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DESIGN AND DEVELOPMENT OF CITRONELLEA OIL MICROEMULSION FOR EFFECTUAL TOPICAL DELIVERY.

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Abstract:

The purpose of this study was to formulate topical microemulsion gel of citronella oil suitable for topical delivery. Citronella oil micro emulsion system with Tween 20 as Surfactant, PEG 200 as cosurfactant and citronella oil as oil was developed for topical delivery. Pseudo ternary phase diagram were constructed to identify the microemulsion region and a suitable composition was identified to formulate the microemulsion. Single isotropic region, which is considered as an O/W microemulsion was found in the pseudo ternary phase diagram developed at various Tween 20 and PEG 200 ratio using phase titration method. The developed microemulsion was characterized for clarity, Zeta potential, Viscosity, Globule size. Centrifugation studies were carried out to confirm the stability of the developed formulation. The formulation was thickened with a gelling agent carbopol 940 and xanthum gum, to yield a gel with desirable properties facilitating the topical application. The developed microemulsion based gel was characterized for pH, Spreadability, Viscosity. Optimized microemulsion based gel formulation was found to exhibit significant antifungal activity against candida Albicansspecies. Thus the present study indicates that developed topical microemulsion gel of citronella oil effective for treatment of fungal infection.

Key Words: Microemulsion, Citronella oil, Topical delivery, Antifungal, Microemulsion based gel, Candidaalbicans.

Introduction:

Microemulsions are clear, stable, isotropic mixture of oil, water and surfactant, frequently in combination with a cosurfactant. Microemulsions have several advantages such as enhanced drug solubility, good thermodynamic stability and higher transdermal permeability over conventional formulations. Many studies reported the use of microemulsion as topical drug delivery vehicles and show their ability to improve transdermal and dermal delivery properties.

Microemulsions have several permeation enhancement mechanisms such as increase in concentration gradient and thermodynamically activity towards skin.[1]

The concept of microemulsion introduced by Hoar and Schulman during 1940s. It is defined as system of water, oil and amphiphile which is an optically isotropic and thermodynamically stable liquid micro dispersion. Although microemulsion can be used to deliver drugs via several routs, these versatile systems have been extensively studies as vehicles for topical routes. Microemulsions are attractive vehicles for drug delivery because of their ease of formulation, thermodynamic stability and solubilisation properties. The present study was conducted to design and evaluate citronella oil micremulsion based dermal gel which provides fast absorption, increase the residence time of drug on skin thereby enhanced bioavailability, prolonged release and enables in reduction in dose.[2, 20]

Citronella oil is widely used as an insect repellent, Aromatherapy, Detergents, Fragrances and personal care product.[3]

Material and Methods[1]:

Citronella oil was purchased from Research Lab Fine Chemicals Industries (Mumbai), polysorbate 20 (Tween 20), polyethylene glycol 200, carbopol 940, xanthum gum and reagents were used as received. All other chemicals and reagents used were of AR and HPLC grades.

Construction of ternary phase diagram[21, 22]

Surfactant Tween 20was blended with cosurfactant PEG200 in fixed weight ratios 1:1, 2:1, and 3:1

Surfactant and cosurfactant mixture (Smix) then mixed with citronella oil at room temperature. For each phase diagram the ratio of oil to Smix varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 then water was added drop wise to each oil and Smix mixture undergo vigorous stirring

After equilibrium the samples were visually checked and determined as clear microemulsion.

Formulation of Microemulsion:

Formulation was further optimized with surfactant/ cosurfactant 3:1.Citronellaoil containing microemulsion were formulated by mixing oil, surfactant and cosurfactant with varying component ratio as described in Table no. 4 (A,B ,C ,D and E). Citronella oil was dissolved in surfactant and cosurfactant mixture (Smix) drop by drop with constant stirring on magnetic stirrer. Citronella oil containing microemulsion was obtained spontaneously on stirring the mixture at ambient temperature. All microemulsions were stored at ambient temperature[4, 5]

Characterization of Microemulsion[6 to 8]

Clarity:

It is observed visually, because microemulsions are clear and transparent.

Dilutability:

The microemulsions formed were diluted in 1: 10and 1: 100, ratios with double distilled water to check if the system shows any sign of separation.

pH:

pH determined by the digital pH meter. The pH values of microemulsion should be 5-6 range, which is an acceptable, non-skin irritating pH value.

Zeta potential:

It found to be or must be negative or neutral. This indicates that droplets of microemulsion having no charge that is system is stable. Zeta potential determined by using Zetasizer, Zeta potential is essentially useful for assessing flocculation since electric charges of particles influences the rate of flocculation.

Centrifugation:

Microemulsion system was centrifuge at 3000rpm for 30 min to determine whether system shows signs of creaming or phase separation. The system was observed microscopically for appearance.

Viscosity:

Viscosity of microemulsion was determined by using Brookfield rotational viscometer at various rpm. Each reading was taken after equilibrium of sample at the end of two min. The samples were repeated three times. Low viscosity is required to make them good in appearance and easy to handle and packed.

Globule size analysis of microemulsion:

Mean globule size of microemulsion should me minimum. It should be below 500nm range. It is acceptable for stable microemulsion.

Refractive index:

Refractive indexes are the net value of the components of microemulsion and indicate the isotropic nature of the formulation. The refractive index of the system was measured by an Abbe Refractivemetery placing one drop of the formulation on the slide in triplicate at 25°C

FORMULATION DEVELOPMENT OF MICROEMULSION BASED GEL[9]

Gelling agent such as carbopol 940(0.5gm) added into the 100ml water and stay to overnight then mix slowly added in the medicated microemulsion with the help of stirrer. The dispersion was neutralized by using trietahanolamine to obtain the gel.

Table 1: Formulation composition of microemulsion[4, 5]

Sr.no.	Formulation code	Citronella oil (ml)	Smix (ml)	Water(ml)
1	A	10	80	10
2	B	20	70	10
3	C	25	65	10
4	D	30	60	10
5	E	40	50	10

Table 2: Composition of selected microemulsion

Smix ratio	Formulation code	Citronella Oil (ml)	S mix (ml)	Water (ml)	Heating /cooling	Centrifugation	Freeze	Result
3:1	A1	10	80	10	pass	Pass	Pass	Selected
	A2	20	70	10	pass	Pass	×	
	A3	25	65	10	×	Pass	Pass	
	A4	30	60	10	pass	Pass	×	
	A5	40	50	10	pass	×	×	

CHARACTERIZATION OF MICROMULSION BASED GEL[10to12]

Spreadability:

Formulation based between two glass slide and 100gm weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight 100gm was added to the pan. The time in seconds required to separate the 2 slides was taken as measure of Spreadability.

Viscosity:

Viscosity of microemulsion based gel was determined by using Brookfield rotational viscometer at various rpm. Each reading was taken after equilibrium of sample at the end of 2 min. The samples were repeated 3 times.

pH:

The pH of microemulsion based gel was measured on digital pH meter standardized using pH 4.0 and 7.0 standard buffers before used. Microemulsion based gel 2.5gm was weighed accurately and dispersed in 20ml water. The measurement was pH of formulation was done in triplicate and mean values were calculated.

***In vitro* antifungal studies:**

The antifungal activity of microemulsion gel was determined using candida species. A single well isolated colony of candida albicans of at least 1mm diameter was picked from the culture plate and was streaked aseptically to agar slant, the slant was incubated 24 hours at 37°C after incubation the inhibition zone diameter was measured.

Results:

Pseudo ternary Phase study:
Pseudo ternary phase diagrams of oil, surfactant/ cosurfactant (S/ CoS) and water were developed using the water titration method. The mixtures of oil and S /CoS at certain weight ratios were diluted with water in a drop of wise manner. For each phase diagram at specific ratio of S/ CoS was formed by overtaxing for 5 min. then each mixture was titrated with water and visually observed for phase clarity and flow ability. The concentration of water at which turbidity to transparency and transparency to turbidity transitions occurred was derived from weight measurements. These values were then used to determine the boundaries of microemulsion domain corresponding to chosen value of oils, as well as S/ CoS mixing ratio. To determine the effect of drug addition on microemulsion boundary, phase diagram were also constructed in presence of drug using drug enriched oil as the hydrophobic component. Phase diagrams were then constructed using CHEMIX software. Phase diagram of 1:1, 2:1, 3:1 ratio of S/CoS are shown in Figure 1, 2, 3respectively.

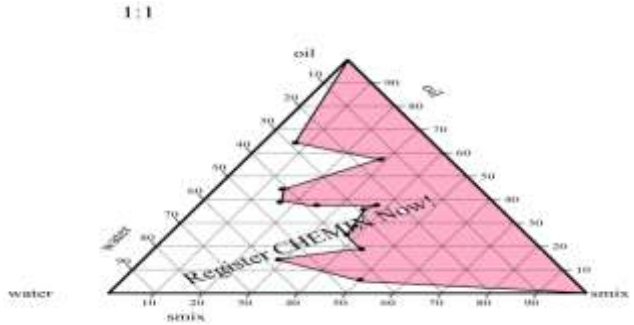

Fig 1: **Surfactant (Tween 20) and cosurfactant (PEG200)**

Surfactant (Tween 20) and cosurfactant (PEG200) was blended in ratio 1:1 to form S mix then citronella oil was added in S mix, water is added drop wise by vigours shaking. For each phase diagram ratio of oil to S mix varied at different concentration.

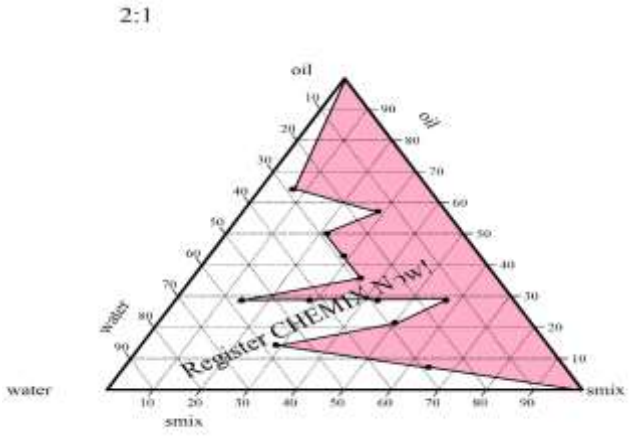

Fig 2: Surfactant (Tween 20) and cosurfactant (PEG200)

Surfactant (Tween 20) and cosurfactant (PEG200) was blended in ratio 2;1 to form S mix then citronella oil was added in S mix, water is added drop wise by vigours shaking. For each phase diagram ratio of oil to S mix varied at different concentration.

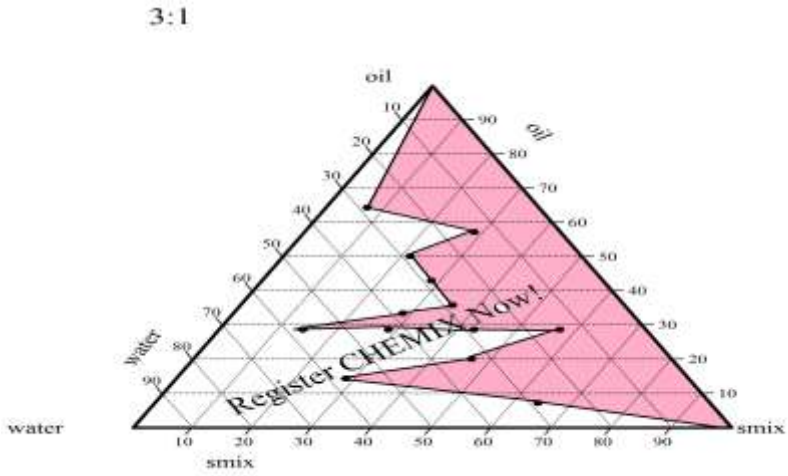

Fig 3: Surfactant (Tween 20) and cosurfactant (PEG200)

Surfactant (Tween 20) and cosurfactant (PEG200) was blended in ratio 3:1 to form S mix then citronella oil was added in S mix, water is added drop wise by vigours shaking. For each phase diagram ratio of oil to S mix varied at different concentration.

Table 3 ratio of surfactant to cosurfactant (Tween 20:PEG200)

Oil to Smix ratio	Citronella oil in ml	S mix (Tween 20:PEG 200) (ml)	Water (ml)
1:9	5.55	50	44.44
2:8	14.28	57.14	28.57
3:7	18.75	43.75	37.5
4:6	25	37.5	37.5
5:5	35.71	35.71	28.57
6:4	37.5	25	37.5
7:3	38.88	16.66	44.44
8:2	57.14	14.28	28.57
9:1	64.28	7.14	28.57

Table 4 ratio of surfactant to cosurfactant (Tween 20:PEG200)

Oil to Smix ratio	Citronella oil in ml	S mix (tween 20:PEG 200) (ml)	Water
1:9	7.14	64.28	28.57
2:8	14.28	57.14	28.57
3:7	21.42	50	28.57
4:6	28.57	42.85	28.57
5:5	35.71	35.71	28.57
6:4	42.85	28.57	28.57
7:3	50	21.42	28.57
8:2	57.14	14.28	28.57
9:1	64.28	7.14	28.57

Table 5 ratio of surfactant to cosurfactant (Tween 20:PEG200)

Oil to Smix ratio	Citronella oil in ml	S mix (Tween 20:PEG200) (ml)	Water
1:9	7.14	64.28	28.57
2:8	14.28	57.14	28.57
3:7	21.42	46.66	33.33
4:6	28.57	42.85	28.57
5:5	35.71	35.71	28.57
6:4	42.85	28.57	28.57
7:3	50	21.42	28.57
8:2	57.14	14.28	28.57
9:1	64.28	7.14	28.57

Phase behaviour:

The figure represents the Pseudo ternary phase diagrams for microemulsions systems along with the ratios of surfactant and cosurfactant, as 1: 1, 2:1 and 3:1. Each of vertices of triangle represents 100 % of each oil, water and surfactant and cosurfactant mixture (Smix). The change in the area of microemulsion region can be very well seen in the ternary phase diagram as the ratio of surfactant to cosurfactant was changed from 1:1 to 3:1.

The area of microemulsion did not show significant change when the surfactant to cosurfactant ratio was 1:1.But when ratio of surfactant to cosurfactant was 2:1 there was increased in microemulsion region, because of high concentration of surfactant. Phase diagram of 1:1 ratio shows larger microemulsion region and turbidity occurs. Therefore 1:1 ratio not consider for further formulation. Phase diagram of 2:1 ratio shows small microemulsion region but turbid or gel mass formed. Phase diagram of 3:1 ratio shows larger microemulsion region and microemulsion obtained after mixing with oil[21, 22] Therefore 3:1 ratio of surfactant to cosurfactant selected for preparation of microemulsion. Pseudo ternary phase diagram was plotted by using CHEMIX software.

Characterization of microemulsion[13, 14]

Thermodynamic testing of microemulsions:

In order to exclude the possibility of meta stable formulations, stress testing is required. Some representative formulations were taken from the o/w microemulsion region of the phase diagram constructed at Smix 3:1 for Tween 20 and PEG 200 and were subjected to the thermodynamic stability tests such as heating cooling cycle, freeze thaw cycle, and centrifugation. Results of

thermodynamically stable formulations were shown in table. Thermodynamic stability test confers long term stability to the microemulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability and will eventually phase-separate. Thermodynamically stable formulations were selected for further studies. In order to find out the stable microemulsion and to discard the unstable or metastable, microemulsions the placebo microemulsions were subjected to following thermodynamic stability studies.

Freeze thaw cycle:

Microemulsions were kept in deep freezer (at -20◦ C) for 24h. After 24h the microemulsions were removed and kept at room temperature. The thermodynamically stable microemulsions returned to their original form within 2-3 min. 2-3 such cycles were repeated.

Centrifugation studies:

Microemulsions after freeze thaw cycle were subjected to centrifugation studies where they were made to undergo centrifugation for 30 min. at 5,000 rpm in a centrifuge. The stable formulations did not show any phase separation or turbidity.

Heating cooling cycle:

Six cycles between refrigerator temperature (40C) and 400C with storage of 48 hours were performed.

Those formulations which were stable at these temperature, subjected to further study.

From the thermodynamic stability testing study of microemulsions, A1 microemulsion selected for further study.

Clarity:

It observed visually, because microemulsions are clear and transparent**.**

Dilutability:

The microemulsions formed were diluted in 1:10 and 1:100, ratios with double distilled water to check if the system shows any sign of separation, no sign of separation observed.

pH:

The pH of microemulsions found in the range of 5-6. The pH of optimized formulation A1 was 5.6. This required for the topical delivery because non skin irritating.

Zeta potential[15]

Zeta potential of A1 optimized formulation was –12.29 mv. The negative zeta potential indicates that a droplet of microemulsion having no charge that system is stable. Zeta potential determined by using Zetasizer. There was no charge on particles, so no flocculation of particles and microemulsion was stable.

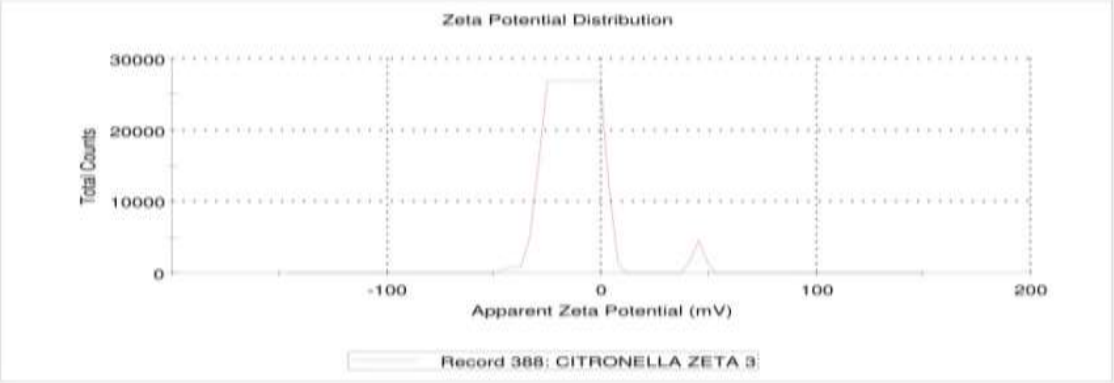

Fig 4: zeta potential

zeta potential of optimized formulation was found to be 12.29mv and it also shows there was no change on particles, so no flocculation of particles.

Centrifugation[16]

The microemulsion system was centrifuge at 3000 rpm. for 30 min to determine whether system shows signs of creaming or phase separation. The system was observed microscopically for appearance. The system does not show creaming or phase separation therefore microemulsion was stable.

Viscosity[17]

Viscosity of A1microemulsion was determined by using Brookfield rotational viscometer at various rpm. Viscosity of A1microemulsion was determined by using Brookfield rotational viscometer at 5, 10 20 and 50 rpm using spindle no.61. Each reading was taken after equilibrium of the sample at the end of two min. The samples were repeated three times. Low viscosity is required to make them good in appearance and easy to handle and packed.

RPM value increases then observed viscosity of microemulsion decreases that shows the applying the shear rate the formulation becomes less viscous. Therefore formulation pseudo plastic flow which is required for topical preparation.

Table 6: Viscosity value of microemulsion:

RPM	CP
5	600
10	300
20	220
50	160

Table 7: Viscosity value of microemulsion based gel

RPM	CP
5	60000
10	45900
20	16590
30	9100
50	5220

Globule size analysis of microemulsion[18]

Malvern particle size analyser used for globule size analysis of microemulsion. For size analysis approximately 0.1 ml microemulsion is added to 10 ml double distilled water in order to obtain the optimum scattering intensity. Average globule size of optimized microemulsion A1 was 193.0 nm. The observed globule size of formulation sufficiently minimum below than 500 nm. It is acceptable for stable microemulsion.

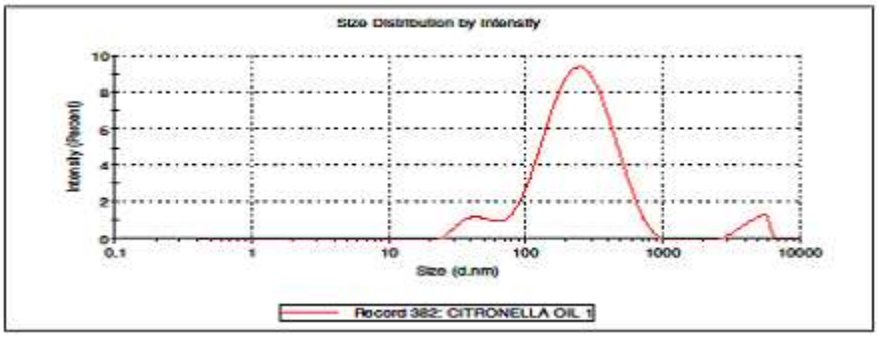

Fig 5: globule size analysis

It was done by using Malvern particle size analyzer and it was found to be193.0 for optimized formulation.

Refractive index:

The refractive index of the system was measured by an Abbe Refractometer by placing one drop of the formulation on the slide in triplicate at 25°C.Refractive index is the net value of the components of microemulsion and indicates the isotropic nature of the formulation. The Refractive index of A1 formulation was -1.47900 to 1.48000. The lowest values of refractive index was seen in A1 formulation, might be due to water content, as water has comparatively lower refractive index.

Characterization of microemulsion based gel[18, 19]

Spreadability:

Formulation placed between two glass slides and 100gm weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight 100 gm was added to the pan. The time in seconds require to separate the two slides was taken as measure of Spreadability. Optimized microemulsion gel shows the good Spreadability.

Viscosity of microemulsion based gel was determined by using Brookfield rotational viscometer at 5, 10 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two min. The samples were repeated three times.

Table 8: Spreadability value of microemulsion based gel[18, 19]

Formulation	Spreadability (gm.cm/sec)
1	5.02
2	5.15
3	4.95

pH:

The pH of microemulsion based gel was measured on digital pH meter standardized using pH 4.0 and 7.0 standard buffers before use. Microemulsion based gel 2.5 gm was weighed accurately and dispersed in 25 ml in water. The measurement of pH of formulation was done in triplicate and mean values were calculated. The pH of microemulsion based gel was 6.0. This pH value of gel optimum for topical delivery for non-sensitizing or nontoxic.

***In vitro* Anti-fungal Studies:**

The antifungal activity of microemulsion gel was determining using Candida species. A single well isolated colony of Candida albicans of at least 1mm diameter was picked from the culture plate and was streaked aseptically to agar slant, the slant was incubated for 24 hrs at 37 0C. After incubation the inhibition zone diameter was measured. The inhibition zone of microemulsion gel was found to be 25 mm. Therefore gel shows the good antifungal activity as compared to the Gentamicin and Nystatin

Fig 6: Antifungal activity of the formulation

This shows the antifungal activity of the formulation. It was done by using Candida albicans species.1 mm peaked from culture media and then streaked aseptically to agar slant, incubated for 24 hours 37 0C.inhibition zone of microemulsion gel was found to be 25mm and then compare with Nystatin.

Conclusion:

Microemulsion based gel formulation containing Citronella oil was successfully prepared with carbopol 940 and xanthum gum as a gelling agent to impart viscosity to the preparation as well as to sustain the action of the drug by increasing residence time. The contents of developed microemulsion based gel were Citronella oil as oil phase, Tween 20 and PEG 200 as surfactant and cosurfactant, double distilled water and carbopol 940 and xanthum gum as gelling agent. The formulated microemulsion and gel was optimized for viscosity, Spreadability, globule size, zeta potential, refractive index, clarity, Spreadability, pH, Antifungal activity. The *in vitro* antifungal activity of Citronella oil microemulsion based system was good as compared to nystatin and gentamycin. Therefore the microemulsion based gel of Citronella oil was prepared to obtain improved patient compliance. The very small droplet size causes a large reduction in the gravity force and the Brownian motion may be sufficient for overcoming gravity. This means that no creaming or sedimentation occurs on storage. Using a transdermal route instead of oral, eliminates systemic side effects, avoids first pass metabolism and maintains plasma drug levels for a longer period of time. The experimental work has demonstrated the design process of Citronella oil microemulsion formulation for topical delivery was capable of forming microemulsions. The effect of phase volume ratio and surfactant concentration supported for enhancement in solubilisation capacity and formation of stable homogenized Citronella oil microemulsion. Preparation of Citronella oil microemulsion using pseudo ternary phase diagram proved to be a sound approach to obtain stable optimized formulation. Pseudo ternary phase diagram suggested the concentration of oil phase; Smix and distilled water which were suitable for formulation microemulsion. Topical microemulsion gel of Citronella oil formulation which effective and stable. Hence Citronella oil based topical microemulsion gel proved the potential for topical delivery.

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