



Formulation and Evaluation of Nanoemulsion Topical Gel Using *Haritamanjari* (*Acalypha indica* L.) For Antibacterial and Antifungal Activity

¹S. Grisilda Heni, ²B. Ramya Kuber, ³S. Uma Naga Hima Deepika, ⁴B. Saanvi Sri

ABSTRACT:

Background: *Haritamanjari* (*Acalypha indica* L.) has been extensively used in Siddha, Ayurveda, and traditional folk medicine in treatment of skin infections, eczema, dermatitis and so on. The nanoemulgel formulation approach was employed to enhance the delivery of the *Haritamanjari* extract. **Objective:** The main aim of the present study was to prepare and evaluate a *Haritamanjari* (*Acalypha indica* L.) nanoemulgel as a topical drug delivery system, with antibacterial and antifungal effects. **Materials and Methods:** The ethanolic leaf extract of *Haritamanjari* was prepared by cold maceration, and subjected to preliminary phytochemical screening. Three nanoemulsion formulations (F1-F3) were then prepared by spontaneous emulsification followed by probe sonication and incorporated into Carbopol 940 gel. The formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, pH, viscosity, spreadability, homogeneity, and extract-equivalent content. The selected formulation was further characterized by FTIR, XRD, *in vitro* extract release, Antibacterial (*S. aureus* and *E. coli*), and Antifungal activity (*C. albicans*). **Results:** F2 was selected as the formulation for further evaluation, based on its overall physicochemical characteristics like particle size 245.73 ± 2.85 nm, PDI 0.327 ± 0.03 , zeta potential -14.60 ± 0.80 mV, and the highest extract-equivalent content $93.17 \pm 0.40\%$. FTIR findings indicated retention of characteristic functional groups without major chemical interaction, while XRD analysis showed reduced peak intensity and broadening in formulation, suggesting reduced crystallinity and increased amorphous character of extract within the formulation. F2 exhibited 73.32 ± 1.59 % cumulative extract release over 7 h, with Korsmeyer-Peppas model indicating anomalous release. Likewise, the nanoemulgel produced inhibition zones of 29.6 ± 1.5 , 23.6 ± 4.7 , and 22.3 ± 2 mm against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. **Conclusion:** The developed *Haritamanjari* nanoemulgel showed potential as topical herbal antimicrobial formulation under experimental conditions. Further marker-based characterization, safety, stability, *in vivo* and clinical studies are required to establish its topical applicability.

KEYWORDS: *Acalypha indica* L, Antibacterial, Antifungal, Antimicrobial, *Haritamanjari*, Nanoemulgel, Nanoemulsion

RECEIVED ON:
01-09-2026

REVISED ON:
23-09-2026; 01-10-2026

ACCEPTED ON:
07-10-2026

Access This Article Online:

Quick Response Code:



Website Link:

<https://jahm.co.in>

DOI Link:

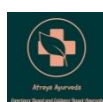
<https://doi.org/10.70066/jahm.v14i9.3037>

Corresponding Author Email:

rkuberpharma@yahoo.com

CITE THIS ARTICLE AS

S. Grisilda Heni, B. Ramya Kuber, S. Uma Naga Hima Deepika, B. Saanvi Sri. Formulation and Evaluation of Nanoemulsion Topical Gel Using *Haritamanjari* (*Acalypha Indica* L) For Antibacterial and Antifungal Activity. Journal of Ayurveda and Holistic Medicine (JAHM) 2026; 14(9):28-42.



1. INTRODUCTION

The largest organ of the human body is skin which serves as the primary barrier against the external environment. Its main functions are to protect against physical, chemical, and microbial invasion, regulation of body temperature, prevent dehydration, sensory perception, immune participation, and synthesis of vitamin D. Due to its large surface area and accessibility, the skin is an attractive route for localized and systemic drug delivery. [1]

Many people worldwide suffer from skin diseases, e.g., bacterial and fungal infections, acne, eczema, psoriasis, dermatitis, and burns. These diseases significantly decrease the quality of life, and the resistance of microorganisms to conventional antimicrobial drugs has increased in recent years, which makes topical therapy with reduced side effects more relevant. [2] Moreover, the rapid rise of antimicrobial resistance is now recognized as a major threat to global health security. [3]

Topical drug administration is considered a highly effective and safe method for treating a variety of skin infections since it allows achieving higher local concentration of the active substance while minimizing the systemic exposure of the body to the drug. This in turn reduces the chances of unwanted adverse effects and increases the therapeutic efficiency of the treatment. [4, 5] However, topical formulations are associated with a number of significant disadvantages, including reduced solubility and stability of the active substance, poor penetration of the drug into the epidermis, and a short duration of action due to the frequent dosing interval. These limitations are mainly overcome by using various advanced drug delivery technologies to improve the pharmacokinetic and pharmacodynamic properties of the drug, such as enhancing its solubility, stability, bioavailability, and controlled release. [5, 6]

Nanotechnology has shown great promise for overcoming the challenges posed by traditional topical formulations. [7] Various nanocarrier systems, like liposomes, ethosomes, niosomes, solid lipid nanoparticles,

polymeric nanoparticles, nanoemulsions, and nanogels, have shown improved drug solubilization, skin permeation, stability, residence time, and sustained release, resulting in enhanced therapeutic outcomes. [8, 9]

Among these, nanoemulsions are kinetically stable colloidal dispersions consisting of oil and aqueous phases stabilized by surfactants and co-surfactants. Their nanosized droplets give a large interfacial surface area, resulting in enhanced drug solubility, stability, dissolution, skin penetration, and bioavailability compared with conventional emulsions. [10] These advantages have made nanoemulsions promising carriers for pharmaceutical, cosmetic, and biomedical applications. [11]

Despite their advantages, the low viscosity of nanoemulsions limits their direct topical application due to poor skin retention. Incorporation into a gel base forms a nanoemulgel, which improves viscosity, spreadability, retention, controlled drug release, and patient acceptability, making it an effective carrier for topical drug delivery. [10, 12]

The growing interest in herbal medicine has accelerated the development of nano-based herbal formulations. Although medicinal plants possess phytoconstituents with antimicrobial, antioxidant, anti-inflammatory, and wound healing activities, their therapeutic use is often limited by poor solubility, instability, and low bioavailability. Nanotechnology based delivery systems enhance the solubility, stability, skin retention, and sustained release of these bioactive compounds. [13]

Acalypha indica Linn. (*Euphorbiaceae*) is a plant with medicinal properties traditionally used in the treatment of skin diseases, wounds, microbial infections, and inflammation. Rich in flavonoids, alkaloids, tannins, phenolics, and saponins, it exhibits significant antimicrobial, antioxidant, anti-inflammatory, and wound healing activities. However, the topical delivery of the phytoconstituents may be limited by poor stability, solubility, and inadequate skin retention, which highlights the need for developing suitable topical delivery system. [14, 15]

Previous studies have investigated different nanotechnology based topical formulations of *Acalypha indica*, including phytosomal gel, [16] phytoniosomal gel [17] and *A. indica* mediated zinc oxide nanoparticle based nanogel. [18] These approaches demonstrate the potential of *A. indica* for incorporation into advanced topical delivery systems. However, they employ different delivery systems and formulation components. The phytosomal gel utilizes a phospholipid-based phytosome system, whereas the phytoniosomal gel employs a niosome-based vesicular system for delivery of phytoconstituents present in *A. indica*. In contrast ZnO nanoparticle-based nanogel incorporates *A. indica* mediated inorganic zinc oxide nanoparticles into the gel matrix. In the present study an oil-in- water nanoemulsion based topical gel containing ethanolic extract of *Haritamanjari* (*A. indica*) was developed using coconut oil, Tween 80, and propylene glycol as formulation components, followed by incorporation into Carbopol 940 gel. The formulation was further evaluated for physicochemical characteristics, *in vitro* release, and antibacterial and antifungal activity. This study contributes primarily at the formulation level by developing and evaluating a topical *A. indica* extract loaded nanoemulgel with antimicrobial activity, rather than proposing a novel delivery mechanism.

Aim: To formulate and evaluate a topical nanoemulgel containing ethanolic leaf extract of *Haritamanjari* (*Acalypha indica* Linn.) for enhanced antibacterial and antifungal activity.

Objectives

Identification and authentication of plant material *Haritamanjari* (*Acalypha indica* L.) and preparation of ethanolic extract for nanoemulsion gel.

Physicochemical evaluation and *in vitro* evaluation of extract, nanoemulsion and nanoemulgel.

2. MATERIALS AND METHODS

Materials: Conical flask, weighing balance, funnel, glass rod, petri dish, beakers, volumetric flask, spatula, Bunsen burner, magnetic stirrer, ultrasonicator, mortar and pestle, zeta sizer, pH meter, Brookfield viscometer, FTIR spectroscopy, X-ray diffractometer, UV spectrophotometer, Laminar air flow chamber, autoclave, incubator,

refrigerator.

Methods: Collection, Identification and Authentication of plant material: *Haritamanjari* leaves were collected from Dharmapuram, Nagari, Andhra Pradesh in the month of January 2026 and dried at room temperature. The identification and authentication of the plant material was carried out by Dr. K. Madhava Chetty, Plant Taxonomist (IAAT: 337), Assistant Professor, Department of botany, Sri Venkateswara University, Tirupati on 07 January 2026. The plant was authenticated as *Acalypha indica* L. (family: *Euphorbiaceae*) and voucher specimen was assigned voucher number 0810.

Extraction of ethanolic extract: *Haritamanjari* (*Acalypha indica* L.) ethanolic extract was obtained by continuous cold extraction process through maceration. 20 g of dried *Haritamanjari* leaves were subjected to maceration using ethanol at an initial plant material-solvent ratio 1:3 (w/v) and kept at room temperature for 72 h with intermittent shaking to facilitate the solvent penetration. After 72 h extract was filtered through adsorbent cotton, filtrate was collected and remaining marc was subjected to second maceration using fresh ethanol using plant material-solvent ratio 1:2 (w/v) for 72 h and the process was repeated once again using the ratio 1:1 (w/v) to ensure exhaustive extraction of phytoconstituents. Three filtrates were pooled together and dried. [19, 20]



Figure 1: Preparation of ethanolic extract: A) fresh leaves and B) concentrated ethanolic extract

Methods of evaluation of ethanolic extract of *Haritamanjari* : The ethanolic extract of *Haritamanjari* was evaluated for parameters like physicochemical characteristics and quantitative estimation (calibration curve).

Preliminary phytochemical testing of ethanolic extract: The ethanolic extract of *Haritamanjari* leaves were subjected to preliminary phytochemical screening using standard qualitative chemical tests for the detection of major classes of phytoconstituents and the observations were recorded. [21]

Determination of calibration curve: Stock solution of *Haritamanjari* extract 5000 µg/mL was made using methanol. Then the suitable dilutions were done so that the concentrations ranged from 50, 100, 150, 200, and 250 µg/mL, respectively. The absorbance was measured at 256 nm using UV-Vis spectrophotometer (Shimadzu - UV-1900i) after wavelength accuracy and photometric performance verified by manufacturer's procedure, calibration curve was plotted. [22]

Table 1: Composition of *Haritamanjari* Nanoemulgel

Ingredients	Properties	F1	F2	F3
<i>Haritamanjari</i> extract (g)	Active ingredient	0.5	1.0	1.5
Coconut oil (mL)	Carrier oil	3	3	3
Tween 80 (mL)	Surfactant	6	6	6
Propylene glycol (mL)	Co-surfactant	3	3	3
Distilled water (mL)	Aqueous phase	q.s. to 50 g	q.s. to 50 g	q.s. to 50 g
Carbopol 940 (g)	Gelling agent	0.5	0.5	0.5
Triethanolamine	pH adjuster	q.s.	q.s.	q.s.

Formulation design and Selection Criteria: Three trial formulations (F1 – F3) were developed by varying concentration of ethanolic leaf extract of *Haritamanjari*, while keeping the other components and processing conditions constant to identify a suitable extract level could provide a desirable nanoemulgel. The formulations were comparatively evaluated. A smaller particle size, PDI < 0.5, relatively higher magnitude of zeta potential, suitable

Preparation of Nanoemulsion: *Haritamanjari* nanoemulsion was prepared by spontaneous emulsification (low energy emulsification) method, followed by probe ultrasonication for better droplet size reduction. The required quantity of *Haritamanjari* leaf extract was incorporated into coconut oil to obtain oil phase. The surfactant mixture (Smix) was formulated by mixing tween 80 and propylene glycol. The oil phase was added to the Smix and mixed using REMI ZMLH magnetic stirrer at 700 rpm for 30 min until uniform mixture was obtained. Distilled water (aqueous phase) was then added gradually with continuous stirring until a homogeneous nanoemulsion was obtained. The resulting nanoemulsion was subjected to probe ultrasonication using rivotex probe sonicator for 30 min under cyclic pulsing with a complete pulse cycle of 30 s (15 s ON + 15 s OFF), corresponding to a 50% duty cycle. The sonicator was operated at a frequency of 20 kHz and 100% amplitude during the 15 s ON phase. The formulation temperature was strictly maintained below 30 °C throughout sonication to minimize temperature induced changes during process. [24-25]

viscosity and good spreadability were desirable, along with skin compatible pH (5.5 – 6.5), good homogeneity and high extract content. Based on the overall combination of these attributes the formulation with the most suitable characteristics was selected for further evaluation.

Characterization of nanoemulsion:

Evaluation of particle size, PDI, zeta potential: Particle size, polydispersity index, and zeta potential of prepared

nanoemulsion were determined using HORIBA Scientific - nanoPartica SZ-100 after routine calibration according to manufacturer's recommended procedure. The nanoemulsion samples were diluted at a ratio of 1:10 (1 mL of nanoemulsion in 10 mL of distilled water) prior to measurement. For particle size analysis cuvette and for zeta potential analysis, folded capillary cells were used. Measurements were performed at $25 \pm 1^\circ\text{C}$ at measurement angle 90° with automatic selection measurement settings. Measurements were performed in triplicate on the same batch as technical replicates, and the results were expressed as mean $\pm$ standard deviation. [26-28]

Preparation of *Haritamanjari* (*Acalypha indica*) Nanoemulgel:

Hydration of gel base: Carbopol 940 was weighed accurately and dispersed in distilled water under continuous stirring to avoid lumps and allowed for 24 h to fully hydrate. [29]

Incorporation of nanoemulsion into gel base: Prepared nanoemulsion was gradually added to the hydrated gel base under gentle stirring using mortar and pestle until a homogeneous dispersion was obtained. Finally, triethanolamine was added dropwise to neutralize Carbopol dispersion resulting in smooth and homogeneous nanoemulgel formation. [29]

Evaluation of Nanoemulgel

Organoleptic evaluation: The prepared nanoemulgel formulations were evaluated for their organoleptic properties like color, appearance, odor, texture and consistency. Approximately 1 g of each formulation was taken and examined under normal daylight. [30]

Determination of pH: pH of the formulated nanoemulgels were measured using digital pH meter (Labholic - LH-10) after calibrating using standard buffer solutions of pH 4.0, 7.0, and 9.2. The pH of the formulation was detected for maintaining the physiological skin pH range (5.5 – 6.5) to minimize the skin irritation. [30]

Determination of spreadability: Approximately 0.5 g of prepared nanoemulgel was placed between two glass slides and constant weight was applied for 5 minutes to allow the gel to spread uniformly. The spread diameter was measured after specified time. [31]

Determination of viscosity: The viscosity of the formulations were measured using a Brookfield viscometer (Brookfield - DV2T LV) after verified using standard viscosity fluid according to manufacturer's procedure, readings were expressed in centipoise (cP). [23]

Determination of Extract-Equivalent content: 0.5 g of nanoemulgel was accurately weighed and transferred into 100 mL of volumetric flask. The formulation was dissolved and the volume was made up to 100 mL using distilled water. The resulting solution was filtered and the filtrate was observed by UV-visible Spectrophotometer (Shimadzu - UV-1900i). The crude ethanolic leaf extract of *Haritamanjari* was used as reference material for preparation of calibration curve over the concentration range of 50 – 250 $\mu\text{g/mL}$. Extract-equivalent concentration was calculated from the calibration equation after applying the appropriate dilution factor and expressed as percentage extract-equivalent content. A formulation blank containing all excipients except the *Haritamanjari* extract was analyzed at 256 nm to assess possible excipient interaction. The measurements were performed in triplicate using the same formulation batch and expressed as mean $\pm$ standard deviation. [32]

Characterization of Selected Nanoemulgel:

Preliminary Phytochemical analysis of selected nanoemulgel: The selected nanoemulgel was tested for major phytochemical classes by qualitative testing. [21]

FTIR analysis: Fourier Transform Infrared (FTIR) spectroscopy (Bruker - ALPHA-T) was carried out after instrument background was recorded, to identify the functional groups present in the extract and to evaluate compatibility between extract and excipients in the selected nanoemulgel formulation. It is analyzed using an FTIR spectroscopy over the spectral range of 4000–400

cm⁻¹. [23]

X-Ray Diffraction Analysis: XRD (PANalytical – Aeris) was performed after instrument alignment was verified to evaluate the crystalline nature of extract and selected nanoemulgel. The dried ethanolic extract and the selected formulation were placed separately on sample holder and scanned over 2θ range of 5°–80° at room temperature using X-ray diffractometer. [33]

In vitro Extract release study: The *in vitro* release of *Haritamanjari* extract was evaluated using Franz diffusion cell. A dialysis membrane measuring 6 × 6 cm with MWCO of 12,000 Da was used and pretreated by soaking in phosphate buffer of pH 7.4 for 24 h prior to the experiment. The diffusion orifice had a diameter of 11.28 mm, corresponding to an effective diffusion area approximately 1.0 cm². The receptor compartment was filled with 20 mL of phosphate buffer of pH 7.4 and was maintained at 32 ± 0.5°C with continuous stirring at 500 rpm. 1 g of selected nanoemulgel was placed onto the dialysis membrane in the donor compartment. Initial sample was taken at 15th min and subsequent samples were taken at 1 h interval for 7 h. At predetermined time intervals 3 mL of sample was withdrawn from the receptor compartment and replaced with fresh phosphate buffer to maintain constant receptor volume. The withdrawn samples were observed using UV-Vis spectrophotometer at 256 nm. The release study was performed in three independent experimental runs using the selected nanoemulgel formulation with freshly prepared buffer and membrane for each run, and the % cumulative extract release values were expressed as mean ± standard deviation (SD). [23]

The release data obtained from *in vitro* extract release studies were fitted to zero order, first order, Higuchi, and Korsmeyer-Peppas models to characterize release behavior and mechanism. The zero order model was represented by $Q_t = K_0t + Q_0$, while first order model was evaluated by $\log(100 - Q_t) = \log Q_0 - K_1t/2.303$. The Higuchi model was expressed as $Q_t = K_H\sqrt{t}$. The Korsmeyer-Peppas model was evaluated using $\log (M_t/M_\infty) = \log K + n \log t$, where n is

release exponent. The coefficient of determination (R^2) was used to assess the linearity of each kinetic model, the release exponent (n) was used to interpret the release mechanism.

Antibacterial activity: Antibacterial activity was assessed using cultures prepared from the reference strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 by the agar well diffusion method. The microbial suspension was adjusted to 0.5 McFarland standard before inoculation. Nutrient medium was prepared and sterilized by autoclaving (Hoverlabs – HV-110-AC) followed by aseptically transferring the nutrient media into sterile plates and allowed to solidify. After solidification of the media, the bacterial suspension was inoculated onto the agar surface by using sterile L shaped glass rod to obtain uniform lawn. GRGR Kuppaimeni gel was selected as marketed comparator as it is commercially available topical Kuppaimeni (*Acalypha indica*) gel. The product was used as received. The quantitative strength was not specified in the label. The uniform sized wells of 6 mm diameter were made using sterile cork borers and 0.5 g of selected nanoemulgel formulation, crude ethanolic leaf extract of *Haritamanjari* (0.5 mL of 20 mg/mL solution), 0.5 g GRGR Kuppaimeni gel (marketed formulation), and 0.5 g of Vehicle control (nanoemulgel without *Haritamanjari* extract contains same proportion of excipients as Nanoemulgel formulation) were introduced into their respective wells and the standard antibiotic (5 µg Ciprofloxacin disc; HiMedia Laboratories) was placed directly on the surface of the inoculated agar medium. The plates were incubated at 37 ± 1°C for 48 h in incubator (Wincom – CHP – 160), and the resulting zone of inhibition was measured. [34]

Antifungal activity: Antifungal activity was evaluated using *Candida albicans* ATCC 10231 by agar well diffusion method. The inoculum was prepared from solidified fungal culture, and the fungal suspension was adjusted to 0.5McFarland standard before inoculation. Sabouraud dextrose agar media was prepared and sterilized. The sterilized agar media was aseptically poured into sterile petri plates and allowed for

solidification. After solidification the *Candida albicans* was inoculated using a sterile cotton swab to obtain a uniform fungal lawn. Wells of 6 mm diameter were made using sterile cork borer, and selected nanoemulgel formulation (0.5 g), crude ethanolic leaf extract of *Haritamanjari* (0.5 mL of 20 mg/mL solution), 0.5g GRGR Kuppaimeni gel (marketed formulation), standard antifungal (0.5 mL of fluconazole 10 mg/mL) and 0.5 g Vehicle control (nanoemulgel without *Haritamanjari* extract contains same proportion of excipients as Nanoemulgel formulation) were introduced into their respective wells and incubated at $37 \pm 1^\circ\text{C}$ for 72 h in incubator. And the resulting zone of inhibition was measured. [34]

For each test microorganism, the experiment was performed in triplicate and the results are expressed as mean $\pm$ standard deviation (SD).

Statistical analysis: The experimental results were expressed as mean $\pm$ standard deviation of three independent antimicrobial assay runs ($n=3$), conducted on three different days using separately prepared microbial cultures and independently prepared test samples at the same specified concentrations. Statistical analysis was performed separately for each test microorganism, and evaluated using one-way ANOVA (Analysis of Variance), followed by Tukey's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant. [22]

3. RESULTS

Extraction of *Haritamanjari* leaf extract: The dried extract obtained after evaporation of solvent weighed 1.343 g (6.71 %).

Organoleptic evaluation of Ethanolic leaf extract of *Haritamanjari* (*Acalypha indica L.*):

The results of organoleptic evaluation of Ethanolic extract of *Haritamanjari* were listed in the [table 2](#).

Calibration Curve of Ethanolic leaf Extract of *Haritamanjari*: The calibration curve was prepared using standard solution in the concentration range of 50–250 $\mu\text{g/mL}$, and the absorbance was measured at 256 nm. The

absorbance values are mentioned in [Table 3](#) and calibration curve obtained was plotted in [Figure 2](#).

Table 2: Results for organoleptic evaluation of Ethanolic leaf Extract of *Haritamanjari*

Parameter	Observation
Color	Dark green
Odor	Characteristic herbal odor
Appearance	Thick, slightly sticky extract
Consistency	Semi solid

Table 3: Absorbance values of Ethanolic leaf Extract of *Haritamanjari*

Concentration ($\mu\text{g/mL}$)	Absorbance at 256 nm
50	0.198 ± 0.002
100	0.389 ± 0.001
150	0.555 ± 0.005
200	0.748 ± 0.002
250	0.923 ± 0.002

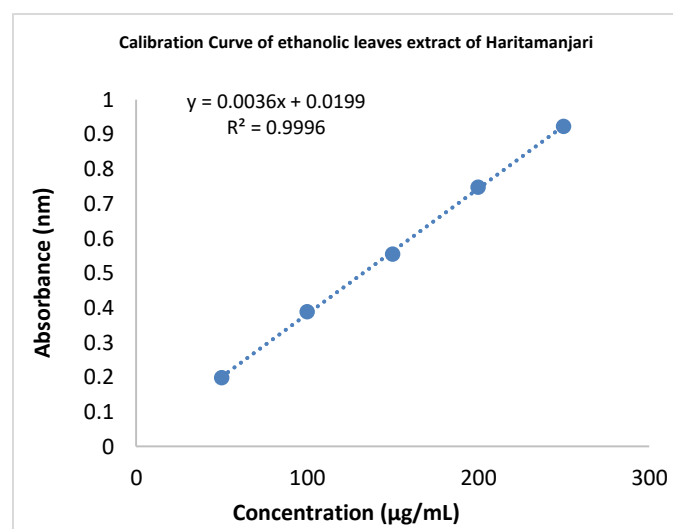


Figure 2: Calibration curve of Ethanolic leaf extract of *Haritamanjari*

The absorbance increased proportionally with increasing extract concentrations, indicating compliance with Beer-Lambert law.

Characterization of Nanoemulsion:

Physicochemical evaluation: The results for particle size, Polydispersity index, and zeta potential of nanoemulsions were mentioned in [table 4](#).

Table 4: Results obtained for Particle size, polydispersity index, and zeta potential of nanoemulsions

Formulations	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	349.3667± 1.58	0.225 ± 0.04	-10.83 ± 0.85
F2	245.7333±2.85	0.327 ± 0.03	-14.6 ± 0.8
F3	400.3667 ± 1.7	0.102 ± 0.02	-3.3 ± 0.37

Table 5 : Results for evaluation of three nanoemulgels

Parameters	F1	F2	F3
Color	Green	Dark green	Dark green
Odor	Characteristic herbal odor	Characteristic herbal odor	Characteristic herbal odor
Texture	Smooth and homogeneous	Smooth and homogeneous	Smooth and homogeneous
Consistency	Semisolid	Semisolid	Semisolid
Homogeneity	Homogeneous, smooth, free from visible coarse particles and lumps.	Homogeneous, smooth, uniform in appearance, free from lumps.	Homogeneous, smooth, slightly thicker consistency, free from visible coarse particles and phase separation.
pH	5.9 ± 0.2	6.0 ± 0.1	6.2 ± 0.1
Viscosity	2210 ± 10 cP	3990 ± 10 cP	3206 ± 11.5 cP
Spreadability	4.68 ± 0.28 cm	5.3 ± 0.3 cm	4.26 ± 0.3 cm
Extract-equivalent content	73.9 ± 0.79	93.17 ± 0.4	86.7 ± 0.75

Extract-equivalent content: The crude ethanolic extract of *Haritamanjari* showed a linear response at 256 nm over concentration of 50 - 250 µg/mL, with the regression equation $y = 0.0036x + 0.0199$ and $R^2 = 0.9996$. The formulation blank showed negligible absorbance i.e., 0.002 at 256 nm. Triplicate analysis of F1, F2, and F3 showed extract-equivalent contents of $73.9 \pm 0.79\%$, $93.17 \pm 0.4\%$, and $86.7 \pm 0.75\%$ respectively. This demonstrated good linearity and repeatability under experimental conditions. As the calibration was performed using the crude extract, the obtained values represent extract-equivalent content rather than the concentration of any specific phytochemical marker.

Selection of formulation: The formulation for further evaluation was selected using a predefined multiparameter comparative approach rather than on the basis of a single response. Although F3 showed the lowest PDI ($0.102 \pm$

By comparing the overall physicochemical characteristics of all nanoemulsions showed a good result. But F2 showed a better particle size i.e., 245.7333 ± 2.85 , while F3 showed good PDI 0.102 ± 0.02 .

Evaluation of Nanoemulgel formulations: The results for the evaluation parameter for Nanoemulgel like organoleptic characterization, homogeneity, pH, viscosity and Extract-equivalent content were presented in [table 5](#).

0.02), F2 exhibited comparatively smaller particle size (245.73 ± 2.85 nm), higher magnitude of zeta potential (-14.60 ± 0.80 mV), better spreadability (5.3 ± 0.3 cm) and the highest extract content ($93.17 \pm 0.40\%$), with acceptable pH (6.0 ± 0.1), viscosity (3990 ± 10 cP) and homogeneity. Hence, F2 was selected for further evaluation.

Evaluation of selected formulation

Preliminary phytochemical analysis of extract and nanoemulgel: Preliminary phytochemical screening of the ethanolic extract and selected formulation F2 revealed the presence of alkaloids, flavonoids, terpenoids, saponins, and glycosides, while carbohydrates and tannins were absent in both samples, indicating the retention of the detectable phytoconstituents after formulation.

FTIR analysis: The FTIR spectrum obtained for extract and selected formulation F2 was shown in [figure 3](#).

Table 6: Comparative FTIR peak table

Functional group	<i>Haritamanjari</i> extract (cm ⁻¹)	F2 nanoemulgel (selected formulation) (cm ⁻¹)	Shift (cm ⁻¹)
O-H stretching	3405.87	3362.89	-42.98
Aliphatic C-H stretching	2922.03, 2852.41	2923.98	+1.95
C=O stretching	1740.62	-----	----
C=C / aromatic skeletal vibration	1589.81	1631.11	+41.30
C-H bending	-----	1384.06	----
C-O stretching	1022.73	1081.88	+59.15

The FTIR spectrum of the ethanolic leaf extract showed the characteristic bands at 3405.87 cm^{-1} (O–H), 2922.03 and 2852.41 cm^{-1} (aliphatic C–H), 1740.62 cm^{-1} (C=O), 1589.81 cm^{-1} (aromatic C=C), and 1022.73 cm^{-1} (C–O), while F2 nanoemulgel showed bands at 3362.89, 2923.98, 1631.11, 1384.06, and 1081.88 cm^{-1} . The O–H stretching band shifted from 3405.87 to 3362.89 cm^{-1} , while the band near 2922.03 cm^{-1} was retained with only minor shift to 2923.98. The other observed changes in peak may be due to hydrogen bonding and physical entrapment of the extract in the Carbopol matrix. Importantly, the characteristic functional groups of the extract were retained in the selected formulation, and no additional bands were observed. This suggests that there was no significant chemical interaction between excipients used in the formulation and the extract, although FTIR alone cannot conclusively establish complete compatibility.

XRD analysis: XRD spectrum of extract and selected nanoemulgel F2 was shown in [figure 4](#).

The XRD pattern of the ethanolic extract exhibited characteristic diffraction peaks at various 2θ values. The XRD pattern of the selected nanoemulgel retained the characteristic peaks, but with reduced intensity and broadening compared with the extract. These changes suggested a reduction in crystallinity and an increase in amorphous nature following incorporation of extract into nanoemulgel formulation.

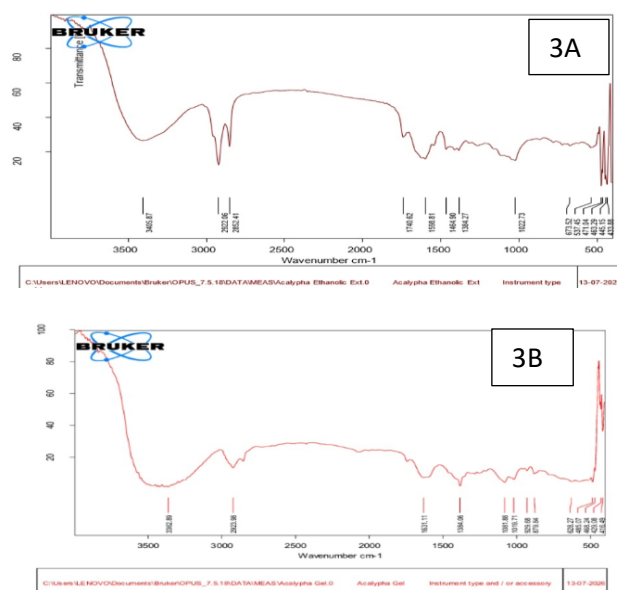


Figure 3: FTIR spectrum of A) ethanolic extract and B) selected formulation F2

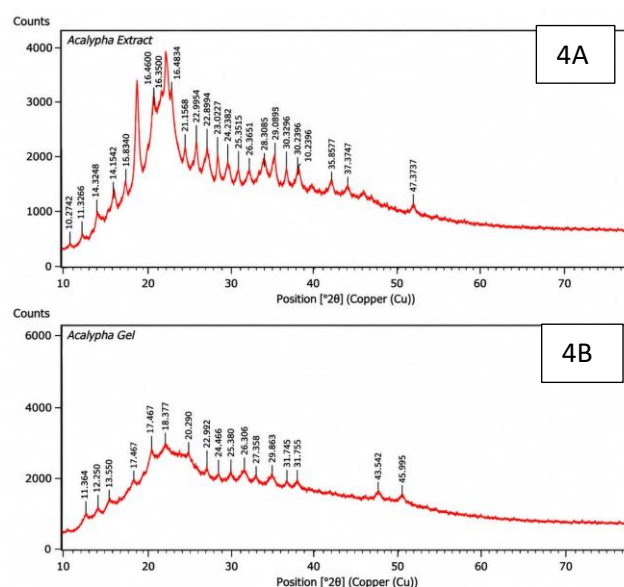


Figure 4: XRD of A) ethanolic extract and B) selected formulation

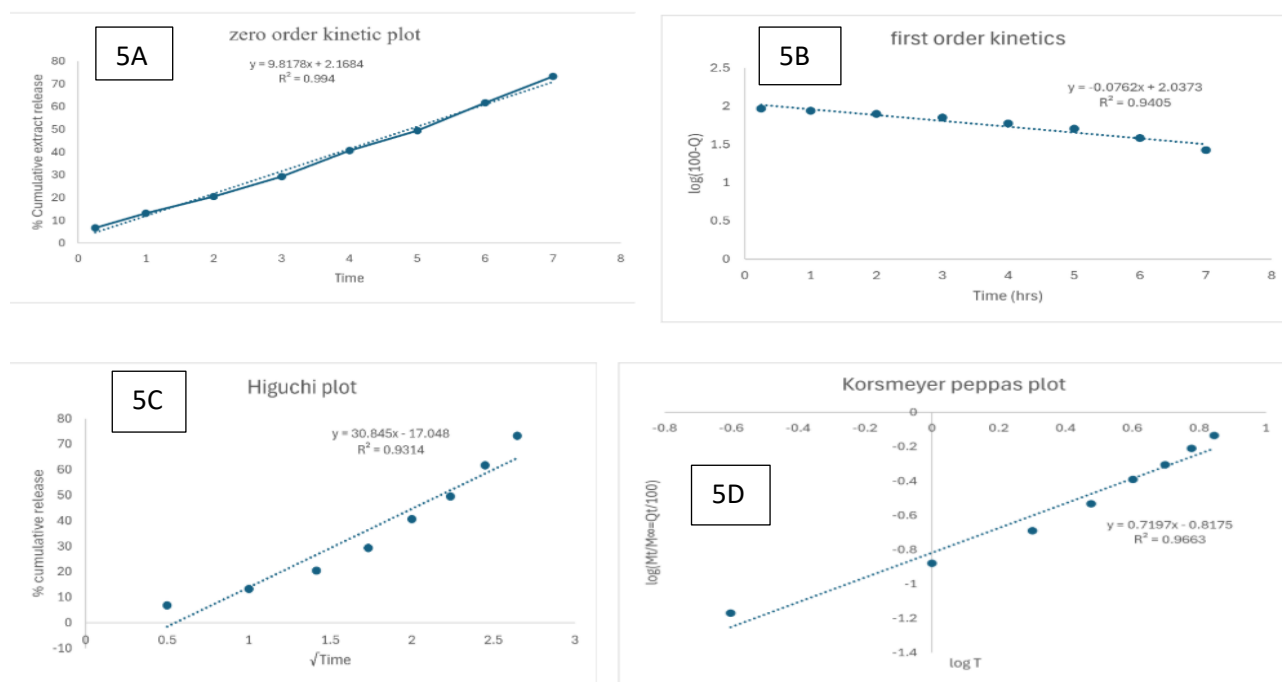


Figure 5: Release kinetic analysis of selected formulation F2: A) zero order, B) first order, C) Higuchi, and D) Korsmeyer-Peppas models.

% *In vitro* Extract release study:

Table 7: % Extract release using *In vitro* franz diffusion study

S.NO.	Time	% cumulative extract release (%) (Mean $\pm$ SD)
1	15 th min (0.25 h)	6.77 $\pm$ 0.43 %
2	1h	13.19 $\pm$ 0.5 %
3	2h	20.43 $\pm$ 0.48 %
4	3h	29.28 $\pm$ 0.97 %
5	4h	40.65 $\pm$ 0.38 %
6	5h	49.42 $\pm$ 0.78 %
7	6h	61.64 $\pm$ 1.1 %
8	7h	73.32 $\pm$ 1.59 %

The *in vitro* extract release study of selected nanoemulgel showed the sustained release profile with 73.32 $\pm$ 1.59 %

Table 8: Antimicrobial activity of selected formulation (F2), Extract, Vehicle control, Standard and marketed gel against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

Samples Placed in well	Zone of inhibition in mm		
	<i>S. aureus</i> (Gram +ve bacteria)	<i>E. coli</i> (Gram -ve bacteria)	<i>C. albicans</i> (Fungi)
Standards - Ciprofloxacin (Antibacterial) and Fluconazole (Antifungal)	36.3 $\pm$ 1.5 ^a	33.6 $\pm$ 1.5 ^a	22.6 $\pm$ 1.5 ^a
Ethanollic extract of <i>Acalypha</i>	16.6 $\pm$ 1.5 ^c	15.6 $\pm$ 2.08 ^c	5.6 $\pm$ 1.5 ^b
Vehicle control	0 $\pm$ 0 ^d	0 $\pm$ 0 ^d	0 $\pm$ 0 ^c
Selected formulation (F2)	29.6 $\pm$ 1.5 ^b	23.6 $\pm$ 4.7 ^b	22.3 $\pm$ 2 ^a
Marketed gel	0 $\pm$ 0 ^d	1 $\pm$ 1 ^d	0.6 $\pm$ 0.5 ^c

cumulative extract release over 7 hr. The release data were fitted to zero order, first order, Higuchi, and Korsmeyer-Peppas kinetic models. Among the models evaluated, zero order showed the highest correlation ($R^2 = 0.994$), followed by Korsmeyer-Peppas model ($R^2 = 0.9663$), first order model ($R^2 = 0.9405$), and Higuchi model ($R^2 = 0.9314$). The Korsmeyer-Peppas release exponent was 0.7197, indicating anomalous/non-fickian transport, suggesting the extract release was influenced by both diffusion and polymer relaxation.

Antimicrobial activity: The zone of inhibition of bacterial strains like *S. aureus* and *E. coli*, and fungal strain *C. albicans* were mentioned in [table 8](#).

Values are expressed as mean $\pm$ SD (n=3). Different superscript letters within each column indicate statistically significant differences according to one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.05$).

One-way ANOVA demonstrated statistically significant differences among the treatment groups for *E. coli* [$F(4,10) = 105.60$, $P < 0.001$], *S. aureus* [$F(4,10) = 595.31$, $P < 0.001$], and *C. albicans* [$F(4,10) = 208.16$, $P < 0.001$]. Tukey's multiple comparison test showed that F2 exhibited significantly greater zones of inhibition than the extract against *E. coli* ($P = 0.0167$), *S. aureus* ($P < 0.001$), and *C. albicans* ($P < 0.001$). F2 also showed significantly greater inhibition than the marketed formulation against all three strains ($P < 0.001$). However, the inhibition zone produced by F2 against *C. albicans* was not significantly different from that of fluconazole ($P = 0.998$).

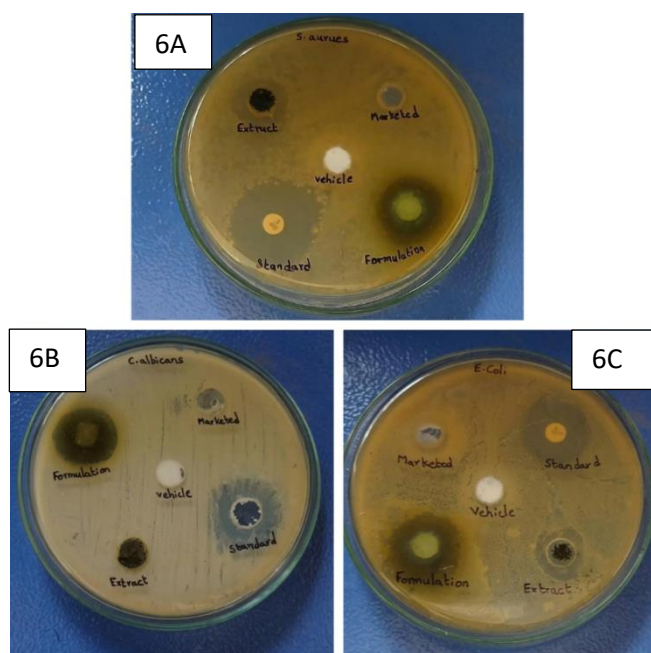


Figure 6: Zone of inhibition for selected formulation F2 against Ethanolic leaf Extract of *Haritamanjari* (*Acalypha indica*), Vehicle control, Standard and marketed gel using A) *Staphylococcus aureus*, B) *Candida albicans*, and C) *Escherichia coli*.

The standard antibacterial agent produced a larger zone of inhibition than F2, while F2 showed greater inhibition than the crude ethanolic extract under present agar well diffusion conditions. This may be associated with improved dispersion of the extract within the nanoemulgel matrix.

However, agar well diffusion alone does not establish enhanced antimicrobial potency or therapeutic superiority. F2 showed an inhibition zone against *C. albicans* comparable to that of fluconazole standard and significantly greater than that of crude extract. MIC/MBC/MFC were not performed in the present study. Consequently, the zone of inhibition data demonstrates growth inhibition under the specific agar diffusion conditions used, but are insufficient to establish antimicrobial potency or superiority. Further MIC/MBC/MFC and dermal safety studies are required to further characterize the antimicrobial and topical applicability.

4. DISCUSSION:

The present study developed *Haritamanjari* (*Acalypha indica*) ethanolic extract loaded nanoemulgel for topical antibacterial and antifungal activity. The UV calibration curve showed good linearity over 50-250 $\mu\text{g/mL}$ ($R^2 = 0.9996$), supporting extract-equivalent estimation under tested conditions. However, this does not represent quantification of a specific phytochemical marker.

Among the nanoemulsions, F2 exhibited the smallest particle size (245.7 ± 2.85), whereas F3 exhibited the lowest PDI (0.102 ± 0.02). The differences may be related to extract concentration and interaction with the oil, surfactant, and co-surfactant system. Comparable nanoscale characteristics have been reported for other herbal nanoemulgels, although direct comparisons are limited by differences in compositions and preparation. F2 showed the highest magnitude of zeta potential ($-14.60 \pm 0.80\text{mV}$), as Tween 80 may provide steric stabilization it should not be alone considered evidence of colloidal stability.

The presence of major phytochemical groups like flavonoids, alkaloids, terpenoids and saponins in both the extract and formulation exhibits the retention of multicomponent phytochemical in the formulation. FTIR showed retention of functional groups of the extract without major change in formulation exhibits compatibility between extract and excipient. XRD pattern showed reduced peak intensity and

broadening after formulation suggesting reduced crystallinity. However, these findings do not establish complete compatibility or complete conversion to amorphous state. F2 exhibited sustained release, by reaching $73.32 \pm 1.59\%$ after 7 hr, with $n = 0.7197$, indicating non-fickian release. Similar non-fickian release has been reported for *A. indica* phytosomal gel, [16] although the delivery system and composition differed.

F2 produced greater inhibition zones than the crude extract under agar well diffusion conditions. This may be associated with improved dispersion and greater interfacial area of the nanoemulsion, potentially facilitating contact between extract constituents and microbial cells. Previous work with *A. indica* has also demonstrated antimicrobial activity in phytosomal gel [16] and in an *A. indica* mediated ZnO nanogel [18]. The antimicrobial activity of *A. indica* may be associated with its reported secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, saponins and terpenoid constituents, which have been reported to possess antimicrobial properties. However, differences in formulation type and composition limit direct comparison with present crude extract nanoemulgel. Coconut oil was used as oil phase and may contribute to antimicrobial activity of the formulation due to its medium chain fatty acids. The vehicle control without *Haritamanjari* extract was also evaluated to assess the contribution of the formulation base to the observed antimicrobial activity.

The study has several limitations, including lack of MIC/MBC/MFC, dermal irritation, cytotoxicity, *ex vivo* permeation, and *in vivo* efficacy assessments. UV analysis measured extract-equivalent content rather than a specific phytochemical marker, and the assay was not subjected to comprehensive validation for specificity, accuracy/recovery, intra and inter day precision, robustness, LOD/LOQ. Total alkaloid content was not quantitatively determined, and GC-MS/LC-MS analysis was not performed for chemical profiling of the extract or formulation. A comprehensive long term stability study was also not performed. A formal design of experiments approach, such as factorial, mixture, or response surface methodology was not employed; preliminary formulation

screening was limited to variation of extract concentration while maintaining the oil, surfactant, and co-surfactant levels constant. Systematic optimization of formulation component ratios using a statistically designed approach may be considered in future studies. Therefore, further marker-based analytical, safety, *ex vivo*, and *in vivo* studies are required before therapeutic implications can be established.

5. CONCLUSION

The present study developed and evaluated a *Haritamanjari* (*Acalypha indica*) nanoemulgel as a potential topical antimicrobial formulation, with antibacterial and antifungal activity evaluated under *in vitro* conditions. The selected formulation F2 exhibited favourable physicochemical properties, like particle size and zeta potential, and also better extract-equivalent content compared to F1 and F3. The selected formulation demonstrated prolonged extract release in the *in vitro* extract release study and produced greater inhibition zones than the crude extract and marketed formulation under experimental agar diffusion conditions. Overall, the findings suggest that the *Haritamanjari* nanoemulgel has potential for further investigation as a topical antimicrobial formulation. However, dermal safety, irritation, cytotoxicity, and *in vivo* therapeutic efficacy were not evaluated in the present study. Further *in vivo* clinical studies are required to establish its safety, efficacy, and therapeutic potential.

Abbreviations:

F1 – Formulation 1; F2 – Formulation 2; F3 – Formulation 3; nm – nanometer; pH – potential of hydrogen; PDI – polydispersity index; mV – millivolts; XRD – X-ray diffraction; h – hour(s) s – seconds; Smix – surfactant mixture; FTIR – Fourier transform infrared; w – weight; v – volume; μg – micrograms; mL – milliliter; g – gram; cP – centipoise; UV – ultraviolet; cm – centimeter; $^{\circ}\text{C}$ – Celsius; SD – standard deviation; mm – millimeter; ATCC – American Type Culture Collection, MWCO – molecular weight cutoff, MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimum fungicidal concentration.

Authors Details:

^{1*}M.Pharmacy Student, Department of Pharmacognosy, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India. ORCID link: <https://orcid.org/0009-0000-7468-2516>

²Professor, Department of Pharmacognosy, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India. **ORCID link:** <https://orcid.org/0000-0002-5885-2535>

³M.Pharmacy Student, Department of Pharmacognosy, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India. **ORCID link:** <https://orcid.org/0009-0004-8640-0175>

⁴Dental Student, CKS Teja Institute of Dental Sciences, Tirupati, Andhra Pradesh, India. **ORCID link:** <https://orcid.org/0009-0002-4216-0611>

Author Contributions:

Conceptualization: SGH, BRK

Data collection and literature search: SGH, BRK, UNHD

Writing – original draft preparation: SGH, BRK, BSS

Reviewing & editing: SGH, BRK

Approval of final manuscript: All authors

Acknowledgements – The authors are grateful to the Institute of Pharmaceutical Technology (IPT), Sri Padmavati Mahila Visvavidyalayam, for providing the necessary laboratory facilities and research infrastructure for carrying out this work. The authors also gratefully acknowledge DST-CURIE, Sri Padmavati Mahila Visvavidyalayam, for providing the necessary facilities and support for the successful completion of the research work.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement: This study did not involve human participants or experimental animals; therefore, Institutional Ethics Committee approval and informed consent were not applicable.

Declaration of Generative AI: The authors declare this manuscript was written without the use of generative artificial intelligence tools. All the content, including text generation, data analysis and references was developed and reviewed by the authors without assistance from AI technologies.

Conflict of Interest – The authors declare no conflicts of interest.

Source of Support – The authors declare no source of support.

Additional Information: Authors can order reprints (print copies) of their articles by visiting: <https://www.akinik.com/products/2281/journal-of-ayurveda-and-holistic-medicine-jahm>

Publisher's Note: Atreya Ayurveda Publications remains neutral with regard to jurisdictional claims in published maps, institutional affiliations, and territorial designations. The publisher does not take any position concerning legal status of countries, territories, or borders shown on maps or mentioned in institutional affiliations.

REFERENCES:

1. Prausnitz MR, Langer R. Transdermal drug delivery. Nature Biotechnology. [homepage on the Internet] 2008;26(11):1261–1268. Available from: <https://www.nature.com/articles/nbt.1504>
2. Hay RJ, Johns NE, Williams HC, et al. The Global Burden of Skin Disease in 2010: An Analysis of the Prevalence and Impact of Skin Conditions. Journal of Investigative Dermatology [homepage on the Internet]

2014 [cited 2026 Sept 20];134(6):1527–1534. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0022202X15368275>

3. World Health Organization. Global research agenda for antimicrobial resistance in human health [Homepage on the Internet]. Available from: <https://www.who.int/publications/m/item/global-research-agenda-for-antimicrobial-resistance-in-human-health>
4. Najafi-Taher R, Jafarzadeh Kohneloo A, Eslami Farsani V, Mehdizade Rayeni N, Reza Moghimi H, Ehsani A, et al. A topical gel of tea tree oil nanoemulsion containing adapalene versus adapalene marketed gel in patients with acne vulgaris: a randomized clinical trial. Arch Dermatol Res [homepage on the Internet] 2022 [cited 2026 Sept 20];314(7):673–679. Available from: <https://link.springer.com/10.1007/s00403-021-02267-2>
5. Benson HAE, Watkinson AC, editors. Topical and transdermal drug delivery: principles and practice. Wiley; 2011. Available from: <https://onlinelibrary.wiley.com/doi/book/10.1002/9781118140505>
6. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. European Journal of Pharmaceutical Sciences [homepage on the Internet] 2001 [cited 2026 Sept 20];14(2):101–114. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0928098701001671>
7. Patra JK, Das G, Fraceto LF, et al. Nano-based drug delivery systems: recent developments and future prospects. Journal of Nanobiotechnology. 2018 [cited 2026 Sept 20];16(1):71. Available from: <https://nanobiotechnology.biomedcentral.com/articles/10.1186/s12951-018-0392-8>
8. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: *In vitro* percutaneous permeation through human skin and *in vivo* anti-inflammatory activity on human volunteers. Journal of Controlled Release [homepage on the Internet] 2005 [cited 2026 Sept 20];106(1–2):99–110. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168365905001689>
9. Singh Y, Meher JG, Raval K, et al. Nanoemulsion: Concepts, development and applications in drug delivery. Journal of Controlled Release [homepage on the Internet] 2017 [cited 2026 Sept 20];252:28–49. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168365917301128>
10. Donthi MR, Munnangi SR, Krishna KV, Saha RN, Singhvi G, Dubey SK. Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery. Pharmaceutics [homepage on the Internet] 2023 [cited 2026 Sept 20];15(1):164. Available from: <https://www.mdpi.com/1999-4923/15/1/164>
11. McClements DJ. Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter [homepage on the Internet] 2012 [cited 2026 Sept 20];8(6):1719–1729. Available from: <https://pubs.rsc.org/sm/article/8/6/1719-1729/384346>

12. Shakeel F, Ramadan W, Ahmed MA. Investigation of true nanoemulsions for transdermal potential of indomethacin: characterization, rheological characteristics, and *ex vivo* skin permeation studies. Journal of Drug Targeting [homepage on the Internet] 2009 [cited 2026 Sept 20];17(6):435–441. Available from: <http://www.tandfonline.com/doi/full/10.1080/10611860902963021>
13. Mathew AE, Abraham A, Abraham E. Lipid-based nanocarriers for transdermal drug delivery: a comprehensive review. International Journal of Pharmaceutical Research and Development [homepage on the Internet] 2025 [cited 2026 Sept 20];7(2):111–115. Available from: <https://www.pharmaceuticaljournal.net/archives/2025.v7.i2.B.169/lipid-based-nanocarriers-for-transdermal-drug-delivery-a-comprehensive-review>
14. Zahidin NS, Saidin S, Zulkifli RM, Muhamad II, Ya'akob H, Nur H. A review of *Acalypha indica* L. (*Euphorbiaceae*) as traditional medicinal plant and its therapeutic potential. Journal of Ethnopharmacology [homepage on the Internet] 2017 [cited 2026 Sept 20];207:146–173. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S037887411632445X>
15. Seebaluck R, Gurib-Fakim A, Mahomoodally F. Medicinal plants from the genus *Acalypha* (*Euphorbiaceae*)—A review of their ethnopharmacology and phytochemistry. Journal of Ethnopharmacology [homepage on the Internet] 2015 [cited 2026 Sept 20];159:137–157. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0378874114007508>
16. Jasmimol.K, Reichal DCR, Saminathan P, S.Manju. Formulation and Evaluation of *Acalypha Indica* Phytosomal Herbal Gel. International Journal of Pharma and Bio Sciences [homepage on the Internet] 2023;14(1):9–20. Available from: <http://ijpbs.net/index.php/journal/article/view/7021>
17. Neelima Devi K, Amaleswari MLNT, Kandapu V, Kammari SC, Kata S, et al. Phytoniosomal gel of *acalypha indica*: a novel topical delivery system for anti-inflammatory activity. International Journal of Research in Pharmacology & Pharmacotherapeutics [Internet]. 2025 Mar. 5 [cited 2026 Oct. 21];14(1):167-75. Available from: <https://doi.org/10.61096/ijrpp.v14.iss1.2025.167-175>
18. Kesavardhini K, Gharban HAJ, Nayak AK, et al. Eco-friendly green synthesis of zinc oxide nanoparticles using *Acalypha indica* L. leaf extract and their incorporation into topical formulations for acne and skin infection treatment. Scientific Reports. 2026 [cited 2026 Sept 20];16(1):26026. Available from: <https://www.nature.com/articles/s41598-026-55356-5>
19. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering [homepage on the Internet] 2013 [cited 2026 Sept 20];117(4):426–436. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0260877413000277>
20. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants. 2015;4(3):196. Available from: <http://www.omicsgroup.org/journals/a-review-on-the-extraction-methods-use-in-medicinal-plants-principle-strength-and-limitation-2167-0412-1000196.php?aid=58448>
21. Thamil Priya V, Balasubramanian N, Shanmugaiah V, Karunakaran C. Phytochemical Properties of *Acalypha indica* (L), and its Antimicrobial Potential against Human Pathogens. Journal of Pure and Applied Microbiology. 2019;14(1):319–326. Available from: <https://doi.org/10.22207/JIPAM.14.1.33>
22. Moghbel A, Farzadeh A, Aghel N, Agheli H, Raisi N. Evaluation of the Effect of Green Tea Extract on Mouth Bacterial Activity in the Presence of Propylene Glycol. Jundishapur Journal of Natural Pharmaceutical Products [homepage on the Internet] 2012 [cited 2026 Sept 27];7(2):56–60. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3941855/>
23. Giri S, Chakraborty A, Mandal C, Rajwar TK, Halder J, Irfan Z, Gouda MM. Formulation and Evaluation of Turmeric- and Neem-Based Topical Nanoemulgel against Microbial Infection. Gels [homepage on the Internet] 2024 [cited 2026 Sept 27];10(9):578. Available from: <https://doi.org/10.3390/gels10090578>
24. Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. Current Opinion in Colloid & Interface Science [homepage on the Internet] 2012 [cited 2026 Sept 21];17(5):246–254. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1359029412000787>
25. Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. Advances in Colloid and Interface Science [homepage on the Internet] 2004 [cited 2026 Sept 21];108–109:303–318. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S000186860300157X>
26. Bhattacharjee S. DLS and zeta potential – What they are and what they are not? Journal of Controlled Release [homepage on the Internet] 2016 [cited 2026 Sept 21];235:337–351. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168365916303832>
27. Danaei M, Dehghankhold M, Ataei S, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics [homepage on the Internet] 2018 [cited 2026 Sept 21];10(2):57. Available from: <https://www.mdpi.com/1999-4923/10/2/57>
28. Honary S, Zahir F. Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 1). Tropical Journal of Pharmaceutical Research [homepage on the Internet] 2013 [cited 2026 Sept 21];12(2):255–264. Available from: <http://www.ajol.info/index.php/tjpr/article/view/88554>
29. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharmaceutical Journal [homepage on the Internet] 2012 [cited 2026 Sept 21];20(1):63–67. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1319016411000624>

30. Dasawanti Y, Reveny J, Sumaiyah S. Formulation and evaluation of nanoemulgel clove leaf oil (*Syzygium aromaticum*) (L.) Merr. & Perry as anti-acne. International Journal of Science, Technology & Management. 2022;3(6):1777–1783. Available from: <https://ijstmjournal.com/index.php/ijstm/article/view/681>
31. Shinde UA, Kanojiya SS. Serratiopeptidase Niosomal Gel with Potential in Topical Delivery. Journal of Pharmaceutics [homepage on the Internet] 2014 [cited 2026 Sept 21];2014:1–9. Available from: <https://www.hindawi.com/journals/jphar/2014/382959/>
32. Jamal Basha D, Srinivas Murthy BR, Prakash P, Anuradha KC. Preliminary Phytochemical Investigation and In Vitro Anti Bacterial Studies of *Polycarpaea aurea* Wight and Arn (Caryophyllaceae) Emulgel. Asian Journal of Pharmaceutical Research [homepage on the Internet] 2018 [cited 2026 Sept 27];8(3):151–157. Available from: <https://asianjpr.com/AbstractView.aspx?PID=2018-8-3-7>
33. Farooq U, Rasul A, Zafarullah M, Abbas G, Rasool M, Ali F, et al. Nanoemulsions as novel nanocarriers for drug delivery across the skin: *In-vitro*, *in-vivo* evaluation of miconazole nanoemulsions for treatment of *Candidiasis albicans*. Designed Monomers and Polymers. 2021;24(1):240–258. Available from: <https://doi.org/10.1080/15685551.2021.1965724>
34. Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis [homepage on the Internet] 2016 [cited 2026 Sept 21];6(2):71–79. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2095177915300150>