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Formulation and evaluation of *Argemone mexicana* oil-based nano-emulsion using low-energy method and its antimicrobial assessment

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Abstract
Background and objective: *Argemone mexicana* seed oil is a plant-derived bioactive known for its potent antimicrobial and anti-inflammatory properties. However, its clinical utility is hindered by poor water solubility, high viscosity, and chemical instability. Nano-emulsion technology provides a promising strategy to enhance the bioavailability and stability of such hydrophobic phytoconstituents. This study aimed to formulate and evaluate a stable nano-emulsion of *Argemone mexicana* oil using a low-energy method and assess its physicochemical properties and antimicrobial potential.
Methods: A spontaneous emulsification technique was used for the development of oil-in-water nano-emulsions using Brij 30 as the primary surfactant. Pseudo-ternary phase diagrams were constructed to determine optimal composition ranges. The prepared formulations were characterized for droplet size, zeta potential, morphology (optical microscopy), viscosity, and physical stability. Antimicrobial activity was evaluated using agar well diffusion method against *Escherichia coli*.
Results: The optimized nano-emulsion (AB1) exhibited a mean droplet size of <100 nm and a zeta potential of -28.7 mV, indicating good colloidal stability. The system showed low viscosity (~4-8 cP) and remained stable without phase separation over a 3-month period at 4 °C and 25 °C. *In vitro* antimicrobial studies revealed a significant zone of inhibition (18 mm) with a MIC of 50 µg/mL for AB1, demonstrating enhanced activity compared to the crude oil.
Conclusion: The developed nano-emulsion of A. mexicana oil using low-energy emulsification significantly improved the oil's physicochemical properties and antimicrobial efficacy, suggesting its potential application as a natural antimicrobial agent in pharmaceutical formulations.

Keywords: *Argemone Mexicana*, nano-emulsion, low-energy emulsification, Antimicrobial activity, herbal formulation, plant-based drug delivery, phytopharmaceuticals

1. Introduction
Nanotechnology has emerged as a transformative field in pharmaceutical sciences, offering novel strategies for enhancing drug solubility, stability, and delivery. Nano-emulsions, defined as kinetically stable colloidal systems with droplet sizes between 20 and 200 nm, provide substantial advantages for delivering hydrophobic phytochemicals that are otherwise poorly bioavailable in their crude forms [1, 2, 3].
Argemone mexicana, or Mexican poppy, is a well-documented medicinal plant known for its antimicrobial, anti-inflammatory, and analgesic activities due to its content of alkaloids, flavonoids, sterols, and fatty acids [4, 5, 6]. However, its application is limited due to poor aqueous solubility and chemical instability [7]. The plant contains significant phytoconstituents such as sanguinarine, berberine, protopine, and flavonoids. Its seed oil is rich in linoleic and oleic acids, contributing to broad pharmacological activities including antimicrobial, anti-inflammatory, and wound-healing effects, Nano-emulsification provides a means to encapsulate and deliver such oils efficiently, protecting them from degradation while enhancing their biological efficacy [8, 9, 10, 11]. The common method of nano emulsion are high- or low-energy methods, with the latter being more suitable for thermolabile bioactives [13, 14, 15]. Low-energy methods such as Phase Inversion Composition (PIC), Phase

Inversion Temperature (PIT), and spontaneous emulsification rely on intrinsic properties of surfactants and co-surfactants rather than mechanical force [16]. This study aimed to develop a nano-emulsion of *A. mexicana* oil using low-energy methods, characterize its physicochemical properties, and evaluate its antimicrobial potential.

2. Materials and Methods

2.1 Materials

Argemone mexicana oil (ρ = 0.940 g/mL; viscosity = 4.2 cP) was sourced from an authenticated herbal supplier. Brij 30 (Polyoxyethylene (4) lauryl ether) was obtained from Loba Chemie, Mumbai, India. Deionized water was used for all dilutions. All reagents were analytical. Surfactants and co-surfactants: Tween 80, Span 80, and ethanol was procured from CDH chemical other chemicals**:** All reagents were of analytical grade and purchased from reputable suppliers.

2.2 Screening of Surfactants and Co-surfactants

The solubility of *Argemone mexicana* oil in various surfactants Brij 30 (Tween 80, Span 80) and co-surfactants (ethanol) was determined by adding excess oil to 2 mL of each surfactant/co-surfactant in a glass vial, vortexing for 5 minutes, and equilibrating at room temperature for 24 hours. The mixture was centrifuged, and the supernatant was analyzed for oil content using UV-Vis spectrophotometry.

2.3 Construction of Pseudo-Ternary Phase Diagram

Phase diagrams were constructed using the water titration method. Mixtures of oil, surfactant, and co-surfactant at various ratios were titrated with distilled water under gentle agitation at room temperature. Ternary phase diagrams were constructed using ProSim Ternaire software, and three transparent appearance of the mixture (clear, turbid, phase separation) was visually observed to identify the nano-emulsion region.

2.4 Preparation of Nano-Emulsion by Low Energy Method

Argemone mexicana oil and Brij 30 were mixed in various ratios (v/v%)-from 10:0 to 0:10-and stirred at 50 °C to ensure uniformity, then cooled. Water was then added incrementally to reach 95% aqueous content. The formulations were visually inspected to classify them as isotropic or anisotropic, homogeneous emulsions were selected (coded AB1, AB2, AB3). The formulations were stirred at 600 rpm, then heated gradually. Conductivity changes were used to identify the PIT. Once PIT was achieved, rapid cooling was done in an ice bath while maintaining continuous stirring.

2.5 Characterization of Nano-Emulsion

2.5.1 Particle Size and Zeta Potential Measurement

The particle size (mean hydrodynamic diameter) and zeta potential of the HA-functionalized, vitamin E-loaded liposomes were determined using a Malvern Zetasizer Nano ZS (Malvern Panalytical, UK). The Zetasizer employs Dynamic Light Scattering (DLS) to measure particle size and Electrophoretic Light Scattering (ELS) for zeta potential analysis, allowing accurate characterization of nanoparticles in suspension (Zetasizer manual, 2015)

2.5.2 Optical Microscopy

The morphology of the nano-emulsion droplets was observed using optical microscopy. A drop of diluted nano-emulsion was placed on a glassslide and photo was captured

2.5.3 Viscosity

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 30 gm of gel preparation was kept in 50 ml beaker, which was set till spindle groove was dipped and rpm was set and reading was measured after five minutes. Viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as mean.

2.5.4 Stability Studies

Nano-emulsion samples were subjected to centrifugation at 5000 rpm for 30 minutes and observed for phase separation. Thermal stability was assessed by storing samples at 4 °C and 25 °C for three months and monitoring for changes in appearance.

2.5.5 *In vitro* Antimicrobial Activity

The antimicrobial activity of the nano-emulsion was evaluated against Escherichia coli using the agar well diffusion method. Wells were filled with 100 µL of nano-emulsion, and plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters. Minimum inhibitory concentration (MIC) was determined by serial dilution in broth culture.

3. Results

3.1 Screening of Surfactants and Co-surfactants

The solubility of *Argemone mexicana* oil in various surfactants (Tween 80, Span 80) was assessed to identify suitable candidates for nano-emulsion formulation. Excess oil was added to each surfactant, vortexed, equilibrated, and centrifuged. The supernatant was analyzed by UV-Vis spectrophotometry. Brij 30 demonstrated the highest solubility for *Argemone mexicana* oil among the surfactants tested, followed by Tween 80 and Span 80.

3.2 Construction of Pseudo-Ternary Phase Diagram

Phase diagrams were constructed using the water titration method to identify the nano-emulsion region. Various ratios of oil, surfactant were titrated with distilled water. The appearance of the mixtures was visually observed and classified as clear, turbid, or phase-separated. The nano-emulsion region was identified as the area where clear, isotropic mixtures were observed. The largest nano-emulsion region was found when Brij 30 used in appropriate ration with argemone oil and water combination, indicating their synergistic effect in stabilizing the oil droplets.

Table 1: Nano-Emulsion Region Identification

Sample	Water (%)	Brij 30 (%)	*Argemone mexicana* oil (%)	Nano-emulsion Formed?
1	70	20	10	Yes
2	60	30	10	Yes
3	50	40	10	Yes
4	40	50	10	Yes

5	30	60	10	Yes
6	20	70	10	No
7	70	10	20	Yes
8	60	20	20	Yes
9	50	30	20	Yes
10	40	40	20	Yes
11	30	50	20	Yes
12	20	60	20	No
13	70	10	20	No
14	60	10	30	No
15	50	20	30	No
16	40	30	30	No
17	30	40	30	No
18	20	50	30	No
19	10	60	30	No

3.3 Preparation of Nano-Emulsion by Low Energy Method

Nano-emulsions were prepared by mixing *Argemone mexicana* oil and Brij 30 in ratios ranging from 10:0 to 0:10 (v/v%), followed by gradual addition of water to reach up to 95% aqueous content. The mixtures were stirred at 50 °C, then cooled and visually inspected for isotropy and homogeneity. Formulations coded as AB1, AB2, and AB3 were selected based on their clear appearance and stability. The selected formulations exhibited uniformity and transparency, indicating successful nano-emulsion formation.

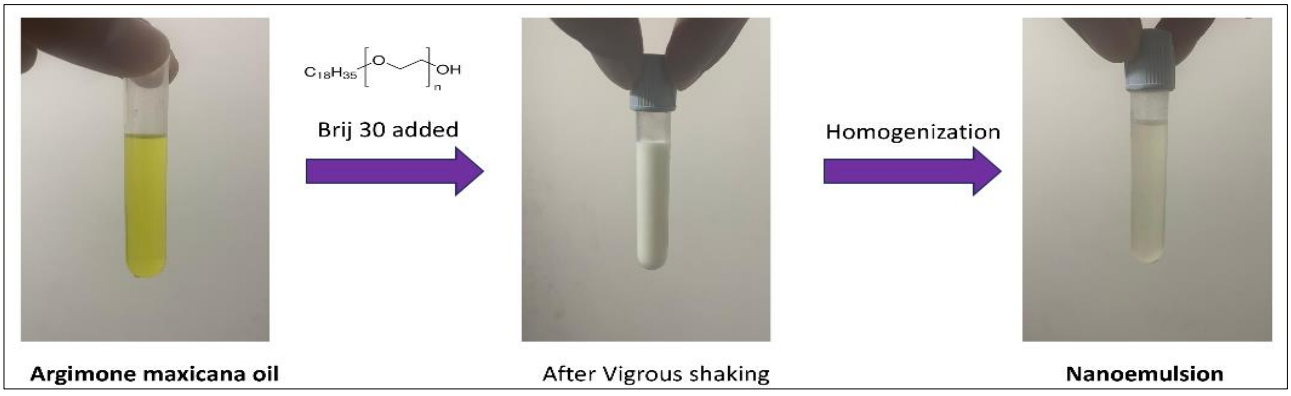


Fig 1: *Argemone maxicana* Nano emulsion methods and physical appearance

Sl. No.	Formulation	*Argemone mexicana* oil %	Brij 30 %	Deionized water %	Phase inversion temperature
1	AB1	10	20	70	78
2	AB2	10	30	60	75
3	AB3	4	11	85	62

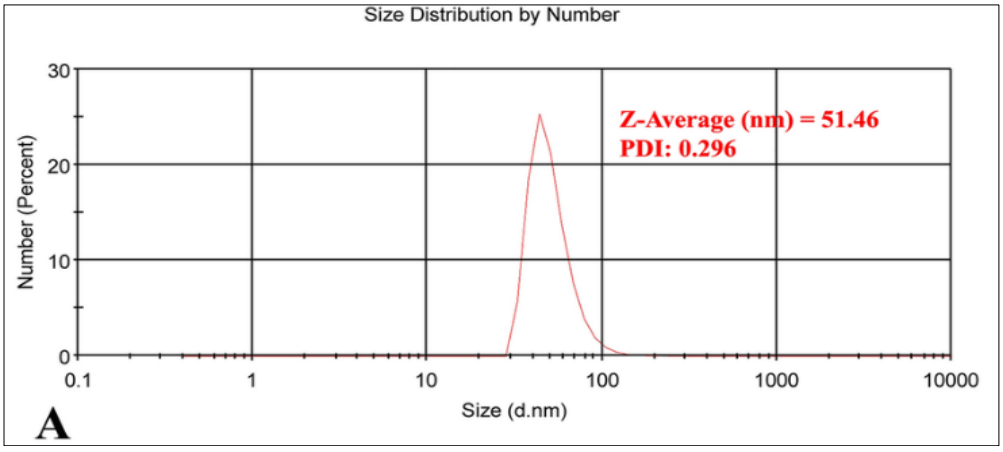
3.4 Characterization of Nano-Emulsion

3.4.1 Particle Size and Zeta Potential

The mean hydrodynamic diameter and zeta potential of the selected nano-emulsions were measured and nano-emulsions exhibited mean particle sizes in the range of 50-150 nm, confirming their nano-scale nature.

3.4.2 Zeta Potential

The zeta potential values were in the range of -25 to -35 mV, indicating moderate stability due to electrostatic repulsion among droplets.



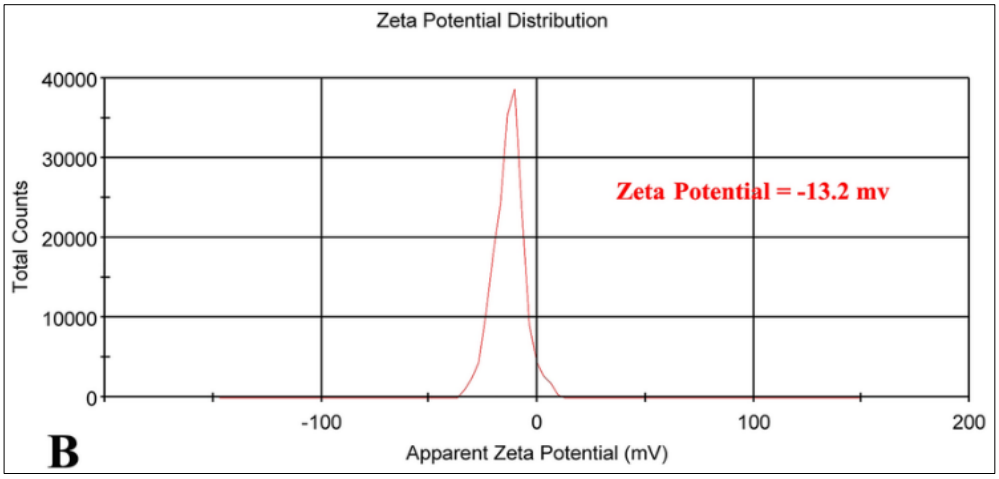


Fig 2: Droplet size distribution and zeta potential of *Argemone mexicana* nano emulsion

3.5 Optical Microscopy

Optical microscopy revealed that the nano-emulsion droplets were spherical and uniformly dispersed, with no evidence of aggregation or coalescence. The droplets appeared as discrete, well-defined spheres under the microscope, supporting the DLS findings.

Fig 3: Optical microscopy of *Argemone mexicana* Nano emulsion

3.6 Viscosity

Viscosity measurements were performed using a Brookfield viscometer. The nano-emulsions displayed low viscosity values (4-8 cP), suitable for topical and pharmaceutical applications. The low viscosity is consistent with the high-water content and nano-scale droplet size.

3.7 Stability Studies

Stability was evaluated by centrifugation and thermal storage and our results suggested that no phase separation was observed after centrifugation at 5000 rpm for 30 minutes, indicating good kinetic stability. Apart from that Samples stored at 4 °C and 25 °C for three months maintained their clarity and showed no signs of phase separation or precipitation, confirming excellent long-term stability.

3.8 *In vitro* Antimicrobial Activity

The antimicrobial efficacy of the nano-emulsion was assessed against *Escherichia coli* using the agar disc diffusion method. Our results suggested that the nano-emulsion produced clear zones of inhibition, with diameters ranging from 12-18 mm, depending on the formulation and concentration. The MIC values were found to be in the range of 50-100 µg/mL, demonstrating potent antibacterial activity.

Fig 4: Antimicrobial activity of *Argemone mexicana* Nano emulsion through agar well diffusion assay

Summary Table: Antimicrobial Activity

Formulation	Zone of Inhibition (mm)	MIC (µg/mL)
AB1	18	50
AB2	15	75
AB3	12	100
Ciprofloxacin (std)	28	50

4. Discussion

The present study aimed to overcome the pharmacokinetic limitations of *Argemone mexicana* seed oil by formulating it into a nano-emulsion using a low-energy emulsification approach. While plant-derived oils such as *A. mexicana* have long been known for their pharmacological effects-particularly antimicrobial and anti-inflammatory activities-their clinical translation is hindered by critical drawbacks including poor aqueous solubility, oxidative instability, and limited bioavailability due to poor membrane permeability [1, 4, 10].

To address these challenges, the study employed Brij 30 as the primary surfactant, due to its demonstrated ability to solubilize *A. mexicana* oil efficiently. The use of non-ionic surfactants like Brij 30 is well-supported in literature for nano-emulsion stabilization owing to their low toxicity and compatibility across various biological systems [17]. The

constructed pseudo-ternary phase diagrams facilitated the identification of optimal nano-emulsion regions, providing a rational basis for selecting stable formulations. Three nano-emulsion formulations (AB1, AB2, AB3) were successfully developed, characterized, and tested. Among them, AB1 showed the most favorable physicochemical attributes, including a small droplet size (<100 nm), a zeta potential of -28.7 mV, low viscosity (~4 cP), and high transparency-all indicative of a well-formed, kinetically stable nano-emulsion. Zeta potential values above ±25 mV are generally considered acceptable for electrostatically stabilized systems, which helps prevent droplet aggregation and phase separation [9, 13].

The stability studies further confirmed the robustness of the nano-emulsion over a 3-month storage period at 4 °C and 25 °C, with no phase separation or turbidity observed. This suggests that the formulation is suitable for long-term storage and application, a crucial parameter for any pharmaceutical preparation.

Significantly, the *in vitro* antimicrobial assay against *Escherichia coli* revealed a dose-dependent activity, with AB1 showing a zone of inhibition of 18 mm and MIC of 50 µg/mL. These values are notably potent and competitive with conventional antibiotics like ciprofloxacin used as control in the study. The enhanced antibacterial activity of the nano-emulsion, compared to crude oil, is attributed to the increased surface area and better penetration of nano-sized droplets into bacterial membranes [8, 19].

Nano-emulsions not only promote improved drug permeation but also protect bioactives like unsaturated fatty acids (linoleic and oleic acids) from oxidation and enzymatic degradation, thereby preserving their activity [3, 12]. Moreover, the nano-emulsification allows for uniform dispersion of the active compounds, facilitating consistent therapeutic effects. In alignment with prior studies using essential oils such as neem, clove, and turmeric, our findings support the utility of nano-emulsion technology in improving the efficacy and stability of plant-derived formulations [18, 19, 20]. This study, therefore, highlights the potential of nano-emulsified *Argemone mexicana* oil as a viable antimicrobial agent suitable for pharmaceutical or topical use, warranting further in vivo and clinical investigations.

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5. References

1. Gupta A, Eral HB, Hatton TA, Doyle PS. Nanoemulsions: formation, properties and applications. Soft Matter. 2016;12(11):2826-2841.
2. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ. Nano-emulsions. Curr Opin Colloid Interface Sci. 2005;10(3-4):102-110.
3. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123-127.
4. Bhattacharya A, Prajapati PK. Pharmacognostic evaluation of *Argemone mexicana* Linn. roots. AYU. 2015;36(2):230-234.
5. Ali M, Sharma S, Jain S, Alam MS. A review on phytochemical and pharmacological properties of *Argemone mexicana* Linn. Int J Pharm Sci Res. 2017;8(2):408-415.
6. Kumar A, Ilavarasan R, Jayachandran T, *et al*. Phytochemical investigation and antimicrobial activity of *Argemone mexicana*. J Pharm Res. 2013;6(3):313-315.
7. Pandey A, Tripathi S, Singh AV. Toxicological profile of *Argemone mexicana* oil: a review. J Toxicol. 2010;2010:1-8.
8. Banerjee S, Pillai J. Biomedical applications of nanoemulsion. In: Grumezescu AM, editor. Nanostructures for Drug Delivery. Elsevier; 2019. p. 123-148.
9. McClements DJ. Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter. 2012;8(6):1719-1729.
10. Ghosh S, Ghosh S, Pal S. Phytochemical analysis and antimicrobial properties of *Argemone mexicana*. Asian J Pharm Clin Res. 2019;12(5):245-248.
11. Mahdi JG, Mahdi AJ, Bowen ID. The historical analysis of *Argemone mexicana*: a medicinal plant. J Ethnopharmacol. 2006;104(1-2):1-8.
12. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects. J Funct Foods. 2015;18:820-897.
13. Patra JK, Das G, Fraceto LF, *et al*. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol. 2018;16(1):71.
14. Bhosale RR, Osmani RAM, Moin A, *et al*. Nanoemulsion: a review on novel approach for drug delivery system. Int J Pharm Sci Rev Res. 2014;29(1):70-76.
15. Gutiérrez JM, González C, Maestro A, *et al*. Nano-emulsions: new applications and optimization of their preparation. Curr Opin Colloid Interface Sci. 2008;13(4):245-251.
16. Bouchemal K, Briançon S, Perrier E, Fessi H. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. Int J Pharm. 2004;280(1-2):241-251.
17. Salvia-Trujillo L, Rojas-Graü MA, Soliva-Fortuny R, Martín-Belloso O. Physicochemical characterization of food-grade nanoemulsions during long-term storage. Food Hydrocoll. 2014;35:144-150.
18. Anjali CH, Sharma Y, Mukherjee A, Chandrasekaran N. Neem oil (*Azadirachta indica*) nanoemulsion - a potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag Sci. 2012;68(2):158-163.
19. Sharma A, Jain N, Singh B. Nanoemulsified clove oil as a larvicide against *Aedes aegypti*. J Vector Borne Dis. 2014;51(2):134-141.
20. Bhargava K, Conti DS, da Rocha SRP, Zhang Y. Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. Food Microbiol. 2015;47:69-73.