www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(12): 401-410

www.thepharmajournal.com
Received: 11-10-2019
Accepted: 15-11-2019

Jeet Gandhi
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical education and research, Gandhinagar, Gujarat, India

Disha Suthar
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical education and research, Gandhinagar, Gujarat, India

Hetal K Patel
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical education and research, Gandhinagar, Gujarat, India

Pragna Shelat
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical education and research, Gandhinagar, Gujarat, India

Punit Parejiya
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical education and research, Gandhinagar, Gujarat, India

Corresponding Author:
Jeet Gandhi
Research Scholar, Kadi Sarva Vishwavidyalaya, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

Development and characterization of lemongrass oil loaded microemulsion based gel for treatment of superficial fungal infections

Jeet Gandhi, Disha Suthar, Hetal K Patel, Pragna Shelat and Punit Parejiya

Abstract
Exploration of natural resources from biological metabolites of plants as an alternative to synthetic chemical moieties has proved to emerge as an ailment to various disorders. One of such are essential oils from plants that are belied to have immense medicinal values and have a potential to treat various disorders. One of a kind is lemongrass oil essential oil which is believed to have excellent mosquito repellent activity, perfumery and reported to have strong antifungal property. The objective of present study is to develop and characterize the topical micro emulsion based formulation containing lemon grass oil. The lemongrass oil formulation developed using pseudo ternary phase diagram and optimized by D-optimal design possessing 1.10% of oil, 6.1% of Smix and 91.3% of water was clear transparent light to pale yellow microemulsion with globule size of 14.60 ± 0.31 nm, zeta potential of 0.94 mV and PDI value 0.00133 indicating a stable microemulsion. The light to pale yellow coloured microemulsion based gel showed a drug release of 99.63 ± 0.41% within 180 minutes with retention of drug in skin layers of about 64.99 ± 0.42% and on the skin about 16. 39 ± 0.25% after 12 hours indicating the retention of drug at site of action. The skin irritation study and stability study indicated a non-irritant and stable MBG formulation at 40°C/75% RH for 6 months. Hence it can be said that the lemongrass oil loaded microemulsion based formulation can be used for treatment of fungal infections.

Keywords: Lemongrass oil, microemulsion based gel, *candida albicans*

1. Introduction
The scenario of current era is filled with occurrences of skin diseases like psoriasis, impetigo, antibacterial infections due to invasion of bacteria like *streptococci, staphylococci, klebsiella, E. coli*, etc. and many more. Fungi are other such invasive organisms that lead to severe fungal infections like superficial and vaginal candidiasis, ringworm, jock-itch, etc [1]. The species of fungi responsible for such infections are *candida albicans, candid glabrata, candida krusei, trichophyton rubrum, trichophyton megantrophes, tinea captis, tinea barbae, meselesia furfur, cryptococcus neoformans* to name a few [2]. The ways to treat the fungal infections are using either of the topical dosage forms flooded in the market like creams, gels, sprays and lotions or the oral medications containing synthetic active pharmaceutical moieties like triazole derivatives viz fluconazole, eberconazole, sertaconazole, ketoconazole, luliconazole or oral antifungal medications like tablets, capsules, suspensions containing fluconazole, itraconazole, micafungin [3].
Along with the treatment benefits, underlies the side effects associated with the triazoles like rash, itch, pruritis, skin hypersensitivity reactions, etc. Also, the long duration of therapy looses the patient compliance with the lapse of time and substantially results in development of resistance against the agents used for curing the infections [4]. The multidrug resistance developed against triazoles, echinocandins and allylamines and the adverse drug reactions has developed a need to explore the resources to heal the underlying infection [5].
Since the medieval ages the saints or Vedic's have been utilizing the natural resources like plants, herbs, shrubs, and parts of trees as an ailment to various disorders. Various parts of plants used for extraction of useful medicinal extracts are leaves, stems, roots, rhizomes, bark, flowers, seeds, buds and fruits due to presence of variety of phyto-chemical constituents present in them [6]. The abundance of these resources makes them useful and non-perishing source of therapeutically active aids [7]. Rhizomes of ginger were directly chewed or the decoction of the rhizomes of ginger are taken as an expectorant. Leaves of eucalyptus are

boiled in water and the vapour of these were inhaled to treat cough and cold [8]. Neem leaves since ages are used as an antibiotic to treat several bacterial infections [9]. Garlic is believed to have excellent antihyperlipidemic activity [10]. Clove buds and the extract is used as an analgesic and in various dental disorders [11]. Seeds of malkangdi are used as a brain tonic. Preparation using flowers of Shankhpushpi were taken to improve the cognition ability. Dried leaves of nagkesar are used in treatment of piles [12].

One such beneficial ailment that can be extracted from plants are the essential oils which have become the crust of research within this decade. The essential oils are believed to have immense medicinal values and have a potential to treat various disorders [13]. The essential oil of clove is supposed to have good antimicrobial properties [14]. Essential oil of peppermint is used as an expectorant, asthma, allergies and in gastric disorders like irritable bowel syndrome, gastritis, etc. The essential oil of chamomile is believed to have soothing and calming action and used as anti-stress medication [15]. The essential oil from garlic bulb is used to fight bad cholesterol and aids in weight loss [10].

Like-wise one such is the essential oil from leaves of lemon grass which is believed to have excellent mosquito repellent activity, perfumery and also reported to have antifungal property [16]. All though it is proven by a lot of research studies, the formulation development using lemongrass oil for its antifungal action is yet not explored. Thus, the aim of present study is to develop and characterize the topical microemulsion based formulation containing lemon grass oil.

D. Ganjewala *et al*. performed an extensive review on the chemical constituents present in essential oils of different cymbopogon species as the different spp. Of essential oils of Cymbopogon spare used in various therapeutic, cosmetics, food and perfumery industries. Cymbopogon essential oils and their constituents have been known to possess impressive antibacterial, antifungal, anti-yeast, insecticidal and insect repellent activities for a long time. The major components of lemongrass were found to be citral (70-90%), Linalool, geraniol, nerol, citranellol, linalyl acetate, geranyl acetate, Limonene, beta pinene, terpeneolone, myrecene, alpha terpineol, etc [17].

Lemon grass oil, a volatile oil extracted from the leaves of Cymbopogon citrus plant is a important essential oil used in many medical preparations. Simsek S. et.al. in his review described the biological properties of lemongrass oil. Lemongrass oil composition includes the citral in its maximum concentration. Investigations performed on lemongrass extracts showed much important therapeutic potentials such as anti-cancer, anti-hypertensive and anti-mutagenicity. Others include non-toxic properties, anti-diabetic, anti-oxidant, anxiolytic, anti-nociceptive and anti-fungal action [18].

Christiane da Bona da Silva et.al. studied the antifungal properties of lemongrass oil and the active citral against *candida albicans* species, a yeast filamentous fungus responsible for superficial mycotic dermal fungal infections like candidiasis. The antifungal assay was performed by disk diffusion assay on different *candida* species including *Candida albicans*, *candida krusei*, *Candida glabrata*, *Candida parapsilosis* and it was observed that lemongrass oil had a broad spectrum of activity against all *candida* species. Also, it was observed that the minimum inhibitory concentration of lemongrass oil and citral were found to be similar [19]

Diogo miron *et. al*. studied antifungal activity of monoterpenes like geranial, neral, citral, nerol and geraniol against dermatophytes and yeasts using microdilution assay method described by CLSI and explored the mechanism of action of these monoterpenes against the active dermatophytes and yeasts using sorbitol protective assay. Out of all the pathogenic yeast and dermatophytes studied, T. rubrum was found the most sensitive to monoterpenes. The sorbitol protection assay showed that the monoterpenes exhibited affinity related to ergosterol and thus the mechanism of action was attributed to ergosterol disruption and cell wall disruption leading to cell membrane destabilization and cell death [20] Similarly, Maria Clerya Alvino Leite et.al. studied the antifungal activity and mechanism of action of citral against *candida albicans*, the results showed the affinity of citral to the ergosterol in cell wall and hence it was confirmed that the mode of action of lemongrass oil for its antifungal action is cell membrane disruption by binding of citral to ergosterol and causing cell membrane disruption and cell death [21].

Microemulsions are considered as thermodynamically unstable clear transparent or translucent systems containing dispersed phase dispersed in a dispersion phase along with a surfactant and co-surfactant where the globule size of dispersed phase is not more than 100 nm. Microemulsions are better in terms of emulsions in terms of duration of action, providing targeted delivery to site of action and stability. Hence the microemulsion of lemongrass oil was a preferred choice rather than a macro emulsion [22].

2. Materials

Lemongrass oil was purchased from Chemical international private limited (Mumbai, India). Gift sample of cremophore EL was gifted by BASF chemicals (Mumbai, India). Isopropyl alcohol was procured from Sulab chemicals private limited (Vadodara, India). Gift sample of Carbopol 934 was obtained from Lubrizol (India). The strains of *candida albicans* ATCC 10321 was procured from MTCC (Chandigarh, India). Glycerine was obtained from Sulab chemicals private limited, (Vadodara, India).

3. Methods

3.1 Preformulation study of oil

Solubility of lemongrass oil was tested in various solvents as per Indian pharmacopeia vol. III. solubility was determined per ml of solvent to a fixed amount of the solute added accordingly. Specific gravity of lemongrass oil was determined using the specific gravity apparatus (Borosil, India). The weight of empty specific gravity bottle was noted and was filled to fixed volume of lemongrass oil and reweighed to obtain the actual weight of lemongrass oil, and then specific gravity was calculated in weight per ml. The refractive index of lemongrass oil was measured using abbe's Refractometer by placing the oil in the sample holder and the reading notation denotes the refractive index of oil. Acid value, Saponification value was performed as per the method described in IP 2014. Assay of lemongrass oil was carried out using UV spectrophotometer (UV series 1800, M/s Shimadzu, Kyoto, Japan). Lemongrass oil was dissolved in methanol and absorbance was measured and the assay was characterized using 99.99% pure citral as standard. Calibration curve of lemongrass oil was taken preparing various concentrations of lemongrass oil i.e. 2, 4, 6, 8, 10 mcg per ml in methanol at 264 nm in a UV visible spectrophotometer (UV series 1800,

M/s Shimadzu, Kyoto, Japan). Drug excipient compatibility study was conducted by physically mixing drug and excipients in 1:1 ratio and subjecting them to 40 ºC / 75% RH for one month and the drug excipient compatibility was evidenced by using FTIR as an analytical characterization tool.

3.2 Preparation of lemongrass oil loaded microemulsion (LGME) [23]

The microemulsion of lemongrass oil was prepared using cremophor EL as a surfactant and isopropyl alcohol as a co-surfactant using phase titration method where the different combinations of surfactant and co-surfactant in different ratio referred as Smix ratio, oil and water were utilized to investigate the phase behaviour by employing simple titration method. The ratio of oil with Smix were altered at 1:1, 1:2, 1:3, 1:4, 2:1, 3:1 and 4:1 to construct a total of 7 ternary phase diagrams. For the construction of pseudo-ternary phase diagram at each Smix ratio, the mixtures containing oil and Smix were prepared with volume ratios ranging between 1:9 and 9:1, and titrated drop by drop with water (Kept in continues stirring) at ambient temperature till the appearance of turbidity. The area of the ternary phase diagram with the maximum area coverage was considered as optimized ratio of Smix and the evaluation of optimized ratio of Smix was performed using CHEMIX software (Chemix Ver.1.1.1, India).

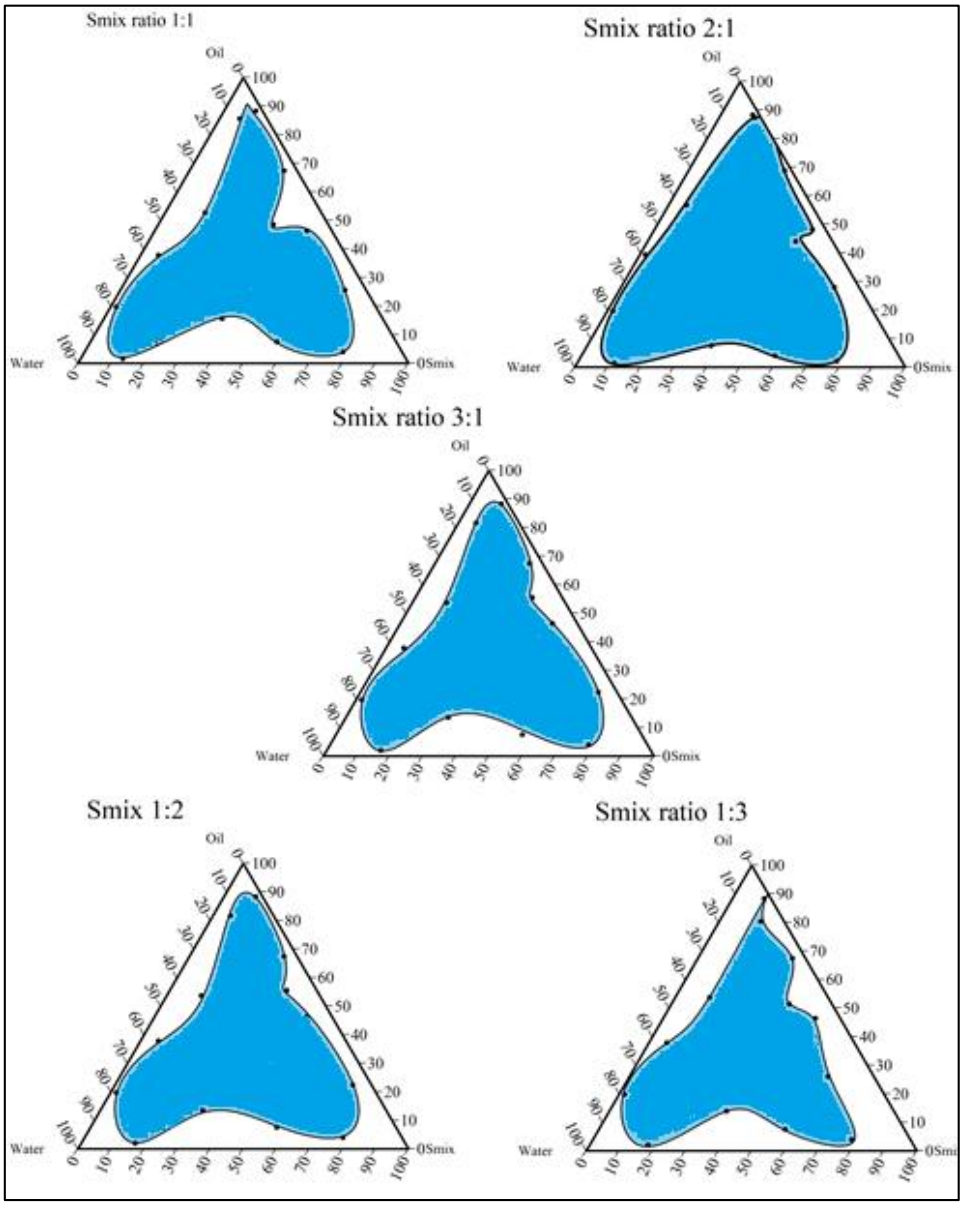

Fig 1: Pseudo-ternary phase diagram

3.3 Optimization using design of experiment [24]

The D-optimal design was selected to optimize and prepare the microemulsion of lemongrass oil in which the three levels of the independent factors i.e. oil (X_1) Smix (X_2) and Water (X_3) were chosen based on the pseudo ternary phase diagram. The range of components varied for the application of D-optimal design were X_1 from 0.5 to 1.5; X_2 between 6 to 16 and X_3 between 80 to 90. Hence, with the different combinations in common and 1 block design 12 runs were obtained including a single replicate run. The response selected were globule size % drug retention and % Drug penetration of the drug from the skin. Table 1 below enlists of experimental runs with the responses.

Table 1: Experimental runs with responses

Runs	Oil	S_{mix}	Water	Globule size (nm)	% Drug permeation	% Drug retention
1	0.336	0.334	0.330	12.79 ± 0.06	18.79 ± 0.13	81.17 ± 0.17
2	0.665	0.166	0.169	15.34 ± 0.04	25.69 ± 0.14	74.34 ± 0.23
3	0.167	0.666	0.166	10.76 ± 0.04	13.64 ± 0.09	86.33 ± 0.27
4	0.000	1.000	0.000	10.97 ± 0.03	10.37 ± 0.17	89.61 ± 0.34
5	1.000	0.000	0.000	19.72 ± 0.01	27.48 ± 0.31	72.49 ± 0.14
6	0.500	0.500	0.000	14.64 ± 0.03	20.41 ± 0.42	79.61 ± 0.26
7	0.500	0.000	0.500	14.84 ± 0.05	17.18 ± 0.05	82.73 ± 0.41
8	0.832	0.168	0.000	14.97 ± 0.01	19.04 ± 0.19	80.79 ± 0.27
9	0.000	0.000	1.000	12.68 ± 0.07	10.56 ± 0.21	88.93 ± 0.19
10	0.166	0.169	0.666	14.47 ± 0.06	13.94 ± 0.34	85.96 ± 0.09
11	0.000	0.000	1.000	12.93 ± 0.04	10.37 ± 0.29	89.04 ± 0.18
12	0.000	0.500	0.500	13.04 ± 0.03	12.18 ± 0.24	87.25 ± 0.37

The variables of design of experiment was evaluated using the design expert 7.0 software (version 7.0, Stat-ease.inc, Minneapolis, USA) which fitted to a cubic model for all the three responses based on the scheff's model. The polynomial equation was formed using the significant coefficients found by applying analysis of variance (ANOVA). The polynomial equation generated using the d-optimal design was represented in form of the counter plots and the optimized formulation was obtained using the polynomial regression equation or the counter plots and its superimposition called overlay plot which helps to find best suitable formulation. A check point was formulated to validate the correctness of design of experiment chosen.

3.4 Preparation of lemongrass oil loaded microemulsion based gel (LGMBG) [25]

The microemulsion based gel was developed by addition of Carbopol to the optimized microemulsion and addition of glycerine to the formulation was to provide the emoliency, humactancy and better texture properties to the gel formulation.

3.5 Evaluation of microemulsion [25]

Microemulsion is evaluated based on its physicochemical parameters like appearance, texture, odour, taste, transparency, Globule size, PDI value, viscosity and pH of microemulsion. Appearance and texture was observed visually while the transparency of the developed microemulsion was characterized using UV visible spectrophotometer. Globule size and PDI value was obtained by a Malvern zeta-sizer. The pH of microemulsion was measured using digital pH meter (Electroquip, India) and viscosity was measured using Brookfield viscometer (Brookfield CTS, USA) where 100 g of gel was loaded in a beaker and the spindle (S4) was plunged in the sample at 100 rpm and the viscosity was recorded accordingly. TEM was used to characterize the droplet size of micro emulsion (IIT, Bombay).

3.6 Evaluation of microemulsion based gel

3.6.1 Physicochemical properties of LGMBG [26]

The physicochemical properties of lemongrass oil loaded microemulsion based gel (LGMBG) like appearance, odour and texture was characterized visually. The pH of LGMBG was characterized using pH meter (Electroquip, India) viscosity was obtained using Brookfield viscometer (Brookfield, USA) using LV 61 spindle. The texture was visually observed and the spreadability test was performed by placing a weighed quantity of gel between two glass plates weighing (5*10) cm and measuring the distance of spreading of the gel. The dilute ability test was performed to confirm the phase of emulsion as well as mark the phase separation if any was observed.

3.6.2 *In-vitro* Drug release study [26]

In-vitro Drug release was performed using a Franz diffusion cell apparatus (25 sq.cm) containing a donor and a receiver compartment and a sampling port. The cellophane membrane (Thermofisher, USA) of 0.2 microns (which was soaked in acetate buffer pH 5.5 six hours before the study) was placed on between the donor and receiver compartment and 1 g of sample was placed on the cellophane membrane which was exposed to the receptor component which contained the acetate buffer pH 5.5. 1 ml of sample was performed at 15, 30, 60,120, 180, 360, 720 hrs and the sink condition was maintained replacing the same of buffer in the receptor compartment and was assayed at 238 UV max using UV visible spectrophotometer (UV series 1800, M/s Shimadzu, Kyoto, Japan).

3.6.3 *Ex vivo* permeation study [27]

Ex vivo permeation study was accomplished by using franz diffusion apparatus. The fresh sample of skin excised from the wistar albino rats weighing (150-250g) and the skin was made free from hair using a suitable hair removing applicator. The same was mounted above the receiver compartment and 1 g of LGMBG was applied on the skin. The receiver compartment was filled with phosphate buffer pH 7.4. The sampling was performed at time intervals of 15, 30, 60,120, 180, 360, 720 hrs and the sink condition was maintained replacing the same of buffer in the receptor compartment. The amount of drug transported from donor compartment to receptor compartment was estimated by UV visible spectrophotometer ((UV series 1800, M/s Shimadzu, Kyoto, Japan) at 238 nm. Cumulative amount of lemongrass oil permeated through the skin was calculated through the equation below.

$$Q_n = C_n \times V_o + \sum_{i-1}^{n-1} C_i \times V_i$$

Where Cn is the drug concentration in receptor medium at each sampling time, V_o is the volume of receptor compartment, C_i is the drug concentration at $_i^{th}$ sampling and V_i is the volume of the sample. The flux values were calculated from slope of the linear graph between the amounts of drug released per unit surface area versus time.

At the end of the study the skin retained on the surface was estimated by scrapping the surface and dissolving it into the media and was estimated using UV visible spectrophotometer

(UV series 1800, M/s Shimadzu, Kyoto, Japan) at 238 nm. Finally, the drug retained within the skin was found by tearing the skin into pieces macerating and homogenizing the fraction and analysing it using UV visible spectrophotometer ((UV series 1800, M/s Shimadzu, Kyoto, Japan) at 238 nm.

3.6.4 Drug content

Drug content of a LGMBG was measured by using UV visible spectrophotometer ((UV series 1800, M/s Shimadzu, Kyoto, Japan) at 238 nm, where the sample of formulation diluted in a methanol against the drug substance as a standard compound. The absorbance was obtained spectrophotometrically and the drug content was calculated on the equation mentioned below.

$$Cs * As = Cu * Au$$

Where, Cu is concentration of standard compound; As is the absorbance of the standard compound. Au is the absorbance of test compound and Cs is the absorbance of the test compound.

3.6.5 Anti-fungal study using plate method [28]

The antifungal assay of the LGMBG was performed using an agar plate well method. The fresh culture of *candida albicans ATCC 10321* is serially diluted to obtain $2.5 * 10^5$ CFU/ ml of organisms and was poured in the saboraud dextrose media. The plate was solidified hole of 6 mm was placed using a suitable borer and formulation weighing 1 g was added 1 g of DMSO and was poured into the well. The Petri plates were then incubated at 25 °C for 48 hours. The zone of inhibition was measured after 48 hours and was compared with clotrimazole (1g clotrimazole added to 1 g DMSO which was added to well) as a standard compound.

3.6.6 Skin irritation study [29]

Skin irritation study was performed on wistar rats weighing 150-250 g whose hairs on the dorsal and ventral side of abdomen were removed using a suitable hair remover. The animals were divided into two groups containing six animals each. The area was marked for 2 X 2 cm and the LGMBG was applied on the sides and the observation was made visually and the scores were given from 1 to 5 based on 1 – no erythema 2- slightly patchy erythema, 3- erythema, 4- patchy erythema and severe erythema.

3.6.7 Stability study [30]

The samples of lemongrass oil loaded microemulsion based gel were subjected to stability in three different conditions i.e. 25 °C/60% RH, 30 °C/75% RH and 40°C/ RH for 6 months. The samples were observed for parameters like Appearance, liquification, bleeding, drug content, pH of 10% dispersion, Viscosity and zone of inhibition.

4. Results and Discussion

Selection of excipients for the lemongrass oil microemulsion was based on the solubility of the oil in surfactants and co-surfactants. Amongst all the chosen surfactants, cremophore EL showed the highest amount of miscibility (2 g/ml) then other surfactants like tween 80, tween 20, span 80 and cremophore RH 40. Isopropyl alcohol was chosen as co-surfactant due to highest solubility compared to all other co-surfactants like propylene glycol and poly ethylene glycol 400. The lemon grass oil loaded micro emulsion was clear, transparent coloured pale yellow to yellow liquid with strong aromatic citrus odour and neutral/ characteristic taste. The solubility of Lemongrass oil is shown in figure 2.

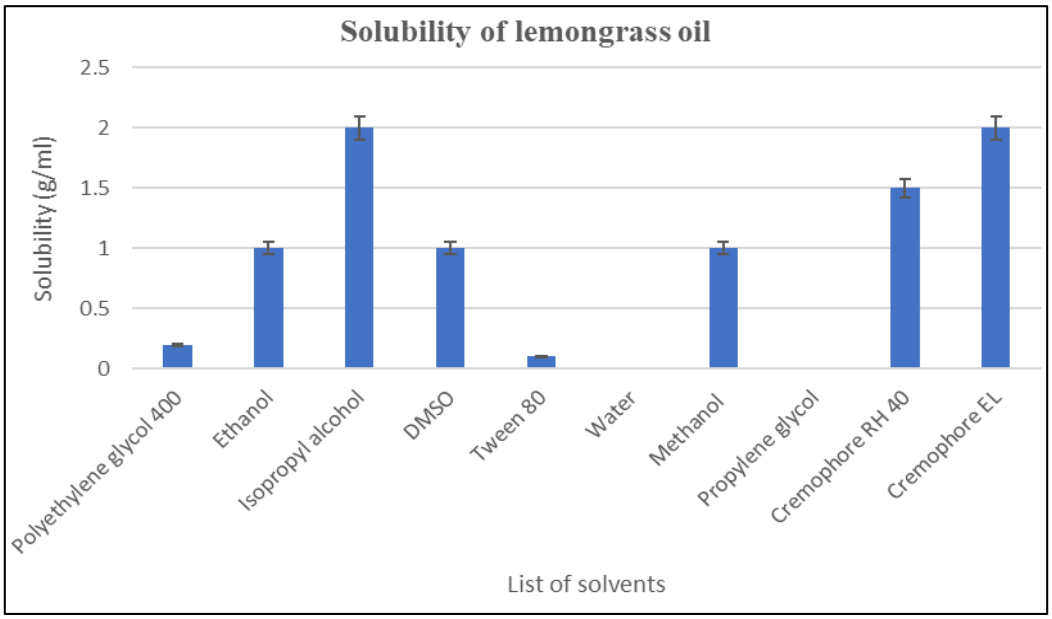

Fig 2: Solubility of lemongrass oil

The specific gravity of lemon grass oil was found to be 0.867 g/ml. The refractive index of lemongrass oil was found to be 1.478°. The acid value and saponification value of lemongrass oil was found to be 0.913 and 28.551, which were within the limits as per the Indian pharmacopeia 2014, which indicated that the oil was not rancid. The assay of lemongrass oil i.e. the citral compound was found to be 72.01%. The calibration curve of lemongrass oil is shown in the figure 3. The drug excipient compatibility study confirmed that there was no interaction between the drug and excipients that proves that the drug and excipients are compatible with each other.

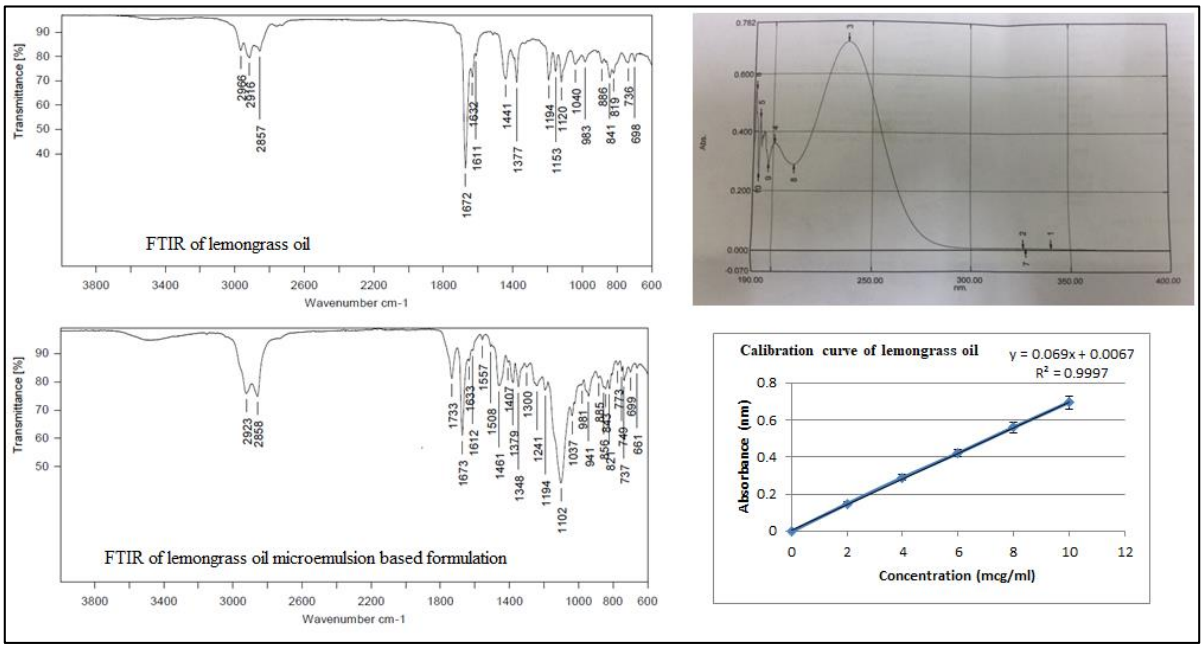

Fig 3: Preformulation study of lemongrass oil

5. Formulation optimization

The D-optimal design is a complex mixture design which is widely used for the formula optimization because of its ability to give the optimized formulation with lesser number of trials when compared to other experimental design. As from the ternary phase diagram for the independent factors Oil (X_1), Smix (X_2) and Water (X_3) the optimized Smix ratio for lemongrass oil microemulsion optimization was found to be 2:1 and the response variables to be evaluated were Globule size (nm),%Drug permeation (%) and% Drug retention (%). For the optimization of lemongrass oil loaded microemulsion 12 experimental trials were obtained running the D-optimal design including 1 repeat trial as shown in table 1.

The selection of the best model from the linear, quadratic or cubic leads to the derivation of correct polynomial regression equation for optimization of the microemulsion. Hence to obtain a suitable polynomial equation predicted sum of residual squares for three response variables (y1 to Y3) to determine suitability of a right model fit and generally the minimum value of PRESS statistic is considered as the best appropriate model. The table 2 below states the statistical data of the three responses evaluated. The analysis of variance evaluated for the responses ensured the r square value near to 1.0 confirming the suitability of the selected model. Also, the Prob.>F value for each of the variable was less than 0.05 indicating the suitability of the model. Following were the polynomial regression equations derived and used to demonstrate the relationship between the formulation components oil (X_1), S_{mix} (X_2), water (X_3), and the response variables Y_1 to Y_3. The following table below shows the summary model statistics and coefficients for the equation mentioned below.

$$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1X_2X_3 + \beta_8X_1X_2(X_1-X_2) + \beta_9X_1X_3(X_1-X_3) + \beta10X_2X_3(X_2-X_3)$$

Table 2: Model summary statistics and constraint values

Coefficient code	Y1	Y2	Y3
Model suggested	Cubic	Cubic	Cubic
β1	19.72	27.49	72.49
β2	10.97	10.38	89.61
β3	12.80	10.47	88.98
β4	-2.83	5.99	-5.98
β5	-5.70	-7.11	7.95
β6	4.60	7.11	-8.21
β7	-34.73	55.50	-49.30
β8	-31.05	-69.12	61.17
β9	25.81	117.66	-117.75
β10	-55.62	-105.08	102.83
R square	0.9995	0.9999	1
Adj. R-sq.	0.9971	0.9993	0.9999
SD	0.13	0.15	0.067
Mean	13.93	16.64	83.19
PRESS	22.96	666.78	69.40

Despite of the polynomial regression equation the other appropriate to confer the optimized formulation are the counter plots depicting the effect of variables on the responses chosen. The counter plots for globule size clearly reflects that with the increase in X_2 (S_{mix}) and X_3 (water) the globule size increased significantly and decrease in the concentration of water and S_{mix} increases the size of globules. Similarly, the rise in the oil concentration of the microemulsion the drug permeation is increased which may be due to the lipophilic nature of the drug that enhances the permeation of oil into the skin and the reverse relationship was seen in the% drug retention that attributed to rise in water level into the microemulsion. The overlapped view of all the counter plot gives the optimal region called as overlay plot which defines a space by a yellow region within which the formulation designed with the factor range will yield the formulation that produces desired responses values to deliver a robust and efficacious formulation.

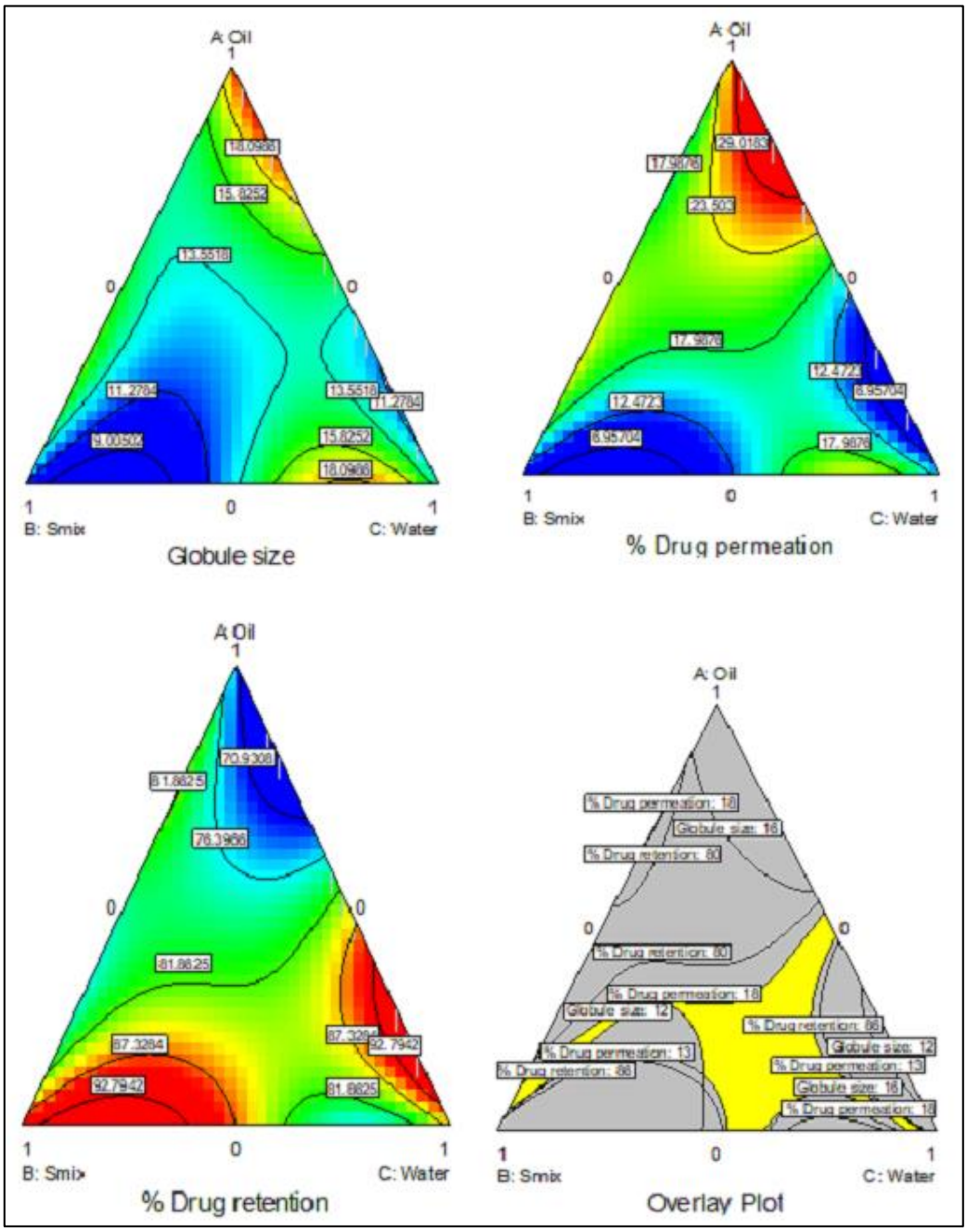

Fig 4: Counter plots for optimization of LGME

The finalized formulation was determine based on the numerical optimization technique provided by design expert software where the determination of the finalized formulation is based on the desirability value. Table No. 2 enlists the different constraint values for the formulation optimization. Based on the desirability criteria one formulation batch constraints along with predicted response values were suggested by the design software and a check point batch was formulated and the responses were compared to the predicted responses provided by the software. The predicted optimized formulation by the software contained 1.10% oil, 6.70 Smix and 92.21% water. The response value provided by the design expert and the experimental values were close and the deviation between the values was less than 1.0% as shown in Table 3. Hence, the D-optimal design was validated for optimization of microemulsion. The microemulsion was then formulated in to microemulsion based gel containing 0.85% carbopol 934 and 1.55% glycerine as a humectant.

Table 3: Predicted and experimental values

Parameters	Coded Values for factors and Check point batch expected Values as per DoE	Coded Values for factors and Experimental batch results
Oil	0.472	0.472
Smix	0.027	0.027
Water	0.501	0.501
Globule Size	14.48 nm	14.60 ± 0.31 nm
% Drug penetration	16.83%	16.11% ± 0.36%
% Drug retention	83.11%	83.64% ± 0.23%

The optimized formulation was clear transparent pale yellow to light yellow coloured microemulsion with strong aromatic citrus odour and characteristic neutral taste and smooth texture. The viscosity of the microemulsion was 0.809 cps and the pH of microemulsion was 5.97. The TEM of the microemulsion showed the dark spherical globules dispersed in the bright background. The% transmittance of microemulsion measured using UV visible spectrophotometer (UV series 1800, M/s Shimadzu, Kyoto, Japan) at 650 nm was 99.68% indicating a clear transparent microemulsion. The conductivity of microemulsion was 144 µS.cm^{-1} indicating oil in water nature of microemulsion. The zeta potential of microemulsion was found to be 0.94 mV and the PDI value was 0.00133 indicating a stable microemulsion. The globule size of LGME was found to be 14.60 ± 0.31 nm.

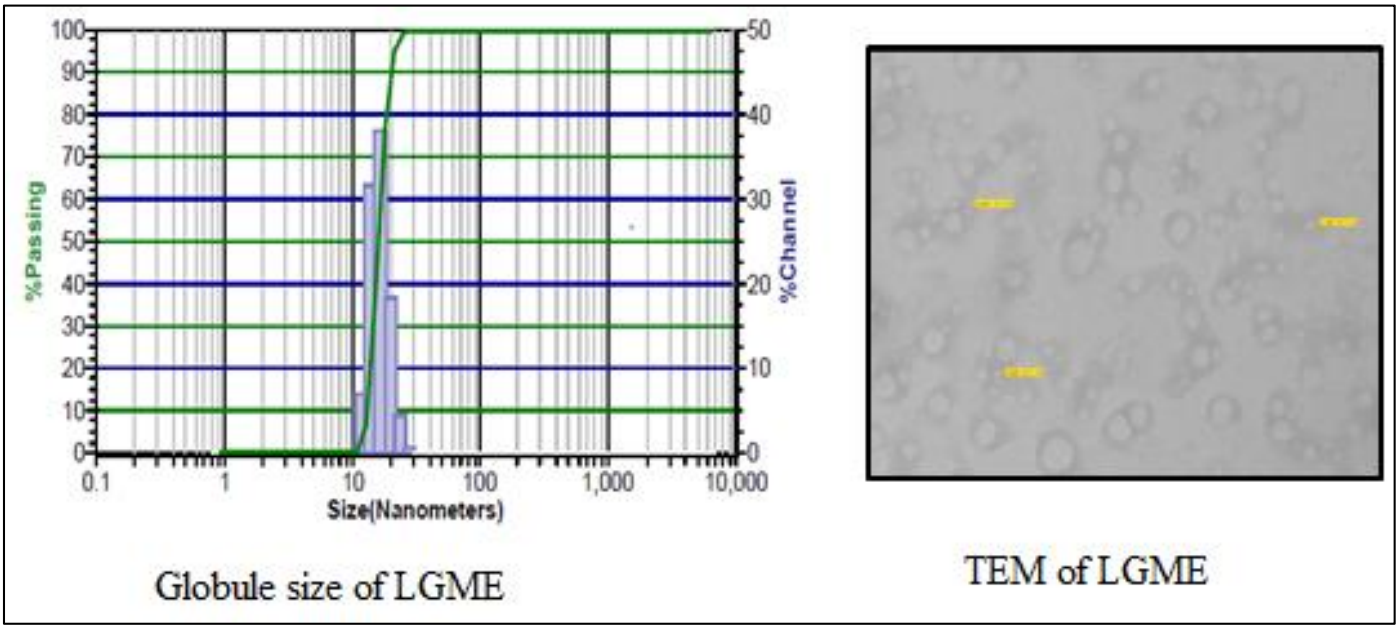

Fig 5: Globule size and TEM of LGME

The lemongrass oil loaded microemulsion based gel was light to pale yellow coloured clear transparent gel with a citrus aromatic odour, neutral taste and smooth texture. The pH of 10% dispersion of the gel was 6.17 and the viscosity was 14.39 ± 0.037 m.pas. sec^{-1}. Spreadability as a texture property, evaluated by spreadability tester was comparable to the marketed gel and the distance travelled by the LGMBG was 8 ± 1 mm while the distance travelled by the distance travelled by the marketed aloe vera gel was 7 ± 1 mm. The *in-vitro* drug release of LGMBG was 99.63 ± 0.41% ensuring complete release of the drug substance from the dosage form within 180 minutes. The drug content of LGMBG was found to be 98.67% ± 0.21%.

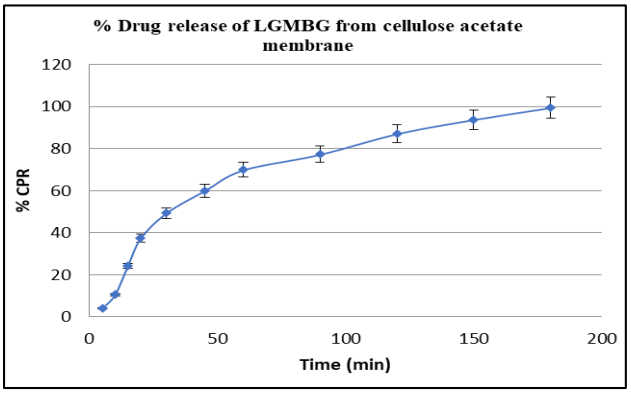

Fig 6: % *In-vitro* Drug release study

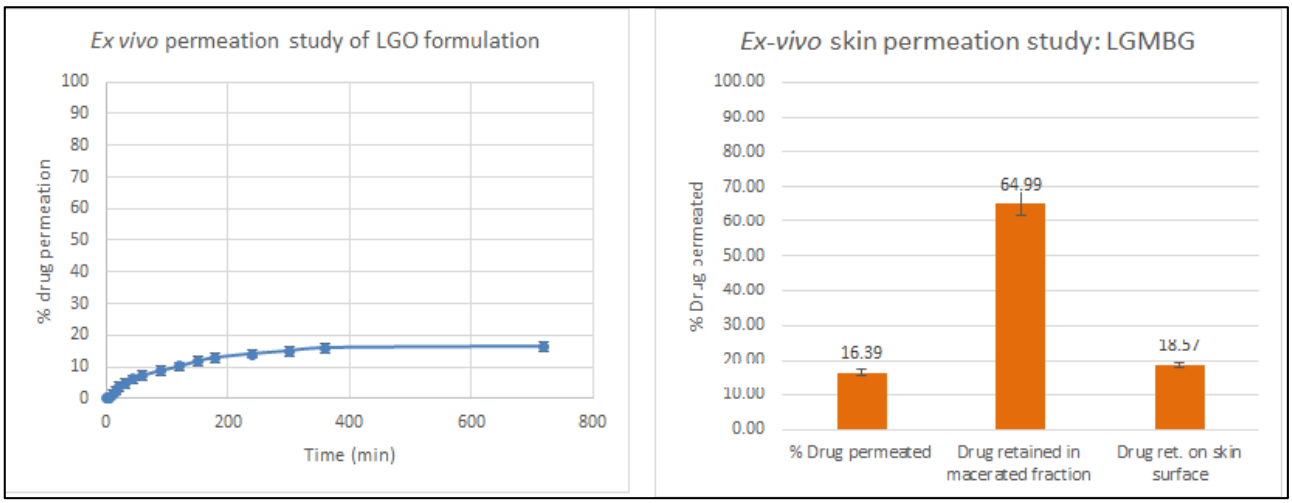

Fig 7: *Ex-vivo drug permeation study*

The *ex-vivo* drug permeation study showed that 16.39 ± 0.25% drug retained on the skin surface while 64.99 ± 0.42% dug was found within the skin layers and 18.57 ± 0.20% drug was permeated through the skin layers to the receptor compartment. This indicates the retention of drug at the site of infection. Thus, carbopol 934 was a suitable delivery carrier for the microemulsion based formulation. The zone of inhibition was found to be 16 mm and the marketed product (clobet gel) was 15 mm against *candida albicans*. Hence it can be said that the LGMBG was efficacious comparable to the available marketed formulation.

Table 4: Skin irritation study of LGMBG

Batch	Control						Formulation					
Animals	1	2	3	4	5	6	1	2	3	4	5	6
1 hr	0	0	0	0	0	0	0	0	0	0	0	0
24 hrs	0	0	0	0	0	0	0	0	0	0	0	0
48 hrs	0	0	0	0	0	0	0	0	0	0	0	0
72 hrs	0	0	0	0	0	0	0	0	0	0	0	0

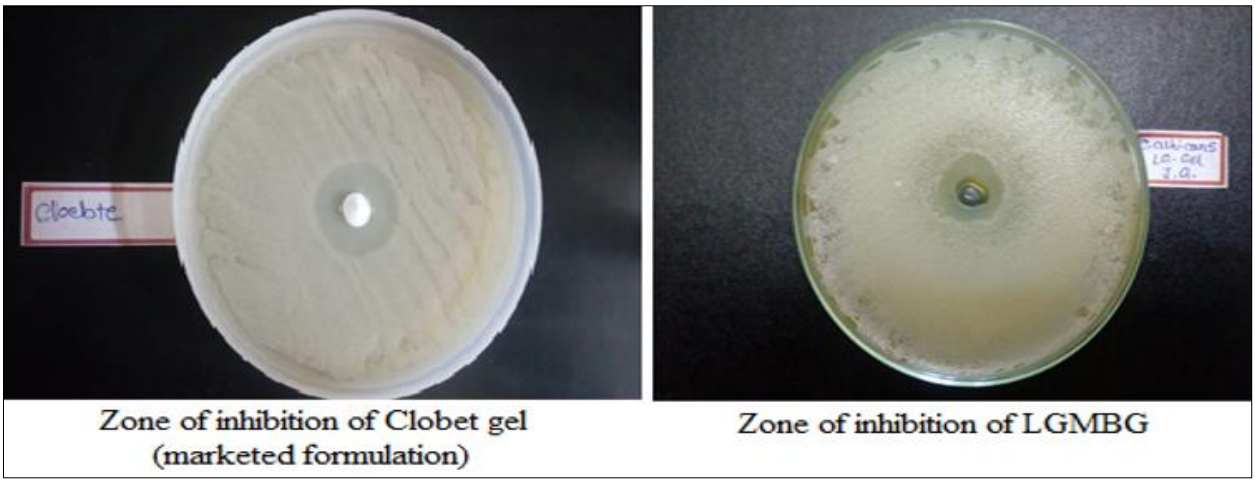

Fig 8: Zone of inhibition of LGMBG

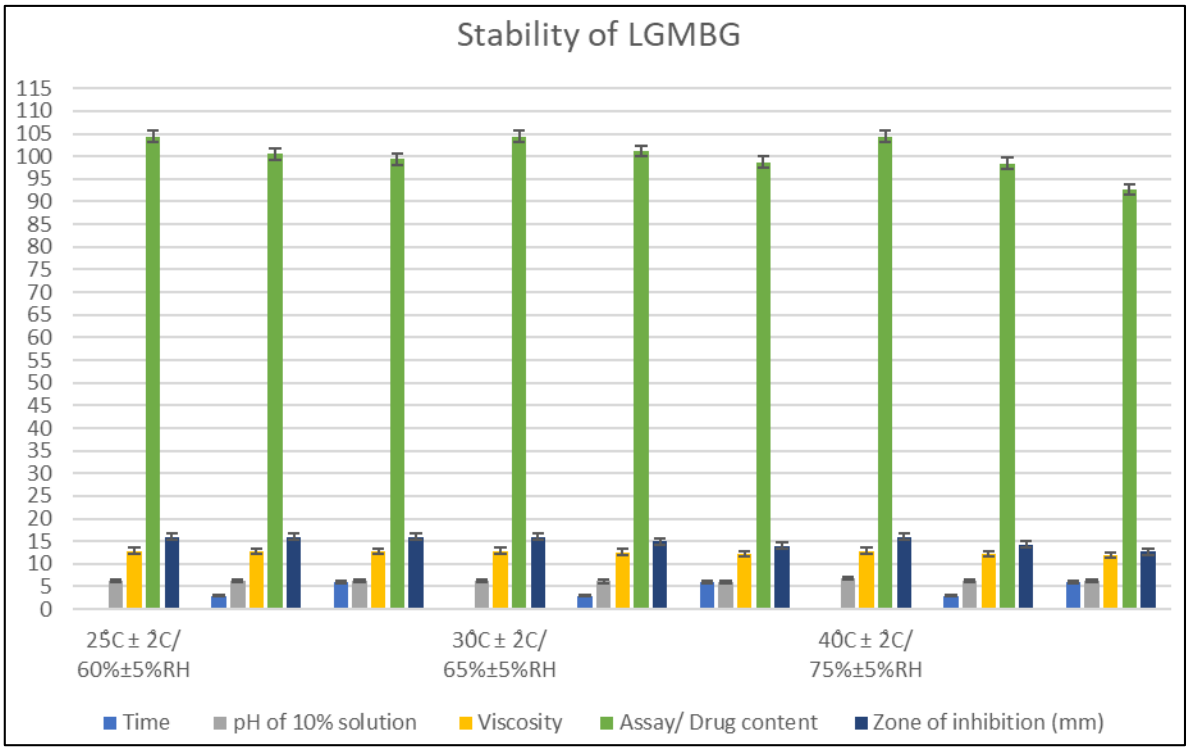

Fig 9: Stability study of LGMBG

The results of skin irritation study indicate that the LGMBG did not provoked any kind of irritable response to the skin. The stability study subjected at various condition indicated a stable microemulsion based gel formulation. The stability study of lemongrass oil MBG indicated a stable formulation without any signs of decolouration or phase separation along with consistent pH and assay of the formulation. Hence, it can be said that the lemongrass oil loaded microemulsion based formulation can be used for treatment of fungal infections.

6. Conclusion

The lemongrass oil formulation developed using pseudo ternary phase diagram and optimized by D-optimal design possessing 1.10% of oil, 6.1% of Smix and 91.3% of water was clear transparent light to pale yellow microemulsion with globule size of 14.60 ± 0.31 nm, zeta potential of 0.94 mV and PDI value 0.00133 indicating a stable microemulsion. The light to pale yellow coloured microemulsion based gel showed a drug release of 99.63 ± 0.41% within 180 minutes

with retention of drug in skin layers of about 64.99 ± 0.42% and on the skin about 16. 39 ± 0.25% after 12 hours indicating the retention of drug at site of action. The comparison of LGMBG with marketed clobet gel proved to have equivalent efficacy against fungal pathogens. The skin irritation study proved no irritation was provoked after the oil was micro emulsified using surfactant and cosurfactant mixture in water and the microemulsion based gel was found stable at 40 °C/75% RH for 6 months indicating its stability. Hence it can be said that the lemongrass oil loaded microemulsion based formulation can be used for treatment of fungal infections.

7. References

1. Achterman RR, White TC. Dermatophyte virulence factors: identifying and analyzing genes that may contribute to chronic or acute skin infections. International journal of microbiology. 2012; 2012:358305.
2. Nirmal K, Karki AA, Rita Charde, Manoj Charde, Bhushan Gandhare. An overview on antifungal therapy. International Journal of Biomedical and Advance Research. 2011; 02(01):69-85.
3. Debjit Bhowmik HG, Pragati Kumar B, Duraivel S, Sampath Kumar KP. Recent advances in novel topical drug delivery system. The pharma innovation. 2012; 1(9):12-31.
4. Bowyer P, Moore CB, Rautemaa R, Denning DW, Richardson MD. Azole antifungal resistance today: focus on Aspergillus. Current infectious disease reports. 2011; 13(6):485-91.
5. Dun E. Antifungal resistance in yeast vaginitis. Yale journal of biology and medicine. 1999; 72(4):281-5.
6. RS Medicinal Plants: A Review. Journal of Plant sciences. 2015; 1(1):50-5.
7. Marimuthu Krishnaveni CR, Kalaivani M, Krishnakumari G. A Phytochemical Study on *Muntingia calabura* L. Stem. Research J Pharm and Tech. 2015; 8(10):1423-8.
8. Ameur Elaissi. ea. Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complement Altern Med. 2012; 12(8):1-15.
9. Kumar VS. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed. 2013; 3(7):505-14.
10. Jo W. Garlic (*Allium sativum* L.) in the management of hypertension and dyslipidemia-A systematic review. Journal of Herbal Medicine. 2019; 4(1):21-8.
11. Xu JG, Liu T, Hu QP, Cao XM. Chemical Composition, Antibacterial Properties and Mechanism of Action of Essential Oil from Clove Buds against Staphylococcus aureus. Molecules. 2016; 21(9).
12. Gupta SKR. Study of medicinal plants in Kathua, J&K. Indian Journal of Plant Sciences. 2016; 5(3):66-78.
13. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy research: PTR. 2007; 21(4):308-23.
14. In Yang WSC. Antifungal Activities of the Essential Oils in *Syzygium aromaticum* (L.) Merr. Et Perry and Leptospermum petersonii Bailey and their Constituents against Various Dermatophytes. The Journal of Microbiology. 2007; 45(5):460-5.
15. NTaVG M. Antifungal investigations on plant essential oils. A review. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(2):19-28.
16. Nazzaro F, Fratianni F, Coppola R, Feo VD. Essential Oils and Antifungal Activity. Pharmaceuticals. 2017; 10(4):86.
17. Ganjewala D. Cymbopogon essential oils Chemical compositions and bioactivities. International Journal of Essential Oil Therapeutics. 2009; 3(1):56-65.
18. Kola Saheed Olorunnisola ea. Biological properties of lemongrass: An overview. International Food Research Journal 2014; 21(2):455-62.
19. Cristiane de Bona da Silva ea. Antifungal activity of the lemongrass oil and citral against *Candida* spp. Brazilian Journal of Infectious Diseases. 2008; 12(1):63-6.
20. Diogo Moron ea. Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. Revista Brasileira de Farmacognosia. 2000; 12(1):660-7.
21. Leite MCA. Evaluation of Antifungal Activity and Mechanism of Action of Citral against Candida albicans. Evidence-Based Complementary and Alternative Medicine. 2014; 1:9.
22. Kale SN, Deore SL. Emulsion Micro Emulsion and Nano Emulsion: A Review. Systematic Reviews in Pharmacy. 2016; 8(1):39-47.
23. Raza K, Negi P, Takyar S, Shukla A, Amarji B, Katare OP. Novel dithranol phospholipid microemulsion for topical application: development, characterization and percutaneous absorption studies. Journal of microencapsulation. 2011; 28(3):190-9.
24. Singh B, AK. Optimizing drug delivery systems using systematic "design of experiments. Part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst. 2005; 21(1):27-105.
25. Anoop Kumar VK. Pharmaceutical Microemulsion: Formulation, Characterization and Drug deliveries across skin. Int J Drug Dev & Res. 2014; 6(1):1-21.
26. Bhrutika Panseria. Formulation, development and evaluation of microemulsion based hydrogel of econazole nitrate. An international journal of pharmaceutical sciences. 2014; 5(2):86-107.
27. Ujwala Shinde ea. Design and Evaluation of Microemulsion Gel System of Nadifloxacin. Indian J Pharm Sci. 2012; 74(3):237-47.
28. Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. FEMS yeast research. 2005; 5(9):867-73.
29. Jianzhong Wang ea. Evaluation of dermal irritation and skin sensitization due to vitacoxib. Toxicology Reports. 2017; 4:287-90.
30. Sabale V. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. Int J Pharm Investig. 2012; 2(3):140-9.