Int. J. Pharm. Investigation, 2026; 16(1):272-282.
https://www.jpionline.org

Original Article

From Ghrita to Microemulsion: Innovative Delivery of Medhya Rasayana for Neuroprotection

Sharada Laxman Deore, Ram Chhatrapati Vighne*, Bhushan Arun Baviskar

Government College of Pharmacy, Amravati, Maharashtra, INDIA.

ABSTRACT

Background: The aim of the current investigation is to develop a microemulsion of bioactive enriched fractions of Indian traditional medicinal plants *Bacopa monnieri* and *Centella asiatica* to enhance neuroprotective efficacy overcoming drawbacks of traditional formulations. **Materials and Methods:** Microemulsion comprising bioactive enriched fractions of *Bacopa monnieri* and *Centella asiatica* are prepared, characterized and evaluated for its neuroprotective and neurocognitive efficacy as well as potency by *in vitro* antioxidant, anti-cholinesterase, anti-inflammatory activities. Particle size, polydispersity index, viscosity, zeta potential, electrical conductivity, drug content, pH measurement, percentage transmittance, FTIR and DSC analysis found promising and demonstrated that the integrity of the microemulsion. **Results and Discussion:** The optimized microemulsion formula exhibited chemical and physical stability during study duration. DSC and FTIR studies confirmed compatibility of excipients and bioactive enriched fractions. Values of electrical conductivity 0.0743 mS/cm, 0.0746 mS/cm, and 0.0745 mS/cm for BM, CA and mix combinations of microemulsions respectively. Oil in water type microemulsion confirmed in staining tests of optimized microemulsion formula. **Conclusion:** Microemulsions of bioactive-enriched fractions *Bacopa monnieri* and *Centella asiatica* are next-generation delivery systems that help in overcoming solubility, stability, and bioavailability challenges of herbal actives, making them more clinically relevant.

Keywords: Centella, Bacopa, Asiaticosides, Bacosides, Acetylcholinesterase.

Correspondence:
Mr. Ram Chhatrapati Vighne
Government College of Pharmacy, Amravati-444604, Maharashtra, INDIA.
Email: rampharmacy14@gmail.com

Received: 08-04-2025;
Revised: 24-06-2025;
Accepted: 19-08-2025.

INTRODUCTION

A clear, single-phase system, known as a microemulsion, was produced by Hoar and Schulman in the 1940s through titration of a watery emulsion with hexanol (Anil *et al*., 2018). Microemulsions have been recognized for their numerous advantages since that time, and a significant advancement in delivery methods has been made (Vo *et al*., 2021). Microemulsion can be considered one of numerous nanotechnology formulations that can be used to deliver drugs across lipophilic barriers. Popular traditional medicinal plants are not only antistress, memory enhancing but also help in regeneration of neural tissues by antioxidant, anti-inflammatory, anti-amyloidogenic, nutritional and immune-supportive effects (Bera *et al*., 2014). Ayurvedic neuroprotective formulations of selected plants *Centella asiatica* and *Bacopa monnieri* are mostly in the form of powder, syrups and tablets which need to be consumed orally in large quantity to achieve desired pharmacological effect which is always inconvenient to geriatric patients (Kahlweit *et al*., 1993; Fanun *et al*., 2010). Hence, microemulsion comprising bioactive enriched fractions-Asiaticosides of *Centella asiatica* and Bacosides of *Bacopa monnieri* will provide enhanced potency with therapeutic efficacy despite increased efforts to develop suitable therapies for neurodegenerative diseases.

MATERIALS AND METHODS

The selected herbs *Centella asiatica* and *Bacopa monnieri* were collected from the Medicinal and Aromatic Plant-AYUSH (MAPA) Garden, Government College of Pharmacy, Amravati, Maharashtra, India. The identification and authentication of both plants were performed by HOD, Pharmacognosy Department, Govt. College of Pharmacy, Amravati based on organoleptic/ macroscopic (organ and sense), microscopic, phytochemical and physicochemical parameters complying with pharmacopeial and equivalent standards. The herbarium voucher specimen number is GCOPA/2021/02.

Reagents and chemicals

Thio Barbituric Acid (TBA), reduced glutathione, oxidized glutathione, and NADPH were procured from Hi-Media Laboratories in Mumbai, India. 5,5-Dithio-bis (2-Nitrobenzoic Acid) (DTNB). All chemicals were obtained from Sigma Aldrich

DOI: 10.5530/ijpi.20260458

(Bengaluru, India) and CDH (Central Drug House Pvt. Ltd., New Delhi, India); the remaining chemicals and reagents used were of analytical quality.

Extraction and Isolation of Bioactive Enriched Fractions

Centella asiatica and *Bacopa monnieri* plants were dried and crushed into a powder. A 100 g sample of each plant powder was extracted using several solvents, including dist. water, 70% ethanol, 95% ethanol, 80% methanol. The solvent was chosen based on the solubility of the selected neuroprotective phytoconstituents. The extraction procedure was carried out using a Soxhlet apparatus at 60ºC for 7-8 hr to guarantee thorough extraction. After the extraction, the filtrates were concentrated and dried at low temperatures. All the resultant extracts were kept in a refrigerator at 4ºC until further processing (Deore *et al.*, 2023). Asiaticosides 12.89% by HPLC and 25.14% by UV spectroscopy found in final bioactive enriched *Centella* extract. Bacosides 13.19% by HPLC and 40% by UV spectroscopy found in final bioactive enriched *Bacopa* extract.

Optimization of microemulsion

Solubility of bioactive enriched fractions was measured in various oils, surfactants and cosurfactants (Table 1).

Preparation of microemulsion

Various trial microemulsion batches were prepared using bioactive enriched fractions at different molar ratios of oil, surfactant, co-surfactant and water (Table 2) by phase titration method. Different optimization batches A, B, C, D, E, F, G, and H prepared, based on ratio of oil phase, surfactant, co-surfactant. Bioactive enriched fractions - CA, BM, and BCM were dissolved in a mixture of oleic acid (oil phase), Tween 80 (surfactant), and ethanol (co-surfactant) according to the composition stated using a magnetic stirrer. Double distilled water was added dropwise with constant uniform and persistent stirring until the solution appeared as clear and transparent.

FT-IR Analysis

The physicochemical compatibility of the bioactive enriched fractions and excipients (oil, surfactant mixture) studied by FTIR (Mishra *et al.*, 2022).

Morphology, Particle size, Polydispersity Index (PDI), Zeta Potential Analysis

Particle Size and polydispersity index measured using Dynamic Light Scattering (DLS) and photon correlation spectroscopy. 1 mL of microemulsion were mixed with 10 mL of water and transferred into a cuvette, and the zeta potential shown in Table 3 was determined using the zeta-sizer (Mandal *et al.*, 2010; Singh *et al.*, 2022).

Pharmacokinetic Evaluation

Permeation study

Ex vivo permeation measured by Franz diffusion cell using a goat buccal mucosa. The donor and receptor compartments of the diffusion cell were carefully separated by the goat buccal mucosa. An isotonic phosphate buffer of pH 6.8 was added to the receptor compartment and maintained at 37±0.2ºC. The hydrodynamics in the receptor compartment kept stable by a stirrer. The donor compartment was filled with the 1 mL microemulsion sample. At regular intervals, samples were taken from the receptor compartment and sink conditions were maintained by adding an equal volume of fresh phosphate buffer (Shah, J. P. *et al.*, 2020).

Differential Scanning Colorimetry

DSC thermograms were recorded on a (DSC-Mettler Toledo) using hermetically sealed aluminum pans under nitrogen purge (50 mL/min). Approximately 5 mg of formulation was weighed into each pan. Samples were equilibrated at -50ºC, heated to 120ºC at 5ºC/min (1st heating), cooled to -50ºC at 5ºC/min, and reheated to 120ºC (2nd heating). Pure drug, physical mixture with excipients, and blank microemulsion were analyzed identically. Onset temperature (Tonset), peak Temperature (Tpeak), and Transition Enthalpy (ΔH) were determined using the instrument software after baseline correction. The absence or reduction of the drug's melting endotherm in the microemulsion relative to the physical mixture was taken as evidence of solubilization/ amorphization. Sub-ambient scans (-50 to 20ºC) were conducted at 2ºC/min to characterize freezable vs. non-freezable water (Shah *et al.*, 2020).

Stability study

Optimized microemulsion, sealed in a glass vials, retained for 6 months to accelerated stability studies at 40±2ºC / 75%±5% RH as per ICH guidelines. Key parameters like appearance, extract content, particle size, zeta potential and pH measured at 0, 1-, 2-3-, and 6-months interval are determined (Mostafa *et al.*, 2014).

In vitro Neuroprotective Studies

Stock solutions of *Bacopa monnieri* Microemulsion (BMM), *Centella asiatica* Microemulsion (CAM), and Both Mix Microemulsion (BCMM) were prepared by dissolving 10 mg of each extract in 10 mL of ethanol, resulting in a final concentration of 1 mg/mL. The stock solution was serially diluted to 10, 20, 30, 40, and 50 µg/mL used for further activities. Neuroprotective potency and efficacy of bioactive enriched fractions was performed by following *in vitro* methods.

In vitro Acetylcholinesterase (AChE) inhibition activity

This study is performed by Ellman's colorimetric method. (Ellman *et al.*, 1961) The chromophore employed was 5,

5-dithio- his-(2-nitrobenzoic acid) (DTNB) at a concentration of 0.01 M. A mixture of 3 mL phosphate buffer (pH 8), 100 µL AChE enzyme, and 100 µL of the sample or standard Donepezil hydrochloride was incubated at 37°C for 5 min to facilitate the binding of the enzyme with the phytoconstituents present in the samples. After 5 min, 100 µL of DTNB was added to the mixture and allowed to stand for a brief period. Subsequently, 20 µL of ATCI was added to initiate the reaction. Readings were taken at 412 nm using a UV Spectrophotometer after 2 min of ATCI addition. For the control, the test compound solution was replaced with 100 µL of water. Calculate the percentage inhibition of acetylcholinesterase by the following formula:

% Inhibition = (Δ Absorbance control)-(Δ Absorbance sample)/(Δ Absorbance control)×100

In vitro Anti-inflammatory activity

Lipoxygenase (LOX) inhibitory activity was measured by monitoring formation of conjugated dienes from linoleic acid at 234 nm. Briefly, 1.0 mL reaction mixtures contained 0.1 M phosphate buffer (pH 7.0), linoleic acid (0.2 mM final), test sample (diluted so final solvent ≤1% v/v) and soybean LOX (added to initiate reaction). Absorbance at 234 nm was recorded every 15 sec for 3 min at 25°C. Percent inhibition was calculated relative to control. A decrease in LOX activity shows that the inflammatory process is less active (Huang *et al.*, 2024). Calculate the percentage inhibition by the following formula:

% LOX Inhibition = (Δ Absorbance control)-(Δ Absorbance sample) / (Δ Absorbance control)×100

In vitro Antioxidant activity

3 mL of each enriched fraction or microemulsion sample (10-50 µg/mL) and 1 mL of DPPH (0.3 mM) solution were combined in a test tube and incubated for 30 min in the dark. After incubation, the absorbance of the mixture was measured at 520 nm. (Khadabadi *et al.*, 2011) The same process was carried out for the standard ascorbic acid samples. % inhibition was calculated by following

% inhibition = (Δ Absorbance Blank-(Δ Absorbance sample/(Δ Absorbance Blank)×100

In vitro Superoxide Dismutase Activity

Take buffer (1.2 mL), PMS stock (0.1 mL), NBT stock (0.3 mL), sample or buffer for control (0.1 mL) and add distilled water to bring the volume close to 2.8-2.9 mL. Keep his mixture at 30°C in water bath for 1-2 min to equilibrate. Add NADH stock (0.2 mL) to each tube to initiates superoxide generation. Immediately mix by gentle inversion or vortex and start timer. Keep tubes at

Table 1: Solubility of bioactive enriched fractions and mixture in oils, surfactants, co-surfactants.

Sl. No.	Types of oils	Solubility in mg/mL		
		EBM	ECA	EBCM
1	Castor oil	10.33±1.572	9.33±1.52	15.964±2.64
2	Soyabean oil	11.710±1.18	8.340±2.172	10.958±1.675
3	Linseed oil	9.6710±0.8511	11.950±1.511	19.497±3.252
4	Oleic acid	21.98±1.672	18.00±2.983	22.964±298
5	Olive oil	10.3611±1.64	16.694 ±2.197	10.641±9.64
6	Peanut oil	19.972±1.29	12.697±257	11.625±2.658
Sl. No.	**Types of surfactants**	**Solubility in mg/mL**		
		EBM	ECA	EBCM
1	Tween 80	17.828±2.8923	15.268±2.641	16.256±1.392
2	Tween 20	1.37±0.52	3.694±2.987	3.652±0.597
3	Span 80	10.00±2.0	13.328±2.551	9.371±2.15
4	Span 20	8.25±2.121	9.361±1.648	6.689±1.975
5	Propylene glycol	8.90±1.725	9.498±2.009	9.158±2.941
6	PEG 400	3.36±1.625	6.265±1.83	11.25±2.35
Sl. No.	**Types of Co-surfactants**	**Solubility in mg/mL**		
		EBM	ECA	EBCM
1	Ethanol	24.298±2.772	19.268±2.031	29.256±0.320
2	Glycerol	14.37±1.52	13.694±2.987	10.692±1.039
3	Propylene glycol	9.00±2.098	8.328±1.921	9.921±1.048
4	Isopropanol	1.25±0.1632	2.361±1.698	3.389±2.629

30°C. Incubate for exactly 90 sec. Add 1.0 mL glacial acetic acid to each tube to terminate reaction and stabilize chromogen. Mix. Add 4.0 mL n-butanol to each tube, vortex vigorously for 30 sec, then centrifuge at 3,000-4,000 × g for 5-10 min to achieve phase separation. The organic (upper) layer contains the blue formazan chromogen. Carefully pipette the upper n-butanol layer (usually ~3.5-4.0 mL) into a fresh tube or measure directly. If emulsions persist, centrifuge again. The intensity of the blue color in the butanol layer is then measured spectrophotometrically at 560 nm. Spectrophotometry was utilized to measure the color intensity of chromogen in the n-butanol layer at 560 nm. The results were expressed in units per milligram of protein (units/mg protein), indicating the specific activity of SOD (Parveen *et al.*, 2016).

SOD % inhibition =Absorbance of Control-Absorbance of treated sample/Absorbance Control×100

In vitro Reduced Glutathione Activity

Reduced glutathione plays an important role in reducing oxidative stress levels. The potential of the selected bioactive rich fractions to increase the level of reduced glutathione was tested using the standard protocol. The absorbance was read within 5 min of the addition 40µL of DTNB at 412 nm against the reagent blank with no sample (Parveen *et al.*, 2016). All the values were expressed as µg mg^{-1} protein. For determination of IC_{50}, 50% inhibition of LOX activity was calculated from

Glutathione % inhibition = Absorbance of Control-Absorbance of treated sample/Absorbance Control×100

Statistical analysis

The results were presented as mean±SD. Statistical comparisons were made by one-way ANOVA followed by Tukey's *t*-test in GraphPad Prism version 10.0, USA. ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Indian traditional medicinal plants, *Bacopa monnieri* and *Centella asiatica* are popular as brain tonic. The novel lipophilic as well hydrophilic carrier system- microemulsion mediated improved neuroprotection is observed due to enhanced bioavailability, permeability, stability and efficacy in *in vitro* studies.

Oil Oleic acid, surfactant Tween 80 and co-surfactant alcohol showed higher solubility for all bioactive enriched fractions. Oleic acid is found to be excellent solubilizer for amphiphilic bioactive enriched fractions. Surfactant Tween 80 contributed for enhanced solubility of bioactive enriched fractions. Co-surfactant alcohol helped to load more bioactive rich fractions by making the interface more flexible and fluid, which is also necessary for forming very small and stable droplets. Thus, based on the choice of these excipients, the developed microemulsion can be useful for oral as well as transdermal application. The particle size of optimized batch H (BMM, CAM, BCMM) was found to be 181.4, 176.4, and 182.7 nm, respectively with zeta potential values of -19.6, -19.7, and -18.6 mV indicating higher stability (Figure 1). Optimized formulation was kept at room temperature for intermediate stability studies for 6 months as per ICH guidelines. No significant physical, chemical and microbial changes observed in the optimized microemulsion batches.

Thermal analysis by DSC of microemulsion (Figure 2) shows no drug melting peak in microemulsion but strong peak in physical mix indicates that drug fully solubilized/amorphous in droplets.

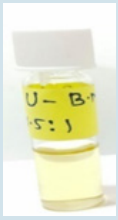
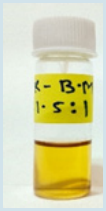
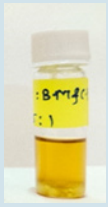
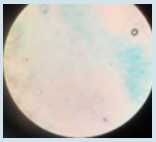
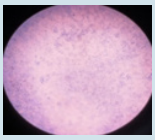
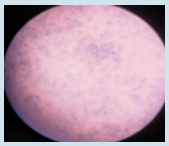
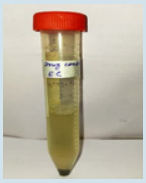
The FTIR spectrum (Table 3) of the microemulsion formulation showed no new peaks, confirming the absence of chemical interactions between drug and excipients. Shift and broadening of characteristic functional groups (O-H, C=O, C-O-C) indicated successful entrapment of the bioactive fraction within the surfactant-oil interface of the microemulsion system. Bioactive extract peaks are masked and or merged, it suggests successful encapsulation inside the microemulsion system Thus, FTIR

Table 2: Optimization Batches of Microemulsion.

Batches	Oil phase	Surfactant	Co-surfactant	Aqueous Phase	Enriched Extract	Different Ratios
Batch A	Oleic acid	Tween 80	Ethanol	Dist. Water	EBM, ECA, EBCM	1:1:1.5:1
Batch B	Oleic acid	Tween 80	Ethanol	Dist. Water		2:1:1:2
Batch C	Oleic acid	Tween 80	Ethanol	Dist. Water		1:3:3:0.5
Batch D	Oleic acid	Tween 80	Ethanol	Dist. Water		1:1.5:3:1.5
Batch E	Oleic acid	Tween 80	Ethanol	Dist. Water		1:1:2:2
Batch F	Oleic acid	Tween 80	Ethanol	Dist. Water		2:2:1.5:2.5
Batch G	Oleic acid	Tween 80	Ethanol	Dist. Water		1:1:2:2.5
Batch H	Oleic acid	Tween 80	Ethanol	Dist. Water		1:1:1.5:3

Batch H has more stability and solubility of enrich drug than other batches. Therefore, this batch select as a finalize batch for pharmaceutical parameter evaluation.

Table 3: Pharmaceutical evaluation of microemulsions.

Parameter	Formulation		
	BMM	CAM	BCMM
Particle size	181.4 nm	176.4 nm	182.7 nm
Polydisperse index	0.633	0.514	0.406
Zeta potential	-19.6	-19.7	-18.6
Viscosity	0.8872cP	0.8872cP	0.8872cP
Electrical Conductivity	0.0743mS/cm	0.0746mS/cm	0.0745mS/cm
Stability			
pH measurement	5.09±6.8	6.0±7.9	6.7±7.6
Dye test/Stanning Test	As water continuous phase and oil is disperse in continuous phase 	As water continuous phase and oil is disperse in continuous phase 	As water continuous phase and oil is disperse in continuous phase 
Optical Clarity/% Transmittance	94.8±1.36	98.6±0.6	98.51±1.65
Centrifugation Assay	No phase separation was observed in the sample after placing it in a centrifuged at 2000 rpm for 30 min at room temperature (27±2°C).		
Drug Content	88.65±2.82 	92.76±3.94 	92.41±2.14 
DSC	Endothermic peak at 100.34°C and 200.15°C	Endothermic peak at 101.54°C and 215.29°C	Endothermic peak at 98.32°C and 207.26°C
FTIR	Plant Extract and microemulsion excipient found to be compatible with each other		

Permeation Study					
	Permeability parameter	Permeation study of microemulsion			
		BMM	CAM	BCMM	Plain suspension
	Area	3.46	3.46	3.46	3.46
	Flux (J) µg/cm2/hr	1.62	0.408	0.684	B-0.234 C-0.105 BC-0.158
	Permeability Coefficient cm/hr	0.00162	0.000408	0.000684	B-0.000234 C-0.000105 BC-0.000158

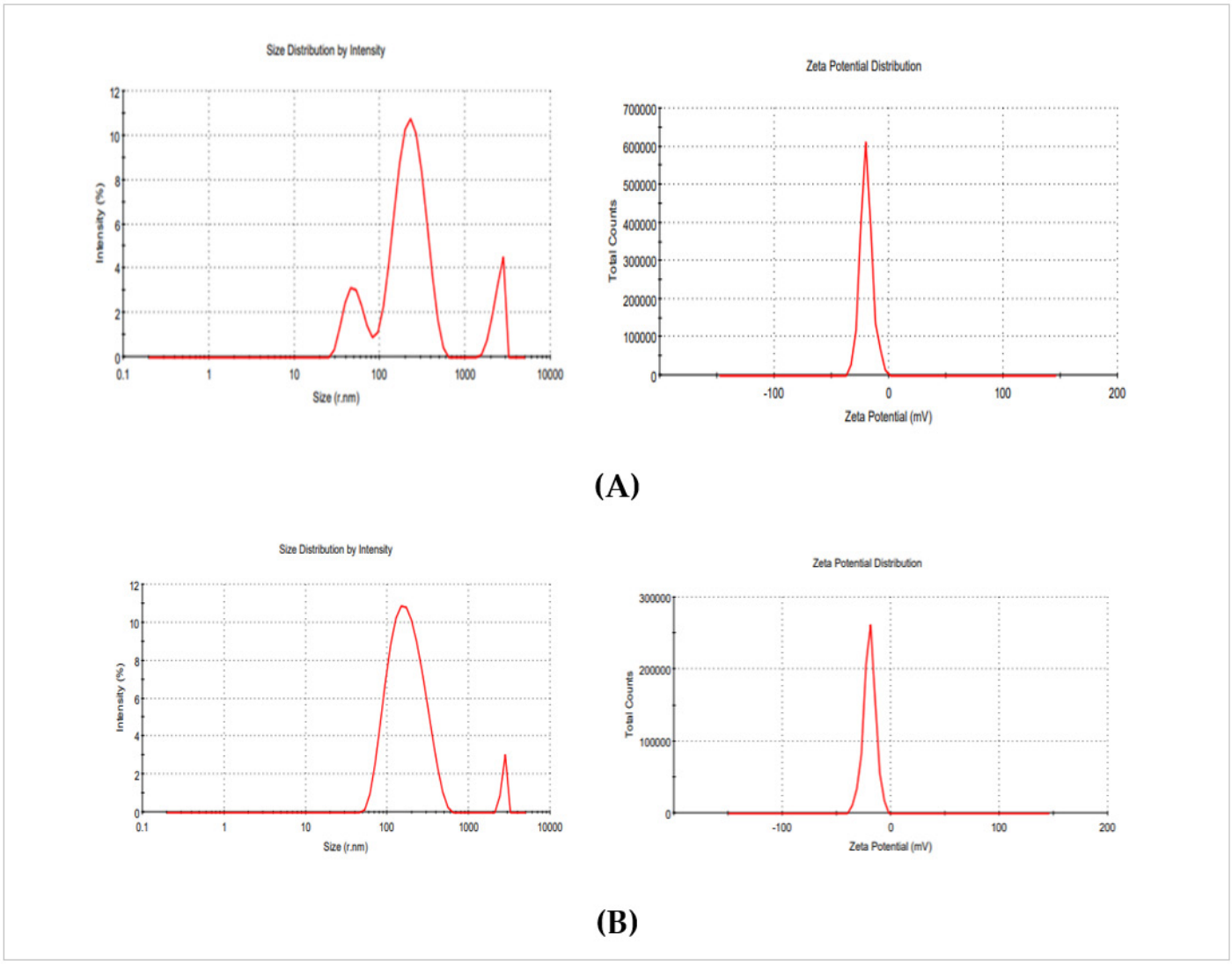


Figure 1: Particle Size Distribution and Zeta-potential of (A) BMM, (B) CAM, (C) BCMM Microemulsion.

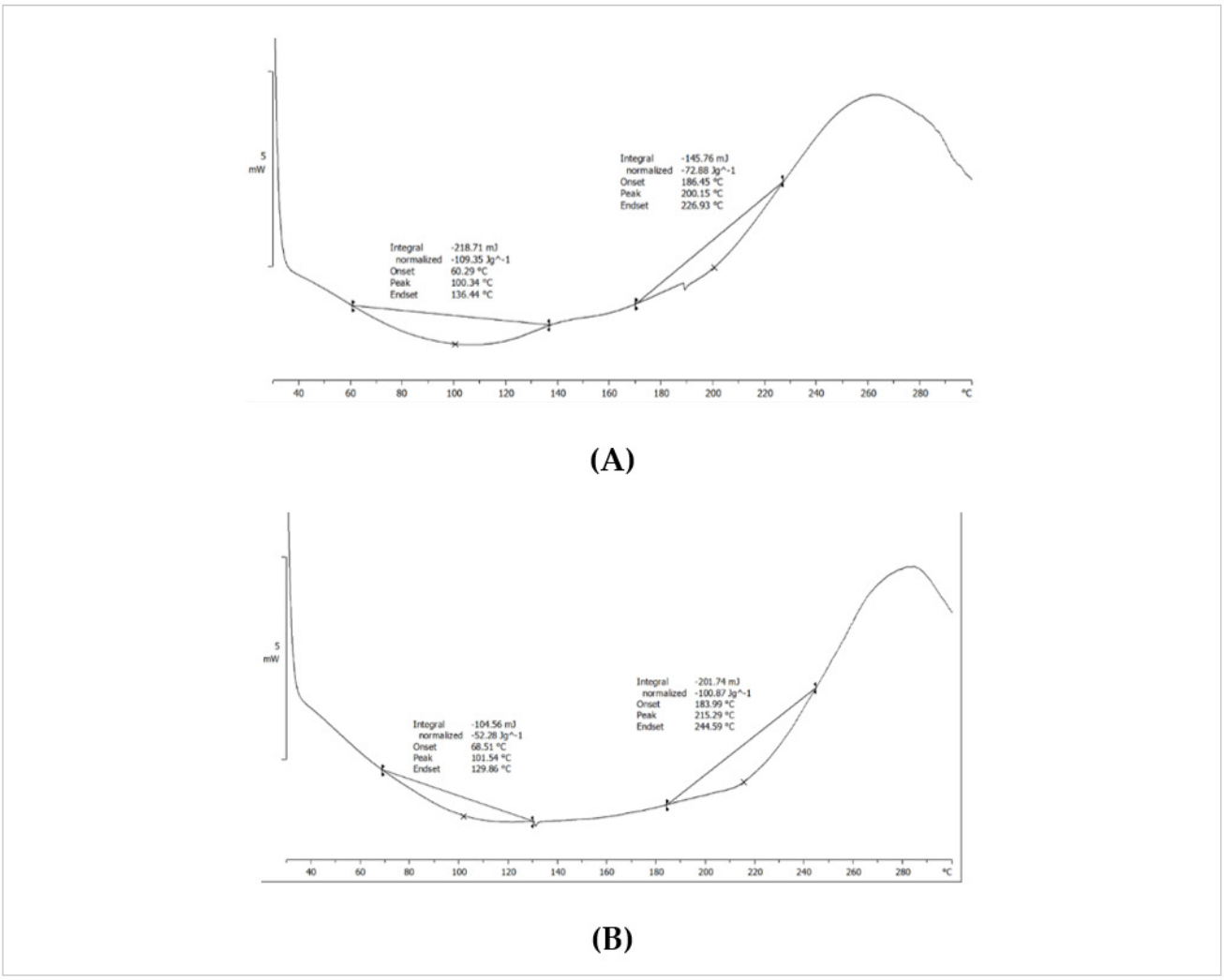
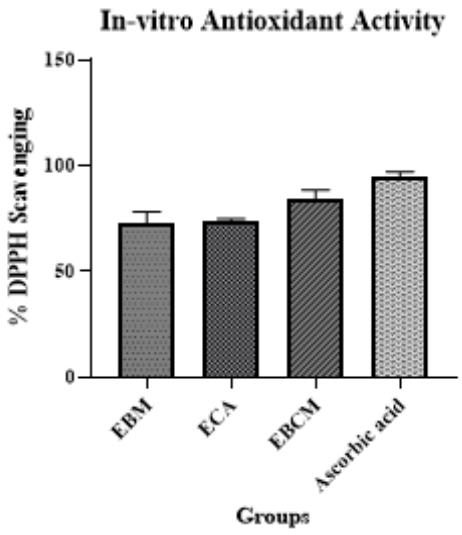
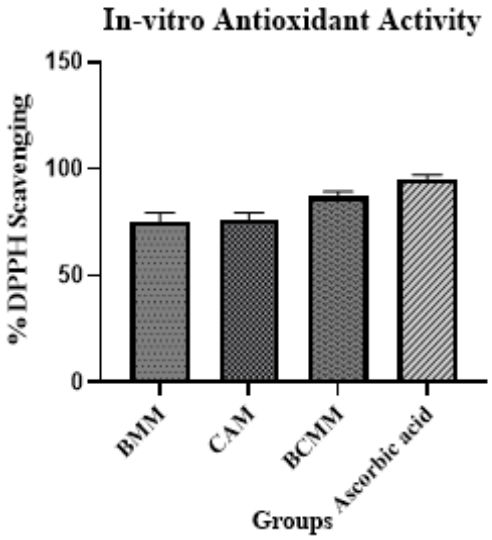
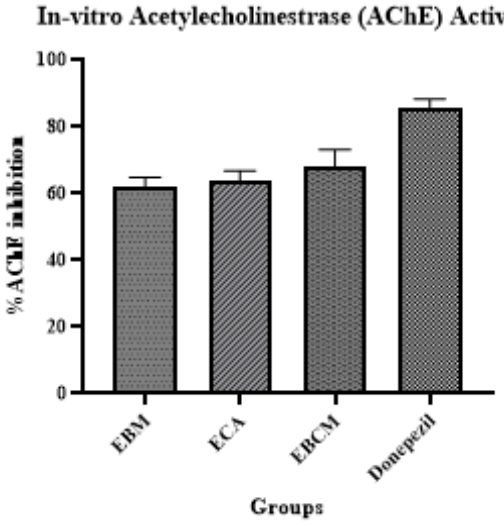
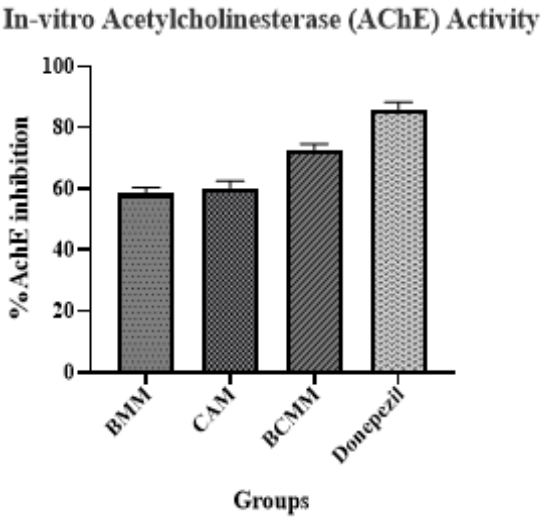


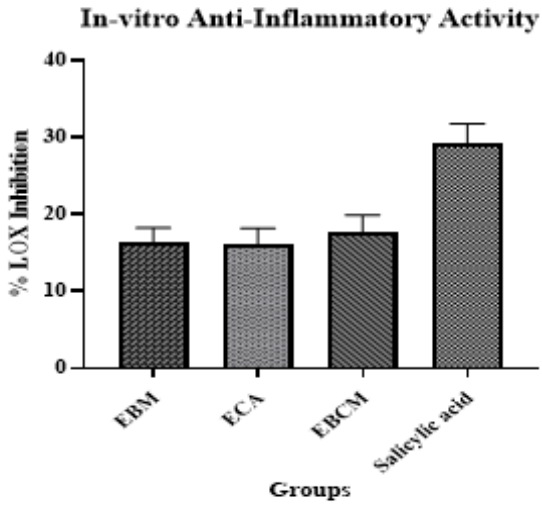
Figure 2: Differential Scanning Calorimetry (DSC) of Batch T: (A) BMM, (B) CAM, (C) BCMM microemulsion.

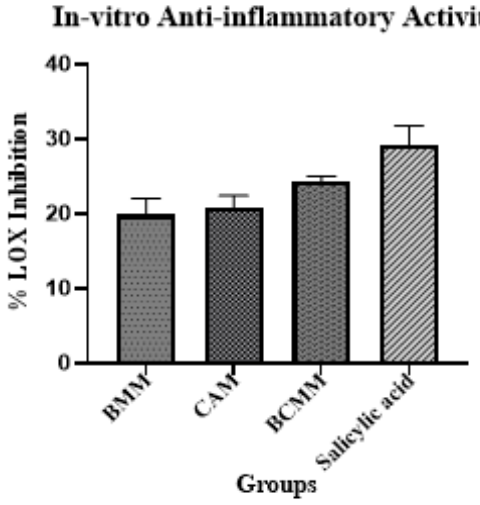

In-vitro Antioxidant Activity
% DPPH Scavenging
150
100
50
0
EBM
ECA
EBCM
Ascorbic acid
Groups


In-vitro Antioxidant Activity
% DPPH Scavenging
150
100
50
0
BMM
CAM
BCMM
Ascorbic acid
Groups


In-vitro Acetylecholinestrase (AChE) Activity
% AChE inhibition
100
80
60
40
20
0
EBM
ECA
EBCM
Donepezil
Groups


In-vitro Acetylcholinesterase (AChE) Activity
% AchE inhibition
100
80
60
40
20
0
BMM
CAM
BCMM
Donepezil
Groups


In-vitro Anti-Inflammatory Activity
% LOX Inhibition
40
30
20
10
0
EBM
ECA
EBCM
Salicylic acid
Groups


In-vitro Anti-inflammatory Activity
% LOX Inhibition
40
30
20
10
0
BMM
CAM
BCMM
Salicylic acid
Groups

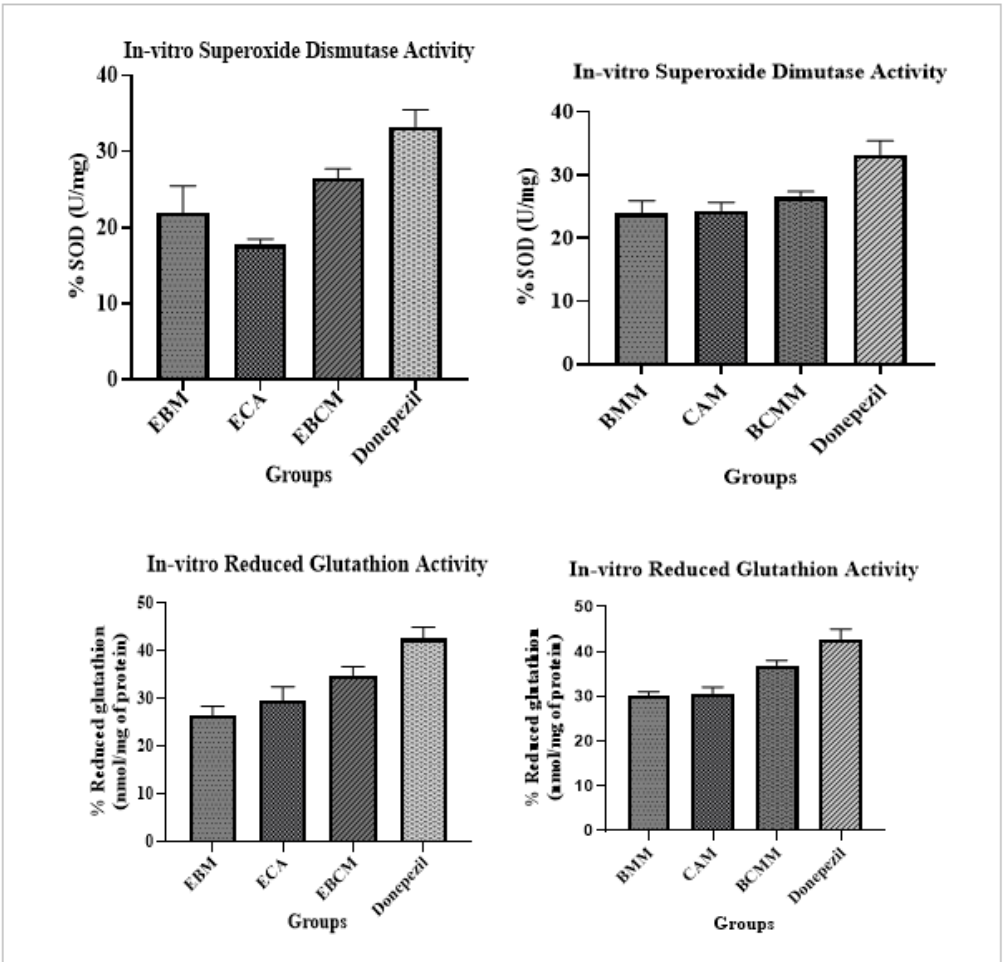


Figure 3: *In vitro* neuroprotective efficacy of bioactive enriched fractions and microemulsion.

confirmed compatibility and encapsulation of the plant bioactive in the microemulsion formulation.

The *ex vivo* permeation study across goat buccal mucosa showed a steady increase in drug diffusion from the microemulsion, with a significantly higher flux compared to the plain suspension (Table 3). This indicates enhanced permeability and potential for improved bioavailability through the buccal route.

The microemulsion formulation remained physically stable during the stability study, showing no signs of phase separation, creaming or precipitation. Extract content above 90% and globule size were within acceptable limits, confirming its suitability for long-term storage. Based on accelerated stability data (40°C/75% RH for 6 months) and extrapolation using the *Arrhenius* equation, the microemulsion formulation exhibited a predicted shelf life of approximately 24 months under long-term storage conditions (25°C/60% RH).

Antioxidant activity is a cornerstone of neuroprotection because it counteracts oxidative stress, preserves neuronal integrity, and enhances cognitive function. Evaluation of antioxidant activity in plant bioactive microemulsions provides direct evidence of their potential to prevent or slow down neurodegenerative processes. Antioxidant Activity (Figure 3) found in this form Ascorbic acid>BCM>CA>BCMM>CAM>BM>BMM.

Acetylcholinesterase inhibition activity is considered a critical neuroprotective marker because it directly reflects the ability of a compound or formulation to restore cholinergic neurotransmission, a central deficit in cognitive impairment and neurodegeneration. By preventing excessive acetylcholine breakdown, AChE inhibitors improve synaptic signaling, enhance cognitive performance, and provide symptomatic as well as disease-modifying benefits. Therefore, evaluating AChE inhibition is indispensable in the pharmacological screening of neuroprotective phytochemicals, bioactive fractions, and advanced drug delivery systems such as microemulsions. The microemulsion shows better inhibition than bioactive rich fraction. Higher inhibitory activity shows at 50 µg/mL. conc. and lowest at 10 µg/mL, whereas Donepezil used as reference standard give inhibition at 84.42%. The highest inhibition (Figure 3) shows at 65.41% (BMM), 67.06% (CAM), 73.57%

(BCMM). Acetyl Cholinesterase inhibition activity found as donepezil>BCMM>MMnd aM>BMM>CA>BM.

Lipoxygenase (LOX) is a rate-limiting enzyme that contributes to inflammation. The inhibition of LOX can reduce LT, leading to in an anti-inflammatory effect. This could lead to less availability of lipid hydroperoxide substrate required for LOX catalysis The LOX activity was monitored as an increase in the absorbance at 234 nm indicating the formation of hydroperoxyl linoleic acid. All the BMM, CAM, BCMM tested inhibited LOX in a concentration dependent manner. Highest inhibitory action shows at 50 µg/mL 18.13% (BMM), 18.26% (CAM), 20.93% (BCMM) when compared with bioactive rich fractions as shown in Figure 3.

In SOD assay, superoxide radicals are generated *in vitro* (commonly by riboflavin-light-NBT or pyrogallol autooxidation methods). The superoxide radicals reduce Nitro Blue Tetrazolium (NBT) to form blue formazan, a chromogen. The amount of formazan formed (and thus color intensity) is inversely proportional to SOD activity, because SOD scavenges the superoxide radicals, preventing NBT reduction. To enhance sensitivity, the chromogen is sometimes extracted into n-butanol. SOD activity decreases significantly ($p<0.05$) with BMM at 23.92±1.76, CAM at 20.57±1.10, and BCMM at 28.42±2.09. In our study, the hydroxyl radical scavenging activity was increased by the microemulsion as compared to bioactive enriched fraction with increasing concentration shown in Figure 3.

Reduced Glutathione is an essential component of nonenzymatic antioxidants because it removes peroxy nitrite (ONOO-) by forming oxidized Glutathione (GS-SG), which is subsequently transformed back into GSH by NADPH-dependent glutathione reductase. Reduced glutathione interacts with DTNB (5,5'-dithiobis nitro benzoic acid) to form a yellow product that absorbs at 412 nm. DTNB was used as a control, and DTNB that includes varying concentrations of BMM, CAM, and BCMM was used as a test sample and compared to the bioactive rich fraction of drug shown in Figure 3. The concentration of GSH decrease significantly ($p<0.05$).

CONCLUSION

It is concluded that, microemulsion of bioactive enriched fractions of *Bacopa monnieri* and *Centella asiatica* mimics Ayurvedic ghimic formulations, offering the same lipid-mediated, brain-targeted delivery of nootropic and neuroprotective phytochemicals but with modern advantages of micro size, stability, and patient-friendly dosage forms. Microemulsions convert bulky, lipophilic, poorly soluble phytochemicals- asiaticosides and bacosides entrapped into bioavailable, brain-accessible novel dosage form. The microemulsion is developed successfully and *in vitro* studies on microemulsion has provided valuable insights into pre-clinical neuroprotective and cognitive efficacy, solubilization, droplet size, stability and preliminary release kinetics, but critical limitations in replicating the complex biological, metabolic, and physiological environments of the human body. Hence, *in vitro* results must be interpreted with caution and supported with *in vivo* pharmacokinetic and pharmacodynamic validation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BM: *Bacopa monnieri* bioactive enriched fractions; **CA:** *Centella asiatica* bioactive enriched fractions; **BCM:** *Bacopa-Centella* mix bioactive enriched fractions; **BMM:** *Bacopa monneri* microemulsion; **CAM:** *Centella asiatica* microemulsion; **BCMM:** *Bacopa-Centella* mix microemulsion; **AChE:** Acetylcholinesterase; **DTNB:** 5,5-Dithio-bis (2-nitrobenzoic acid); **ATCI:** Acetyl thiocholine iodide; **AChE:** Acetylcholinesterase; **LOX:** Lipoxygenase; **DPPH:** 2,2 diphenyl picryl hydrazine; **SOD:** Superoxide dismutase; **mV:** Millivolt; **nm:** nanometer; **GSH:** Reduced glutathione; **BMS:** *Bacopa monnieri* bioactive enriched fractions suspension; **CAS:** *Centella asiatica* bioactive enriched fractions suspension; **BCMS:** *Bacopa-Centella* mix bioactive enriched fractions suspension.

REFERENCES

Anil, L., & Kannan, K. (2018). Microemulsion as drug delivery system for peptides and proteins. Journal of Pharmaceutical Sciences and Research, 10(1), 16–25.

Bera, A., Mandal, A., & Kumar, T. (2014). Physicochemical characterization of anionic and cationic microemulsions: Water solubilization, particle size distribution, surface tension, and structural parameters. Journal of Chemical and Engineering Data, 59(8), 2490–2498. https://doi.org/10.1021/je500274r

Deore, S. L., Kide, A. A., Baviskar, B. A., Khadabadi, S. S., & Shende, B. A. (2023). Memory protecting herbs: *Centella asiatica* and *Bacopa monnieri* in the fight against Alzheimer's disease. Revista Brasileira de Farmacognosia, 33(6), 1263–1273. https://doi.org/10.1007/s43450-023-00455-x

Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Fanun, M. (2010). Formulation and characterization of microemulsions based on mixed nonionic surfactants and peppermint oil. Journal of Colloid and Interface Science, 343(2), 496–503. https://doi.org/10.1016/j.jcis.2009.12.008

Huang, X., Li, Y., Sabier, M., Si, J., Wang, P., Shen, Y., Zhang, X., & Liu, J. (2024). Guidelines for the *in vitro* determination of anti-inflammatory activity. eFood, 5(3), e160. https://doi.org/10.1002/efd2.160

Jadhav, S. T., Salunkhe, V. R., Bhinge, S. D., Honmane, S. M., & Jadhav, A. S. (2024). Development and evaluation of imiquimod-loaded nanoemulsion-based gel for the treatment of skin cancer. Future Journal of Pharmaceutical Sciences, 10(1), 93. https://doi.org/10.1186/s43094-024-00660-y

Kahlweit, M., Busse, G., & Winkler, J. (1993). Electric conductivity in microemulsions. The Journal of Chemical Physics, 99(7), 5605–5614. https://doi.org/10.1063/1.465953

Kaur, G., & Mehta, S. K. (2017). Developments of polysorbate (Tween) based microemulsions: Preclinical drug delivery, toxicity and antimicrobial applications. International Journal of Pharmaceutics, 529 (1–2), 134–160. https://doi.org/10.1016/j.ijpharm.2017.06.059

Khadabadi, S. S., Deore, S. L., & Baviskar, B. A. (2011). Experimental phytopharmacognosy-A comprehensive guide. In Nirali Prakashan.

Mandal, S., Mandal, S. D., Surti, N., & Patel, V. B. (2010). Development of microemulsion formulation for the solubility enhancement of flunarizine. Der Pharmacia Lettre, 2(3), 227–236.

Mishra, A. K., & Sahoo, P. K. (2022). Characterization of nanophytopharmaceuticals. In M. Rudrapal (Ed.),Phytoantioxidants and nanotherapeutics (pp. 239–265). Academic Press. https://doi.org/10.1002/9781119811794.ch12

Mostafa, D. M., Ammar, N. M., Abd El-Alim, S. H., & El-Anssary, A. A. (2014). Transdermal microemulsions of *Glycyrrhiza glabra* L.: Characterization, stability and evaluation of antioxidant potential. Drug Delivery, 21(2), 130–139. https://doi.org/10.3109/10717544.2013.834419

Parveen, R., Shamsi, T. N., Kumar, H., & Fatima, S. (2016). Phytochemical analysis and *in vitro* biological characterization of aqueous and methanolic extract of *Bacopa monnieri*. International Journal of Pharmacy and Pharmaceutical Sciences, 8(12), 90–96. https://doi.org/10.22159/ijpps.2016v8i12.14739

Shah, J. P., & Ray, S. (2020). Development studies on microemulsion gels containing herbal excipients as permeation enhancers. Journal of Pharmaceutical Research, 19(1), 8.

Singh, M., & Jindal, D. (2022). Recent advances in nanoformulation-based intranasal delivery of bioactive compounds and biologics for neurodegenerative disorders (NDDs). In International Conference on Nanotechnology for Sustainable Living and Environment (pp. 61–76). Springer Nature. https://doi.org/10.1007/978-981-19-5869-0_6

Talegaonkar, S., Azeem, A., Ahmad, F. J., Khar, R. K., Pathan, S. A., & Khan, Z. I. (2008). Microemulsions: A novel approach to enhanced drug delivery. Recent Patents on Drug Delivery and Formulation, 2(3), 238–257. https://doi.org/10.2174/187221108786241679

Vo, T.-V., Chou, Y.-Y., & Chen, B.-H. (2021). Preparation of microemulsion from an alkyl polyglycoside surfactant and tea tree oil. Molecules, 26(7), 1971. https://doi.org/10.3390/molecules26071971

Cite this article: Deore SL, Vighne RC, Baviskar BA. From Ghrita to Microemulsion: Innovative Delivery of Medhya Rasayana for Neuroprotection. Int. J. Pharm. Investigation. 2026;16(1):272-82.