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DEVELOPMENT AND EVALUATION OF HERBAL ANTIMICROBIAL TOPICAL EMULGEL FORMULATION

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ABSTRACT:

Background: Skin diseases are a common problem among young adults. Skin diseases are a major health problem affecting a high proportion of the population in India. Acne and Cutaneous Mucormycosis are among them. Cutaneous Mucormycosis infections are invasive, often lethal, and they primarily affect immunocompromised patients. Skin Trauma is an important mode of acquiring the disease. The disease can be very invasive locally and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, fascia, and bone. The diagnosis of Cutaneous Mucormycosis is often difficult because of the nonspecific findings of the infection. Adequate therapy usually includes antifungal agents and extensive debridement; in spite of which, death and limb amputation frequently result. Acne vulgaris, a common skin disorder speculated in the age group of 15-25 years.*Propionibacterium acnes* (P. acne) is considered as the major skin bacteria that causes the inflammation have generally been treated by antibiotics. Within the light of the growing threat of antibiotic resistance, natural plant products are applied as a safer alternative.

Objective: The objective of the present research work was to investigate the herbal formulation containing curcuminoids and neem seed oil for its antimicrobial potential against Propionibacterium acne and Rhizopus oryzae.The Curcuminoids and Neem oil loaded Microemulsion based Emulgel was prepared to stop spreading of infection and to provide relief from the associated symptoms of Cutaneous Mucormycosis and agent of acne vulgaris.

Methods: In India the most common causative agent of Mucormycosis and Acne is *Rhizopus oryzae* and *Propionibacterium acne.* Therefore, we choose Curcuminoids and Neem oil as an antimicrobial agent that had already reported antimicrobial properties against the various different species. The screening of oil phase for microemulsion formulation was selected based on the solubility study of Herbal components. The screening of surfactant and co-surfactant was selected based on the % transmittance for the formulation for stable microemulsion. The prepared Curcuminoids and neem oil loaded microemulsion was characterized by globule size or particle size, polydispersity index, Zeta potential and viscosity. The optimized Microemulsion was converted to Emulgel using 974 P NF as a gelling agent. The Prepared Emulgel was characterized by different evaluation parameters and antifungal study is under investigation.

Results: The result showed that the average particle size or globule size of Microemulsion was 11.6 nm, polydispersity index was 0.131, Zeta potential was -34.7 mV and viscosity was found to be 500 centipoise. The texture of formulated emulgel was found to be greasy, with mild odour and easily

spreadable. The viscosity of the prepared Emulgel was found to be under standard limits. The In-vitro release study showed satisfactory drug release after 5hrs.
Conclusion: The herbal herbal antimicrobialgel was successfully formulated and can be used for the treatment of acne, cutaneous mucormycosis and related symptoms.Further preclinical and clinical studies needs to be undertaken to prove its exact mechanism of action and safety.
KEYWORDS: Cutaneous Mucormycosis, Acne, Herbal, Topical, Emulgel, Antifungal, Anti-acne, *Rhizopus oryzae, Propionibacterium acne.*

INTRODUCTION:
Mucormycosis (previously called zygomycosis) is a serious but rare fungal infection caused by a group of molds called mucormycetes. These molds live throughout the environment. Mucormycosis mainly affects people who have health problems or take medicines that lower the body's ability to fight germs and sickness. It most commonly affects the sinuses or the lungs after inhaling fungal spores from the air. It can also occur on the skin after a cut, burn, or other type of skin injury. [1] There are different types of Mucormycosis Viz.

1. Rhinocerebral (sinus and brain) mucormycosis is an infection in the sinuses that can spread to the brain. This form of mucormycosis is most common in people with uncontrolled diabetes and in people who have had a kidney transplant.

2. Pulmonary (lung) mucormycosis is the most common type of mucormycosis in people with cancer and in people who have had an organ transplant or a stem cell transplant.

3. Gastrointestinal mucormycosis is more common among young children than adults, especially premature and low birth weight infants less than 1 month of age, who have had antibiotics, surgery, or medications that lower the body's ability to fight germs and sickness.

4. Cutaneous (skin) mucormycosis occurs after the fungi enter the body through a break in the skin (for example, after surgery, a burn, or other type of skin trauma). This is the most common form of mucormycosis among people who do not have weakened immune systems. Cutaneous and subcutaneous disease may lead to necrotizing fasciitis, which has a mortality approaching 80%. Can look like blisters or ulcers, and the infected area may turn black.

5. Disseminated mucormycosis occurs when the infection spreads through the bloodstream to affect another part of the body. The infection most commonly affects the brain, but also can affect other organs such as the spleen, heart, and skin. [2]

The causative microbe includes *Rhizopus species*, *Mucor species*, *Rhizomucor species*, *Syncephalastrum species*, *Cunninghamella bertholletiae*, *Apophysomyces species*, and *Lichtheimia species* (formerly Absidia). [3]

Cutaneous disease can be very invasive locally and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, fascia, and even bone. Secondary vascular invasion may also lead to hematogenously disseminated infection of the deep organs. Other symptoms include pain, warmth, excessive redness, or swelling around a wound.[4]

Topical drug delivery can be defined as the application of a drug containing formulation to the skin to treat cutaneous disorder directly. Emulgel is emerging field for the topical drug delivery, and till date it has less marketed product, so it is interesting and challenging to focus on emulgel.

Many widely used topical agents like ointment, cream, lotion have many disadvantages. They have very sticky causing uneasiness to the patient when applied. Moreover they also have lesser spreading coefficient and need to apply with rubbing. And they exhibit the problem of stability also. Due to all these factors within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations.

Gels having various advantages has still limitation in the delivery of hydrophobic drugs so to overcome this limitation and adore the delivery in the form of gel for the hydrophobic drug, the concept for emulgel was introduced where the hydrophobic drugs are incorporated in emulsion and then to gel. As the name suggest, they are the combination of gel and emulsion.
Both oil-in-water and water-in-oil type of emulsion used as a vehicle to deliver various drugs to the skin. They also have a high ability to penetrate the skin. The presence of the gelling agent in water phase converts a classical emulsion into an Emulgel. [5,6]

Acne is a disease characterized by inflammatory and non-inflammatory lesions. The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions. *Propionibacterium acnes* have been recognized as pus-forming bacteria triggering an inflammation in acne. [7] For mild acne, topical treatments such as retinoids, benzoyl peroxide,and topical antibiotics are used alone or in combination. For moderate acne with inflammatory papules or deeper cystic lesions, an oral antibiotic is commonly added. For severe or treatment-resistant acne, isotretinoin is the optimal therapy. Oral contraceptives can also be used in women who exhibit hormonally induced acne breakouts.

The objective of the present research work was to investigate the herbal formulation containing curcuminoids and neem seed oil for its antimicrobial potential against *Propionibacterium acne and Rhizopus oryzae.*

MATERIALS AND METHODS

Materials:

Curcuminoids was obtained as a gift sample from Sahaydri Naturals & Phytochemicals, Pune. Neem oil was purchased from Aditya Mint, Mumbai. Carbopol 974 P NF were used of Lubrizol Company. Dialysis membrane was procured from Hi media, Mumbai. All other chemicals used were of analytical grade and were used without any further chemical modification.

Methodology:

1. Selection and Procurement of Herbal Drug Extracts.

Curcuminoids extracts from Turmeric and Neem Seed Oil were selected based on their potential antifungal activity reported in Literature. Curcuminoids was procured from Sahyadri Phytoconstituents Pune, and Neem oil was procured from Aditya Mint, Mumbai. [8,9]

2. Preliminary Phytochemical Analysis of Curcuminoids and Neem Oil Extract.

Herbal Components were evaluated for the presence of various Phytoconstitutents, phytochemical screening was carries out using simple chemical test to detect the presence of secondary metabolite such as alkaloids, tannins, flavonoids, saponins, and glycoside etc. using the Standard procedure reported in previously published literature.[10,11,12]

3. Preparation of Herbal Emulgel

Different formulations were prepared using varying amount of gelling agent and penetration enhancers. The method only differed in process of making gel in different formulation. The preparation of microemulsion was same in all the formulations. The gel phase in the formulations was

prepared by dispersing Carbopol 974 P NF in distilled water with constant stirring at a moderate speed using overhead stirrer.
In another beaker polyethylene glycol 400 was taken and the drug was added into it and stirred to completely dissolve the drug. Required amount of emulsifiers was added. Creamphor El and Tween 80 were used as emulsifiers. Then the Aqueous phase was added with continuous stirring.
The obtained microemulsion was mixed with the gel in 1:1 ratio with gentle stirring then the pH was adjusted to 6–6.5 using tri ethanol amine (TEA) to obtain the emulgel. The composition of different formulations has been discussed in Table 1. [13,14,15]

Table 1: Composition of emulgel formulations.

Trial Batches			
Carbopol 974 P NF	1 % w/w	2 % w/w	3 % w/w
Curcuminoids	0.1 gm	0.1 gm	0.1 gm
Neem Oil	0.1 gm	0.1 gm	0.1 gm
PEG 400	4.41 gm	4.41 gm	4.41 gm
Creamphor EL	1.47 gm	1.47 gm	1.47 gm
Tween 80	2.94 gm	2.94 gm	2.94 gm
Distilled Water	q.s. 10gm	q.s. 10gm	q.s.10gm

q.s: Quality Sufficient

4. Evaluation of Herbal Emulgel

4.1. Physical Examination

The prepared emulgel formulations were inspected visually for their color, appearance, consistency homogeneity, and Phase separation.

4.2 Measurement of pH

One gram of Emulgel was dissolved in 100ml distilled water and stirred until it forms uniform dispersion, kept aside for 2 hours. The pH of the formulation was determined by using a digital pH meter. The pH meter was calibrated with a standard buffer solution having pH 4 and 7 before use. pH meter electrode was washed with distilled water and then dipped into the formulation to measure pH and this process was repeated 3 times.[16]

4.3 Spreadability

Spreadability was determined by the Arvouet-Grand Method which consists of two 20 X 20 cm horizontal plates. By this method, spreadability was measured on the basic ability of emulgels to spread between the horizontal plates. The spreadability of emulgels was determined by pressing 1 g of a sample between two horizontal plates, the upper of which weighed 125 g. The spread diameter was measured after 2 min. Measured the spreadability of formulations was done in triplicate and the average value was calculated. [17]

Spreadability was calculated using the following formula:

$$S=M \times L / T$$

Where
S= Spreadability
M = Weight tied to the upper horizontal plate
L = Length spread by the emulgel on the horizontal plate
T = Time taken in sec

4.4 Viscosity/Rheological Study

The viscosity of the formulated batches was determined using a Brookfield viscometer with spindle no. 6 at a different speed using a 25 ml beaker. The formulation whose viscosity was to be determined was added to a beaker. The spindle was allowed to move freely into the emulgel and the reading was noted in triplicate was noted. Viscosity in centipoise (cP) was measured. [18]

4.5 Drug Content Determination

Drug content in terms of total Curcuminoids in Emulgel was determined by RP-HPLC. The drug content was determined by dissolving a known quantity of Emulgel formulation in Methanol, filtered, and sonicated. The Peak area was measured against the Standard Peak for drug content. [19]

4.6 In vitro Release Studies

In-vitro release study was performed by dialysis method. One gram of prepared Emulgel was placed into dialysis bags and transferred in a small beaker containing 50 ml of PBS (pH 7.4) under shaking conditions (200 rpm, 37 °C). At each 1 h interval, 2 ml of PBS was removed and stored for quantification. The same volume of fresh PBS buffer at the same temperature was added immediately to maintain constant release volume. The length of the dialysis tubing was kept consistent for all methods to ensure that the surface area available for dialysis remained constant. The amount of drug in emulgel was measured by the UV–vis spectrophotometer (UV-1800, Shimadzu) method as described above. Release profile data of the drug was plotted using equations of the calibration curve. [20,21,22]

4.7 Skin Irritation Study

Skin irritation study was done using a patch test. The test was performed on healthy volunteers in the age group of 21-25 years. The dorsal surface of the skin was cleaned with 70% alcohol before the application of the formulation. A patch of the emulgel was applied to 4x5 cm of the forearm. The regions were covered with a surgical dressing where the application of Emulgel was. The patches were removed after 48h and the forearm was washed with physiological salt. The cutaneous reactions were evaluated by monitoring the reactions of erythema and edema at the end of 15 minutes, 1 hour, and 24 hrs after the removal of the patch of the test sample. [23]

4.8 Antioxidant Assay by DPPH Method

Standard & test samples were prepared by properly diluting the stock solution and added to 3 ml of methanol and 50 µl of 0.1mM DPPH solution was added and incubated in a dark room at room temperature for 4 minutes. Absorbance was measured at 516 nm using methanol as a blank. A decrease in absorbance in the presence of test samples at different concentrations was noted. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (without extract) using the formula. [24,25]

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

4.9 Antifungal Study

In the present study, the prepared Emulgel was evaluated for antifungal activity; an agar well diffusion method was applied. For the antifungal assay, *Rhizopus oryzae* were spread uniformly on Potato Dextrose Agar plates. The test sample and control sample was introduced in the wells under sterilized conditions and incubated at 37°C for 24 h. The zone of inhibition (mm) around the well was measured. [26]

4.10 Anti-acne Study

The efficacy of anti-acne emulgel formulas was performed using the agar diffusion method with perforation technique against P. acne. A total of 20µl bacterial suspension was fed into sterile petri dishes. The test medium was homogenized and allowed to solidify. Media that has been solidified, then perforated to make holes for sample reservoir. A total of 50 mg of each concentration was introduced into the reservoir on the test medium. The test media were incubated at 37°C for 24h. The inhibitory diameter formed was measured using a caliper.

4.11 Stability studies

The prepared emulgels were packed in aluminum collapsible tubes (5g) and subjected to stability studies at 5°C /60% RH, and 40°C /75% RH for a period of 1 month. Samples were withdrawn after one month and checked for their physical characteristics like appearance, homogeneity, pH, viscosity, and spreadability.

RESULTS AND DISCUSSION:

1. Phytochemical Analysis:

Table 2: Phytochemical screening of Selected Herbal components

Test		Curcuminoids	Neem Oil
Carbohydrates	Molich test	+	-
	Felhings test	+	+
Proteins	Millons test	+	+
Steroids	Salkowaski test	+	-
	Liberman bruchard	-	-
Glycosides	Bontrager test	-	-
Saponin	Foam test	+	+
Flavonoids	Shinoda test	+	+
Tannins and Phenolic Compound	$Fecl_3$ test	+	+
Alkaloids	Dragendroff test	+	+
	Mayers test	+	+
	Wagners test	+	+

The preliminary phytochemical analysis indicated the presence of various secondary metabolites such as Alkaloid, Tannin, Flavonoid, Saponin, and Glycoside etc.

2. Evaluation of Herbal Emulgel

2.1 Physical Evaluation

The prepared emulgel formulations were opaque, yellow, viscous preparation with a greasy, homogeneous appearance with good consistency and without any gritty particles.

The physical observation of prepared emulgel formulations is shown in Table 3.

Table 3: Physical examination of Emulgel formulation

Carbopol Content	1 %	2 %	3 %	Marketed Formulation
Physical Appearance	Opaque	Opaque	Opaque	Opaque
Color	Yellow	Yellow	Yellow	White
Odour	Mild	Mild	Mild	Mild
Texture	Greasy	Greasy	Greasy	Greasy
Phase Separation/ Precipitation	No	No	No	No
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.61	6.21	6.73	6.82
Spreadability	2.59gmcm/Sec	2.43gmcm/Sec	2.00gmcm/Sec	1.86gmcm/Sec
Viscosity	2000cP	6000cP	16000cP	21000cP

2.2 Measurement of pH

The pH values of all prepared Emulgel formulations ranged from 6.21 to 6.73 which matched the requirements of topical preparations for skin, thus avoiding skin irritation. The pH values of emulgel formulations are shown in Table 3.

2.3 Spreadability

The spreadability of the emulgel formulation depends on its viscosity. The greater the viscosity the longer will be the time taken for the spread on the skin. The values of spreadability indicate that the prepared emulgel is easily spreadable by a small amount of shear. The spreadability of all prepared batches is shown in table 3.

2.4 Viscosity

The measurement of the viscosity of the prepared Emulgel was done with a Brookfield Viscometer. The emulgels were rotated at 2 min and 0.5 rotations per minute with spindle 6 and the corresponding dial reading was shown in Table 3.

2.5 Drug Content Determination

The content of curcuminoids in the emulgel formulations were determined using RP-HPLC. Curcuminoids content in the emulgel formulations was 99.1%. This percentage agreed with the acceptable range according to the USP (85-115%).

2.6 In-Vitro Release Study

The cumulative % drug release profile of the optimized emulgel formulation has been shown in figure 1 and as shown in the figure of in vitro release studies, the release profile of curcuminoids and neem seed oil showed a high amount of drug release 63.90 % and 61.69% respectively after 5 hours in-vitro cumulative % drug release data of formulations was shown in table no. 4.

Table 4: *In vitro* drug release of emulgel formulations

	% CR	
Time	**Curcuminoids**	**Neem Oil**

0	1.26	5.68
1	7.96	14.09
2	22.80	27.41
3	34.03	37.78
4	46.11	52.72
5	63.90	61.69

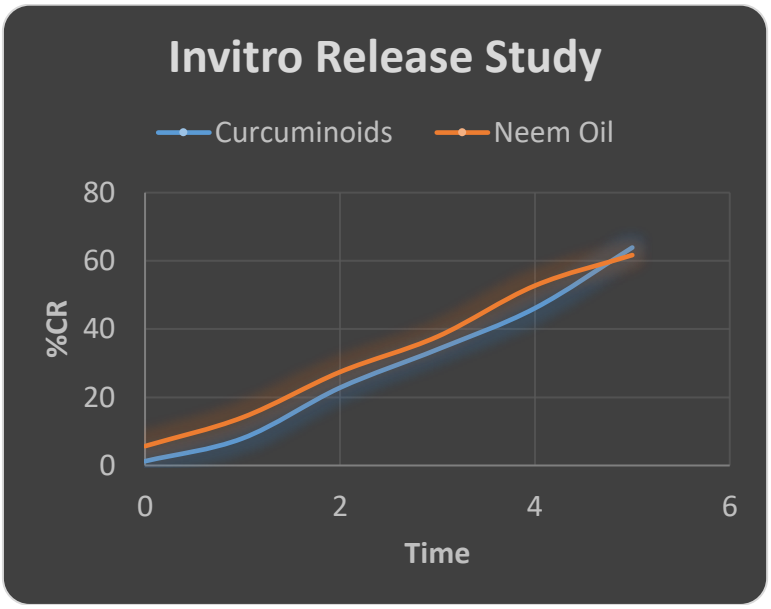


Fig No. 1: *In-vitro* release study of emulgel

2.7 Skin Irritation Study

It was found that optimized emulgel formulation does not produce any cutaneous allergic reaction like edema or erythema. This indicated that the emulgel is safe for human use in the concentration used.

Table 5: Result of skin irritation test

Volunteers	**Erythema/Edema**
A	Nil
B	Nil
C	Nil
D	Nil
E	Nil
F	Nil

2.8 Antioxidant Assay by DPPH Method

The prepared emulgel was subjected to radical scavenging activity with the help of the DPPH assay. As the outermost covering of the body, the skin cells are susceptible to oxidative stress due to continuous exposure to ultraviolet light and other various environmental harmful conditions.

All the test Samples showed concentration-dependent increases in radical scavenging capacity. The IC 50 value of the optimized emulgel formulation was recorded at (60.15 µg/mL). While the IC50 value of standard ascorbic acid (2.89 µg/mL), is presented in table 6.

Table 6: % Inhibition of Standard Ascorbic Acid and Optimized emulgel formulation

Ascorbic Acid		**Emulgel Formulation**	
Conc. (µg/ml)	**% Inhibition**	**Conc. (µg/ml)**	**% Inhibition**
1.5	34.72	30	20.83
2.5	48.63	40	33.33
3.5	58.83	50	46.29
4.5	61.11	60	55.31
5.5	74.3	70	55.09
6.5	87.5	80	63.42
		90	64.81
		100	68.51
IC_{50}	**2.89**	**IC_{50}**	**60.151**

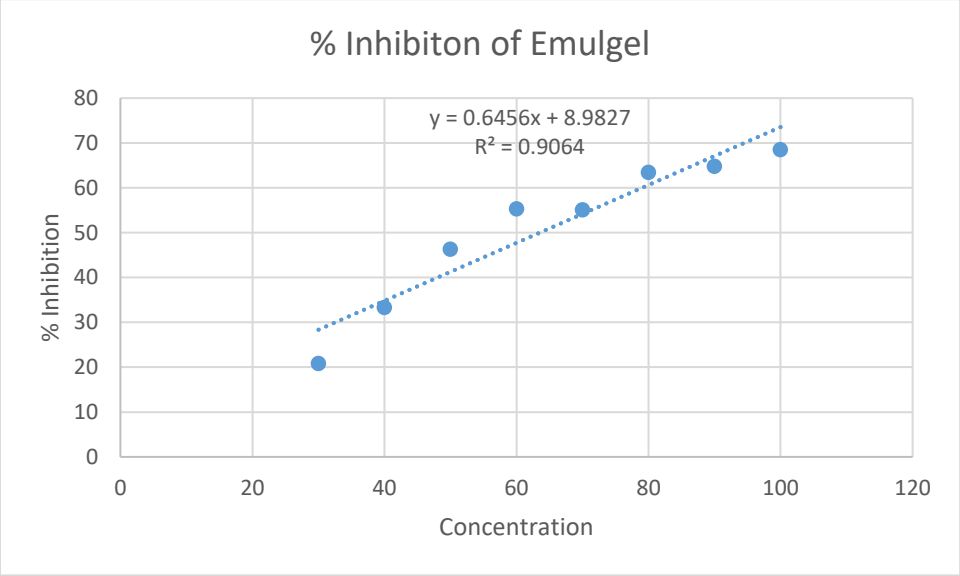


Fig 2: Standard Curve of % Inhibition of Optimized Emulgel Formulation

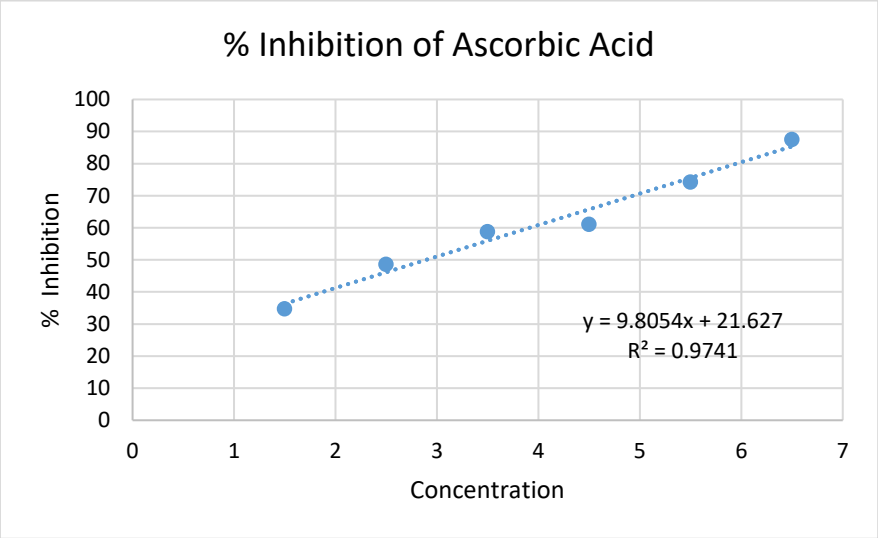


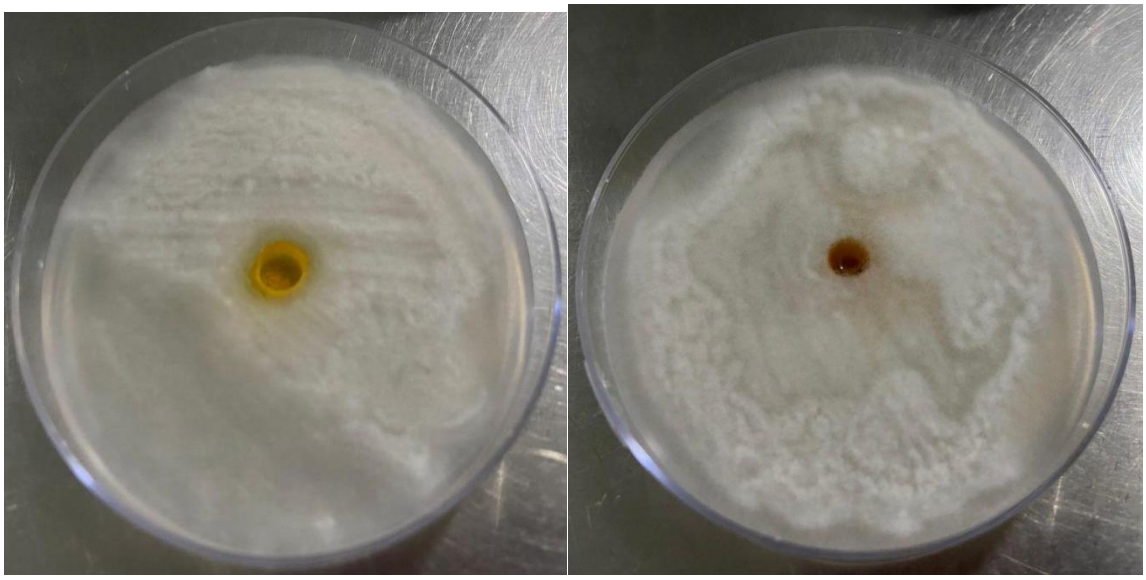
Fig 3: Standard Curve of % Inhibition of Standard ascorbic acid

2.9 Antifungal Study

The zone of inhibition measured is summarized in table number 7. From the table, it is evident that the prepared emulgel does not show any zone of inhibition. This curcuminoid extract shows remarkable activity against *Rhizopus oryzae* as shown in figure number 4(A). The higher activity of curcuminoid extract compared to neem seed oil and emulgel formulation can be viewed in figures number 4.

Table 7: Zone of inhibition against *Rhizopus oryzae*

Sample	Micro-organism	Zone of inhibition (mm)
Curcuminoid Extract	*Rhizopus oryzae*	11.90 mm
Neem Seed oil	*Rhizopus oryzae*	No zone
Emulgel Formulation	*Rhizopus oryzae*	No zone



A B

C

Fig 4: Zone of inhibition against *Rhizopus oryzae* (A) Curcuminoids extract; (B) Neem Seed Oil; (C) Herbal Topical Emulgel

2.10 Anti-acne Study

The formulated emulgel has a potential in the treatment of acne because *Propionibacter acnes* has been reported as common bacteria in causing acne skin infections. The formulated herbal emulgel shows remarkable activity against *Propionibacter acnes* as shown in figure number 5. The formulated emulgel showed zone of inhibition of 9.8 mm after incubated at 35°C for 24h.

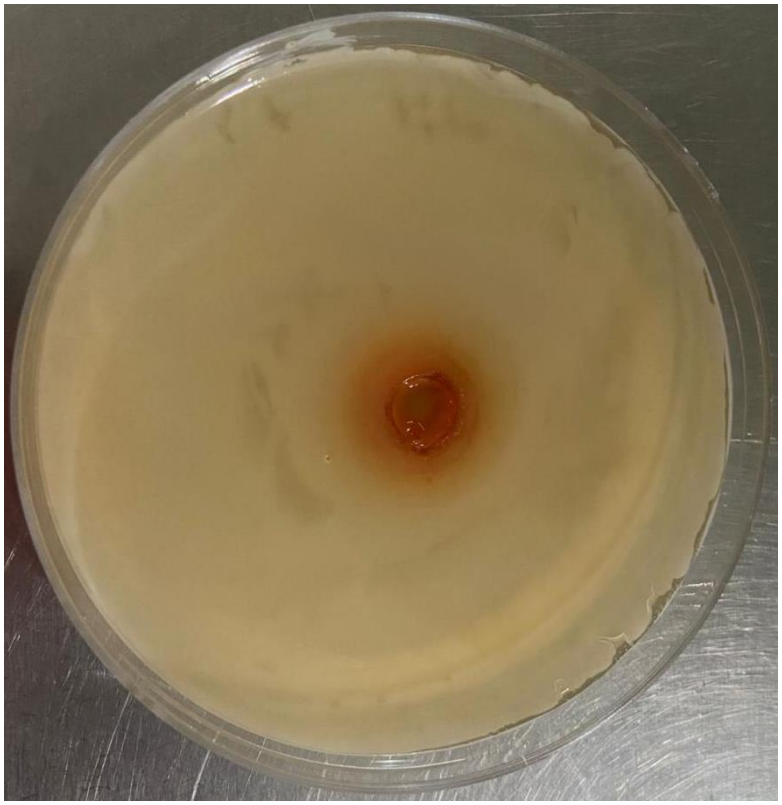


Fig no. 5: Zone of inhibition against *Propionibacter acnes*

2.11 Stability Study

The Stability study of optimized emulgel formulations was carried out at storage conditions of 50C and 400C for a period of one month. Samples were withdrawn at the time interval and after one month and the results are tabulated in Table No. 8. During the study period, the formulations [kept at 50C & 400C] were found to be homogenous and free from microbial growth.

Table no. 8: Stability data of optimized emulgel formulation

Parameter Evaluated	Initial	5°C & 60%RH	40°C& 75%RH
Physical Appearance	Yellow in Colour, Greasy in texture	No Change	No Change
Homogeneity	Homogenous	Homogenous	Homogenous
pH	6.73	6.69	6.59
Viscosity	16000cp	15800	15500
Spreadability	2.00 gmcm/Sec	2.03 gmcm/Sec	2.09 gmcm/Sec
Drug Content	99.50%	96.43%	93.26%

CONCLUSION:

In the current research project, Herbal Topical Emulgel was formulated successfully using Curcuminoids and Neem seed oil. In the coming years, topical drug delivery will be used extensively to impart better patient compliance. Since emulgel is helpful in enhancing spreadability, adhesion, viscosity, and extrusion, this novel drug delivery become popular. Moreover, they will become a solution for loading hydrophobic drugs in water-soluble gel bases for long-term stability. In the present investigation, topical herbal emulgel was prepared by using carbopol 974p NF and showed

acceptable physical properties, pH, drug content, and viscosity. The developed Herbal topical emulgel formulation can be used as an alternative to synthetic formulations. The prepared Herbal topical formulation will help to treat the associated symptoms of Cutaneous Mucormycosis. Skin irritation study reveals that developed herbal emulgel didn't showed any erythema and edema. Therefore, formulation was found to be safe and non-irritant to the skin. From Antifungal activity study it can be concluded that, herbal emulgel does not show any zone of inhibition but the individual extract of curcuminoids showed good zone of inhibition against *Rhizopus oryzae*. The antiacne activity and antioxidant activity gives this emulgel the status of a drug having pharmacological agent, whereas the presence of neem seed oil imparts benefits like preservative, heal wounds, reduce post-acne scars and minimize skin inflammation etc. Thus the present research work suggests that herbal emulgel formulation holds a potential against acne and can prove to be a safe and efficacious remedy for treating this dermatological disorder. However an elaborate protocol for the clinical trials is needed to be designed and implemented to check the mechanism of anti-acne activity. The antioxidant activity also proves this emulgel to be a cosmeceuticals as antioxidant activity prevents skin ageing and wrinkles which directly add up to its cosmetic value. Stability studies revealed no significant differences before and after storage for the selected formula.

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