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"Formulation and evaluation of microemulsion based gel of *Calendula officinalis* extract and its anti-microbial activity"

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ABSTRACT

The present study focuses on the formulation and evaluation of a micro emulsion-based gel containing *Calendula officinalis* extract, targeting its potential as an antimicrobial agent. Micro emulsions offer a unique advantage in enhancing the solubility and bioavailability of hydrophobic plant extracts, thereby improving their therapeutic efficacy. Qualitative phytochemical screening of *Calendula officinalis* showed the presence of active metabolites such as Alkaloids, flavonoids, glycosides, carbohydrates, saponin, tannins and phenols. The micro emulsion was prepared using oil, surfactant, and co-surfactant systems, followed by its incorporation into a gel matrix.

The formulated micro emulsion-based gel was characterized for physicochemical properties such as pH, viscosity, spread ability, and stability. The viscosity of microemugel is found to 6891 ±0.37 cps. The pH of microemugel is 6.5 and spread ability is 11.18, indicating that microemugel has high release and permeability. The microemugel's creation and shape were verified using SEM images. Furthermore, the antimicrobial efficacy of the gel was assessed against common pathogenic microorganism including *Escherichia coli* through in vitro studies. The results demonstrated significant antimicrobial activity, suggesting the potential application of this formulation in topical antimicrobial therapy. The study concludes that the micro emulsion-based gel of *Calendula officinalis* extract exhibits promising characteristics for the development of effective antimicrobial treatments.

Keywords: *Calendula officinalis*, Microemulsion, Gel formulation, Antimicrobial activity, Topical therapy

1. INTRODUCTION

Micro emulsions, which range in size from 5 to 200 nm and have very low interfacial tension, are thermodynamically stable isotropically transparent dispersions of two immiscible liquids, such as oil and water, supported by an interfacial coating of surfactant molecules. Micro emulsions have garnered significant interest as possible drug delivery systems due to their distinct solubilization characteristics. They can be used as topical administration systems or to improve the bioavailability of active pharmaceutical ingredients (API) that are

poorly soluble in water[1]. Micro emulsion-based gels are garnering significant attention in the pharmaceutical and cosmetic industries due to their enhanced stability, solubility, and bioavailability of active ingredients. These systems combine the unique properties of micro emulsions, such as thermodynamic stability and the ability to solubilize hydrophobic compounds, with the convenience and application benefits of gels, which offer improved adhesion to the skin and controlled release of active agents[2]. *Calendula officinalis*, commonly known as marigold, is renowned for its broad spectrum of pharmacological properties, particularly its antimicrobial, anti-inflammatory, and wound-healing activities. The bioactive compounds in *Calendula officinalis*, including flavonoids, saponins, and triterpenoids, exhibit potent antimicrobial activity against various pathogenic microorganisms, making it a valuable candidate for topical formulations aimed at treating skin infections and promoting wound healing[3,4]. The development of a micro emulsion-based gel containing *Calendula officinalis* extract is a promising approach to harness and enhance its therapeutic benefits. Micro emulsions, as drug delivery systems, can improve the solubility and stability of hydrophobic bioactive compounds, facilitating better penetration through the skin barrier and ensuring a sustained release of the active ingredients. This leads to improved therapeutic efficacy and patient compliance[5, 6].

This study aims to formulate and evaluate a micro emulsion-based gel of *Calendula officinalis* extract, with a focus on its antimicrobial activity. The formulation process involves optimizing the micro emulsion system for maximum stability and efficacy, incorporating it into a gel base, and assessing its physical properties, stability, and antimicrobial efficacy. The ultimate goal is to develop a novel topical formulation that leverages the advantages of micro emulsion systems to deliver the therapeutic benefits of *Calendula officinalis* more effectively.

2. MATERIAL AND METHOD

2.1 Plant collection

300 grams of *Calendula officinalis*, a therapeutic herb, were collected, dried, and refrigerated to prevent contamination. A plant taxonomist confirmed the plant's identity and purity after confirming its purity.

41 -52| MD HAMID ANSARI: Research Scholar in School of Pharmacy, LNCT University, Bhopal, (Madhya Pradesh), India.

2.2 Extraction

The study used the Soxhlet apparatus and a continuous hot percolation process to extract plant material from *Calendula officinalis* powder. The process involved soxhlation with non- polar solvents, drying, and extraction multiple times until no color change was observed.

After the weighing of dried extract, we calculated the % yield for each extract using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use[7].

2.3 Phytochemical investigation

By performing a thorough qualitative phytochemical analysis, the experiment was designed to determine whether or not various phytoconstituents were present. The precipitate formation or color intensity was utilized to gauge how the body would react to various tests. Standard operating procedures were employed[8].

2.4 Formulation of micro emulsion

In order to ascertain the extract's solubility in different oils (oleic acid, Capryol 90, olive oil), surfactants (Cremophor RH 40, Tween 80), and co-surfactants (polyethylene glycol 400), an excess of medication was first added to 2 mL of chosen excipients in 5 mL stopper vials, followed by vortex mixing. The selection of oil, surfactant, and co-surfactant was done on the basis of their drug solubility. With four components—olive oil as the oil phase, tween 80 as a surfactant, propylene glycol as a co-surfactant, and distilled water as the aqueous phase—the micro-emulsion of the corresponding composition, as indicated in table 1, was created. Various ratios of surfactant to co-surfactant were intended for each batch. E.S/CoS, the ratio of surfactant to co-surfactant, was selected, and a matching combination was prepared. Oil was added to the mixture. Using a magnetic stirrer, each liquid was thoroughly stirred until a homogeneous dispersion or solution was achieved. In these formulations, double-distilled water was employed to avoid the addition of surface-active contaminants[9, 10].

Table 1: Composition of micro emulsion formulation

S. No	Ingredients	FORMULATION CODE				
		ME1	ME2	ME3	ME4	ME5
1.	Extract (1:1 ratio) (mg)	100	100	100	100	100
2.	Propylene Glycol (ml)	0.1	0.2	0.3	0.4	0.5
3.	Tween 80 (ml)	1.0	1.0	1.0	1.0	1.0
4.	Olive oil (ml)	2.0	2.0	2.0	2.0	2.0
5.	Stirring Time (min.)	30	30	30	30	30
6.	Distilled water	1.5	1.5	1.5	1.5	1.5

2.5 Evaluation parameter of extract loaded micro emulsion

2.5.1 Particle size

One of the most crucial parameters for characterizing a micro emulsion is its particle size. The Malvern Zeta sizer (Malvern Instruments) was used to measure the size of the micro emulsion. The sample was put in a disposable sized cuvette after the dispersions were diluted with Millipore filtered water to the proper scattering intensity at 25°C[11].

2.5.2 Zeta potential

In order to ascertain the particle charge and movement velocity of the particles in an electric field, the zeta potential was measured. In the current study, Zeta sizer Malvern equipment was used to examine the micro emulsion after it had been diluted ten times

with distilled water. Prior to zeta potential tests, all samples underwent a 5-to 15-minute sonication[12]**.**

2.5.3 Scanning Electron Microscopic (SEM)

The extract-loaded micro emulsion was morphologically examined using a scanning electron microscope. A thin layer of metal was applied to the micro emulsion, such as platinum, palladium, or gold. The specimen was exposed to an electron beam, causing secondary electrons to develop. Only electrons scattered at a 90° angle were chosen and processed using Rutherford and Kramer's Law to obtain surface topography photographs[13].

2.6 Formulation of Micro emulsion loaded Gel

First, carbopol-934 was mixed evenly using a magnetic stirrer set at 600 rpm after being submerged in 50 mL of warm water (A) for two hours. To create a stiff gel, 50 milliliters of warm water (B) was combined with carboxymethyl cellulose and methyl paraben in a different container and continuously agitated. Continuous stirring was used to combine mixtures A and B. After adding tri-ethanol amine (drop wise) to the dispersion to bring the pH down, the optimized formulation micro emulsion was added to create gel. Propylene glycol, a permeability enhancer, was introduced at this point. After the final dispersion was worked out, a lump-free, smooth gel was produced[14,15].

Table 2: Composition of gel formulation

S. No	Excipients	Quantity
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm
3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	micro emulsion	10 ml
6.	Tri-ethanolamine	q.s
7.	Water	100 ml

2.7 Characterization of micro emulsion loaded Gel

2.7.1 Physical appearance and pH

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation[16]**.** The formulation's pH was measured using a digital pH meter. After stabilizing and calibrating the meter, the probe was rinsed with water and dried. A pH reading was taken by submerging the probe's tip in the sample. The readings should match within the meter's accuracy range. Three copies of samples were examined, and minor pH variations were corrected by adding tri-ethanolamine solution drop by drop until the pH was the same as skin[17].

2.7.2 Viscosity and Spread ability

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 61 at 100 rpm at the temperature of 25^0C[18]. A topical gel that is placed or rubbed on the skin's surface should have a sufficient spreading coefficient. A glass slide containing one gram of the formulation was used to assess this. A second, identically sized glass slide was positioned above it, and a 50 mg mass was added to it such that the gel was sandwiched between the two slides and spread out at a specific distance. The amount of time it took the gel to move that far from its original position was recorded. Spread ability was calculated using the subsequent formula.

$$S= M*L/T$$

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T- Time for spreading gel in sec[19].

2.8 Antibacterial activity by Well diffusion assay

2.8.1 Preparation of Nutrient Agar Media

One liter of distilled water was used to dissolve 28 grams of nutritional media. The media's pH was measured prior to sterilization. The medium was autoclaved for 15 minutes at 121 degrees Celsius and 15 pounds of pressure. After adding nutritional media to plates, they were put in a laminar air flow until the agar solidified.

2.8.2 Well Diffusion Assay

The *E. coli* bacterial suspension was standardized to 108 CFU/ml and collected from a shaker. Inoculums were transferred to a sterile Agar Media Plate, and three wells were drilled into the inoculation material. The micro emulsion loaded gel, gel (0.5 and 1 mg/ml), and extract (1 mg/ml) solution were added to the wells. The sample was loaded in 100 µl and incubated for 18-24 hours at 37 degrees Celsius. A clear zone was formed around the well,

indicating the chemicals' antimicrobial activity. The zone of inhibition (ZOI) was measured in millimeters using a ruler held on the back of the inverted Petri plate. The diameters of the well and the zone of total inhibition were also measured[20].

2.9 Stability study

After being packed and put in the stability test chamber, the micro emulsion loaded gel formulation was tested for three months at two accelerated testing temperatures: $25^0C \pm 2^0C$ and 60 ±5% RH, and $40^0C \pm 2^0C$ and 70 ±5% RH. Viscosity and pH investigations were performed on the formulations at intervals of 30, 45, 60, and 90 days (3 months). In compliance with the rules set forth by the International Conference on Harmonization (ICH), the formulation underwent a three-month period of accelerated storage to assess its stability. The formulation for the pH and viscosity studies' change in assessment parameters was examined. All Results were compared against final formulation of 0 days as the reference.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Calendula officinalis* is shown in Table: 3

Table 3: Percentage Yield of crude extracts of *Calendula officinalis* extract

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Calendula officinalis*	Pet ether	299	1.51	0.50%
2		Methanol	289.12	6.49	2.24%

3.2 Preliminary Phytochemical study

Table 4: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present

3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Present	Present
5.2	Lead Acetate test	Present	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

3.3 Characterization of Micro emulsion

3.3.1 Particle Size and Zeta potential

Table 5: Particle size and Zeta potential determination

S. No	Formulation code	Particle size (nm)	PI Value	Zeta potential
1.	ME1	210.3 nm	0.327	-9.2 mV
2.	ME2	240.3 nm	0.010	-4.6 mV
3.	**ME 3**	**199.7 nm**	**0.312**	**-5.1 mV**
4.	ME 4	276.4 nm	0.212	-6.3 mV
5.	ME 5	290.4 nm	0.213	-7.0 mV

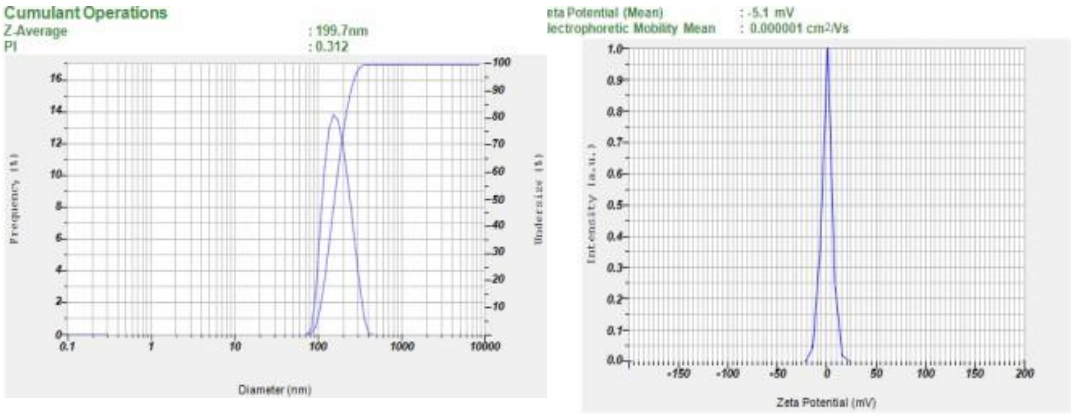

Figure 1: Particle size and Zeta potential(F3)

The particle size is one of the most important parameter for the characterization of Micro emulsion. The average particle size of the prepared extract loaded Micro emulsion was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of extract loaded Micro emulsion was found to be range 199.7 nm to 290.4 nm.

Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. If the particles in Micro emulsion have a large positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating for Micro emulsion. Zeta potential of all formulations was found to be range -4.6 mV to -9.2 mV with peak area of 100% intensity. These values indicate that the formulated Micro emulsion is stable.

3.3.2 Scanning electron microscope (SEM) of F3 Formulation (Optimized)

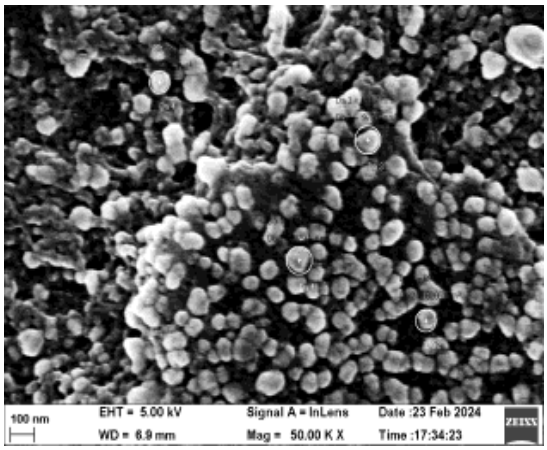

Figure 2: Scanning electron microscope

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared Micro emulsion. Micro emulsion were prepared and dried well

to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared Micro emulsion at 50.00 KX magnification showed that the Micro emulsion were smooth surface morphology and spherical shape. The spongy and porous nature of Micro emulsion was clearly observed in the SEM images.

3.4 Characterization of Micro emulsion loaded gel

3.4.1 Physical appearance

Table 6: Physical appearance

S. No	Parameter	Result
1.	Colour	Reddish colour
2.	Odour	Odourless
3.	Appearance	Reddish colour
4.	Homogeneity	Homogeneous

An evaluation of the gel, including colour, odor, appearance and homogeneity, was conducted. Gel was discovered to have Reddish colour to it when tested. Gel does not have a distinctive odor and has a Reddish colour appearance, according to research conducted on it.

3.4.2 Viscosity of Gel, PH determination and Spread ability

Table 7: Viscosity, pH & Spread ability

S. No	Formulation	Viscosity (cps)	pH	Spread ability (g.cm/s)
1.	Gel	6891±0.37	6.5	11.18

The viscosity was measured by the Brookfield viscometer spindle no. 61 at 100rpm. The viscosity of Gel was found to be 6891 centipoises respectively. The pH of the gel formulation was found to be 6.5, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time. The physicochemical properties of prepared gel formulation were in good agreement. One of the essential criteria for a Gel is that it should possess good spread ability. Spread ability depends on the viscosity of the formulation and physical characteristics of the polymers used in the formulation. A more viscous formulation would have poor spread ability. Spread ability is a term expressed to denote the extent of area on which the gel readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends upon its spreading value. The spread ability of Gel formulation is found to be 11.18 g.cm/s.

3.5 Results of antimicrobial activity of micro emulsion loaded gel

3.5.1 Antimicrobial activity of Formulation and extract against *E.coli*

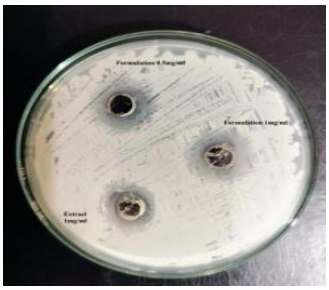

Figure 3: Antimicrobial activity

Table 8: Antimicrobial activity of Formulation and plant extract against *E.coli*

S. No.	Sample Name	Zone of Inhibition (mm)
1.	Extract	10 mm
2.	Emulgel (0.5mg/ml)	12 mm
3.	Emulgel (1mg/ml)	14 mm

3.6 Stability study

Table 9: Stability Study of optimized formulation (Micro emulsion gel)

S. No	Time (Days)	$25^0C \pm 2\ ^0C$ and 60 ± 5% RH		$40^0C \pm 2\ ^0C$ and 70 ±5% RH	
		Viscosity	pH	Viscosity	pH
1.	0	6891	6.5	6891	6.5
2.	30	6894	6.7	6895	6.7
3.	45	6897	6.9	6906	7.2
3.	60	6902	7.1	6911	7.4
4.	90	6903	7.4	6917	7.7

Formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions ($25^0C \pm 2\ ^0C$ and 60 ± 5% RH) and ($40^0C \pm 2\ ^0C$ and 70 ±5% RH). Evaluation parameters including viscosity and pH studies were not altered significantly. Results of assay and other evaluation criteria at periodic time points of stability studies are summarized in Table 9. The result of accelerated stability studies of Micro emulsion gel formulation is shown in above table. No major changes were observed.

4. CONCLUSION

The formulation and evaluation of a micro emulsion-based gel containing *Calendula officinalis* extract has demonstrated significant potential as an antimicrobial agent. The optimized micro emulsion gel showed desirable physicochemical properties, including stability, homogeneity, and appropriate viscosity for topical application. Importantly, the antimicrobial activity tests revealed that the micro emulsion gel exhibited effective inhibition against a spectrum of pathogenic microorganisms. These findings suggest that the developed micro emulsion-based gel of *Calendula officinalis* could serve as a promising candidate for treating various skin infections and promoting wound healing. Future studies should focus on in vivo evaluations and the exploration of the mechanism of action to further validate its clinical efficacy and safety.

50 -52| MD HAMID ANSARI: Research Scholar in School of Pharmacy, LNCT University, Bhopal, (Madhya Pradesh), India.

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