RESEARCH ARTICLE

Anti-Inflammatory Activity of *Arquita ancashiana* Nanoemulgel: *In vitro* and *In vivo* Evaluation

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ABSTRACT
The present study investigates the anti-inflammatory potential of *Arquita ancashiana* extracts and their nano formulations. The ethanol and aqueous extracts were obtained using Soxhlet & maceration extraction methods respectively, yielding semi-solid dark green residues with a percentage yield of 20% and 20.5%, respectively. In membrane stabilization assay, the ethanol extract exhibited a higher stabilization effect (IC_{50} = 475.76 µg/mL) in comparison to aqueous extract (IC_{50} = 815.14 µg/mL), though both remained less potent than the standard Diclofenac sodium (IC_{50} = 199.62 µg/mL). A nano emulsion of *Arquita ancashiana* was successfully formulated using a surfactant: co-surfactant ratio of 3:1, and thermodynamic stability studies confirmed its stability. Physicochemical characterization revealed optimal globule size (85.46–91.57 nm), high zeta potential (-14.7 to -38.5 mV), and favorable viscosity. The nano emulgel was evaluated using a carrageenan-induced paw edema model, demonstrating significant anti-inflammatory activity, though slightly less effective than Diclofam Gel. ELISA analysis revealed a marked reduction in TNF-α levels in the nano emulgel-treated group, further supporting its anti-inflammatory potential. These findings suggest that *Arquita ancashiana* nano emulgel could serve as a promising natural alternative for inflammation management.

Keywords: *Arquita ancashiana*, anti-inflammatory activity, nanoemulsion gel, membrane stabilization assay, carrageenan-induced paw edema

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INTRODUCTION
Inflammation is a crucial biological response to harmful stimuli, including pathogens, injuries, and irritants, essential for immune defense and tissue repair[1]. However, prolonged or chronic inflammation is linked to several diseases, such as arthritis, cardiovascular disorders, diabetes, and cancer. The increasing prevalence of inflammatory conditions worldwide is linked to lifestyle changes, environmental pollutants, and aging populations. While conventional anti-inflammatory drugs like NSAIDs and corticosteroids are widely used, their long-term application often results in adverse effects such as gastrointestinal irritation, immunosuppression, and organ toxicity[2]. This has led to growing interest in plant-derived therapies as safer alternatives with multi-targeted mechanisms and fewer side effects.

Natural products have been extensively explored for their anti-inflammatory potential due to presence of flavonoids, alkaloids, terpenoids, and polyphenols. Phytochemicals modulate key inflammatory pathways by inhibiting TNF-α, IL-1β, IL-6, and COX enzymes[3,4]. Several medicinal plants, including *Annona muricata* and *Acanthus mollis*, have demonstrated significant anti-inflammatory effects[5,6], reinforcing the therapeutic promise of botanical sources. Plant-derived compounds also exhibit antioxidant properties, which contribute to their efficacy in managing inflammation and related conditions, positioning them as attractive candidates for further research and pharmaceutical development[5].

Arquita ancashiana, a traditionally used medicinal plant shows excellent anti-inflammatory effects. Preliminary studies indicate that presence of flavonoids, alkaloids, saponins, and tannins, shows pharmacological activity. In vitro membrane stabilization assays have demonstrated the plant's ability to inhibit haemolysis in heat-induced erythrocyte membrane tests, indicating its role in cellular protection under inflammatory conditions. *In vivo* models have shown significant reduction in inflammation, with effects comparable to standard anti-inflammatory drugs like Diclofenac sodium. These findings suggest that may exert its effects by suppressing key inflammatory mediators and enhancing antioxidant enzyme activity.

To enhance the bioavailability and therapeutic application of *Arquita ancashiana*, its ethanolic extract was formulated into a nanoemulsion-based gel (nanoemulgel)[7-9]. The membrane stabilization assay demonstrated that the ethanolic extract exhibited superior anti-hemolytic activity compared to the aqueous extract. The optimized nanoemulgel formulation showed desirable physicochemical characteristics, including small globule

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Table 1: Formulation batches composition of Extracts and excipients.

Formulation Batch	Extracts of *Arquita ancashiana* in gm	Smix		Oil (Oleic acid) %	Water %
		Surfactant (Tween 80) %	Co-surfactant (Ethanol) %		
F1	1	22.5	7.5	10	60
F2	1	26.25	8.75	15	50
F3	1	30	10	20	40

Table 2: Composition of Extract Loaded Nano Emulsion Gel

Ingredients (%)	Gel Formulation Batches		
	NEGF1	NEGF2	NEGF3
Nano emulsion	90 (F1)	90 (F2)	90 (F3)
Carbopol 940	1.5	1.5	1.5
Stearic acid	5	5	5
Ethanol	3	3	3
Triethanolamine (TEA)	0.65	0.65	0.65
Methyl paraben	0.02	0.02	0.02
Water	2	2	2

size, high zeta potential, and optimal viscosity, making it a promising candidate for topical application in inflammation management[10-12]. While these findings highlight the therapeutic potential of *Arquita ancashiana*, further studies are needed to fully characterize its bioactive compounds, evaluate its safety, and explore its clinical applications in inflammatory conditions.

MATERIAL AND METHODS

Materials

The leaves of *Arquita ancashiana* were collected in March 2021 and authenticated by Dr. Vikas B. Awale, Head of the Department of Botany, Dr. Pantagrao Kadam Mahavidyalaya, Sangli. The taxonomic identity of the plant was authenticated, and a Plant herbarium specimen was Stored in the laboratory for future reference.

Extraction procedure

Aqueous & ethanolic Extraction of leaves of by Arquita ancashiana Maceration

To extract compounds from *Arquita ancashiana* leaves, two different methods can be used: maceration for aqueous extraction and Soxhlet extraction for ethanol-based extraction[13,14]. In maceration, the coarsely ground plant material is soaked in a solvent, allowing it to fully absorb the liquid. The mixture is then left undisturbed in a sealed container for at least three days, with occasional stirring or shaking to maximize extraction. Once the process is complete, the liquid containing the extracted compounds is separated from the solid plant material through filtration or decantation. The solvent is then evaporated using an oven or water bath, leaving behind the final extract. On the other hand, Soxhlet extraction is a more advanced technique that uses a specialized glass apparatus to continuously circulate hot ethanol through the plant material. The plant material is placed inside a porous thimble within the extraction chamber, and as the ethanol is heated, it evaporates, rises into a condenser, and then drips back onto the plant material. This cycle repeats until all the desired compounds have been extracted, at which point the solvent is removed, leaving behind the concentrated extract. While this method is highly effective for extracting compounds from materials with insoluble impurities, it is not suitable for heat-sensitive plant components.

Table 3: Results of Ethanolic & Aqueous Extraction of *Arquita ancashiana*

Extract	Solvents	Colour	Nature	% yield w/w
Arquita ancashiana	Aqueous	Dark green	Semi solid	20.5
	Ethanol	Dark green	Semi solid	20

Table 4: Anti-inflammatory action of *ethanol and aqueous extract of Arquita ancashiana*

Extracts	Conc (µg/mL)	Mean	% stabi-lization	IC_{50}
Blank		0.263 ± 0.03		
Standard (Diclo-fenac sodium)	100	0.163 ± 0.02	37.90	199.62
	200	0.12 ± 0.01	54.37	
	400	0.09 ± 0.02	65.78	
	800	0.07 ± 0.01	73.38	
Aqueous extract of arquita ancashiana	100	0.21 ± 0.02	18.88	815.14
	200	0.18 ± 0.02	31.56	
	400	0.156 ± 0.01	40.43	
	800	0.14 ±0.02	46.77	
Ethanol extract of arquita ancashiana	100	0.17 ±0.02	35.36	475.76
	200	0.143 ±0.015	45.50	
	400	0.133 ±0.015	49.30	
	800	0.11 ±0.036	58.17	

The in vitro membrane stabilizing activity of Arquita ancashiana extracts

The membrane-stabilizing activity of *Arquita ancashiana* extract was evaluated using an in vitro model with bovine red blood cells (RBCs)[15-17]. Fresh cow blood was collected in an anticoagulant solution composed of NaCl, citric acid, sodium citrate, and dextrose followed by centrifugation at 3000 rpm for 10 min to isolate packed RBCs. To ensure purity, the cells were washed several times with normal saline (0.85% NaCl) until a clear supernatant was obtained. A 2% v/v RBC suspension was subsequently prepared for the study. To assess the anti-inflammatory potential, reaction mixtures were prepared containing hypotonic saline, phosphate buffer, test solutions (500 and 1000 µg/mL), and a 10% RBC suspension. These mixtures were incubated at 56°C for 30 minutes. Following incubation, the samples were cooled and centrifuged, and the absorbance of free haemoglobin in the supernatant was measured at 560 nm. % membrane stability was then calculated using the standard formula.

% Inhibition=100−(At−Ac)/Ac×100)

Table 5: Composition of Oleic acid, Tween 80, Ethanol and water at Km=1

Smix: Oil	% W/W		
	Smix	Oil	Water
1:9	30.42	9.25	60.33
2:8	25.63	13.48	60.89
3:7	21.75	17.44	60.81
4:6	42.28	33.29	24.43
5:5	40.36	40.31	19.33
6:4	33.48	52.45	14.07
7:3	26.78	60.58	12.64
8:2	18.62	72.68	8.7
9:1	11.54	77.52	10.94

Table 6: Formula Compositions of Oleic acid, Tween 80, Ethanol and water at Km=2

Smix:Oil	% W/W		
	Smix	Oil	Water
1:9	25.47	7.24	67.29
2:8	23.39	11.38	65.23
3:7	17.54	21.47	60.99
4:6	40.68	34.86	24.46
5:5	37.41	43.52	19.07
6:4	30.63	56.32	13.05
7:3	21.44	65.47	13.09
8:2	15.39	78.12	6.49
9:1	9.28	86.38	4.34

Table 7: Formula compositions of Oleic acid, Tween 80, Ethanol and water at Km=3

Smix:Oil	% W/W		
	Smix	Oil	Water
1:9	20.82	8.65	70.53
2:8	16.35	9.48	74.17
3:7	13.48	17.64	68.88
4:6	36.84	33.47	29.69
5:5	33.46	42.18	24.36
6:4	28.63	53.29	18.08
7:3	17.42	63.72	18.86
8:2	13.55	73.46	12.99
9:1	11.47	82.67	5.86

Where,
At = Test absorbance
Ac = Control absorbance
Diclofenac sodium was used as the standard drug, with 100% lysis indicating complete erythrocyte disruption and 0% stability representing total membrane preservation.

Preparation of topical emulgel formulation

Preparation of Nano Emulsion of Extracts of Arquita ancashiana Using High Pressure Homogenizer

Based on the constructed phase diagrams, the Km value at which the largest microemulsion region was observed (Km = 3) was taken in to consideration. To identify the optimal composition of oil, surfactant, and co-surfactant, three specific points within the nanoemulsion region were selected. For preparing nanoemulsions containing *Arquita ancashiana* extract, Oleic acid was used as the oil phase, while Tween 80 and Ethanol served as the surfactant and co-surfactant, respectively. These components were chosen based on the pseudo-ternary phase diagram, as outlined in Table 1. The extract-loaded nanoemulsion was formulated using a two-step high-pressure homogenization technique. Initially, a coarse emulsion was formed by dispersing the extract powder into the oil phase, followed by stirring at 5000 rpm using an overhead mixer. The surfactant and co-surfactant were then incorporated into the mixture and vortexed at 300 rpm for 10 minutes to ensure uniform blending. Subsequently, the oil phase was introduced dropwise into the aqueous phase through a 0.2 µm syringe orifice at a controlled rate of 10 mL/min while continuously stirring with a magnetic stirrer. The mixture was further stirred using an overhead stirrer at 500 rpm for one hour to ensure proper emulsification. In the second step, the coarse emulsion underwent high-pressure homogenization, where it was processed for five cycles at 1000 bar to achieve the desired nanoemulsion[18, 19].

Table 8: Thermodynamic stability studies

Formulation	H/C	Cent.	Friz. Thaw	Inference
F1	✓	✓	✓	Passes
F2	✓	✓	✓	Passes
F3	✓	✓	✓	Passes

Formulation of Carbopol 940 plain gel

To formulate a plain gel base, Carbopol 940 polymer (0.75 wt%) was dispersed in distilled water and allowed to swell in the dark at 25 ± 2°C for 24 hours. This process facilitated air bubble removal and ensured polymer equilibrium. Following the swelling phase, the pH of the polymeric solution was adjusted to 6 using triethanolamine, yielding a clear and transparent gel[20].

Formulation of nanoemulgel

Formulations confirmed to be within the nano-size range and were subsequently incorporated into a gel matrix to get nanoemulsion gel. Gel was prepared by blending the nanoemulsion with a plain Carbopol 940 gel. Specifically, the plain gel and nanoemulsion were mixed at a 1:1 (wt%) and stirring was done at 120 rpm at RT till clear and homogeneous nanoemulsion gel was obtained[21,22]. Composition of nano emulsion gel formulations (NEGF) (Table 2).

Evaluation of Extract Loaded Nano emulsion Gel:

Determination of pH

pH was measured by mixing 1 g of each formulation with 20 mL DW. pH was measured by digital pH meter. Measurements were performed in triplicate, and the results were expressed as the mean ± SD[23].

Viscosity

Table 9: Characterisation results

Formulation	Globule size (nm)	Zeta potential (mV)	% Transmittance	Viscosity (cps)
F1	91.57	-14.7	92.74	18.27 ± 1.2
F2	87.63	-27.6	94.59	16.54 ± 1.5
F3	85.46	-38.5	96.28	14.73 ± 1.8

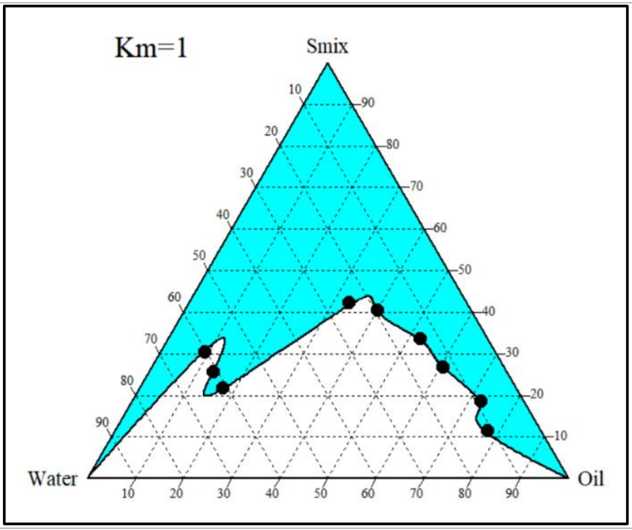

Figure 1: Pseudo ternary phase diagram of Oleic acid, Tween 80, Ethanol and water at Km=1

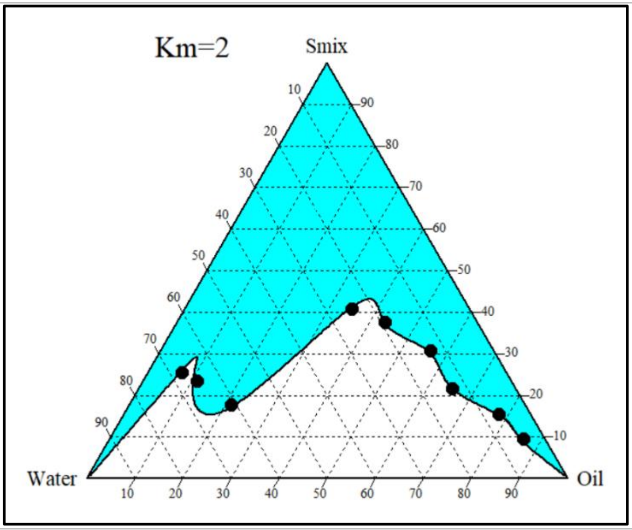

Figure 2: Pseudo ternary phase diagram of Oleic acid, Tween 80, Ethanol and water at Km=2

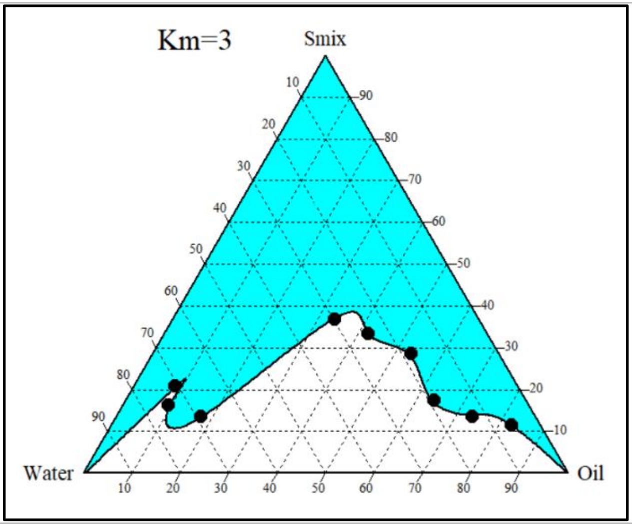

Figure 3: Pseudo ternary phase diagram of Oleic acid, Tween 80, Ethanol and water at Km=3

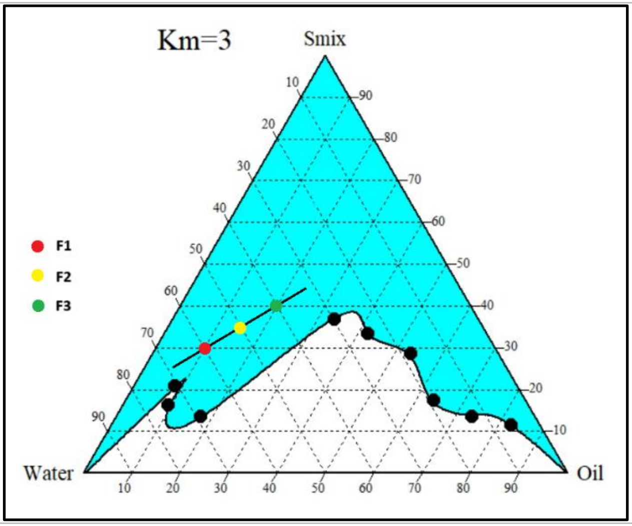

Figure 4: Selection of Oil, surfactant and co-surfactant concentrations from micro emulsion region

The viscosity of the formulations was determined using a Brookfield viscometer R/S+ with spindle CC 14. A 100 mg gel sample was placed in the sample holder, and the spindle was lowered to equilibrate for 5 minutes. The viscosity was then measured at room temperature with a shear rate of 10/s and a spindle speed of 10 rpm[23].

Spreadability

5 g sample of the developed gel was placed between two slides and allowed to rest for 5 minutes. The diameter of the spread circles was then measured in centimeters, and the spreadability was calculated using the following formula. $S = ML/T$[24, 25].

Where,

S: Spreadability (g·cm/s),

M: mass (g),

L: Length (cm),

T: Time (s)

Extrudability

The prepared gel formulations were filled into clean collapsible tubes with 5 mm openings and sealed using heat. To assess extrudability, a constant load of 500 g was applied to the tube, allowing the gel to be released. The extruded gel was then collected, weighed, and its extrudability percentage was determined [24, 25].

In vivo evaluation of anti-inflammatory action by carrageenan-induced inflammation model

The study was conducted at Biocyte Institute of Research and Development, Sangli, with approval (IAEC/Sangli/2022-23/10.) To induce inflammation, all groups of rats received a subcutaneous injection of 0.1 ml of a freshly prepared 1% carrageenan solution into the right hind paw footpad, while the left hind paw was used as a control. Paw edema was measured at 0, 30, 60, 90, 120, and 150 minutes using a digital Vernier caliper, and the percentage of edema inhibition was determined[26, 29]. After the experiment, blood samples were collected from the retro-orbital plexus, and plasma was separated for TNF-α estimation. Additionally, paw tissue TNF-α levels were assessed using ELISA (Krishgen Biosystem Kits). Paw tissues were stored at -20°C for 5 hours, homogenized in PBS (pH 7.4), and centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was analyzed using ELISA, where

Table 11: Changes in paw thickness

Groups	Dose	Min				
		30	60	90	120	150
Saline		7.53 ± 0.31	8.02 ± 0.11	8.01 ± 0.30	8.00 ± 0.30	8.03 ± 0.28
Diclofam Gel	2gm/paw	6.65 ± 0.68 11.68 %	6.74 ± 0.17 15.94 %	5.33 ± 0.30 33.56 %	4.66 ± 0.27 41.89 %	3.79 ± 0.26 52.72 %
Nano Emulgl	2gm/paw	7.19 ± 0.68 4.42 %	6.94 ± 0.52 13.52 %	6.67 ± 0.20 16.73 %	6.20 ± 0.24 22.47 %	5.02 ± 0.18 37.46 %

Table 10: Results of pH, viscosity, spreadability and extrudability of Nano emulsion gel

Batch	pH	Viscosity (cps)	Spreadability (g cm/s)	Extrudability (%)
F1	5.78 ± 0.42	9743 ± 14.6	21.62 ± 1.46	86.48 ± 0.63
F2	5.45 ± 0.18	9861 ± 12.5	20.83 ± 1.71	88.37 ± 0.52
F3	5.75 ± 0.23	9886 ± 15.8	19.78 ± 1.16	0.18

Table 12: Standard curve of Rat TNF α on Elisa reader.

S no.	Group	Pg/ml	Abs (OD)
1	Standard 7	2000	1.59
2	Standard 6	1000	0.88
3	Standard 5	500	0.52
4	Standard 4	250	0.39
5	Standard 3	125	0.32
6	Standard 2	62.5	0.21
7	Standard 1	31.25	0.11
8	Standard 0	0.0	0.1

a pre-coated microtiter plate with monoclonal antibodies specific to TNF-α was used. Samples were pipetted into wells, followed by washing, biotinylated antibody binding, and streptavidin-peroxidase substrate detection. Absorbance was recorded at 490 nm, and TNF-α concentrations were calculated using a standard curve.

Inhibition (%)=(Change in Control−Change in Treatment)/ Change in Control ×100

Statistical analysis

All data were expressed as mean ± SD. *In vitro* anti-inflammatory investigations utilized and IC_{50} values were determined using linear regression analysis in Microsoft Excel. In vivo statistical analysis was conducted using GraphPad Prism® version 9.0, applying two-way ANOVA for paw diameter evaluation and one-way ANOVA for TNF-α estimation.

RESULTS

Aqueous & ethanolic Extraction of Arquita ancashiana

The extraction of *Arquita ancashiana* was carried out using aqueous and ethanol solvents, yielding semi-solid extracts with a dark green appearance. The percentage yield was 20.5% for the aqueous extract and 20% for the ethanol extract (table 3).

In vitro membrane stabilizing activity

Diclofenac sodium, used as the standard, exhibited a concentration-dependent increase in membrane stabilization, with a maximum stabilization of 73.38% at 800 µg/mL and an IC_{50} value of 199.62 µg/mL. Ethanolic extract demonstrated greater activity in comparison to

Table 13: Estimation of Rat TNF α Elisa on given tissue Sample

S no.	Group	Abs	Pg/mL ±SD
1	Normal Control	0.161	008.17 ±1.62
2	Disease Control	1.28	1545.21 ±1.24
3	Standard (Diclofam gel)	0.39	322.67 ±1.84
4	Nano Emulgel	0.65	679.81 ±1.27

aqueous extracts. The ethanol extract showed 35.36% stabilization at 100 µg/mL, which increased to 58.17% at 800 µg/mL, with an IC_{50} value of 475.76 µg/mL. Aqueous extract exhibited a lower stabilization effect, with 18.88% at 100 µg/mL and 46.77% at 800 µg/mL, resulting in an IC_{50} value of 815.14 µg/mL (table 4).

Preparation of Nano Emulsion of Extracts of Arquita ancashiana Using High Pressure Homogenizer

The nanoemulsion of *Arquita ancashiana* extract was prepared using a high-pressure homogenizer, utilizing the ethanolic extract due to its superior membrane stability activity observed in the in vitro assay. The higher stabilization percentage and lower IC_{50} value indicated better efficacy, making it the preferred choice for further formulation and evaluation.

Construction of pseudo ternary phase diagram

Based on the solubility study results, Oleic acid, Tween 80, and Ethanol were chosen as the oil, surfactant, and co-surfactant for nanoemulsion formulation. Nine different surfactant-to-oil ratio combinations were evaluated at varying Km values (1:1, 2:1, and 3:1) to construct phase diagrams for extract-loaded nanoemulsions (table 5). The o/w nanoemulsion boundary layer was identified in each phase diagram, with the shaded regions representing the nanoemulsion area. The pseudo-ternary phase diagrams at the respective Km values are presented in Figures 1, 2, and 3. As the concentration of surfactant and co-surfactant increased, the nanoemulsion region also expanded.

Preparation of Nano Emulsion of Extracts of Arquita ancashiana Using High Pressure Homogenizer

The largest nanoemulsion region was observed at a 3:1 ratio. Therefore, the optimal surfactant-to-co-surfactant ratio for formulating the extract-loaded nanoemulsion was chosen as 3:1. 3 batches of nano emulsion containing extract of *Arquita ancashiana* and *Paederia foetida* were prepared successfully and were used for further evaluation (Figure 4).

Evaluation of Extract Loaded Nano Emulsion

Thermodynamic stability studies

The results of the thermodynamic stability studies for the nanoemulsion are summarized in Table 8. The formulation successfully passed the heating-cooling cycle test and was subsequently subjected to the centrifugation test. No phase separation was observed after centrifugation, allowing it to proceed to the freeze-thaw stress test. Following this test, the nanoemulsion demonstrated good stability, showing no signs of phase separation, creaming, or cracking.

Percentage Transmittance, globule size, zeta potential and viscosity

Results are presented in Table 9. % Transmittance of extract loaded nano emulsion was found in between 92.74 to 96.28. This indicates that prepared nano emulsions are clear and no turbid. Globule size and zeta potential of all formulations were found to be in between 85.46 to 91.57 nm and -14.7 mV to -38.5 mV respectively. As the oil concentration in the formulations increased, a slight increase in globule size was observed. However, the difference in globule size among the formulations was minimal. The viscosity of all formulations ranged from 14.73 ± 1.8 to 18.27 ± 1.2 cP.

Evaluation of Extract Loaded Nano emulsion Gel

The results for pH, viscosity, spreadability, and extrudability are summarized in Table 8. The pH of the nanoemulsion gel formulations ranged from 5.45 ± 0.18 to 5.78 ± 0.42, aligning with the skin's natural pH, making them suitable for topical use without causing irritation. Viscosity plays a vital role in gel formulations, influencing drug release, spreadability, stability, and ease of application. It is also affected by the choice of polymers and excipients used. The viscosity of the nanoemulsion gel formulations was recorded between 9743 ± 14.6 cP and 9886 ± 15.8 cP. Spreadability, crucial for effective application, determines how easily the formulation spreads over the skin. Optimal spreadability allows the gel to be dispensed with minimal shear stress. It is temperature-dependent, increasing as temperature rises, while viscosity decreases with cooling. The spreadability of the prepared nanoemulsion gel formulations ranged from 19.78 ± 1.16 g cm/s to 21.62 ± 1.46 g cm/s. Extrudability, which measures how easily the gel can be released from the tube, was also evaluated. The esomeprazole-loaded nanoemulsion gel formulations demonstrated good extrudability, with values between 86.48 ± 0.63% and 89.55 ± 0.18%, ensuring easy application (table 10).

In vivo evaluation of anti-inflammatory effect

Anti-inflammatory efficacy of Diclofam Gel and Nano Emulgel was evaluated by measuring the change in paw thickness at different time intervals (30, 60, 90, 120, and 150 minutes) following treatment. The control group (Saline) exhibited a consistent increase in paw thickness over time, ranging from 7.53 ± 0.31 mm at 30 minutes to 8.03 ± 0.28 mm at 150 minutes. In contrast, the Diclofam Gel (2 gm/paw) group demonstrated a significant reduction in paw thickness, with values decreasing from 6.65 ± 0.68 mm at 30 minutes to 3.79 ± 0.26 mm at 150 minutes, corresponding to a 52.72% inhibition. Similarly, the Nano Emulgel (2 gm/paw) group showed a time-dependent reduction in paw thickness, decreasing from 7.19 ± 0.68 mm at 30 minutes to 5.02 ± 0.18 mm at 150 minutes, resulting in a 37.46% inhibition (table 11 and figure 5). These findings suggest that both formulations exhibit anti-inflammatory activity, with Diclofam Gel showing greater efficacy compared to Nano Emulgel. For six rats in each group, every value signifies mean ± SEM. Table 12 shows the standard curve of Rat TNF α on Elisa reader. Table 13 and figure 6 shows the estimation of Rat TNF α Elisa on given tissue Sample The ELISA analysis for TNF-α levels in the rat tissue samples demonstrated a significant elevation in the Disease Control group, with a TNF-α concentration of 1545.21 ± 1.24 pg/mL, indicating severe inflammation. In contrast, the Normal Control group had a substantially lower TNF-α level of 8.17 ± 1.62 pg/mL, confirming the absence of inflammation. Treatment with Diclofam Gel effectively reduced TNF-α levels to 322.67 ±

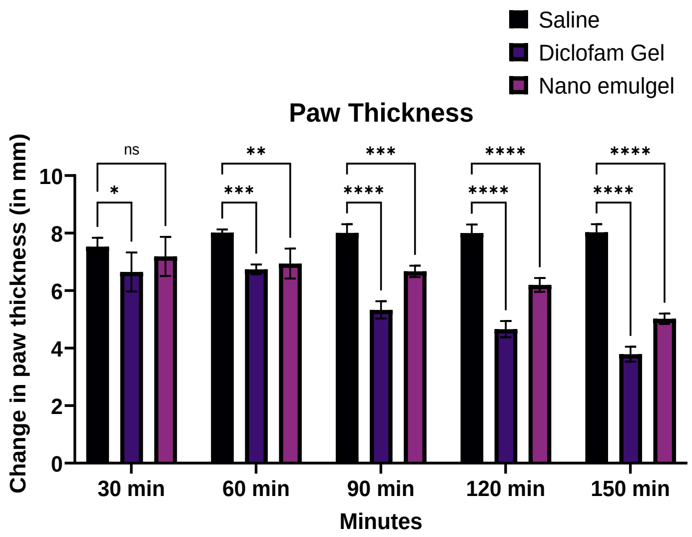

Figure 5: Effect of Diclofam Gel and Nano Emulgel on paw thickness (mean ± SEM. n=3)

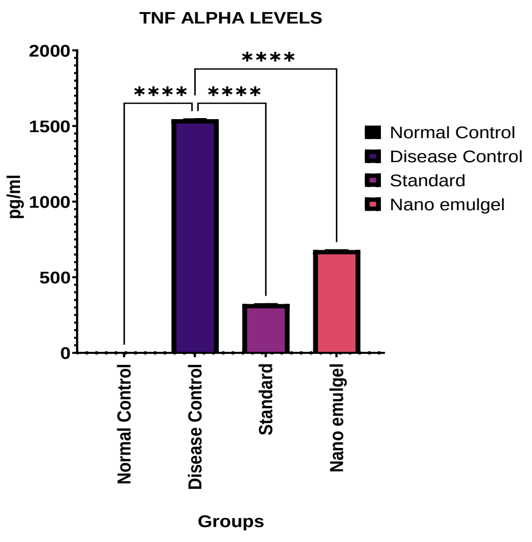

Figure 6: Nano emulgel reduced TNF levels suggestively in carrageenan injected paw

1.84 pg/mL, demonstrating its strong anti-inflammatory potential. The Nano Emulgel formulation also significantly decreased TNF-α levels, reaching 679.81 ± 1.27 pg/mL, though it was less effective than Diclofam Gel. Comparatively, the Nano Emulgel-treated group showed a 55.99% reduction in TNF-α levels relative to the Disease Control, whereas Diclofam Gel achieved a 79.12% reduction. While the Nano Emulgel formulation exhibited promising anti-inflammatory activity, its efficacy was lower than that of the standard treatment, suggesting the need for further optimization to enhance its therapeutic potential.

DISCUSSION

Present research highlights potential of *Arquita ancashiana* extracts, particularly in nano formulations, for anti-inflammatory applications. The ethanol extract demonstrated superior membrane-stabilizing activity in comparison to aqueous extract, which may be due to presence of more bioactive compounds in ethanol-based extracts. While both extracts exhibited notable anti-inflammatory activity, Diclofenac sodium remained the most potent, underscoring the need for further optimization of extract concentrations for enhanced efficacy. Nano emulsions were successfully formulated using a surfactant-to-co-surfactant ratio of 3:1, ensuring stability, optimal particle size, and improved bioavailability. Thermodynamic stability studies confirmed that the formulations remained stable under stress conditions, and physicochemical evaluations indicated that they possessed desirable transmittance, viscosity, and extrudability properties. In vivo studies further validated the anti-inflammatory efficacy of the nano emulgel formulation. Although Diclofam Gel showed a greater reduction in paw thickness, the nano emulgel demonstrated substantial anti-inflammatory effects. ELISA analysis revealed a significant reduction in TNF-α levels in the nano emulgel-treated group, indicating its potential for long-term anti-inflammatory activity. These findings suggest that nano formulations of *Arquita ancashiana* could serve as an effective alternative to conventional anti-inflammatory treatments.

CONCLUSION

The study successfully demonstrated the anti-inflammatory potential of *Arquita ancashiana* extracts and their nano formulations. The ethanol extract exhibited a higher membrane stabilization effect (IC_{50} = 475.76 μg/mL) than the aqueous extract (IC_{50} = 815.14 μg/mL), indicating better anti-inflammatory activity. Nano emulsions were successfully formulated and exhibited excellent stability, optimal globule size, high transmittance, and appropriate viscosity. In vivo studies confirmed a significant reduction in paw edema with the nano emulgel, though Diclofam Gel showed greater inhibition. ELISA analysis further indicated that the nano emulgel reduced TNF-α levels, reinforcing its potential for anti-inflammatory applications. These results suggest that *Arquita ancashiana* nano emulgel could be a promising candidate for inflammation treatment, warranting further investigation into its long-term efficacy and safety.

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