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Original Article

Preparation and Evaluation of Multitargeted Berberine Microemulsion

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ABSTRACT

Background: Berberine, a phytochemical, possesses remarkable potential for multitargeted drug therapy in microbial infections, wound healing and anti-inflammatory applications. However, its clinical utility in skin and other diseases is constrained due to its high aqueous solubility and inadequate dermal and intestinal absorption, resulting in low absolute bioavailability. **Objectives:** The current investigation endeavors to develop a microemulsion loaded with berberine to enhance its solubility and therapeutic effects specifically for skin diseases, while comparing its efficacy with a simple emulsion. **Materials and Methods:** In this study, an optimized oil-in-water (o/w) type of emulsion and microemulsion were formulated utilizing berberine isolated from Berberis aristata roots, along with soy lecithin, oleic acid, Tween 80, and ethanol. The microemulsion and emulsion were subjected to characterization employing parameters such as FTIR, particle size, zeta potential, transmittance, viscosity, and stability. The formulations were assessed for their therapeutic effects through *in vitro* antioxidant, anti-inflammatory, and anti, microbial assays, encompassing six strains of micro, organisms comprising bacteria and fungi. **Results:** The investigation successfully generated a thermodynamically stable o/w type of microemulsion, which exhibited improved transparency and formed a clear solution. Characterization results, including particle size, zeta potential, viscosity, and conductivity measurements, fell within the acceptable range. The therapeutic evaluation demonstrated a substantial enhancement in the *in vitro* antioxidant, anti-inflammatory, and anti, microbial activity of berberine microemulsion compared to the plain berberine solution. **Conclusion:** This study concludes that berberine can be effectively incorporated into a microemulsion system. When topically applied, the microemulsion form exhibits significantly higher therapeutic efficacy against skin diseases compared to the conventional emulsion of berberine.

Keywords: Emulsion, Berberine extract, Antioxidant, Anti-inflammatory, Anti-microbial, DPPH, Topical.

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INTRODUCTION

Berberine, an alkaloidal phytoconstituent of significant medicinal value,[1] has been utilized in Asian countries for centuries to treat skin and wound-related ailments, particularly in berberine-rich plant parts like stems and roots.[1] The diverse therapeutic properties of berberine, including anti, microbial, anti-inflammatory, antioxidant, and wound healing effects, have captured the interest of researchers worldwide.[2-5] However, the clinical application of berberine in topical and oral formulations faces challenges such as poor oral and dermal absorption, high hydrophilicity, and limited bioavailability.[6,7]

To overcome these limitations, the development of a novel drug delivery system is necessary to enhance the lipophilicity and membrane permeability of highly water-soluble drugs. Microemulsion, a well-established approach in phytopharmaceutical development, offers effective solutions to these challenges. Microemulsions are stable, isotropic liquid solutions characterized by particle sizes ranging from 1 to 200 nm.[8] This technology has demonstrated its potential in improving the bioavailability of poorly bioavailable phytoconstituents by solubilizing and delivering hydrophobic phytochemicals, while increasing interfacial area and reducing interfacial tension.[9]

Hence, the objective of our study was to formulate a microemulsion of berberine, a complex molecule, to enhance its solubility and therapeutic efficacy against various skin diseases, including rashes, skin infections, psoriasis, leprosy, chronic wounds, and diabetic wounds, where berberine has shown therapeutic potential. The developed berberine microemulsion

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was compared to an emulsion (o/w) of berberine in terms of solubility and therapeutic efficiency to assess its effectiveness.

MATERIALS AND METHODS

Materials

The plant material, *Berberis aristata* roots, was procured from local Ayurvedic herb vendor in Amravati. Standard berberine was obtained from Yucca Enterprises, Mumbai. Oleic acid and soy lecithin were acquired from Sisco Research Laboratories Pvt. Ltd., Chennai. Tween 80, ethanol, and distilled water were purchased from CDH Chemicals, New Delhi.

Extraction and Isolation of Berberine

Prior to extraction, the roots of *Berberis aristata* were taxonomically authenticated at the Department of Pharmacognosy, Government College of Pharmacy, Amravati (Deposited herbarium Voucher No. 11052022/GCOPA). Subsequently, the roots were thoroughly washed, dried, and powdered. Different extracts were prepared using water, 70% ethanol, and 95% ethanol for analytical purposes. To isolate pure crystals of berberine, 100 g of the powdered material was moistened with an alkaline solution of aqueous ammonia and alcohol, followed by a resting period of 1 hr. The mixture underwent defatting using a Soxhlet apparatus with petroleum ether for duration of 6 hr. After defatting, the remaining marc was completely dried and subjected to extraction in a Soxhlet apparatus using 95% ethanol for 6 hr. Once the extraction process was completed, the resulting extract was concentrated using a rotary evaporator. The concentrated dried extract was mixed with boiling water, followed by filtration. The filtrate was then acidified using 5% HCl, and the solution was allowed to cool, leading to the formation of crystals of berberine hydrochloride.

Qualitative analysis of isolated Berberine

Berberine crystal samples were subjected to qualitative analysis using the Thin-Layer Chromatography (TLC) technique. Precoated silica gel plates from Merck were employed as the stationary phase for the analysis. Equal volumes of the berberine samples were spotted onto the plates. Subsequently, the plates were placed in a developing chamber saturated with a mobile phase comprising methanol, acetic acid, and water in a ratio of 8:1:1 (v/v/v). The plates were allowed to develop at room temperature for duration of 30 min. The developed plate was then examined under wavelengths of 254 nm, 365 nm, and daylight.[10]

Quantitative estimation of Berberine by UV spectrophotometer

To perform quantitative estimation, a stock solution of standard berberine obtained from Yucca Enterprises, Mumbai was prepared in ethanol. The stock solution was prepared by dissolving the standard berberine in a 10 mL volumetric flask, resulting in a concentration of 1 mg/mL. Serial dilutions were carried out using this stock solution to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. The absorbance of each concentration was measured at a wavelength of 345 nm. To determine the unknown concentrations, a calibration curve was constructed by plotting the absorbance values against the corresponding concentrations.

Solubility study

To determine the optimal combination of oil, surfactant, and co-surfactant for formulation, a saturated solubility study was conducted. Various oils (Castor oil, Olive oil, Oleic acid, Soybean oil), surfactants (Span 20, Span 80, Tween 80), and co-surfactants (PEG 400, PEG 600, Ethanol, Propylene glycol) were considered in the study. In the experimental procedure, separate vials were prepared for each oil, surfactant, and co-surfactant, and an excess amount of berberine was added to each vial. The mixtures were allowed to solubilize for 30 min and then placed on a shaker for 48 hr to ensure complete solubility. Subsequently, the solubilized samples were subjected to centrifugation at 3000 rpm for 15 min. From the resulting supernatant, 0.1 mL was pippetted out and diluted with ethanol. The samples were then analyzed using a UV spectrophotometer at a wavelength of 345 nm, with a blank sample used for comparison.[11,12]

Formulation and optimization of Berberine loaded emulsion and microemulsion

The emulsion (o/w) of berberine extract was prepared using a homogenizer according to the method described in the reference 8. In this process, berberine was dispersed in oleic acid as the oil phase, while soy lecithin was used as the emulsifying agent. A small amount of water was added drop wise to the mixture during homogenization, which was carried out for 5 min. The formulation was optimized in batches (Batch A, Batch B, and Batch C) to identify the most suitable formulation (Table 1).[8]

For the preparation of the microemulsion, the phase titration method was employed. The required quantity of berberine was dispersed in a suitable amount of oil, followed by homogenization. Then, a precise weight of surfactant: co-surfactant blend was gradually added to the mixture with continuous stirring. The mixture was thoroughly stirred using a magnetic stirrer while double distilled water was added drop by drop with continuous stirring. Batch-wise optimization was conducted, and batches A, B, C, D, E, and F were prepared as outlined in Table 2. The final amounts of the oil phase, surfactant, and co-surfactant were determined based on the results of the trial batches and solubility study. The prepared microemulsion was stored in a tightly sealed glass container at room temperature for 48 hr, during which any signs of phase separation were monitored and noted.

Table 1: Optimized batches of emulsion (o/w).

Sl. No.	Ingredients	Emulsion A	Emulsion B	Emulsion C
1	Isolated Berberine crystals	10 mg	20 mg	30 mg
2	Distilled water	1.5 mL	2.5 mL	3.5 mL
3	Soy lecithin	10 mg	15 mg	20 mg
4	Oleic acid	10 mL	10 mL	10 mL

Characterization of Microemulsion and o/w emulsion of Berberine

FTIR analysis to study drug interaction

FTIR analysis was carried out to investigate the interactions among berberine, oleic acid, surfactant, and co-surfactant in the microemulsion formulation. The analysis was conducted using an FTIR spectrophotometer from Shimadzu. To facilitate comparative analysis of the interactions, a berberine marker compound was employed as the control.[11,12]

Determination of size distribution and zeta potential

The particle size distribution and zeta potential of the developed microemulsion formulation were assessed using Dynamic Light Scattering (DLS) with a NANO ZS Malvern Instrument equipped with Zeta sizer software version 6.32. For particle size measurement, the microemulsion was appropriately diluted with redistilled water at a ratio of 1:10 (v/v) and analyzed at a temperature of 25ºC. The zeta potential of the microemulsion was determined using the same instrument at a temperature of 25ºC.[11,12]

PH measurement

The pH value of the microemulsion and emulsion (o/w) was determined using a digital pH meter (ANALAB) that was calibrated using pH 4 and 7 buffer solutions.

Viscosity measurement

The rheological properties of the microemulsion and emulsion (o/w) were examined using a Brookfield viscometer equipped with spindle SC-3. The purpose of this evaluation was to determine the system type, specifically whether it was o/w (oil-in-water) or w/o (water-in-oil). The viscosity of the samples was measured at a temperature of 25ºC, with a shear rate of 50 rpm.

Optical clarity (Percentage transmittance)

The percent transmittance of the microemulsion and o/w emulsion was determined at a wavelength of 345 nm using a UV spectrophotometer. Ethanol was employed as the blank solution for baseline correction.[11,12]

Conductivity measurement

The electrical conductivity of the microemulsion and emulsion was assessed using a digital conductivity meter (ANALAB) equipped with conductivity cells having a cell constant of 1.02. The meter was calibrated using a 1N KCl solution with a known standard KCl conductance value of 12.90 µ Siemens/meter. Conductivity measurements were employed to determine the type of microemulsion, with a low conductance value indicating water-in-oil (w/o) microemulsion and a high conductance value indicating an oil-in-water (o/w) microemulsion.[11,12]

The physical stability of microemulsion and emulsion

The physical stability of the microemulsion and emulsion was evaluated in relation to temperature variations. Both formulations were exposed to different temperatures (4ºC, 25ºC, and 37ºC) and observed for any indications of phase separation, flocculation, or precipitation.[11,12]

Staining test/dye test

To determine the nature of the continuous phase, a water-soluble dye and a methylene blue solution (10 µL) were introduced into both the microemulsion and emulsion formulations. In an o/w emulsion, where the continuous phase is water, the dye would uniformly dissolve throughout the system. Conversely, in a w/o emulsion, where the continuous phase is oil, the dye would aggregate and remain concentrated on the surface of the system.[13]

Dilutability test

The microemulsion and emulsion samples were diluted with double distilled water and oil, respectively, in a ratio of 1:10. This dilution was performed to assess the occurrence of any phase separation within the systems.[13]

Storage stability

The storage stability of the microemulsion and o/w emulsion was investigated over a specified period of time. Prepared microemulsion and o/w emulsion samples weighing 10 g each were stored in disposable conical tubes. The stability of both formulations was monitored through visual inspection on a daily basis initially and subsequently by measuring turbidity on a monthly basis for a total duration of 2 months at room temperature (25ºC).[13]

Drug content estimation

To quantify the drug content in the microemulsion, an equivalent of 10 mg of berberine was dissolved in an appropriate volume of

ethanol (1.5 mL). The resulting solution was thoroughly mixed to achieve complete dissolution of the drug in ethanol. Subsequently, the solution was subjected to analysis using a spectrophotometer at a wavelength of 345 nm. The drug content was determined by employing the following equation:[13]

Drug content (DC) (%)=(Actual amount of Berberine in formulation-Theoretical amount of Berberine in formulation)/ Actual amount of Berberine in formulationx100

In vitro Biological Evaluation

In vitro Antimicrobial Activity

The antimicrobial activity of the developed microemulsion and emulsion formulations was evaluated following a modified method by Suzilla W. Y *et al.*, 2020.[14]

To prepare the test samples, 10 mg of the optimized microemulsion formulation was dissolved in 10 mL of a 10% Dimethyl Sulfoxide (DMSO) solution resulting in a concentration of 1 mg/mL. Serial dilutions of 100 µg, 200 µg, 300 µg, 400 µg, and 500 µg were prepared from the stock solution. Six strains of microorganisms, including *Staphylococcus aureus* (NCIM 2079), *Staphylococcus epidermidis* (NCIM 5270), *Pseudomonas vulgaris* (NCIM 2813), *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2065), and *Candida albicans* (NCIM 3100), were selected for assessing the antimicrobial potential of the berberine-loaded microemulsion. The experimental procedure was conducted as follows:

All test tubes and glassware were autoclaved to ensure sterility. To prepare the culture media, 0.434 g of nutrient broth was dissolved in 35 mL of distilled water, and 2.8 g of nutrient agar was dissolved in 100 mL of distilled water. Both solutions were sterilized in an autoclave at 15 lb pressure for 20-30 min. After sterilization, the nutrient broth was allowed to cool in a laminar airflow cabinet, while the nutrient agar was poured into Petri plates in a laminar airflow cabinet and allowed to solidify. Next, 5 mL of nutrient broth was transferred into each sterile test tube. A loopful of two-week-old bacterial, yeast, and fungal isolates were inoculated into the respective nutrient broth tubes, and the tubes were incubated for 48-72 hr. After incubation, the organisms were inoculated onto the entire surface of prepared Mueller-Hinton agar (38 g/L) on culture plates using sterile swab sticks. Wells with a diameter of 6 mm were created in the agar plates using a sterile cork borer. Each concentration of the diluted samples (20 µL) was transferred into the wells. Amoxicillin at a concentration of 40 mg/L was used as a positive control for antibacterial activity. The plates were then incubated at 37°C for 16-18 hr for bacteria and 48-72 hr for fungi. Finally, the results were determined by measuring the diameter of the Inhibition Zone (IZD in mm) around the wells.

In vitro Antioxidant activity

The purpose of this assay was to evaluate the antioxidant potential of the developed microemulsion and emulsion formulations. In this study, a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in ethanol (25 µg/mL) was prepared. Briefly, 2.5 mL of the DPPH solution was mixed with 0.25 mL of various concentrations of standard berberine (10-50 µg/mL) and berberine microemulsion/ emulsion solution in ethanol (10-50 µg/mL), along with 2.25 mL of ethanol. The resulting solution was then incubated in the dark for 20 min at room temperature. After incubation, the absorbance of the solution was measured at 517 nm using a UV-2500 spectrophotometer (Shimadzu Co., Ltd., Tokyo, Japan). A separate measurement was taken for the DPPH radicals without the presence of the drug as a control. The absorbance values of the samples were compared to those of the blank control. The assay was performed in triplicate, and the results were averaged.

To calculate the antioxidant activity, the following formula was used:

% Inhibition=(Absorbance of blank-Absorbance of sample)/ Absorbance of blank x100

This calculation provided the percentage of inhibition, which represents the antioxidant activity of the sample.

In vitro Anti-inflammatory activity

The purpose of this study was to evaluate the anti-inflammatory activity of the microemulsion and emulsion using a protein

Table 2: Optimized batches of microemulsion.

Sl. No	Formulation code	Drug Mg/mL	Oil (Oleic acid)	Surfactant (Tween 80)	Co-surfactant (Ethanol)	Distilled water
1	Batch A	9.40 mg	2 mL	1 mL	1 mL	2 mL
2	Batch B	20 mg	1 mL	3.5 mL	3.5 mL	0.5 mL
3	Batch C	20 mg	1 mL	1.5 mL	3 mL	1.5 mL
4	Batch D	20 mg	1 mL	1 mL	3 mL	1.5 mL
5	Batch E	10 mg	1 mL	1 mL	1.5 mL	2 mL
6	Batch F	20 mg	1 mL	3 mL	3 mL	0.8 mL

denaturation assay with hen egg albumin. The experimental procedure was as follows:

A reaction mixture was prepared by combining 2.8 mL of normal saline solution, 0.2 mL of fresh albumin solution, and 2 mL of the microemulsion solution. The mixture was incubated for 20 min at 37°C and then heated at 51°C for 20 min. After cooling the mixture, the optical density was measured at 600 nm using a suitable spectrophotometer.

To calculate the percentage of inhibition, the following formula was used:

% inhibition=((Abs Control-Abs Sample)/Abs Control)x100

This calculation determines the percentage of inhibition by comparing the optical density values of the control solution and the sample solution.

Statistics

All data was expressed in Mean±SD. Data was analysed by one way ANOVA using MS excel. *p* values of data below 0.05 considered significant

RESULTS AND DISCUSSION

From 100 g of *Berberis aristata* powder, approximately 13.65% of yellow berberine crystals were isolated (refer to Figure 1a and b). To compare the isolated berberine crystals with other trial extracts (water, 70% ethanol, and 95% ethanol) and standard berberine, a comparative qualitative analysis was performed using TLC analysis (refer to Figure 1c, d, e). Berberine spots were observed and confirmed in all extracts, displaying both high and low intensity, with an R_f value of 0.63. The isolated berberine crystals exhibited a higher intensity spot compared to the other extracts. Consequently, the decision was made to utilize the isolated berberine crystals for further formulation and analysis.

A screening process was carried out to determine the most suitable oils, surfactants, and co-surfactants for the microemulsion formulation. Oleic acid, Tween 80, and ethanol demonstrated the highest solubilization capacities, with values of 145.72±4.76 mg/mL, 37.82±2.89 mg/mL, and 34.298±2.782 mg/mL, respectively. In the Oleic acid/Tween-80/ethanol/water system, the maximum solubility of berberine was found to be 10 mg/mL at a temperature of 30°C.

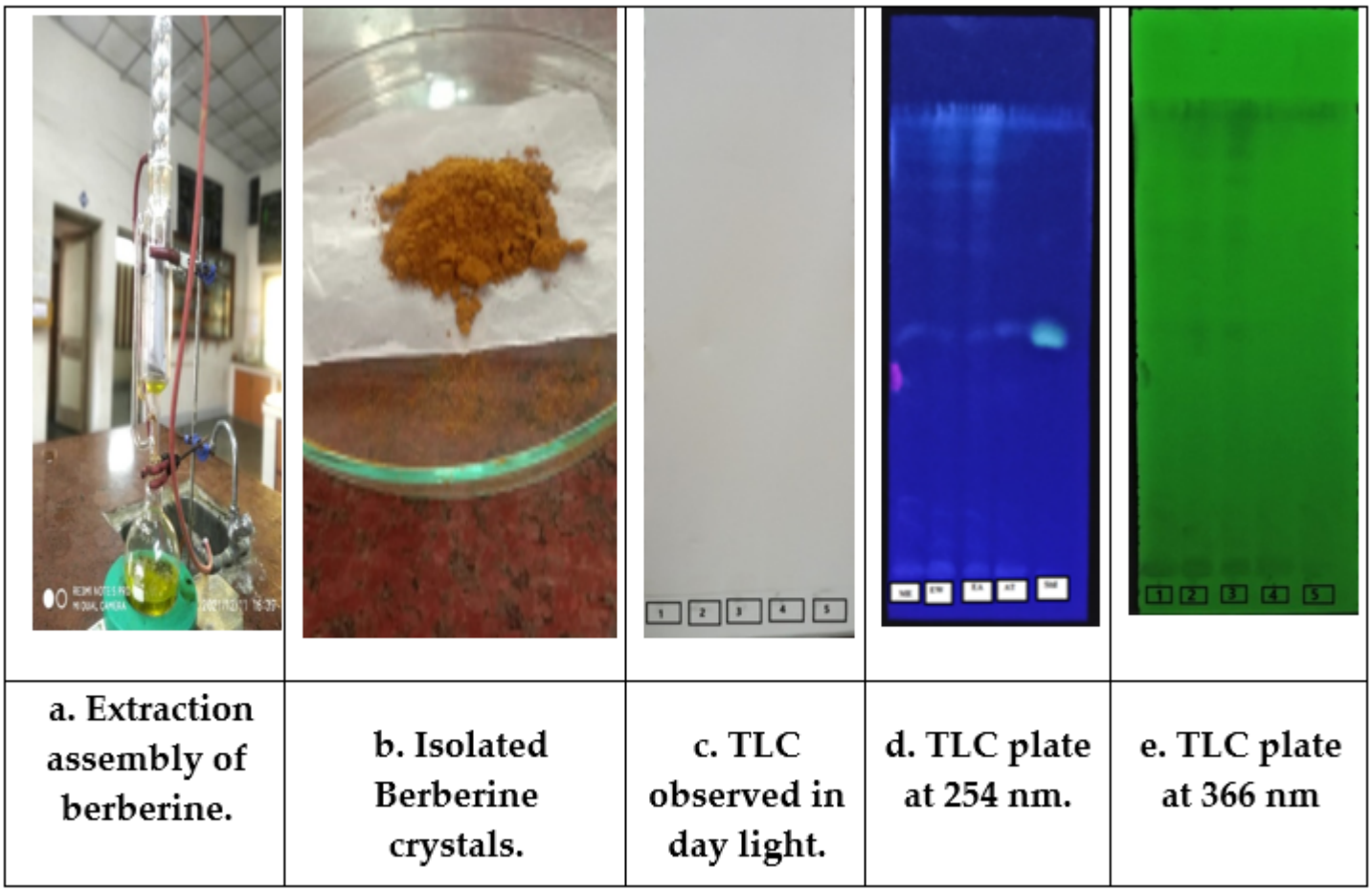

Figure 1: Isolation and confirmation of berberine from *Berberis aristata* roots.

Microemulsion formulation and physicochemical characterization outcomes

During the process of batch-wise optimization of the microemulsion formulation, Batch E exhibited superior characteristics compared to other batches (A, B, C, and D) in terms of clarity and transparency (refer to Figure 2). Physicochemical characterization, including the dye test (refer to Figure 3) and dilutability test, indicated that batches B, C, D, and E belonged to the oil-in-water (o/w) type, while batches A and F belonged to the water-in-oil (w/o) type. Batch E demonstrated favorable viscosity (120.23±1.5 cp), conductivity (201.10±2.03 ms/cm), and physical stability at temperatures of 4°C, 25°C, and 37°C, surpassing the other batches (refer to Table 3 and Figure 4). Moreover, Batch E exhibited higher values for drug content and transmittance percentage compared to the other batches (refer to Table 3). Consequently, Batch E was chosen for further analysis and therapeutic evaluation.

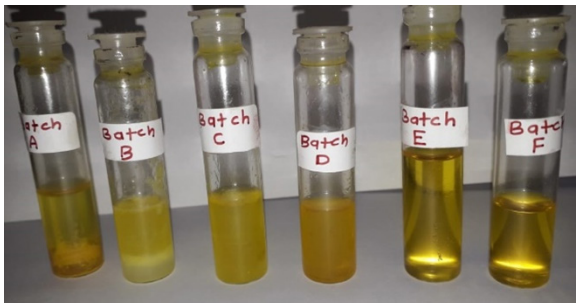

Figure 2: Microemulsion batches A, B, C,D,E

FTIR analysis was conducted on microemulsion Batch E, oleic acid, Tween 80, and unformulated isolated berberine crystals to investigate the possible entrapment of the berberine drug within the system. In the FTIR spectra of berberine (refer to Figure 5c), intense absorption peaks were observed at 1610.56 cm^{-1} (C=C stretching), 1506.41 cm^{-1} (N-O stretching), 1338.60 cm^{-1} (O-H bending), 1107.14 cm^{-1} (C-O stretching), and 1039.63 cm-1 (C-O stretching). In the spectra of the berberine microemulsion

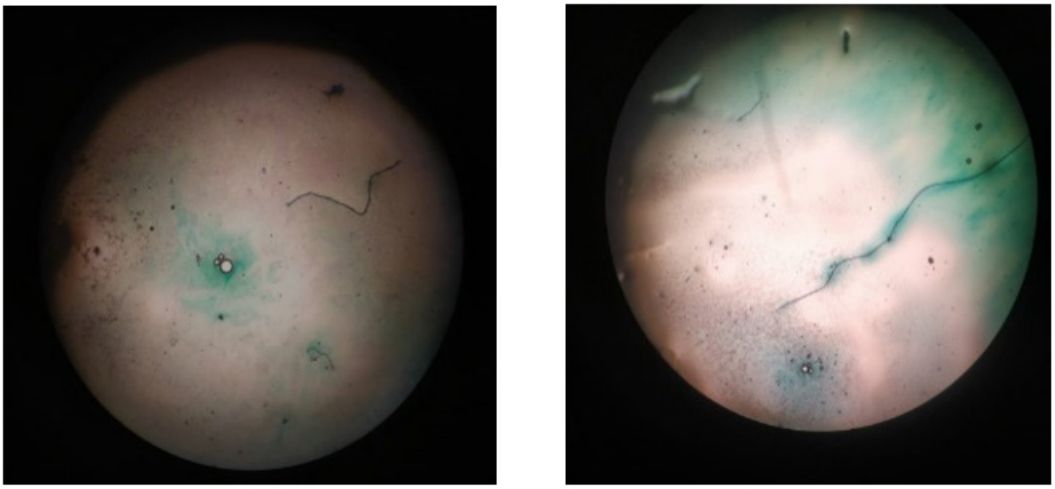

Figure 3: Staining test (dye test) of microemulsion.

Table 3: Summary of characterization of developed microemulsion formulation of isolated berberine crystals.

Sl. No.	Parameters of Characterization	Batch A	Batch B	Batch C	Batch D	Batch E	Batch F
1	pH	5.11±0.51	5±2.10	6±0.32	5.50±0.60	5.09±6.8	6±0.54
2	Viscosity (cp)	122.12±4.03	136.02±6.11	124.23±4.03	123.03±6.05	120.23±1.5	130.20±4.03
3	Conductivity (ms/cm)	110.32±1.08	98.03±3.89	106.13±2.34	108.22±1.78	201.10±2.03	111.08±1.12
4	Dye Test	w/o type	o/w type	o/w type	o/w type	o/w type	w/o type
5	Dilutability Test (Distilled water in 1:10 ratio)	w/o type	o/w type	o/w type	o/w type	o/w type	w/o type
8	Physical Stability at (4°C, 25°C, and 37°C)	Stable	Unstable	Unstable	Unstable	Stable	Unstable
9	% Drug Content at 100 µg/mL concentration	91.03±1.29	96.11±3.21	94.60±3.82	95.30±12.92	95.30±12.92	93.43±9.01
10	% Transmittance	93.23±4.29	89.10±1.04	90.23±2.87	89.45±6.03	98.30±5.01	98.18±1.16

Data analysed by one way ANOVA; $p< 0.05$, data found statistically significant.

formulation (refer to Figure 5d), the characteristic vibrational changes in the absorption peaks of berberine were diminished or absent, indicating successful entrapment of berberine in the oleic acid (refer to Figure 5a), Tween 80 (refer to Figure 5b), and water microemulsion system.

The particle size of microemulsion Batch E was determined to be 116.2 nm (refer to Figure 6), indicating rapid diffusion of the drug. The zeta potential (refer to Figure 7) exhibited a negative value of -9.43, indicating good stability and a lower likelihood of globule aggregation. Based on the comprehensive physicochemical characterization results, Batch E was selected as the final formulation for subsequent analysis of therapeutic efficacy.Entrapment efficiency by IR spectroscopy.

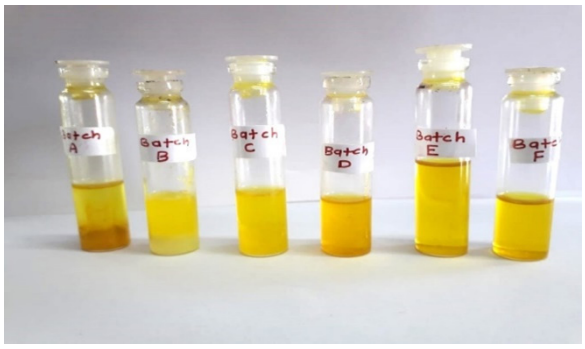

Figure 4: Storage stability of Microemulsion batches.

Emulsion formulation and physicochemical characterization outcomes

The isolated berberine crystals were utilized to develop an emulsion formulation through a batch-wise optimization process. Comprehensive physicochemical characterization was conducted to ensure that the formulation met the required criteria. Among the evaluated batches (A, B, and C), batch B exhibited superior characteristics in terms of clarity and transparency compared to the other emulsion batches A and C. This conclusion was based on various parameters, including viscosity (126.10±3.02), conductivity, percentage transmittance, and physical stability at temperatures of 4°C, 25°C, and 37°C (as presented in Table 4).

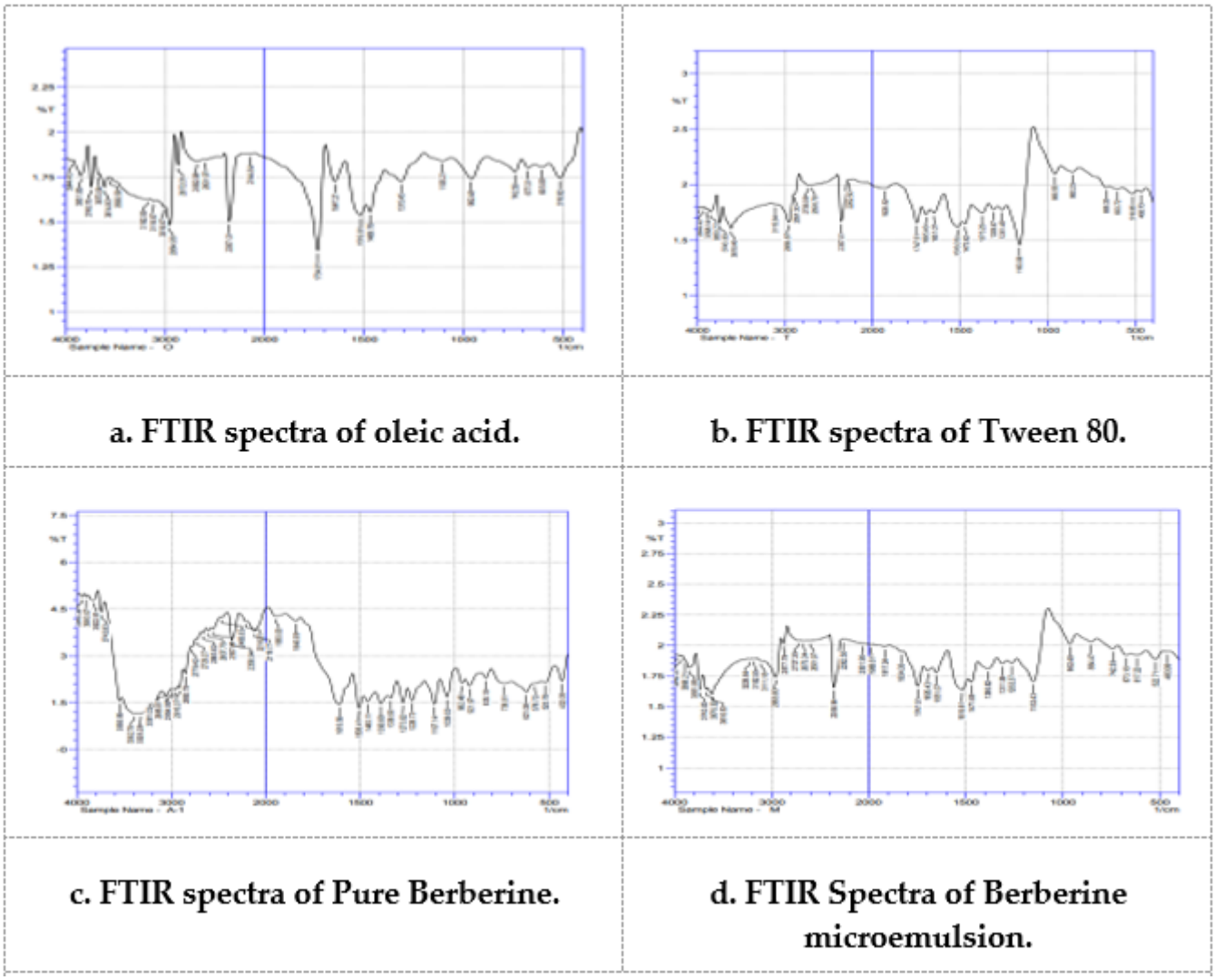

Figure 5a, b, c, d: Entrapment efficiency by IR spectroscopy.

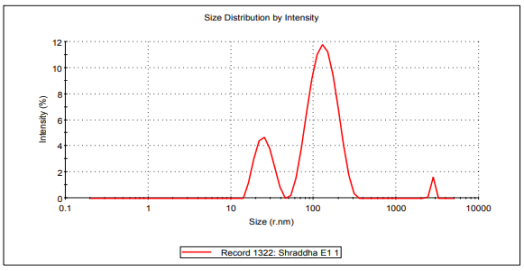

Figure 6: Particle size distribution of Berberine microemulsion.

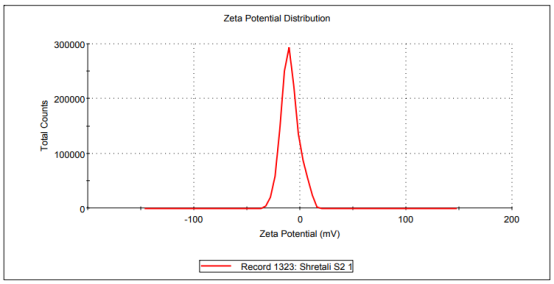

Figure 7: Zeta potential of Berberine microemulsion.

Additionally, the percentage of drug content increased in the order of Batch A, B, and C, as the concentration of berberine in the formulation increased. Notably, Batch B demonstrated a drug content of 89.22±1.01%. Furthermore, Batch B exhibited superior percentage transmittance compared to Batch A and C. Considering these findings, Batch B was chosen as the final emulsion formulation for further analysis of *in vitro* therapeutic efficacy.

In vitro Biological evaluation of Berberine Microemulsion and emulsion

Due to the well-known antioxidant and anti-inflammatory properties of berberine, *in vitro* assays were conducted to evaluate the antioxidant, anti-inflammatory, and antibacterial activities of the formulated microemulsion and emulsion. The results of these assays were compared with an unformulated isolated berberine crystal formulation.

In the antimicrobial assay (Figure 8), the berberine microemulsion exhibited a significant enhancement in antibacterial activity compared to the berberine crystal solution and berberine emulsion against highly pathogenic microbes, including *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Staphylococcus*

Table 4: Summary of characterization of developed emulsion formulation of isolated berberine crystals.

Sl. No.	Physicochemical Parameters of Characterization	Batch A	Batch B	Batch C
1	pH	5.5±0.53	5.1±1.18	6±0.14
2	Viscosity (cp)	119.10±1.02	106.10±3.02	129.02±5.91
3	Conductivity (ms/cm)	105.12±2.04	98.32±3.21	122.43±1.10
4	Dye Test	w/o type	o/w type	o/w type
5	Dilutability Test (Distilled water in 1:10 ratio)	w/o type	o/w type	o/w type
8	Physical Stability at (4°C, 25°C, and 37°C)	Phase separation observed	Stable	Stable
9	% Drug Content at 100 µg/mL concentration	87.03±0.23	89.22±1.01	91.10±2.35
10	% Transmittance	92.76±0.12	93.10±3.14	80.23±0.03

Data analyzed by one way ANOVA; $p>0.05$, data found statistically significant.

Table 5: Zone of inhibition of standard drug ampicillin, Berberine solution, Berberine microemulsion.

Microbial strain	Zone of inhibition (mm) of Ampicillin (500 µg/mL)	Zone of inhibition (mm) unformulated Berberine solution (500 µg/mL)	Zone of inhibition (mm) Berberine microemulsion (500 µg/mL)	Zone of inhibition (mm) Berberine emulsion (500 µg/mL)
S. aureus (NCIM 2079)	30.67±3.51	28.33±5.51	30±8.89	24.01±5.21
E. coli (NCIM 2065)	35±5	26.33±7.09	29.67±1.53	23.54±3.11
P. vulgaris (NCIM 2813)	31.67±6.03	28.33±3.21	30.33±8.74	23.01±5.08
S. epidermidis (NCIM 5270)	40.67±4.04	26±3.61	30.33±6.51	24.26±6.51
B. subtillis (2063)	40.33±1.53	31±5	33.33±7.51	25.05±5.09
C. albicans (NCIM 3100)	41±3	27.33±3.21	31.67±7.02	22.90±7.02

epidermidis, Bacillus subtillis, and *Candida albicans* (as presented in Table 5). The berberine microemulsion demonstrated larger inhibition zones against these microbes at a concentration of 500 µg/mL. However, the *in vitro* activity of the microemulsion was found to be comparable to or slightly lower than that of the standard antibiotic Ampicillin.

Dose-dependent free radical scavenging activity was observed in the unformulated isolated berberine crystal solution, berberine microemulsion, and emulsion (as shown in Figure 9). Comparison of the results revealed that the berberine microemulsion formulation exhibited a higher percentage of free radical inhibition at a concentration of 50 µg/mL compared to the isolated berberine

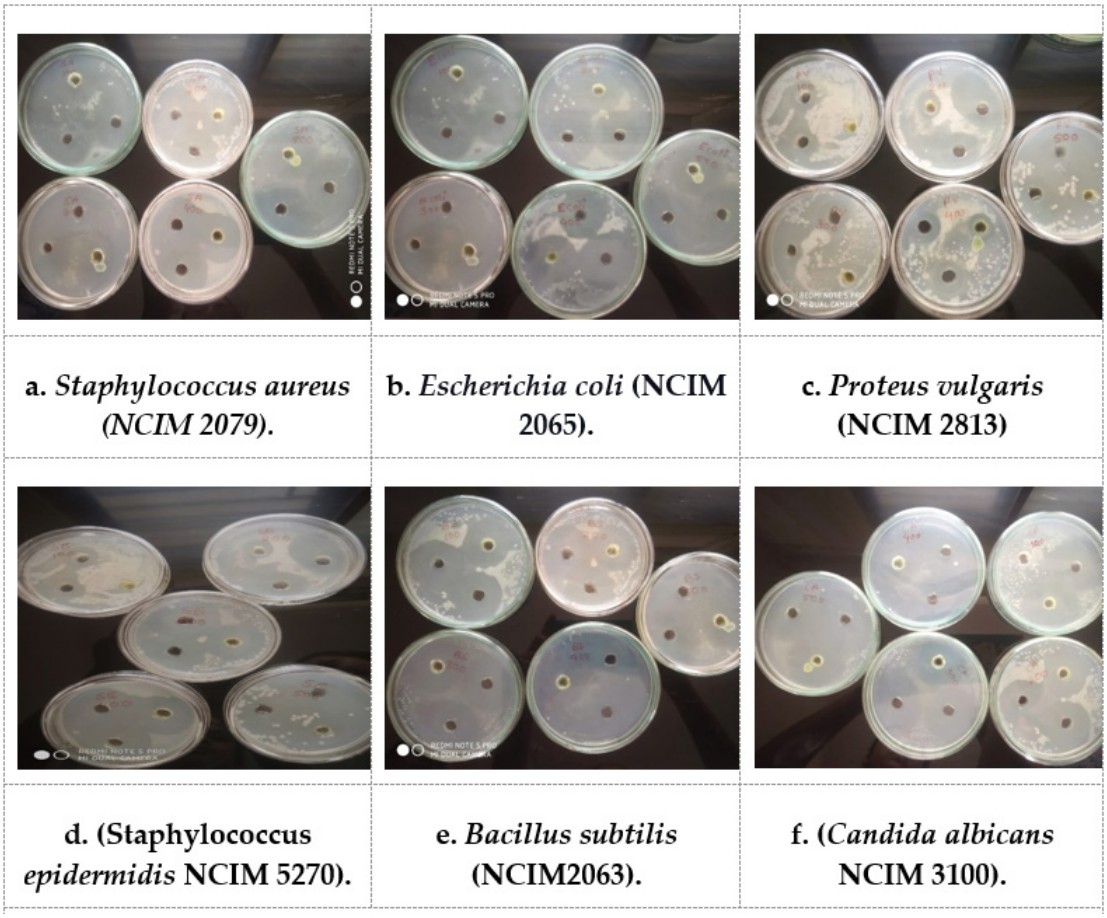

Figure 8a, b, c, d, e, f: Antimicrobial activity results.

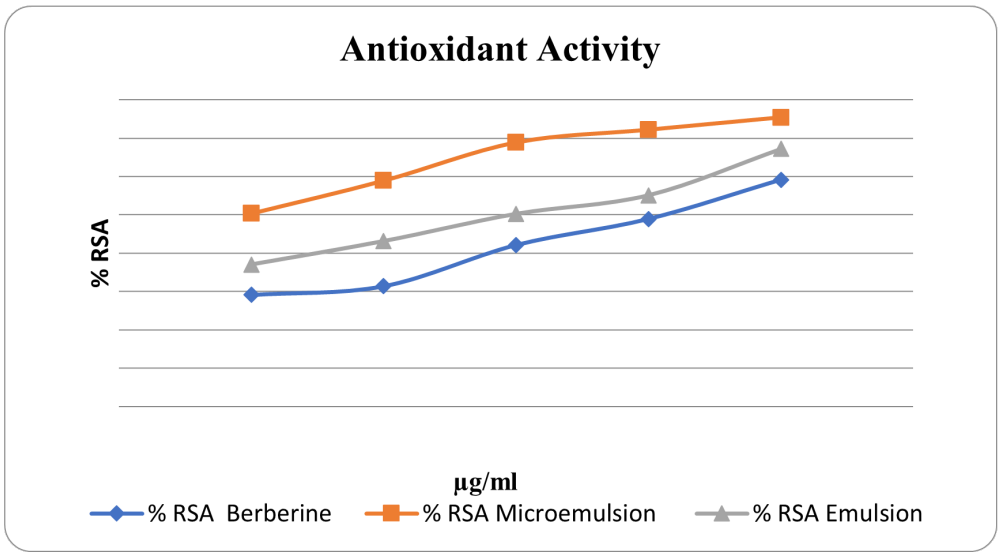

Figure 9: *In vitro* Anti-oxidant efficacy of isolated berberine crystal solution, microemulsion and emulsion. Data analysed by one way ANOVA; $p<0.05$, data found statistically significant.

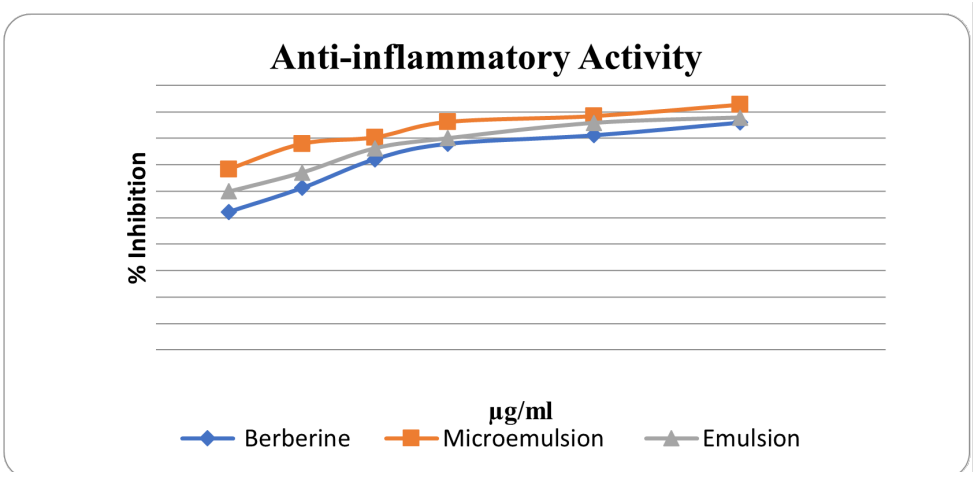

Figure 10: *In vitro* Anti-inflammatory efficacy of berberine crystal solution, microemulsion, emulsion. Data analysed by one way ANOVA; $p < 0.05$, data found statistically significant.

crystals and emulsion. This suggests that the microemulsion formulation improved the antioxidant activity of berberine. In the anti-inflammatory activity assay, dose-dependent inhibition of protein denaturation was observed with the unformulated isolated berberine, berberine microemulsion, and emulsion. The berberine microemulsion exhibited significant inhibition of the protein denaturation process at a concentration of 800 µg/mL, surpassing the activity of the unformulated berberine crystals and emulsion formulation. This indicates that the microemulsion formulation can significantly enhance the anti-inflammatory properties of conventional forms of berberine (as shown in Figure 10).

CONCLUSION

Based on the comprehensive physicochemical characterization, *in vitro* antioxidant, anti-inflammatory, and antibacterial studies, it can be concluded that the microemulsion formulation significantly enhances the therapeutic efficacy of the poorly water-soluble conventional form of berberine compared to the simple emulsion formulation. The o/w type berberine microemulsion exhibited thermodynamic stability, clarity, and improved therapeutic potential, making it suitable for topical and oral applications in the treatment of chronic and acute skin diseases such as leprosy, psoriasis, rashes, and diabetic wounds, among others. However, further *in vivo* investigations are necessary to elucidate the mechanism of action of this NDDS formulation and to validate the *in vitro* findings presented in this research work.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared; **O/W:** Oil in Water; **DPPH:** 2,2-diphenylpicrylhydrazyl; **HCl:** Hydrochloric Acid; **TLC:** Thin Layer Chromatography; **PEG:** Polyethylene Glycol; **rpm:** Revolution Per Minute; **DLS:** Dynamic Light Scattering; **NANO ZS:** Nano Zeta Sizer; **KCl:** Potassium Chloride; **DMSO:** Dimethyl Sulfoxide; **NCIM:** National Collection of Industrial Microorganisms; **SD:** Standard Deviation; **NDDS:** Novel Drug Delivery System.

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