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Formulation and Evaluation of Herbal Microemulgel Loaded with Extract of *Cedrus deodara* (Roxb.) G. Don and their Anti-inflammatory Screening

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ABSTRACT
The aim of this research was to create a novel formulation which poses distinctive challenges. Researchers in this study are particularly interested in exploring the perspective 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's *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 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 volatile oil extracted from the wood of *C. deodara* was examined for its topical anti-inflammatory activity. It produced significant inhibition of carrageenan-induced rat paw edema and of both exudative–proliferative and chronic phases of inflammation in adjuvant arthritic rats. The findings suggest that the newly developed herbal microemulgel containing *C. deodara* extract holds potential for future applications in combating inflammation.
Keywords: *Cedrus deodara* (Roxb.) G. Don, Herbal, Microemulgel, Anti-inflammatory activity, HRBC membrane, Deodarin

1. INTRODUCTION

Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain. Non-steroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activities through the inhibition of cyclooxygenase (COX), the enzyme that makes prostaglandins (PGs).[1] They share, to a greater or lesser degree, the same side effects, including gastric and renal toxicity. Recent research has shown that there are at least two COX isoenzymes. COX-1 is constitutive and makes PGs that protect the stomach and kidneys from damage. COX-2 is induced by inflammatory stimuli, such as cytokines, and produces PGs that contribute to the pain and swelling of inflammation. This selective COX-2 inhibitors should be anti-inflammatory without side effects on the kidney and selective COX-2 inhibitors may have other side effects and perhaps other therapeutic potential, For instance, COX-2 (and not COX-1) is thought to be involved and moreover, NSAIDs delay the progress of Alzheimer's disease (AD). Selective COX-2 inhibitors may demonstrate new important therapeutic benefits as anticancer agents, as well as in preventing premature labor and perhaps even retarding the progression of AD. Naturally occurring flavones and flavonoids inhibits the expression of the inducible forms of COX and NOS, interleukins, tumor necrosis factor-a (TNF-α) and adhesion molecules in inflammatory cells and tissues. Aspirgenin and itsglycosides have been shown good anti-inflammatory activity with minimum side effects of other anti-inflammatory products through arachidonic acid pathway.[2]

This is outstanding part with in the recognition of the value of ancient therapeutic systems and medicinal plants identification from aboriginal pharmacopeia's that have significant healing power, either in their natural state or as the source of new pharmaceuticals. U.S. Pharmacopeia also listed herbal drugs and described about their properties, uses, dosages and test for purity. Generally, these formulations are having moderate efficacy and less toxic when compared to most pharmaceutical agents. In the western world, in particular, the developing concept that 'natural' is better than 'chemical' or 'synthetic' has led to the evaluation of Neo-western herbalism that is the basis of an ever expanding industry.[3]

According to the World Health Organization (WHO) 80% (4 billion people) of the world population relies on traditional medicines for some aspect of primary health care requirements. Almost 25% of the modern pharmacopeia's still contain plant derived drugs, synthetic analogues constructed on plant isolated prototype compounds. Herbal medicine occupies a major role in all indigenous people's traditional medicine and a common element in Ayurveda, homeopathic, naturopathic, traditional oriental, Unani and Indian Siddha medicine. Worldwide the value of medicinal plants increasing due to the awareness of people towards natural products and their benefits. Medicinal plants have traditional importance in sociocultural, supernatural, and medicinal arena of rural and tribal lives of India due to its no side effects and available at affordable prices to the poor. Extensive research undertaken by major pharmaceutical companies on plant materials gathered from the forests, medicinal plant culture fields and other places for their potential medicinal value.[4]

Indian System of (ISM) uses medicinal plants in preventive, promotive, and curative applications. The India ethnobiological survey (Ministry of Environment & Forests,

Government of India) reveals that there are over 8000 species of plants being used by the people of India. WHO stated almost 120 plant-derived pharmaceutical drugs are utilised in developed and developing countries, about 74% of modern medicines have direct or indirect connection with traditional medicine history.[5,6]

A significant number of patients opt for herbal therapy, drawn by its wide-ranging benefits. Herbal medicines demonstrate favorable 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 *Cedrus deodara* is included in this list. *Cedrus deodara* is 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.[7]

The objectives of the proposed research endeavor involve formulating and evaluating a herbal microemulgel enriched with *C. deodara* extract, and exploring both *in vivo* and *in vitro* anti-inflammatory activity.

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

2.4. Formulation of microemulgel

Different batches of microemulgel were prepared by using gelling agents and by varying their concentration as per the **Table 1**. The emulsion was prepared using 3% w/w to 5% w/w of *C. deodara* extract. The gelling agents were soaked in water and triethanolamine was added to adjust the pH. The emulsion was added to the gel and stirred vigorously to form microemulgel.[8]

Table 1: Composition of Cedar wood oil microemulgel.

S. No.	Code	Cedar wood oil (% w/w)	S_{mix} (% w/w)	Soy lecithin (% w/w)
1	CW1	3	86.0	12.5
2	CW2	3	85.0	12.5
3	CW3	3	85.5	15.0
4	CW4*	4	85.5	12.5
5	CW5	5	85.5	10.0
6	CW6	4	86.0	10.0
7	CW4*	4	85.5	12.5
8	CW7	4	85	15.0
9	CW8	4	86.0	15.0
10	CW9	5	85.0	12.5
11	CW10	5	85.5	15.0
12	CW11	3	85.5	10.0
13	CW4*	4	85.5	12.5
14	CW12	4	85.0	10.0
15	CW13	5	86.0	12.5

2.5. Evaluation of microemulgel

The prepared herbal microemulgel was evaluated for pH, spreadability, viscosity, consistency, appearance color, washability, and appearance.

2.6. Physical appearance

The Physical appearance includes study of color, homogeneity, consistency, appearance, etc. Color was noted by visual observation. Homogeneity of microemulgel was checked by rubbing the microemulgel between fingers. The appearance of microemulgel was checked by visual observation. microemulgel was applied to skin to check its consistency.

2.7. Spreadability

The microemulgel was sandwiched between 2 petri plates and the diameter of circle of spreaded microemulgel was used to determine the spreadability. 1 g of microemulgel was weighed and placed on a petri plate. Other petri plate was placed on its top and weight of 50 g was placed on the top of petri plate for about 60 sec. After completion of 60 sec the diameter of circles formed from the spreaded microemulgel were measured in triplicate. The average of the reading was calculated. The reading was put into the following formula:

S = M × L T

S: Spreadability; **M:** Mass; **L:** Diameter; and **T:** Time

2.8. Viscosity

The viscosity of microemulgel was estimated using Brookfield viscometer. 1 g of microemulgel sample was taken. The spindle was rotated at the speed of 50 rpm. Readings were taken in triplicate and averages of readings were calculated. [9]

2.9. pH

The pH of the formulated microemulgel batches were measured using digital pH meter. 0.5 g was dissolved in 10 mL of distilled water and electrode was dipped in it to measure the pH. [10]

2.10. Stability studies

The stability studies of microemulgel were carried out by keeping the samples at 5°C, 25°C/60% RH, 30 °C/ 65% RH, and 40 °C/75% RH for a period of 3 months. The samples were tested at interval of 15 days. They were evaluated for its appearance, pH, viscosity and spreadability.

2.11. *In vitro* anti-inflammatory Human Red Blood Cell (HRBC) Membrane Stabilization Method

The Human Red Blood Cell (HRBC) membrane stabilization method was used to evaluate the anti-inflammatory activity of extract of *C. deodara* heart wood. 2-3 mL of blood was collected from healthy individual. The equal quantity of Alsever's solution was added to blood. iso-saline was added to the above mixture. This mixture was centrifuge for 5 min to get HRBC suspension. Equal amount of sample was added to HRBC suspension. 100, 200, and 300 µg/mL of concentration of sample were prepared. It was incubated at 37°C for 30 mins. The mixtures were centrifuged for 5 mins. Alsever's solution and blood were taken as negative control. Aspirin was taken as a standard. The supernatant solution obtained from centrifugation was used to carry out estimation using UV-Vis spectrophotometry at wavelength 560 nm.[11]

2.12. Inhibition of Protein Denaturation

Around 0.02 mL of sample was taken. 0.2 mL of 1% Bovine Albumin was added. 4.78 mL of PBS (Phosphate buffer saline) was added to the mixture. This mixture was mixed and it was incubated for 15 mins. It was heated for 5 mins on water bath at 70°C. The mixture was cooled. UV-Vis spectrophotometry was used to check absorbance at wavelength 660 nm. Control used was phosphate buffer. Ibuprofen and prednisolone were taken as a standard. The percentage inhibition of protein denaturation was calculated.[12]

2.13. Carrageenan-induced rat paw edema method

The animal study was approved by the Institutional Animal Ethical Committee (IAEC) of the Institute of Pharmaceutical Education and Research (IPER), Wardha (Protocol No. 533/PO/ReReBt /S02/CPCSEA/IPER/IAEC/2023-2024/32) 4-8 weeks old Albino Wistar rat weighing about 150-250 g of either sex were used for the study. Animals were divided in to 3 groups each containing 6 rats and each group were placed separately in polypropylene cages under standard condition on temperature (25±2°C) light (12:12 hrs light: dark cycle) and were provided with standard pellet diet and water *ad libitum*.

Carrageenan (100 µL) 1% w/v solution in saline, was administered by subplanter injection to induce inflammation to all rats. Group-I was control wherein animals did not received any treatment. The animals in Group-II were treated with a single dose of microemulgel (0.5 g) topically 20 mins prior to carrageenan administration. The animals in Group-III received commercial diclofenac gel 20 mins prior to the carrageenan administration. Paw thickness was measured just before the carrageenan injection, that is, at "0 hour" and then at 1st, 2nd, 3rd, 4th, and 24th hour after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" and paw thickness at respective hours.[13]

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 hr 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 hr in Soxhlet apparatus. The extract obtained was used to study the phytochemical and physico-chemical parameters. The presence of alkaloids, tannins, carbohydrate, flavonoids and terpenoids were determined by performing phytochemical test. The *C. deodara* showed presence of alkaloids, tannins, carbohydrates, flavonoids, and terpenoids (**Table 2**).

Table 2: Phytochemical Screening of *Cedrus deodara*.

S. No.	Phytochemical class	Test	Results
1		Dragendroff's test	+
	Alkaloids	Mayer's test	+
		Hagers's test	+
		Wagner's test	+
2	Carbohydrates	Molisch's test	+
		Fehling's test	+
		Benedict's test	+
3	Flavonoids	Shinoda test	+
		Sulfuric acid test	+
4	Tannins	5% $FeCl_3$	+
		Lead acetate	+
5	Terpenoids	Salkowski's test	+
6	Saponins	Foam test	+
7	Steroids	Salkowski's test	+

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°C-45°C for 30 sec, and then a 50 mg mixture was diluted to 50 mL with distilled water and left to stand for 2 hr. 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 co-surfactants, a mixture of co-surfactant (100 mg), selected surfactant (200 mg), and oil (300 mg) was heated at 40-45°C for 30 sec, and the procedure was carried out similarly to the surfactant screening. Isopropyl myristate was identified as the superior co-surfactant (**Table 3**).

Table 3: 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 and Myristate (S_{mix}). (**Table 4** to **Table 7**) 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 (**Figure 1** to **Figure 4**).

Table 4: 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

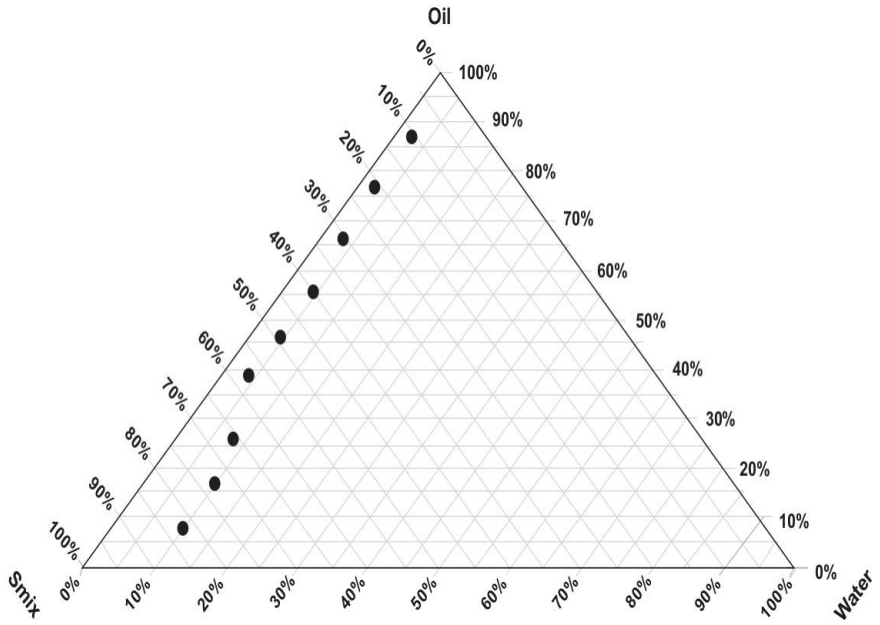

Figure 1: Ternary phase diagram of concentration for S_{mix} 1:0.

Table 5: 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

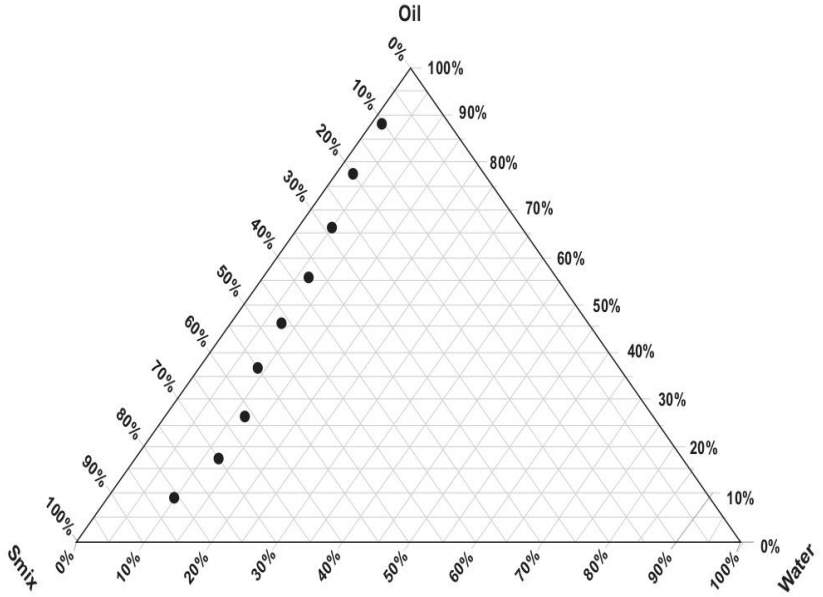

Figure 2: Ternary phase diagram of concentration S_{mix} 1:1.

Table 6: 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

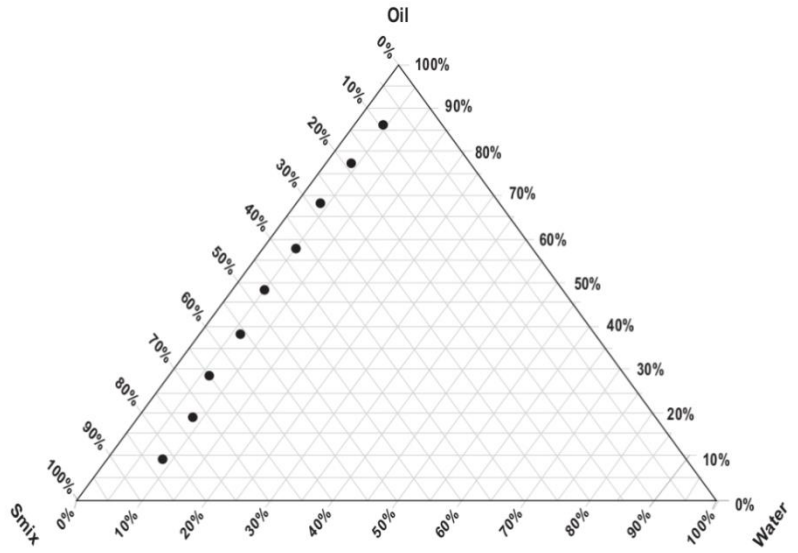

Figure 3: Ternary phase diagram of concentration S_{mix} 2:1.

Table 7: 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

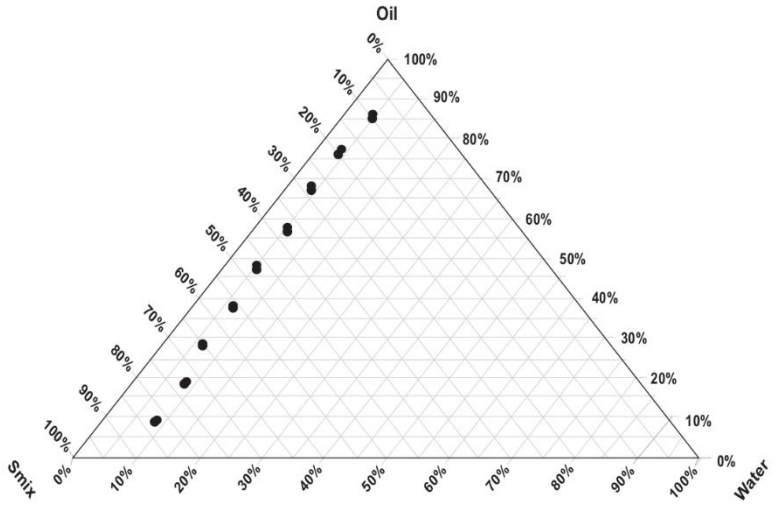

Figure 4: Ternary phase diagram of concentration S_{mix} 1:2.

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 soya lecithin 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 8**).

Table 8: Preparation of microemulgel.

S. No.	Cedar wood oil (% w/w)	S_{mix} (% w/w)	Soy lecithin (% 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.

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 (**Figure 5** and **Figure 6**).

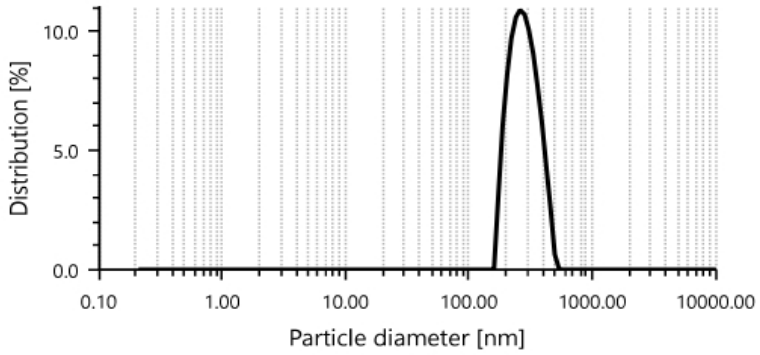

Figure 5: Particle size analysis.

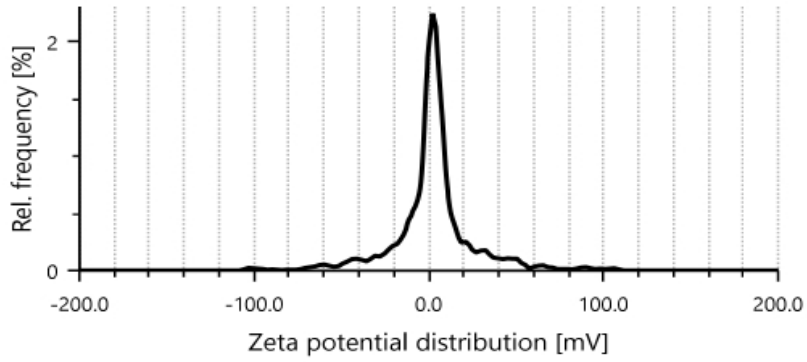

Figure 6: Zeta potential determination.

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 and viscosity

Different formulations are tested for spreadability and viscosity and the results obtained are given below (**Table 9**).

Table 9: Spreadability study.

S. No.	Cedar wood oil (% w/w)	S_{mix} (%w/w)	Soy lecithin (%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

3.3.5. 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.

3.3.6. 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.

3.3.7. *In vitro* anti-inflammatory activity

3.3.7.1. Stabilization Method

The anti-inflammatory efficacy of both the extract and the refined formulation was evaluated using *in vitro* methods, known as 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.

3.3.7.2. Heat-Induced rat Erythrocyte Membrane

When topically applied within a dose range of 100, 200, 300, and 400 $\mu g\,mL^{-1}$, *C. deodar* microemulgel showed a concentration-dependent anti-inflammatory activity and protected the erythrocyte membrane exposed to heat. *C. deodar* microemulgel concentration for 50% inhibition (IC_{50}) was determined on a dose-response curve, plotted as Log (concentration) against percentage inhibition, keeping the hemolysis produced within the control group at 100% (**Figure 7**). The IC_{50} value for *C. deodar* microemulgel was found as per **Table 10** as compared with the ibuprofen and prednisolone.

Table 10: % Inhibition of hemolysis.

S. No.	Concentration (µg/mL)	% Inhibition of Hemolysis		
		Cedar Wood Microemulgel (%)	Ibuprofen (%)	Prednisolone (%)
1	100	27.87	6.12	30.12
2	200	54.69	69.75	63.22
3	300	66.63	71.81	94.45
4	400	81.07	95.36	95.94

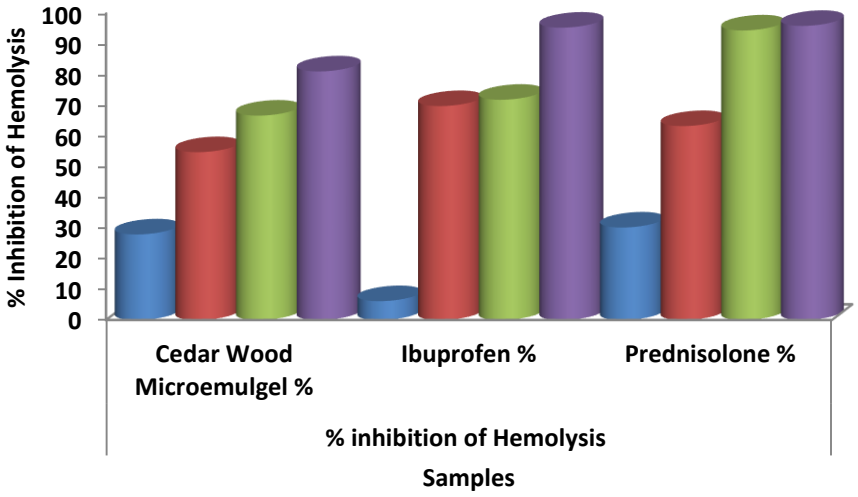

Figure 7: Hemolytic potentials of *C. deodar* microemulgel.

3.3.8. Inhibition of Protein Denaturation

The heat-treated bovine serum albumin denaturation method was used to investigate the anti-inflammatory activity of *C. deodar* microemulgel. When administered at 1, 10, 100, and 1000 $\mu g\,mL^{-1}$, *C. deodar* microemulgel showed a concentration-dependent reduction in albumin denaturation. *C. deodar* microemulgel offered 50% inhibition (IC_{50}) to heat-treated bovine albumin (**Figure 8**). Treatment with ibuprofen and prednisolone showed a similar protection to heat-treated bovine serum albumin denaturation with IC_{50} values as showing in **Table 11**.

Table 11: % Inhibition of Protein Denaturation.

S. No.	Concentration (µg/mL)	% Inhibition of Protein Denaturation		
		Cedar Wood Microemulgel (%)	Ibuprofen (%)	Prednisolone (%)
1	1	20	52.13	97.78
2	10	50.04	68.28	97.86
3	100	47.49	79.37	96.05
4	1000	98.8	86.89	86.85

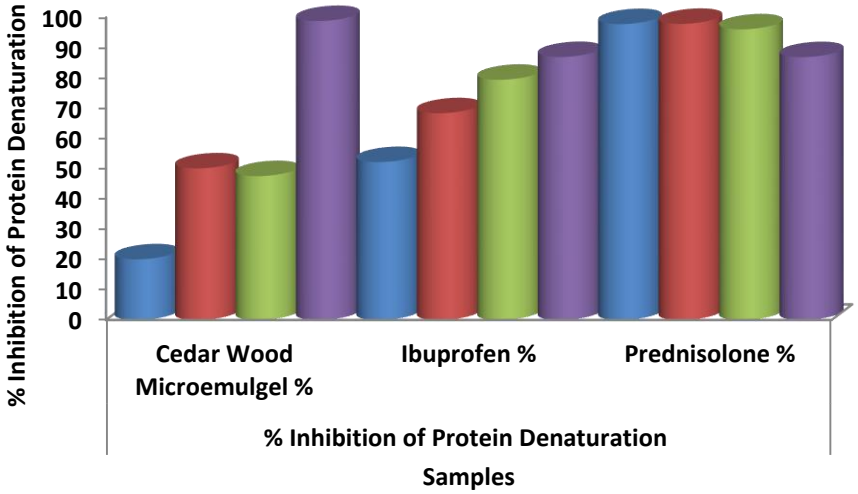

Figure 8: % Inhibition of Protein Denaturation.

3.3.9. *In vivo* anti-inflammatory activity

It was observed that, after treatment with microemulgel consisting Cedar wood oil shows significant (p<0.01) reduction in rat paw volume at 2 hr, 3 hr, and 4 hr after induction of inflammation using carrageenan, when compared with negative control group. Further, it shows its anti-inflammatory effect upto 24 hr (**Figure 9**). Also, diclofenac sodium gel was used as standard marketed preparation. It showed significant (p<0.01) decrease in paw volume, when compared with negative control group (**Figure 10**). The observations of this study concluded that, the microemulgel prepared using Cedar wood oil shows potent anti-inflammatory effect as that of a standard marketed gel consist of diclofenac sodium (**Table 10**, **Table 11**, and **Table 12**).

Table 10: Group of sample treatment.

S. No.	Groups	Treatment
1	Group-I (Negative Control)	Carrageenan (100 µL) 1% w/v (Planter region)
2	Group-II (Test)	Carrageenan (100 µL) 1% w/v (Planter region)and treated with microemulgel
3	Group-III (Standard)	Carrageenan (100 µL) 1% w/v (Planter region) and treated with diclofenac sodium (5 mg/kg; p.o.)

Table 11: Effect of samples on Carrageenan-induced rat paw volume.

Time (hr)	Group I - Negative Control	Group II - Cedarwood oil	Group III - Diclofenac sodium
0	0.26±0.05	0.33±0.05	0.26±0.05
1	0.83±0.08	0.86±0.05	0.80±0.06
2	0.96±0.08	0.56±0.05**	0.35±0.05**
3	0.96±0.09	0.36±0.05**	0.35±0.05**
4	0.86±0.05	0.35±0.05**	0.23±0.05**
24	0.36±0.08	0.30±0.06	0.23±0.05**

Values are expressed as Mean±SD. Data were analyzed by one way ANOVA followed by Dunnett test. (n=6); ** p<0.01 compared with negative control group. GraphPad® InStat software was used for statistical evaluation.

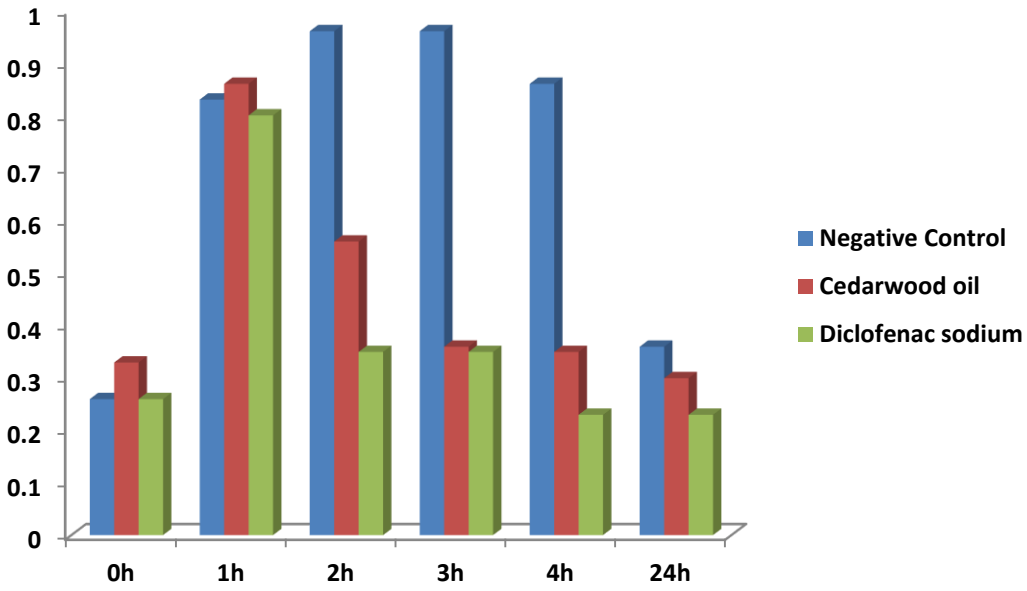

Figure 9: Effect of samples on Carrageenan-induced rat paw edema.

Table 12: Effect of *C. deodara* on Carrageenan-induced rat paw edema and percent inhibition.

Treatment	Topical dose (mg/kg)											
Time and % Inhibition	0 hr	% inhibition	1 hr	% inhibition	2 hr	% inhibition	3 hr	% inhibition	4 hr	% inhibition	24 hr	% inhibition
Negative Control	0.26	---	0.83	---	0.96	---	0.96	---	0.86	---	0.36	---
Cedarwood oil	0.33	78.78	0.86	3.61	0.56	41.37	0.36	61.93	0.35	59.61	0.30	18.18
Diclofenac sodium	0.26	78.78	0.8	3.61	0.35	63.79	0.35	63.66	0.23	73.07	0.23	36.36

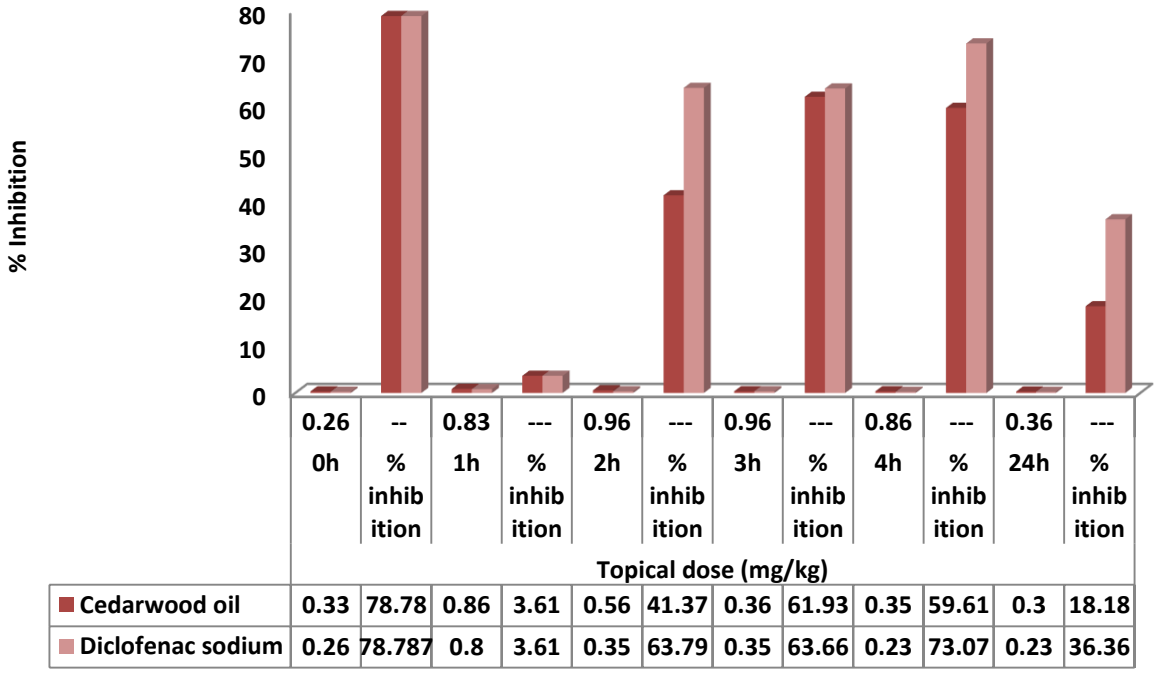

Figure 10: Effect of samples on Carrageenan-induced rat paw edema based on percent inhibition.

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 surfactant, along with n-butanol as the co-surfactant, 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 accelerated conditions.

The microemulgel of *C. deodara* exhibited a transparent appearance with a light yellow and emitted an aroma reminiscent of alcohol. The spreadability of the formulation was in the range of 31.56 ± 0.63 to 37.12 ± 0.25 gm·cm/sec. It shows the size of 90.4 ± 0.01 cps viscosity. This optimum size of *C. deodara* microemulgel showed 100 nm. The 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 hr 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. Developing 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 selecting the appropriate active ingredients and optimizing their concentrations for maximal efficacy. Additionally, it ensures a product stability over the specified duration and maintaining efficacy is crucial. This was assessed through accelerated stress studies conducted as per 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 author affirms no conflicts of interest pertinent to this article.

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