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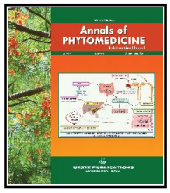

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A novel method: Developing and accessing a herbal microemulgel containing *Cedrus deodara* (Roxb.) G. Don extract for its *in vitro* anti-inflammatory effects

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Abstract

The aim of this research is to create an innovative formulation, which poses unique challenges. Researchers in this study are particularly interested in exploring the potential of natural bioactive compounds. Various plant extracts have demonstrated therapeutic effects. *Cedrus deodara* (Roxb.) G. Don, is indigenous to North India, Pakistan, Afghanistan, Tibet, and Nepal. It is rich in various bioactive elements like deodarin, cedodarin, taxifolin, myrcene, iso-pimpillin, α-pinene, β-pinene, cedrin, deodarone, sesquiterpenes, cedrinoside, dihydromyricetin, matairesinol, and atlantone. Traditionally, people have used it to address a wide range of health concerns, including inflammation, ulcers, pain management, blood sugar control, infections, sleep issues, skin conditions, blood disorders, cell death issues, and fever. Recent investigations, both in laboratory settings and within living organisms, have underscored its anti-inflammatory and pain-relieving characteristics. The intended study seeks to formulate and evaluate an herbal microemulgel incorporating an extract from *C. deodara* to investigate it is *in vitro* anti-inflammatory potential. The extraction process involved the use of a hydroalcoholic solvent on *C. deodara* heartwood, followed by an analysis of the extract's phytochemical composition. Subsequently, four formulations of the herbal microemulgel were prepared for further examination and evaluated for various parameters including pH, spreadability, viscosity, consistency, appearance, color and washability. The maximum release of *C. deodara* microemulgel was 98% at 48 h which indicated that *C. deodara* microemulgel has good topical release properties. *C. deodara* microemulsion gel showed 89.89% drug content. The study evaluated the *in vitro* anti-inflammatory effects of both the *C. deodara* extract and the formulated microemulgel using HRBC membrane stabilization assay and protein denaturation assay. The findings suggest that the newly developed herbal microemulgel containing *C. deodara* extract holds potential for future applications in combating inflammation.

1. Introduction

For centuries, humans have relied on medicinal plants for treating illnesses, recognizing their significance in drug discovery. Various phytoconstituents within plants contribute to diverse therapeutic effects. With approximately 45,000 plant species spread across India, the country is renowned as the hub of medicinal plants. Wild sources play a crucial role in supplying medicinal plants to industries. Incorporating traditional folk treatments can inspire the development of innovative medications. Phytoconstituents largely cater to fulfilling the primary healthcare needs of individuals (Lopes, 2014). The constituents derived from plants play a pivotal role in healing ailments. Plant-derived medications are more cost-effective in comparison to modern synthetic drugs. Various plant parts are utilized in the treatment of diverse diseases (Kalogirou *et al*., 2019).

A significant number of patients opt for herbal therapy, drawn by its wide-ranging benefits. Herbal medicines demonstrate favourable compatibility with the human body and are cost-effective, offering fewer side effects in comparison. Inflammation can be effectively addressed through the utilization of plant extracts. Traditional records highlight the anti-inflammatory properties of various plants including *Curcuma longa* Linn. *Zingiber officinalis* Roscoe. *Borago officinalis* Linn. *Oenothera biennis* Linn. (Evening primrose), *Harpagophytum procumbens* (Devil's claw), and *C. deodara* are included in this list. *C. deodara* indigenous to North India, Pakistan, Afghanistan, Tibet, and Nepal, contains various bioactive constituents such as deodarin, cedodarin, taxifolin, myrcene, iso-pimpillin, α-pinene, β-pinene, cedrin, deodarone, sesquiterpenes, cedrinoside, dihydromyricetin, matairesinol, and atlantone. Its therapeutic uses range from treating inflammation, ulcers, pain, hyperglycemia, infections, insomnia, skin disorders, blood disorders, apoptosis, to fever. Recent studies, both *in vivo* and *in vitro*, have emphasized its anti-inflammatory and pain-relieving properties. Microemulgels, a type of formulation primarily utilized for topical delivery, combine the features of emulsions and gels, offering dual control release properties. This constitutes a recent advancement in topical novel drug delivery system (NDDS) technology. Microemulgels are commonly employed in alleviating various pains and aches such as muscle aches, backaches, and arthritis (Benbow and Campbell, 2019).

The objectives of the proposed research endeavour involve formulating and evaluating a herbal microemulgel enriched with *C. deodara* extract, specifically targeting it is *in vitro* anti-inflammatory activity.

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The oral route is the preferable choice for drug administration but oral and intramuscular injections cause severe toxicity and side effects in patients. Some of the noxious effects produced by these routes include GI toxicity, renal failure and at high doses, liver enzymes are also raised (Moore *et al.,* 1999). To cope with these challenges of invasive techniques of delivery of drugs, a novel transdermal drug delivery system is introduced. This delivery system has many advantages over the oral and intramuscular injections. High absorption rates, ease of preparation thermodynamic stability, avoidance of first-pass metabolism and ease of application, make it a more beneficial delivery system than the conventional type of drug delivery systems. Its non-invasive route and local effectiveness make it more compliant to the patients (Brogden *et al*., 1981). Taking emulsions, a step further, transdermal microemulsion gels offer superior drug delivery for poorly soluble medications by significantly increasing their solubility.

The *C. deodara*, commonly found in the Himalayan ranges spanning Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, and Arunachal Pradesh, also extends its presence to central India. This majestic tree boasts a tall stature with straight, wide branches and small leaves. Standing nearly 60 m tall, it showcases horizontal, slender branches adorned with drooping tips. The leaves are long and pointed, resembling needles, typically clustered densely with occasional solitary ones, measuring 2.5-5 cm in length and featuring a glaucous green hue. The bark, exhibiting vertical and diagonal clefts, presents a greyish or reddish-brown shade (Tiþa *et al*., 2013). Known for its bitter, spicy, mildly pungent, and oily attributes, every part of the *C. deodara* contributes to its distinctive characteristics. Belonging to the Pinaceae family, the Cedrus genus boasts a primarily tropical and subtropical distribution across the globe. Despite the male and female cones of the plant growing on separate branches, it is monoecious. Its fruits, oval-shaped and measuring 3-6 inches in length, exhibit a brown coloration and are coated with tough or arid material. Flowers typically emerge during autumn. Optimal growth is observed in trees thriving on well-drained soils, with a preference for environments rich in atmospheric moisture. While shade promotes growth, young trees remain susceptible to frost and chilly winds.

Traditionally, cedarwood is employed in managing a diverse array of health issues including inflammatory conditions, indigestion, insomnia, hiccupping, fever, urinary issues, bronchitis, itching, swelling (elephantiasis), swollen lymph nodes (tuberculous glands), skin depigmentation (leukoderma), eye inflammation (ophthalmia), haemorrhoids (piles), psychological ailments, as well as diverse skin and blood disorders. These therapeutic benefits stem from the essential oils and gums derived from the plant. Moreover, the wood and bark are utilized for their diuretic, diaphoretic, carminative, and astringent properties. Additionally, cedarwood is used to treat conditions such as antipyresis, rheumatoid arthritis, piles, palsy, epilepsy, prolapse, skin ailments, lung disorders, and urinary issues. One common application involves its use as an antiseptic gel, notably in the preparation of V-gel (Amal *et al*., 2020).

Numerous studies have demonstrated the diverse biochemical effects of *C. deodara* across various *in vivo* and *in vitro* experiments. Different components of *C. deodara* have shown efficacy in anti-inflammatory, analgesic, immunomodulatory, antispasmodic, antihyperglycemic, anticancer, molluscicidal, insecticidal, antiapoptotic, antibacterial, antisarcoptic, anxiolytic, and anticonvulsant activities. Moreover, it has exhibited antiulcer and antigastric properties (Kaur and Mehta, 2017).

Creating a microemulsion gel from *C. deodara* can improve drug solubility and skin penetration, leading to an optimized formulation. This conversion from a basic microemulsion to a microemulgel addresses challenges in standard topical drug delivery systems by offering a wide interfacial area for drug absorption (Ita, 2017).

2. Materials and Methods

2.1 Collection and authentication of plant materials

The raw material, *C. deodara* heartwood, was gathered from Mansuri, Haripur, Kullu Himachal Pradesh. The authentication of the plant material was performed under the supervision of Dr. N. M. Dongarwar, associated with the Department of Botany at R. T. M. Nagpur University, Nagpur, utilizing a botanical specimen sheet. The authentication number for the *C. deodara* specimen sheet is 13031.

2.2 Processing of the plant material

Following authentication, *C. deodara* underwent drying and subsequent powdering. This powdered material was then utilized to extract the desired substance.

2.3 Extraction of plant material

C. deodara powder underwent maceration with 70% alcohol for 48 h, followed by extraction of the marc using a Soxhlet apparatus with 70% ethanol. The extracts obtained from both processes were then combined. Subsequently, this combined extract underwent further concentration (Neubert and Reinhart, 2011).

2.4 Formulation of microemulgel

Several batches of microemulgel were created, each containing different gelling agents and varying concentrations. The emulsion was prepared with 2% *C. deodara* extract. Gelling agents were hydrated in water, and triethanolamine was used to regulate the pH. Then, the emulsion was blended into the gel with vigorous stirring to produce the microemulgel formation. The USP was generously provided by Martin Dow Pharm. Ltd. Lahore, while Tween 80, isopropyl myristate (IPM), n-butanol, Carbopol 940, and triethanolamine were procured from Sigma-Aldrich Pakistan. All solvents and materials used were of analytical grade, with distilled water being utilized throughout the formulation processes (Shripathy Dharmasthala *et al.,* 2019).

2.4.1 Construction of microemulsion phase diagrams

Pseudoternary phase diagrams were generated utilizing the aqueous titration technique for four weight ratios (1:0, 1:1, 1:2, and 2:1) of Labrafac CC Myristate (S_{mix}). Each phase diagram involved thoroughly mixing Cedrus oil and the specified S_{mix} ratio in various weight ratios, ranging from 1:9 to 9:1, of oil and S_{mix} were prepared in separate glass vials. Nine different combinations (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were meticulously crafted to precisely delineate the phase boundaries in the phase diagrams. Each oil and S_{mix} combination underwent slow titration with the aqueous phase, followed by visual inspection to identify transparent and easily flowable o/w microemulsions (Piao *et al*., 2010).

2.4.2 Preparation of microemulgel

The phase diagram featuring S_{mix} 1:1 exhibited the largest microemulsion (ME) region. Based on this, concentrations of oil (ranging from 3 to 5% w/w) and S_{mix} (ranging from 85 to 87% w/w) were selected and combined to form various ME combinations using the Box-Behnken design. Water was gradually added dropwise with stirring at room temperature to each oil and S_{mix} combination (1:1 ratio) to create the ME formulations (Kotta *et al*., 2015).

2.5 Characterization of microemulgel

2.5.1 Thermodynamic stability

The formulations underwent a series of stability tests

I. **Heating-cooling cycle:** Each formulation was exposed to six cycles of alternating heating and cooling at temperatures of 4°C and 45°C. The formulations were stored at each temperature for a minimum of 48 h, and any signs of serious instability such as phase separation, precipitation, or cracking were observed.

II. **To assess:** for potential phase separation, the formulations underwent centrifugation at 3,500 rpm for 30 min.

III. **Freeze-thaw cycle:** Formulations underwent three freeze-thaw cycles, alternating between temperatures of -21°C and +25°C. The formulations were stored at each temperature for at least 48 h to assess stability under these conditions (Zhao *et al*., 2014).

2.5.2 Globule size and zeta potential

The microemulgel (MEs) were analysed to determine their globule size and zeta potential through Zeta Sizer Litesizer DLS 500 Dadasaheb Balpande College of Pharmacy, Besa Nagpur.

2.5.3 Organoleptic evaluation

Physical appearance assessment encompasses the examination of various factors such as color, homogeneity, consistency, and overall appearance. The colour evaluation was conducted through visual observation. The homogeneity of the microemulgel was assessed by gently rubbing it between the fingers. Visual observation was employed to evaluate the overall appearance of the microemulgel. To assess consistency, the microemulgel was applied to the skin and observed (Butani *et al.,* 2014).

2.5.4 Spreadability

To determine the spreadability of the microemulgel, it was placed between two petri plates, and the diameter of the circle formed by the spread microemulgel was measured. Initially, a gram of microemulgel was weighed and deposited onto a petri plate. Another petri plate was placed over it, and a weight of 50 g was applied to the top plate for about 60 sec. Following this interval, the diameter of the circles formed by the spread microemulgel was measured three times. These measurements were averaged and then utilized in the subsequent formula:

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

where,

S = spreadability

M = mass

L = diameter

T = time

2.5.5 Viscosity

To evaluate the viscosity of the microemulgel, a sample weighing 1 g was utilized. The Brookfield viscometer's spindle was set to rotate at a speed of 50 rpm. Three readings were taken, and their average was calculated (Sattar *et al.,* 2023).

2.5.6 pH

The pH of the formulated microemulgel batches was assessed with a digital pH meter. 0.5 g of the microemulgel was dissolved in 10 ml of distilled water, and the pH was measured by immersing the electrode in the solution.

2.5.7 Stability studies

Stability assessments of the microemulgel were carried out by storing the samples under various conditions: 5°C, 25°C with 60% relative humidity (RH), 30°C with 65% RH, and 40°C with 75% RH, spanning a duration of 3 months. Evaluations were conducted at 15-day intervals.

2.5.8 Particle size analysis

The droplet size analysis of the microemulgel was conducted using a scanning electron microscope. A calibrated micrometer slide was utilized, containing a drop of the microemulsion, which was then observed under the eyepiece. The droplet size was subsequently determined through this observation.

2.5.9 Electrical conductivity

To verify whether the microemulgel was oil-in-water or water-in-oil microemulsion, an electrical conductivity test was conducted. The conductivity (μ) of the formulated sample was measured using a conductivity meter. This measurement was facilitated by a probe and a meter. By applying voltage between two electrodes in a probe submerged in the microemulsion, the drop in voltage resulting from the resistance of the microemulsion was utilized to calculate its conductivity (Vijayalakshmi *et al.,* 2023).

2.5.10 Dye solubility test

To discern the type of emulsion (whether it was o/w or w/o), 10 μl of methylene blue, a water-soluble dye, was introduced into the emulsion. This addition aimed to observe whether the dye spread uniformly throughout the emulsion or formed clusters.

2.5.11 Fourier transform infrared spectra analysis (FTIR)

An FTIR scanning microscope was employed to identify the functional groups present in the preparation. As infrared (IR) rays permeated through the sample, the absorbed radiations were transformed into vibrations or stretching, generating spectrum signals ranging from 4000 cm^{-1} to 400 cm^{-1}. These signals provided insights into the functional groups and the types of bonds present, thus constituting a molecular fingerprint of the sample (Soumya *et al.,* 2022).

2.5.12 Release study

The release rate of *C. deodara* from various microemulgel formulations was assessed using a Franz diffusion cell equipped with a rabbit membrane. The abdominal skin of the rabbit was shaved and excised, and the integrity of the subcutaneous fat was examined. The membrane was positioned with the mucous membrane facing upward and the

epidermis downward, next, it was firmly positioned the Franz diffusion cell was set up with the donor and receiver compartments securely positioned. Subsequently, 9 ml of pH 6.8 phosphate buffer was introduced into the cell, and external magnetic bars, driven at 300 rpm, ensured continuous stirring of the receptor fluid during the experiment. *C. deodara* microemulsion was introduced into the donor chamber. At time intervals of 0.5, 1, 2, 3, 4, 5, and up to 48 h, 0.5 ml samples were withdrawn from the receiver chamber for spectrophotometric analysis at 354 nm. These samples were promptly replaced with an equal volume of buffer solution.

2.5.13 Drug content

1 ml of emulsion was mixed with 9 ml of pH 6.8 buffer solution and stirred for 30 min. The mixture was then left at room temperature for 24 h, followed by an additional 30 min stirring session. Subsequently, the solution underwent centrifugation at 4000 rpm for 30 min, yielding a clear supernatant (Yadav *et al*., 2018). From this supernatant, 1 ml was withdrawn and added to 9 ml of buffer solution. The resulting solution was analysed using a UV spectrophotometer at 354 nm, and the absorbance was measured. The concentration of *C. deodara* was determined using a standard calibrated curve of the drug.

2.5.14 The *in vitro* anti-inflammatory evaluation using the human red blood cell (HRBC) membrane stabilization method

The anti-inflammatory potential of *C. deodara* heartwood extract was assessed *via* the human red blood cell (HRBC) membrane stabilization method. Blood samples, approximately 2-3 ml, were drawn from healthy individuals and combined with an equal volume of Alsever's solution. Following this, iso-saline was introduced to the mixture, and centrifugation for 5 min facilitated the extraction of the HRBC suspension. Subsequently, equal volumes of the sample were incorporated into the HRBC suspension at concentrations of 100, 200, and 300 µg/ml. Incubation at 37°C for 30 min ensued, followed by another centrifugation cycle for 5 min. Negative controls comprised Alsever's solution and blood, while aspirin served as the standard. The supernatant from centrifugation underwent estimation *via* UV spectroscopy at a wavelength of 560 nm (Hashem *et al*., 2011).

3. Results

3.1 Extraction

The *C. deodara* heartwood was gathered, dried in the shade, and mechanically powdered to produce coarse powder. A measured amount of this coarse powder (1 kg) was then extracted with petroleum ether at 60°C-70°C for 72 h by hot percolation employing a Soxhlet apparatus. The residual material remaining after the extraction with petroleum ether was dried and later subjected to extraction with ethanol 70% at (60°-70°C) for up to 48 h in the Soxhlet apparatus. A residue is obtained after concentrating the alcoholic extract kept in a desiccator.

3.2 Formulation of microemulgel

3.2.1 Pre-formulation studies

Surfactants and co-surfactants underwent screening based on their emulsification ability and percentage transmittance. In the surfactant screening, 300 mg each of oil and surfactant were heated at 40-45°C for 30 secs, and then a 50 mg mixture was diluted to 50 ml with distilled water and left to stand for 2 h. The percentage transmittance was subsequently measured at 688 nm. Labrafac CC was selected as the surfactant due to its higher transmittance.

Similarly, for the screening of cosurfactants, a mixture of co-surfactant (100 mg), selected surfactant (200 mg), and oil (300 mg) was heated at 40-45°C for 30 secs, and the procedure was carried out similarly to the surfactant screening. Isopropyl myristate was identified as the superior co-surfactant.

Table 1: List of surfactant/co-surfactant along with transmittance

Surfactant/co-surfactant	% Transmittance
Kolliphor PS 20	46.95
Kolliphor PS 80	42.63
Labrafac CC	94.81
Isopropyl myristate	86.65

Pseudoternary phase diagrams were generated utilizing the aqueous titration method for four ratios by weight (1:0, 1:1, 1:2, and 2:1) of Labrafac CC Myristate (S_{mix}). Each phase diagram involved the thorough mixing of cedrus oil and specific S_{mix} ratios in varying ratios by weight ranging from 1:9 to 9:1 across different glass vials. A total of 9 combinations of oil and S_{mix} (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were created to precisely define the demarcations of the phases established in the phase diagrams. Gradual titration with the water phase was executed for each combination of oil and S_{mix}, and visual inspection was conducted to identify transparent and easily flowable o/w microemulsions.

Table 2: For S_{mix} 1:0

S. No.	Oil (w/w%)	S_{mix} (w/w%)	Water (w/w%)
1	8.49	81.99	9.51
2	17.09	73.33	9.57
3	26.25	65.88	7.86
4	39.85	59.11	4.11
5	46.21	49.63	4.14
6	55.84	39.97	4.17
7	66.34	30.46	3.18
8	77.18	20.64	2.16
9	87.39	10.42	2.17

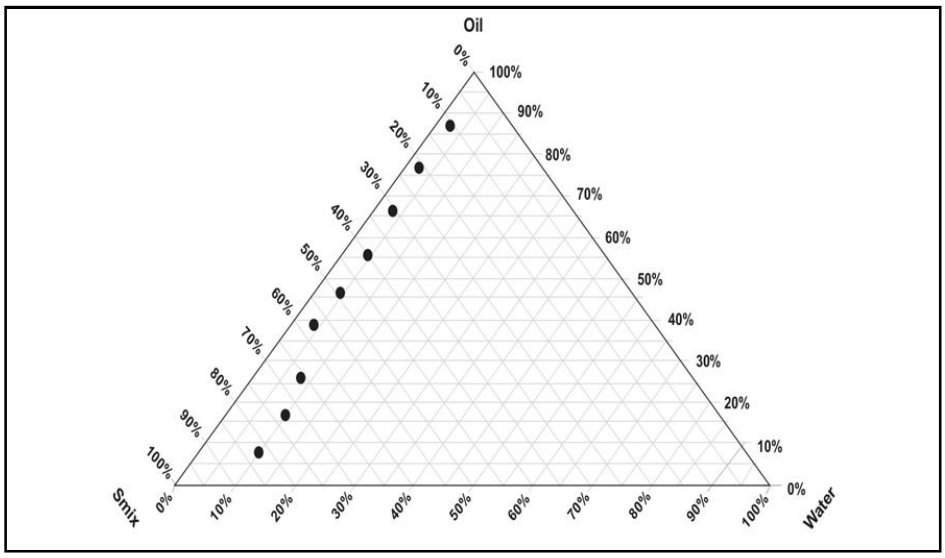

Figure 1: Ternary phase diagram of concentration for S_{mix} 1:0.

Table 3: For S_{mix} 1:1

S. No.	Oil (w/w%)	S_{mix} (w/w%)	Water (w/w%)
1	8.95	81.02	10.02
2	17.39	69.94	12.66
3	26.30	61.87	12.18
4	36.24	54.62	9.13
5	45.74	46.05	8.205
6	55.44	37.202	7.25
7	66.20	28.49	5.29
8	77.35	19.39	3.25
9	88.04	9.84	2.19

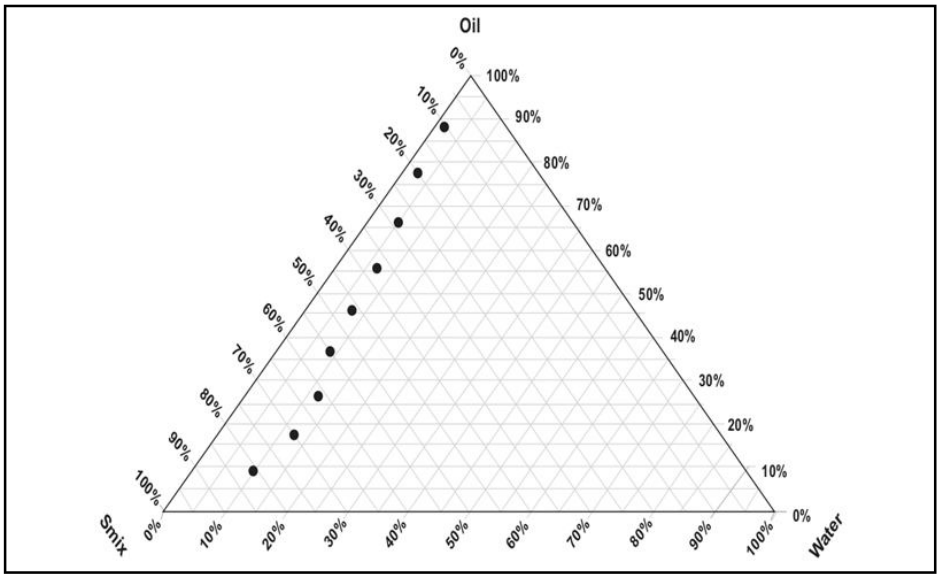

Figure 2: Ternary phase diagram of concentration S_{mix} 1:1.

Table 4: For S_{mix} 2:1

S. No.	Oil (w/w%)	S_{mix} (w/w%)	Water (w/w%)
1	9.34	82.27	8.37
2	18.78	72.79	8.41
3	28.46	65.14	6.39
4	37.93	55.68	6.37
5	47.80	46.83	5.35
6	57.28	37.36	5.35
7	67.42	28.26	4.31
8	76.94	18.75	4.31
9	85.42	9.25	5.31

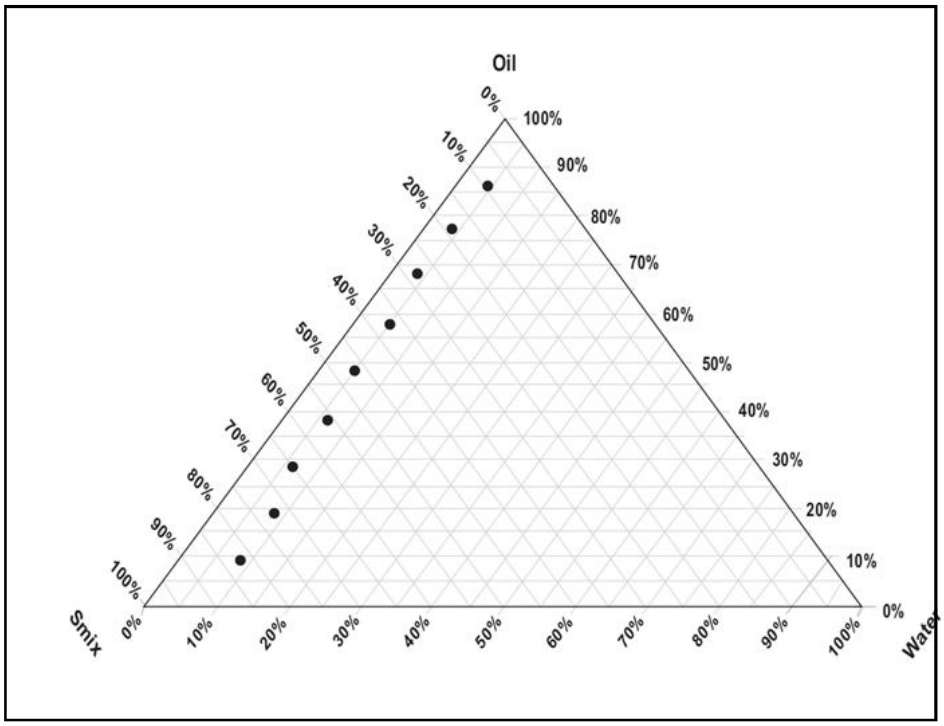

Figure 3: Ternary phase diagram of concentration S_{mix} 2:1.

Table 5: For S_{mix} 1:2

S. No.	Oil (w/w%)	S_{mix} (w/w%)	Water (w/w%)
1	09.65	81.78	8.64
2	19.19	72.21	7.44
3	28.86	63.56	7.56
4	38.76	54.77	6.51
5	48.16	45.35	6.47
6	58.08	36.48	5.42
7	68.15	27.48	4.36
8	77.44	18.22	4.33
9	87.56	09.16	3.27

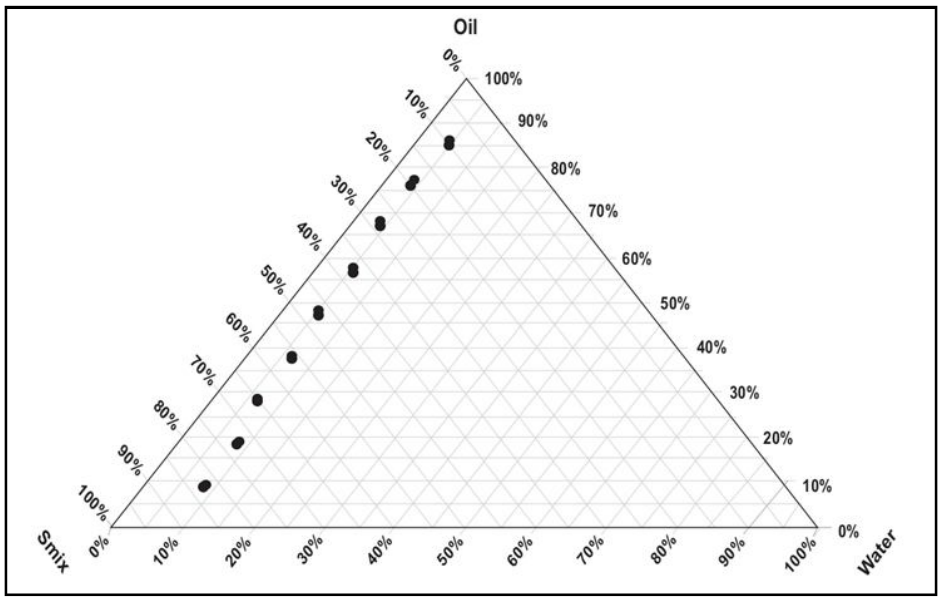

Figure 4: Ternary phase diagram of concentration S_{mix} 1:2.

3.2.2 Development of microemulgel phase diagram

The phase diagram analysis revealed that the highest concentration region occurred at a ratio of 1:1 for surfactant to co-surfactant. This concentration ratio facilitated optimal micelle formation, attributed to the maximal solubilizing capacity of the surfactant and co-surfactant at this specific ratio.

3.2.3 Preparation of microemulgel

Amount in % w/w for gelling phase carbopol was added to the volume of water mentioned in the composition of the microemulsion. Carbopol was swollen in water overnight and neutralized by triethanol amine to the swollen gel, oil and S_{mix} were added and mixed thoroughly.

Table 6: Preparation of microemulgel

S. No.	Cedar wood oil %w/w	S_{mix} %w/w	Soylecithin %w/w	Viscosity (Poise)
1	3	86.0	12.5	08.96 ± 0.55
2	3	85.0	12.5	08.76 ± 0.25
3	3	85.5	15.0	09.57 ± 0.57
4	4	85.5	12.5	14.40 ± 0.25
5	5	85.5	10.0	17.68 ± 0.56
6	4	86.0	10.0	13.86 ± 0.23
7	4	85.5	12.5	14.40 ± 0.25
8	4	85.5	15.0	15.67 ± 0.56
9	4	86.0	15.0	16.75 ± 0.75
10	5	85.0	12.5	18.75 ± 0.15
11	5	85.5	15.0	20.33 ± 0.38
12	3	85.5	10.0	07.65 ± 0.98
13	4	85.5	12.5	14.40 ± 0.25
14	4	85.0	10.0	13.25 ± 0.45
15	5	86.0	12.5	18.90 ± 0.66

3.3 Characterisation of microemulgel

3.3.1 Thermodynamic stability

Heating cooling passed three cycles, the freeze thaw cycle passed six cycles and a centrifugation test was done on the prepared microemulgel in that there was no bad effect on the microemulgel and no phase separation and no detrimental effect was seen in the prepared microemulgel.

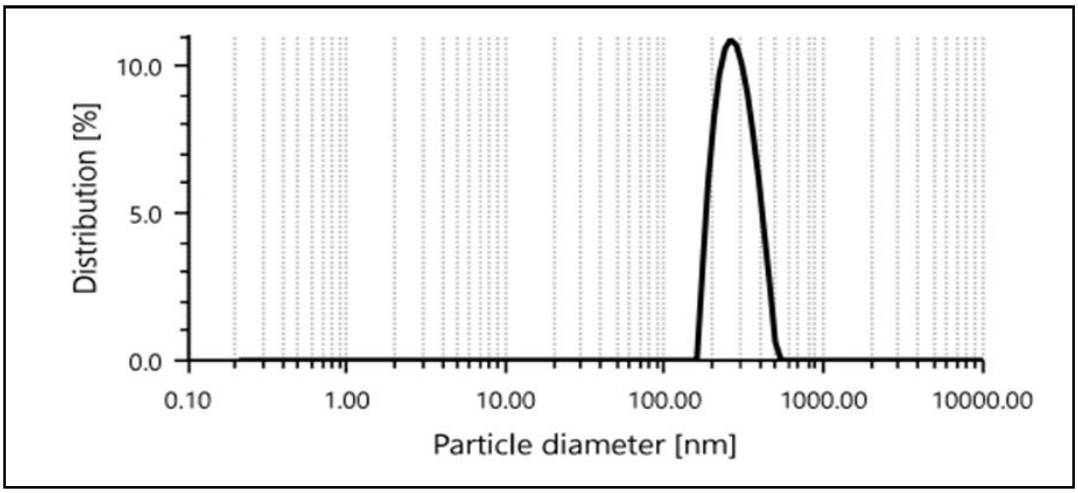

Figure 5: Results of particle size.

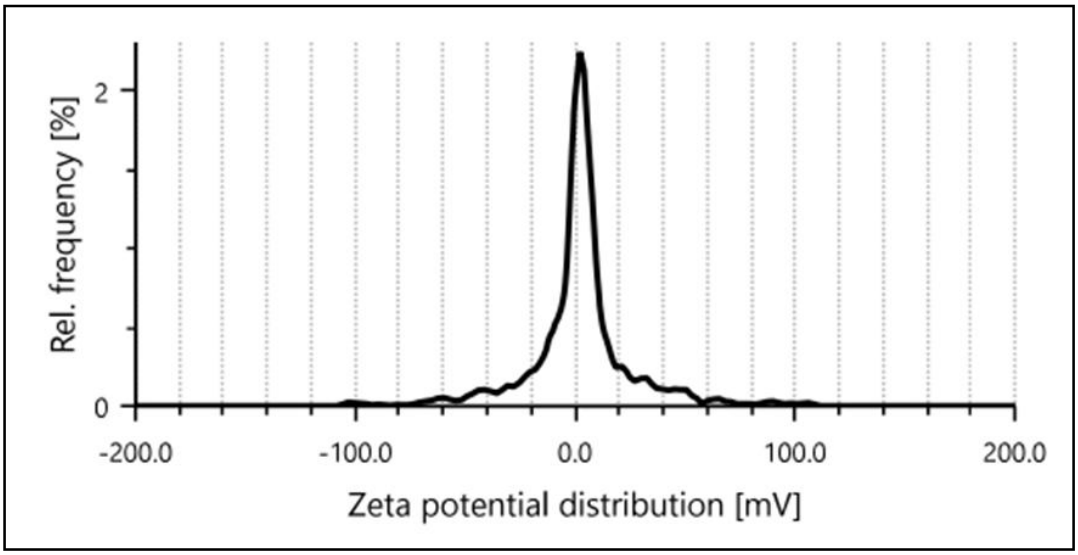

Figure 6: Results of zeta potential.

3.3.2 Globule size and zeta potential

Differential light scattering technology (Particle Analyzer-Litesizer TM 500) was used to determine the mean particle size. Each microemulgel was diluted with water to make it 1% weight per weight. The 1% w/w solution was diluted 100 times more. After that, the dilute evaluation of a particle's repulsion or attraction is called zeta potential. Its measurement provides information on the electrostatic dispersion measurement mechanism.

3.3.3 Organoleptic evaluation

The microemulgel of *C. deodara* exhibited a transparent appearance with a light yellow and emitted an aroma reminiscent of alcohol. Notably, the microemulsion remained stable without undergoing phase separation upon the addition of gel to the formulation, confirming its stability.

3.3.4 Spredability

Different formulations are tested for spreadability and viscosity and the results obtained are given below.

3.3.5 Measurement of viscosity of *C. deodara* microemulgel

The viscosity of the *C. deodara* microemulgel was measured at 90.4 ± 0.01 cps. The formulation displayed Newtonian flow characteristics, as there was no notable change in viscosity when subjected to moderate force such as stirring. This suggests that the microemulgel possesses the ability to withstand minor stress, ensuring that its viscosity remains relatively stable during both handling and storage.

3.3.6 Measurement of pH of *C. deodara* microemulgel

The pH of the microemulgel was 6.5 which showed that the increased S/CO ratio has increased the pH of the microemulgel.

3.3.7 Stability study

The stability study suggested that the formulation was physically and chemically stable when stored at 5°C, 25°C with 60% relative humidity (RH), 30°C with 65% RH, and 40°C with 75% RH over a period of 3 months.

3.3.8 Measurement of droplet size distribution

The size of the droplets was 100 nm. This optimum size of *C. deodara* microemulsion gel showed that surfactant, co-surfactant, and oil phases were highly compatible with each other.

Table 7: Spreadability study

S. No.	Cedar wood oil %w/w	S_{mix} %w/w	Soylecithin %w/w	Viscosity (Poise)	Spreadability (g. cm/S)*
1	3	86.0	12.5	08.96 ± 0.55	36.67 ± 0.85
2	3	85.0	12.5	08.76 ± 0.25	36.98 ± 0.73
3	3	85.5	15.0	09.57 ± 0.57	36.45 ± 0.59
4	4	85.5	12.5	14.40 ± 0.25	35.23 ± 0.63
5	5	85.5	10.0	17.68 ± 0.56	33.56 ± 0.55
6	4	86.0	10.0	13.86 ± 0.23	35.67 ± 0.15
7	4	85.5	12.5	14.40 ± 0.25	35.23 ± 0.63
8	4	85.5	15.0	15.67 ± 0.56	34.98 ± 0.55
9	4	86.0	15.0	16.75 ± 0.75	34.25 ± 0.56
10	5	85.0	12.5	18.75 ± 0.15	32.23 ± 0.89
11	5	85.5	15.0	20.33 ± 0.38	31.56 ± 0.63
12	3	85.5	10.0	07.65 ± 0.98	37.12 ± 0.25
13	4	85.5	12.5	14.40 ± 0.25	35.23 ± 0.63
14	4	85.0	10.0	13.25 ± 0.45	35.98 ± 0.45
15	5	86.0	12.5	18.90 ± 0.66	32.15 ± 0.59

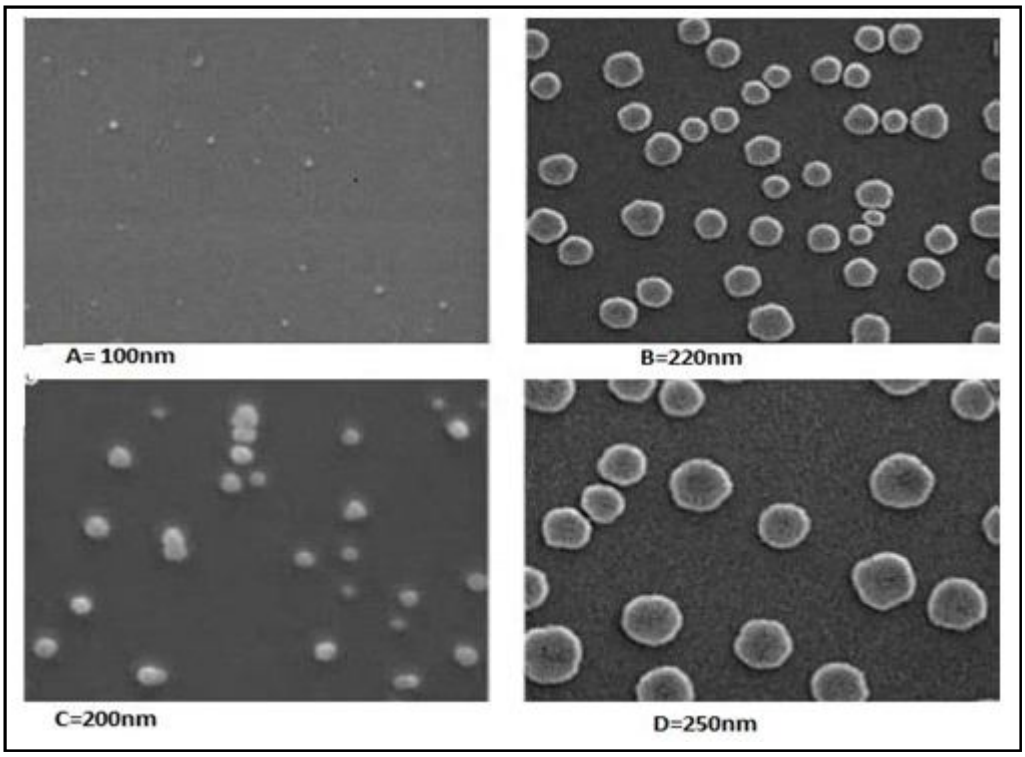

Figure 7: Particle size of *C. deodara* microemulgel (100 nm).

3.3.9 Measurement of electrical conductivity of *C. deodara* microemulgel

The conductivity of the *C. deodara,* a microemulsion gel measured 1 µs/cm, indicating its classification as an o/w microemulsion gel. In comparison, pure water typically exhibits a conductivity of 0.05 µs/cm. The elevated conductivity observed in the microemulgel can be attributed to the presence of dissolved salts and oils. A higher concentration of dissolved salts and oils in the microemulgel leads to increased conductivity values. In the case of the *C. deodara* microemulgel, its higher conductivity is a result of the high solubility of oil, surfactant, co-surfactant, and water within the formulation.

3.3.10 Dye solubility test

Dye spread uniformly in the microemulgel so, it was concluded that the continuous phase was water. Therefore, the *C. deodara* microemulgel was an o/w type of microemulsion.

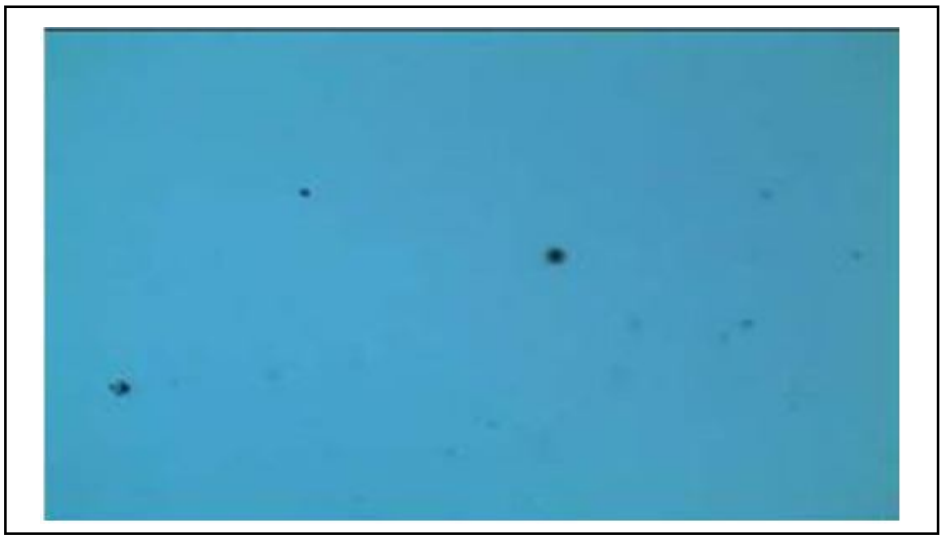

Figure 8: Methylene blue dye distributed showing o/w microemulgel.

3.3.11 FTIR studies

The distinctive peaks of pure *C. deodara* observed in the FTIR the spectrum exhibited at 1632.87 cm^{-1} (C=O), 3338.18 cm^{-1} (NH), and 1434.56 cm^{-1} (CN), as depicted in Figure 9. These peaks were also evident in the *C. deodara* microemulsion, without any additional auxiliary peaks or notable peak shifts. This absence of significant changes indicated the absence of chemical deterioration. Additionally, DSC analysis was employed to assess compatibilities between *C. deodara* and various excipients. The results revealed no incompatibilities between the excipients and *C. deodara*, as both exhibited characteristic peaks in the microemulgel thermograms.

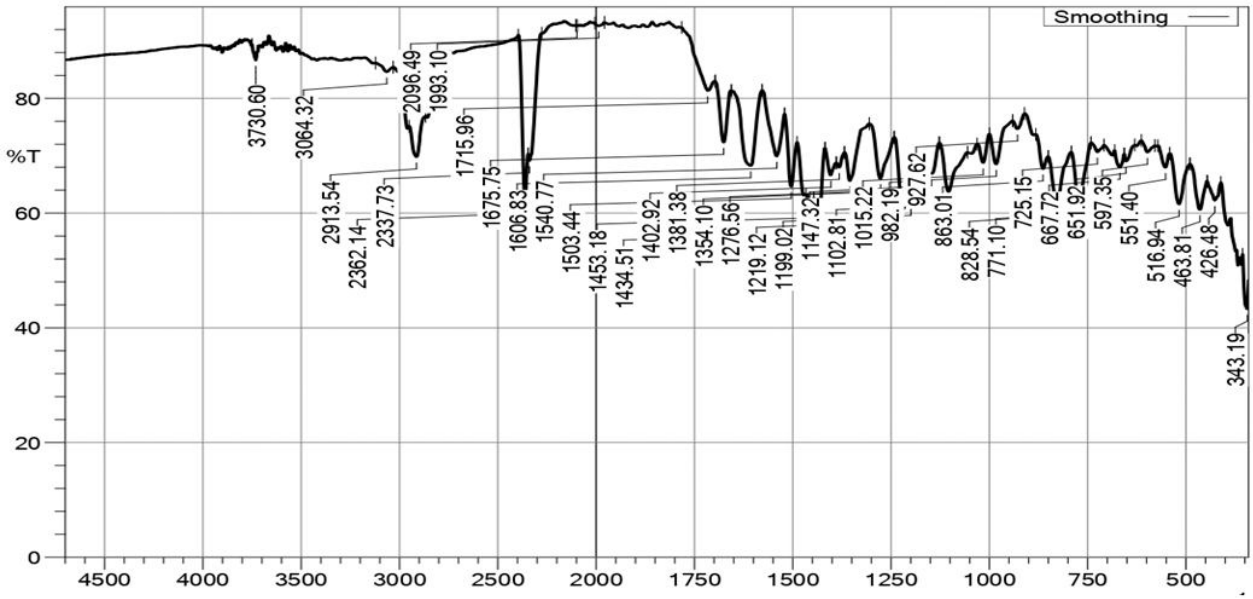

Figure 9: The graph has displayed data from the FTIR measurement of percentage transmittance (%T), corresponding to *C. deodara* peak at 1526.38 cm^{-1}.

3.3.12 Release studies

The maximum release of *C. deodara* microemulgel was 98% at 48 h which indicated that *C. deodara* formulation has good topical release properties.

In the FTIR spectrum, characteristic peaks of pure *C. deodara* were identified at 1632.87 cm^{-1} (C=O), 3338.18 cm^{-1} (NH), and 1434.56 cm^{-1} (CN), as shown in Figure 9. These peaks were similarly observed in the *C. deodara* microemulgel, with no additional auxiliary peaks or significant peak shifts noted. This consistent presence indicated the absence of chemical deterioration. Moreover, DSC analysis was utilized to evaluate the compatibility between *C. deodara* and various excipients. The findings demonstrated no incompatibilities between the excipients and *C. deodara*, as both displayed characteristic peaks in the microemulgel thermograms.

Table 8: Kinetic release from *C. deodara* microemulsion gel

Values	Zero order	First order	Higuchi model	Korsmeyer peppas	n value
Rsqr	0.9401	0.9839	0.9079	0.9927	0.749
AIC	79.3411	63.6078		84.4943	50
MSC	2.6476	3.9587		2.2182	4.5929

Table 9: Impact of microemulgel and standard drug on HRBC membrane hemolysis and membrane stabilization

Concentration (µg/ml)	% Haemolysis of microemulgel	% Stabilization of microemulgel	% Haemolysis of aspirin	% Stabilization of aspirin
100	16.21	83.76	11.45	88.36
200	11.26	88.85	8.12	91.73
300	4.76	95.18	2.86	97.25

3.3.13 Drug content

C. deodara microemulsion gel showed 89.89% drug content. This high drug content demonstrated that the drug was highly soluble and compatible with the excipients.

3.3.14 *In vitro* anti-inflammatory activity

The anti-inflammatory efficacy of both the extract and the refined formulation was evaluated using *in vitro* methods. The HRBC membrane stabilization method. Both the extract and the formulated microemulgel demonstrated promising anti-inflammatory activity. The results indicated notable inhibition of protein denaturation by the extract.

4. Discussion

In this current investigation herbal microemulgel containing extract of *C. deodara* was developed for the management of inflammation. Among the oils and surfactants tested, *C. deodara* exhibited the highest solubility in IPM oil and tween 80 surfactants, along with n-butanol as the cosurfactant, compared to the other options. The combination of IPM, tween 80, and n-butanol resulted in clear and stable microemulsions due to their superior compatibility among the tested surfactant-co-surfactant-oil combinations. The formulation was physically and thermodynamically stable when studied with acerated conditions.

The microemulgel of *C. deodara* exhibited a transparent appearance with a light yellow and emitted an aroma reminiscent of alcohol. The spreadibility of the formulation was in the range of 31.56 ± 0.63 to 37.12 ± 0.25. It shows the size of the droplets around 100 nm and 90.4 ± 0.01 cps viscosity. This optimum size of *C. deodara* microemulgel showed 100 nm. Dye spread uniformly in the microemulgel.

The pH of microemulgel was 6.5 which showed that the increased S/CO ratio has increased the pH. In comparison, pure water typically exhibits a conductivity of 0.05 µS/cm. The FTIR spectra show peaks at 1632.87 cm^{-1} (C=O), 3338.18 cm^{-1} (NH), and 1434.56 cm^{-1} (CN). The maximum release of *C. deodara* microemulgel was 98% at 48 h which indicated that *C. deodara* microemulgel has good topical release properties. *C. deodara* microemulsion gel showed 89.89% drug content. Both the extract and the formulated microemulgel demonstrated promising anti-inflammatory activity. The results indicated notable inhibition of protein denaturation by the extract.

5. Conclusion

Due to their perceived safety and reduced adverse effects compared to synthetic alternatives, natural medicines enjoy widespread acceptance. Herbs are recognized for their safety, effectiveness, and versatility, leading to an increasing demand for herbal formulations in the global market. The development of an herbal microemulgel using hydroalcoholic extracts from medicinal plants represents a promising initiative. The comprehensive findings of this study indicate significant anti-inflammatory activity exhibited by both the extract and the formulated microemulgel. Notably, the extract demonstrated substantial inhibition of protein denaturation, highlighting the importance of not only selecting the appropriate active ingredients but also optimizing their concentrations for maximal efficacy. Additionally, ensuring product stability over the specified duration and maintaining efficacy is crucial. This was assessed through accelerated stress studies conducted in accordance with ICH guidelines. The pH and water content remained within acceptable limits throughout the study period, underscoring the stability of the formulated product.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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