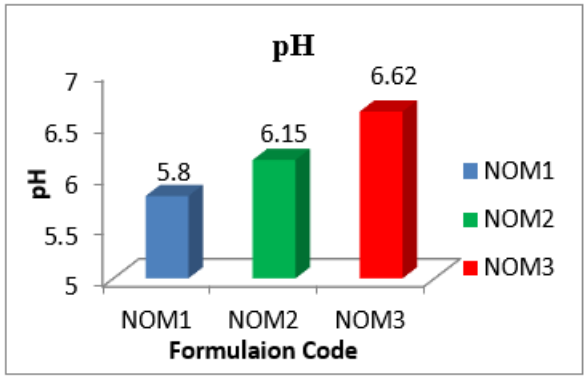

Fig.8: pH of NOM1- NOM3

Percentage Drug content: For the determination of drug content, about 1 mL of each microemulsion formulation was transferred to a 10 mL volumetric flask and dissolved in methanol. It was diluted appropriately and analysed by spectrophotometry at 272 nm.

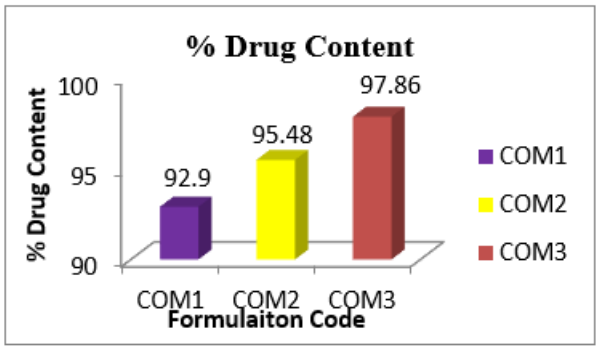

Fig.9: % Drug content of COM1-COM3

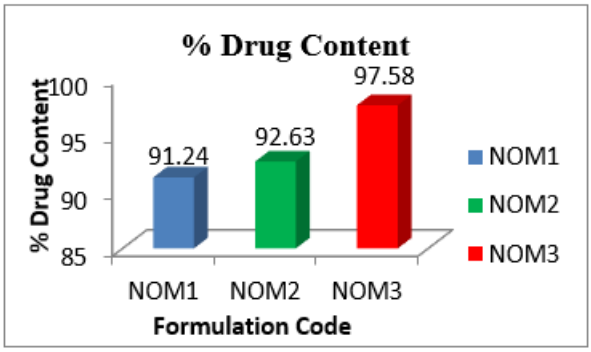

Fig.10: % Drug content of NOM1-NOM3

Measurement of globule size and zeta potential: The average globule size and zeta potential of the optimised microemulsions were measured using a Malvern ZetaSizer instrument at a temperature of 25°C.[12]

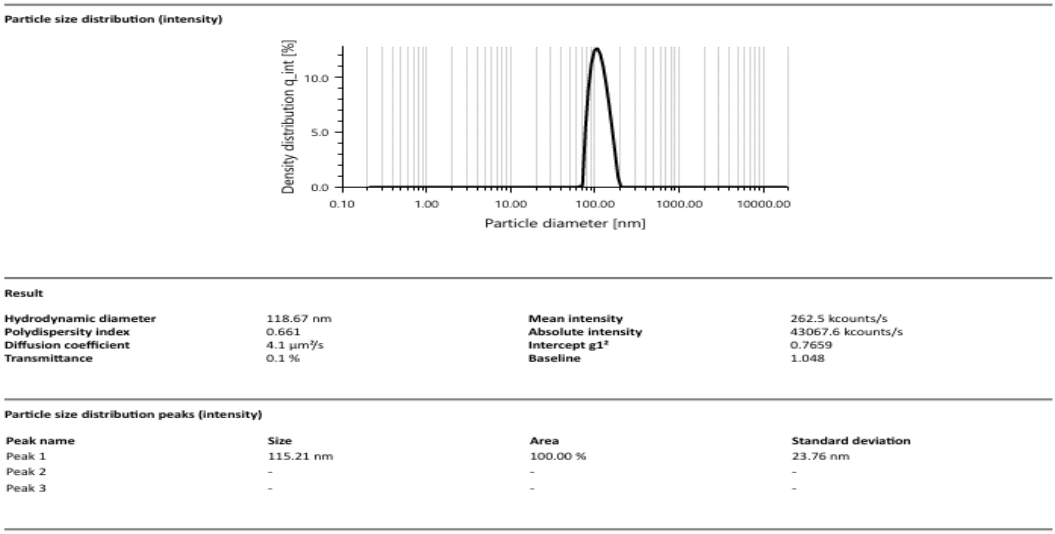

Fig.11: Globular size of COM3

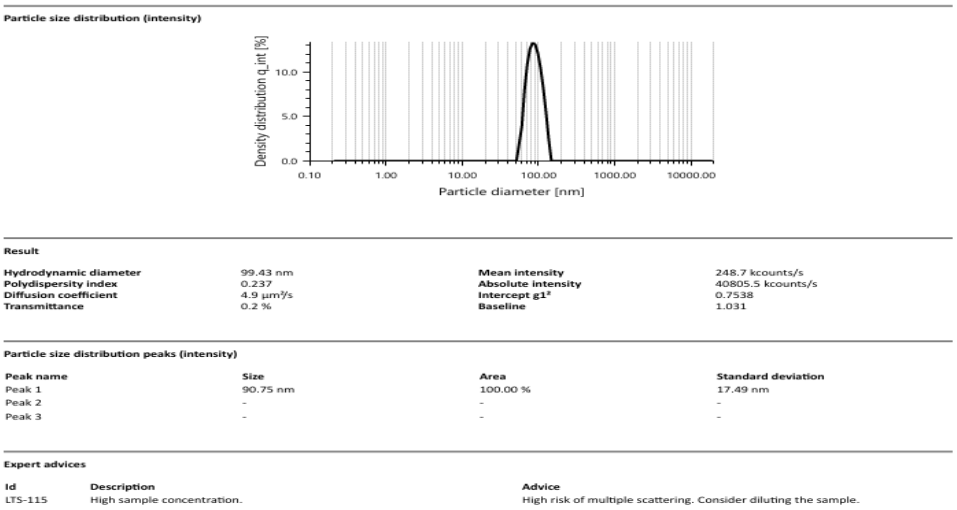

Fig.12: Globular size of NOM3

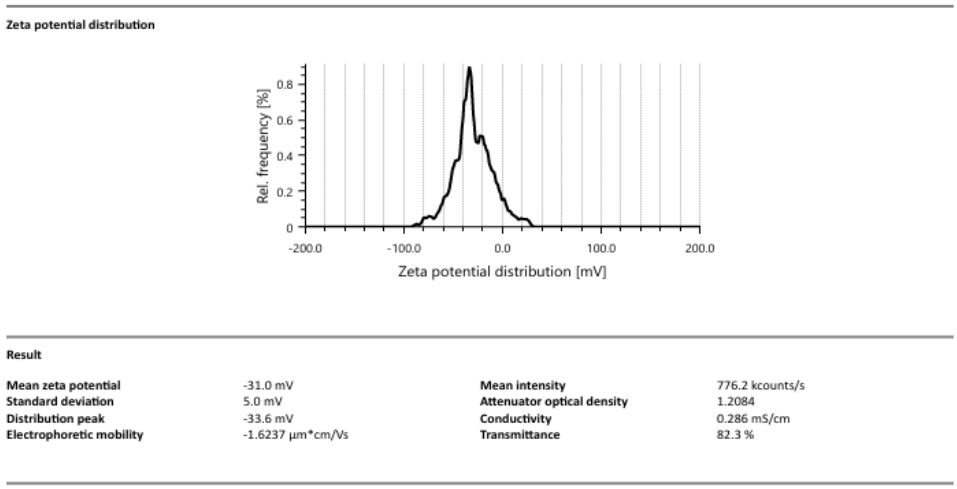

Fig.13: Zeta potential of COM3

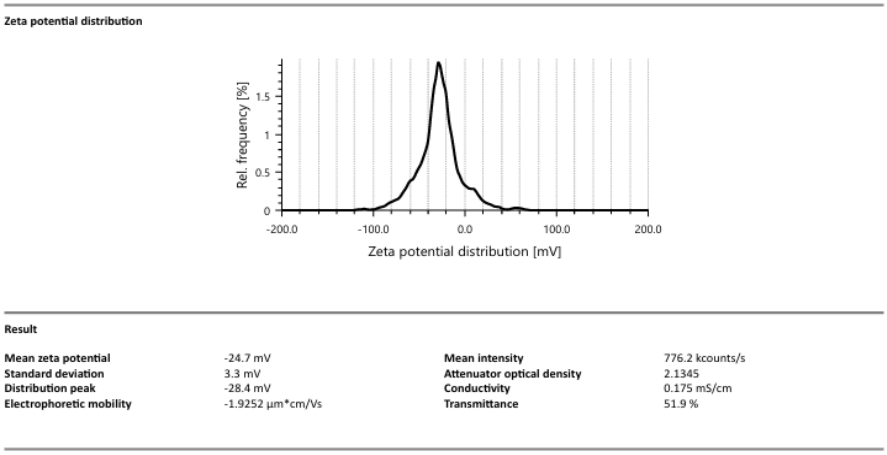

Fig.14: Zeta potential of NOM3

Surface morphology: Surface morphology of the optimised microemulsion formulations COM3 and NOM3 was determined by using a scanning electron microscope (SEM).[13]

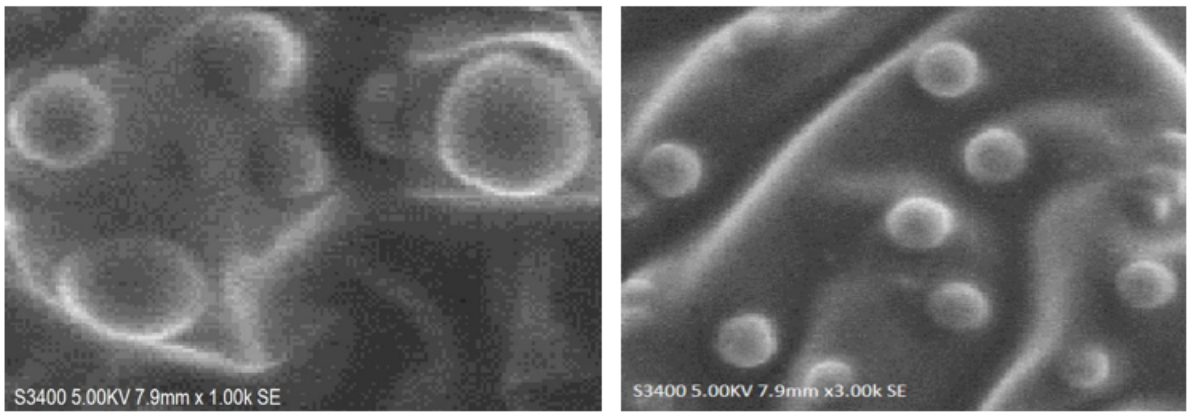

Fig.15: SEM of COM3 and NOM3 Microemulsion

In vitro diffusion study: The medium used was phosphate buffer at pH 6.8. Assembly of a diffusion cell for *in vitro* diffusion studies. The diffusion cell was designed according to the dimensions given. A diffusion cell with an effective diffusion area of 3.14 cm2 was used for *in vitro* permeation studies. The egg membrane was carefully mounted on the cell to avoid the entrapment of an air bubble beneath it. Intimate contact between the egg membrane and the receptor fluid was ensured by clamping the egg membrane tightly.14

The diffusion cells were placed on the receptor compartment with a magnetic stirrer. Then add 1 g of microemulsion to the donor compartment and 200 mL of phosphate buffer (pH 7.4) to the receptor compartment. The stirrer speed and temperature were kept constant throughout the experiment. Using a 1 ml pipette, 1 ml of sample was withdrawn at 60 min intervals (0 to 12 hours) from the receptor compartment, and the same volume was replaced with receptor medium to maintain sink conditions. The samples were appropriately diluted, and the absorbance was measured at 272 nm using a UV spectrophotometer.[15]

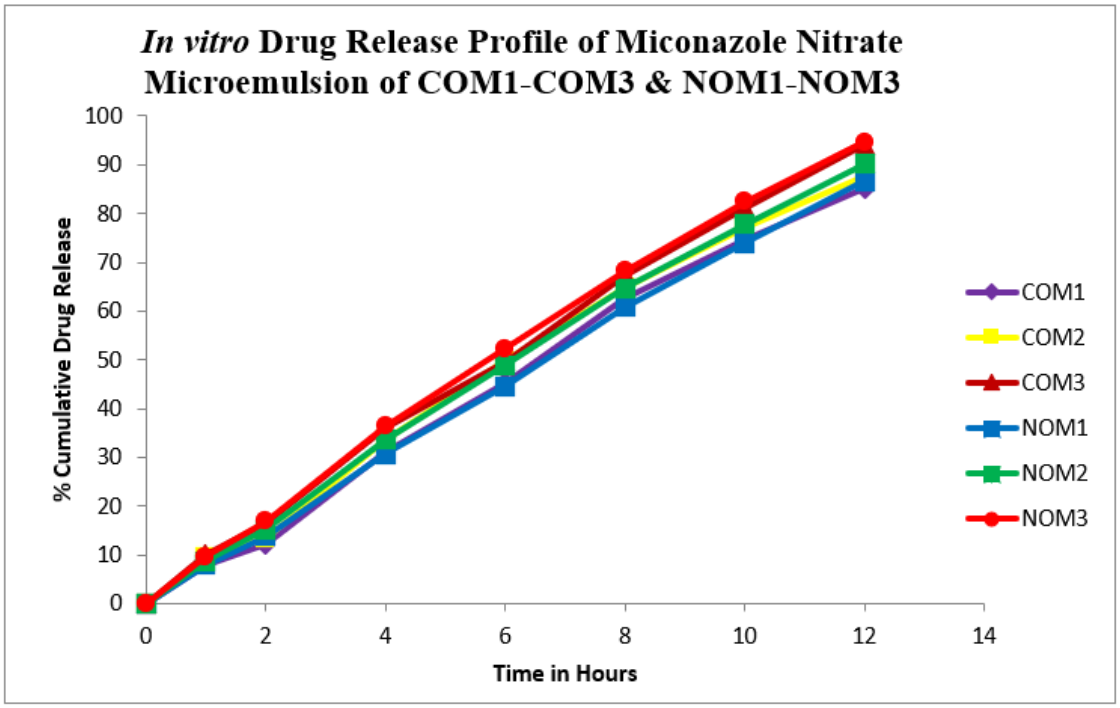

Fig.16: Comparison of Percentage Cumulative Drug Release of COM1-COM3

EVALUATION OF MICONAZOLE NITRATE MICROEMULSION GEL

Viscosity and Rheological studies: A Brookfield digital viscometer (Model LVDV–E, USA) was used to determine the viscosity and rheological properties of the microemulsion-based gel using spindle no. 6. 10 g of sample was placed in a small sample holder, and the viscosity of the gel was measured at 25°C.[16]

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

Determination of Percentage drug content: For the determination of drug content, 1 g of the gel formulation was weighed into a 10 mL volumetric flask and dissolved in methanol. It was diluted appropriately and analysed by spectrophotometry at 272 nm. [17]

***In vitro* release studies:** An *in vitro* drug release study was performed using a diffusion cell. The egg membrane was placed between the receptor and donor compartments. Microemulsion gel equivalent to 0.2 g was placed in the donor compartment, and the receptor compartment was filled with phosphate buffer pH 6.8. The diffusion cells were maintained at 37 ± 0.5℃ with stirring at 100 rpm throughout the experiment. At fixed time intervals, 5ml of sample was withdrawn at 1, 2, 4, 6, 8, 10, and 12 hrs, and the same volume was replaced with receptor fluid solution to maintain sink conditions. The collected samples were analysed using a UV spectrophotometer at λ max 272nm.[18]

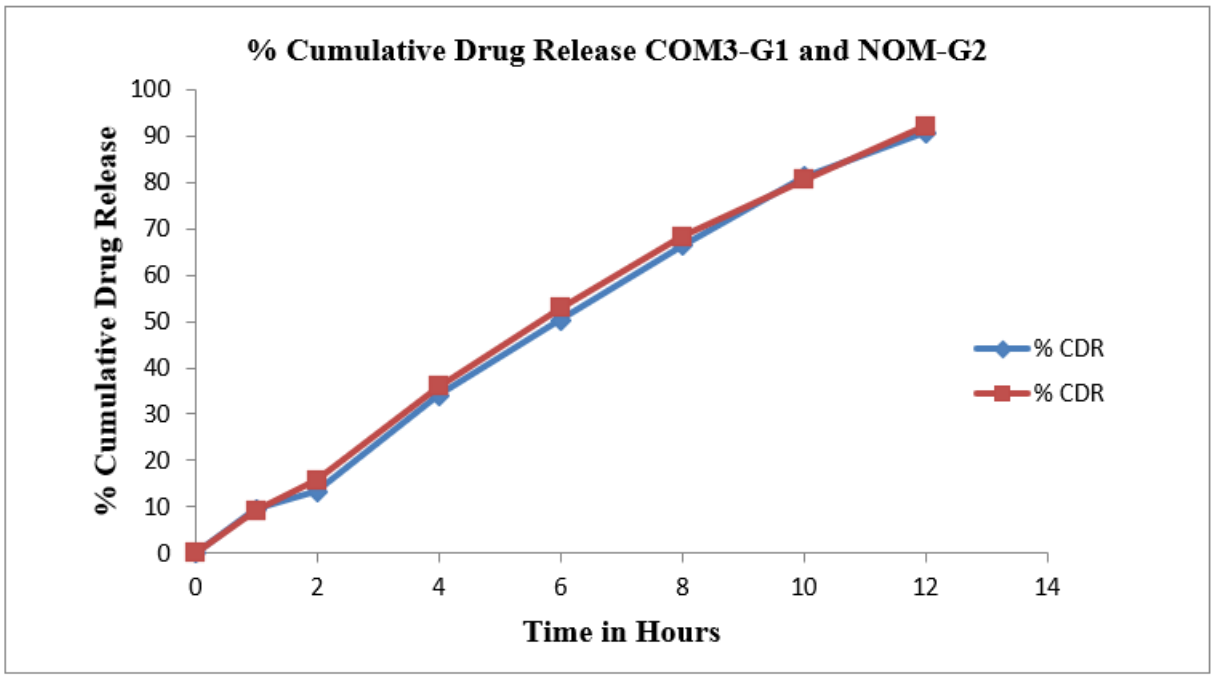

Fig.17: Percentage Cumulative Drug Release of COM3-G1 and NOM-G2

***In vitro* antifungal 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 (*Candida Albicans*) was spread on agar plates. 5 mm diameter holes were made in the agar plates using a sterile bore. 500µg/ml drug, 30µl of formulations (COM3 & NOM3) 20µl of essential oils (CO & NS) and 60mg of gels (COM3-G1 & NOM3-G2) were added into each hole separately. The plates were maintained at +4˚C for 4 hours to allow the solution to diffuse into the agar medium. All plate cultures containing *Candida albicans* were incubated at 28˚C for 48 hours. Zones of microbial growth inhibition around the well were measured and recorded after incubation.[19]

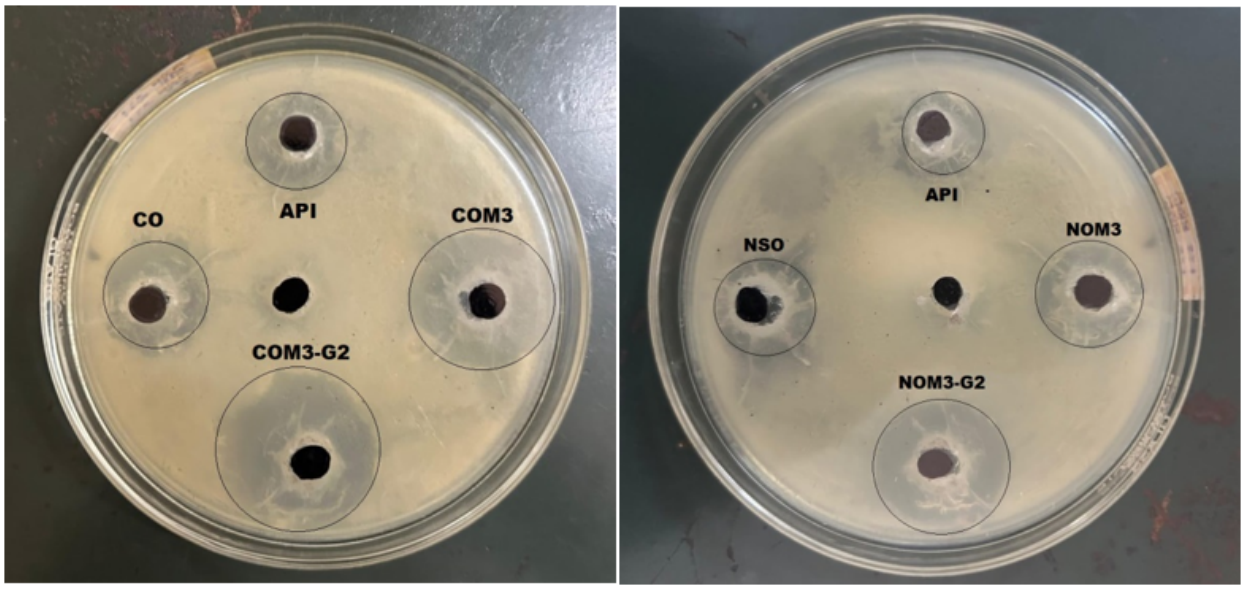

Fig. 18: Antifungal activity against *Candida albicans* using the agar well diffusion method.

***Ex vivo* skin permeation studies:** *An Ex vivo* drug permeation study was conducted using a Franz diffusion cell containing 150 ml of phosphate buffer (pH 6.8) using an excised goat skin. The goat's skin was obtained from a local slaughterhouse within 15 minutes of the goat's sacrifice. After removing the hairs, the skin was stored on ice-cold phosphate buffer (pH 6.8). The skin was immediately immersed in Ringer's solution. The freshly excised skin was mounted on the diffusion cell, and a 20 mg microemulsion gel containing an equivalent dose was placed on it. Throughout the study, the buffer solution in the chamber was maintained at 37±1°. At predetermined time intervals (1, 2, 4, 6, 8, 10, 12 hours), 1 ml of the sample was withdrawn and replaced with an equal volume of phosphate buffer. The samples were appropriately diluted and filtered, and absorbance was measured spectrophotometrically at 272 nm using a UV/Vis Spectrophotometer, taking phosphate buffer (pH 6.8) as the blank.

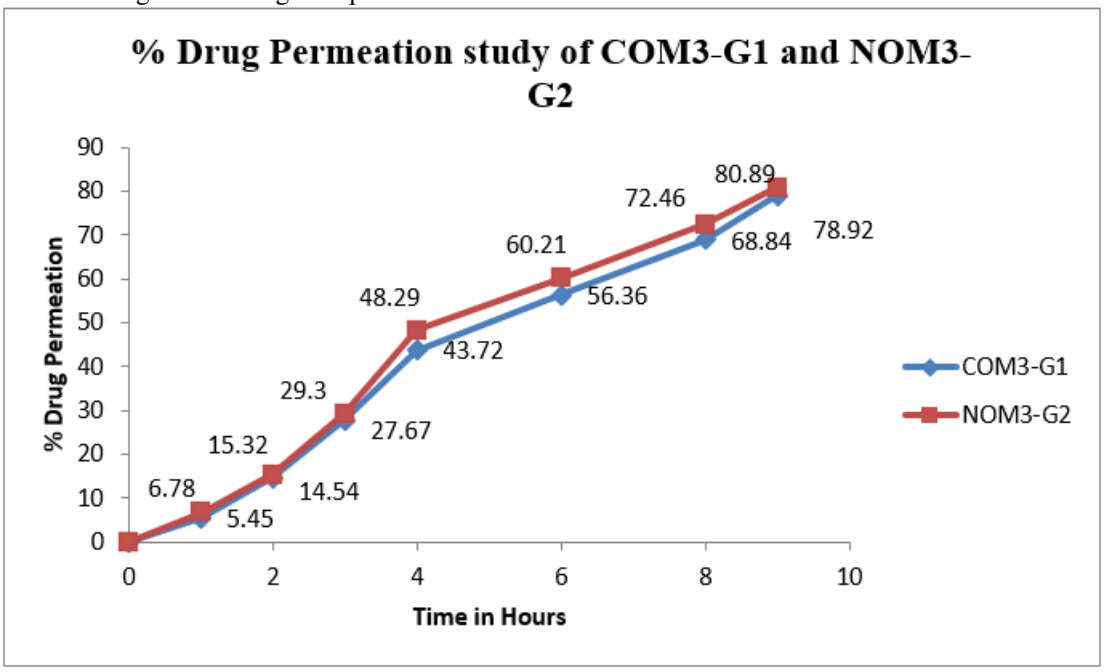

Fig.19: *Ex vivo* drug permeation profile of COM3-G1 and NOM3-G2.

Fig. 20: *Ex vivo* skin permeation studies

Spreadability: Spreadability was performed using two 7.5 cm glass slides. 350 mg of Microemulgel was weighed accurately and placed on one slide. Another glass slide was placed above it from a height of 5 cm. A weight of 5 g was placed on the upper slide, and after 1 minute, the diameter of the circle formed was measured in cm. The observed diameter indicates the type of gel. [20]

RESULTS AND DISCUSSION

For formulation development, a pseudoternary phase diagram was constructed using Tween 80 and propylene glycol at Smix ratios (1:1, 2:1, and 3:1) to identify the microemulsion region. Increasing the surfactant ratio expanded the microemulsion zone. Based on this, suitable proportions of oil, Smix, and water were selected for formulation. The prepared microemulsions were clear, transparent, and stable, with % transmittance above 90%, confirming the nanometric droplet size. The viscosity ranged from 13–19 cps, showing Newtonian flow, while pH values (5.9–6.6) were within the skin-compatible range.

The drug content was uniform (91–98%), and zeta potential values indicated stability without aggregation. The globule size (around 90 nm) and SEM images confirmed the presence of smooth, spherical droplets. No phase separation was observed after centrifugation, confirming good physical stability, and i*n vitro* drug release studies revealed sustained release for 12 hours, with 93.98% release from COM3 and 94.98% from NOM3. Nigella seed oil-based formulation showed slightly higher release than the Cinnamon oil-based formulation.

Stability studies showed no significant changes, confirming the formulation's stability. Optimised microemulsions (COM3 and NOM3) were converted into 1% Carbopol 934-based gels (COM3-G1 and NOM3-G2). These gels showed good spreadability, a suitable viscosity, and a pH (6.4–6.6) compatible with the skin. The optimised formulations COM3, NOM3, COM3-G1, and NOM3-G2 showed higher antifungal activity with inhibition zones of 23 mm, 21 mm, 26 mm, and 25 mm, respectively, compared to 19 mm for the pure drug, 16 mm for Cinnamon oil, and 17 mm for Nigella seed oil. It indicates a strong synergistic effect that enhances antifungal efficacy. *Ex vivo* permeation showed sustained drug release, with cumulative percentage release of 90.69% for COM3-G1 and 92.18% for NOM3-G2, confirming prolonged retention and controlled release. The drug content was above 94%, and the gels exhibited sustained release over 12 hours. All formulations exhibited high cumulative drug release (86–95%)**.** The kinetic modelling revealed that among the formulations (COM1-COM3), COM3 provided the best fit to the zero-order model (R^2 = 0.9972), indicating concentration-independent drug release. The Peppas model also showed a high correlation (R^2 = 0.9968), suggesting controlled release behaviour. The ‘n’ value (0.9237) indicates a non – Fickian transport mechanism, and among the formulations (NOM1-NOM3), NOM3 showed strong linearity in zero-order kinetics (R2 0.9962) along with a high Peppas correlation (R2 0.9977). The ‘n’ value (0.9394) further confirms non-Fickian transport, indicating a combination of diffusion and polymer relaxation mechanism. Among the gel-based

formulations, COM3-G1 and NOM3-G2 also demonstrated following zero-order kinetics with high correlation coefficients (R2 ≈ 0.9930-0.9939). The Peppas model indicated 'n' values of 0.9715 and 0.9649, respectively, suggesting super case II transport mechanisms. Among all formulations, NOM3 showed the highest drug release (94.73%), suggesting it as the optimised formulation for controlled drug delivery. Stability studies showed no major changes during storage.

Table no. 2: Data for different kinetic models resulting from the model fitting.

Formulation code	%CDR	Zero order	First order	Higuchi	Peppas	'n' values
COM1	85.042	0.9831	0.9685	0.9286	0.9891	1.0077
COM2	87.709	0.9652	0.9615	0.9383	0.9857	0.9576
COM3	93.985	0.9972	0.9047	0.9392	0.9968	0.9237
NOM1	86.570	0.9986	0.9456	0.9290	0.9976	0.9862
NOM2	90.140	0.9964	0.9385	0.9374	0.9976	0.9672
NOM3	94.730	0.9962	0.9024	0.9424	0.9977	0.9394
COM3-G1	90.690	0.9939	0.9463	0.9332	0.9851	0.9715
NOM3-G2	92.180	0.9930	0.9382	0.9431	0.9951	0.9649

Table no. 3: Intermediate stability studies at 30ºC±2ºC and 65±5% RH

Parameter	Duration in months					
	0		3		6	
	COM3	NOM3	COM3	NOM3	COM3	NOM3
Drug content	97.86	97.58	97.24	96.57	93.46	95.84
%CDR	95.68	93.89	94.20	92.86	93.12	91.65

Table No 4: Report of Antifungal Activity against *Candida albicans*

Sl. No.	Samples	Quantity Used	Zone of Inhibition in mm	Sensitivity
1.	Miconazole Nitrate	500µg/ml	17	sensitive
2.	COM-3	30µl	23	sensitive
3.	NOM-3	30µl	21	sensitive
4.	COM-3 GEL	60mg	26	sensitive
5.	NOM-3 GEL	60mg	25	sensitive
6.	Cinnamon oil	20µl	16	sensitive
7.	Nigella seed oil	20µl	17	sensitive

CONCLUSION

Pseudo-ternary phase diagrams (showing component ratios for microemulsion formation) guided the preparation of microemulsions. Water was added to various oil-to-Smix (surfactant and co-surfactant mixture) ratios. COM3 and NOM3 formulations were selected for their high transmittance, suitable viscosity, high drug content, and pH values near 6.2, all of which support enhanced drug release. These formulations underwent stability testing and were used to prepare gels with 1% w/w Carbopol 934 (gelling agent). The resulting gels demonstrated good spreadability, appropriate pH, high drug content, and acceptable viscosity. *In vitro* studies showed that COM3-G1 and NOM3-G2 provided sustained drug release. Both the optimised microemulsion and gel formulations showed stronger antifungal activity against *Candida albicans* than the pure drug and oils alone, indicating a synergistic effect. The larger inhibition zones reflected improved drug penetration and efficacy. Finally, *Ex vivo* permeation studies confirmed sustained drug release and prolonged retention, supporting effective topical delivery and potentially replacing the use of synthetic antifungal agents completely.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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