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Development and Evaluation of a Plant-Derived Antimicrobial Nanoemulsion for Enhanced Treatment of Drug Resistant Skin Pathogens

Adarsh Satish Singh, S. V. Raut and Bony Shashikant Dasari*

Department of Microbiology, MM College of Arts, NM Institute of Science, HRJ College of Commerce Bhavan's College Autonomous, Munshi Nagar Andheri West, Mumbai Maharashtra 400058, India

**Corresponding author*

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A B S T R A C T

Skin and soft tissue infections caused by drug-resistant pathogens represent a growing global health concern, particularly due to the increasing prevalence of antimicrobial resistance (AMR). Among the major causative organisms, *Staphylococcus aureus* (including methicillin-resistant strains) and *Pseudomonas aeruginosa* are frequently implicated in chronic and biofilm-associated infections. In this context, plant-derived bioactive compounds offer a promising alternative due to their broad-spectrum antimicrobial, anti-inflammatory, and antibiofilm properties. However, phytochemicals such as Eugenol and Curcumin exhibit poor aqueous solubility, instability, and limited bioavailability, restricting their clinical application. The present study focuses on the development and physicochemical characterization of an oil-in-water Nanoemulsion incorporating selected plant-derived phytochemicals to enhance antimicrobial efficacy against resistant skin pathogens. The integration of phytochemicals into a nanoemulsion-based delivery system is anticipated to enhance solubility, promote effective skin penetration, and improve interaction with microbial membranes, thereby overcoming conventional resistance mechanisms and biofilmassociated tolerance. This research highlights the potential of plant-based Nanoemulsion technology as an innovative and sustainable therapeutic strategy for the management of drugresistant cutaneous infections, offering a promising alternative to conventional antimicrobial therapies.

Introduction

Antimicrobial resistance (AMR) has become a profound global public health crisis, significantly undermining the effectiveness of existing therapeutic regimens and threatening decades of medical advancement. The rapid emergence of multidrugresistant (MDR) bacterial strains is primarily driven by irrational antibiotic usage, prolonged exposure to sub-therapeutic doses, and the adaptive genetic mechanisms of pathogens. Among these, methicillin-resistant *Staphylococcus aureus* (*MRSA*) has emerged as a predominant etiological agent of skin and soft tissue infections, exhibiting resistance to β-lactam antibiotics and frequently demonstrating cross-

resistance to multiple antimicrobial classes. The persistence of MRSA in both community and hospital settings necessitates the development of alternative and innovative therapeutic strategies (World Health Organization [WHO], 2023).

In recent years, phytotherapy has gained renewed scientific interest due to the broad-spectrum antimicrobial potential of plant-derived bioactive compounds. Medicinal plants synthesize diverse secondary metabolites, including Flavonoids, Phenolics, Alkaloids, Terpenoids, and Tannins, which exert antimicrobial activity through mechanisms such as membrane disruption, enzyme inhibition, efflux pump suppression, and interference with nucleic acid synthesis. Despite promising in vitro efficacy, the therapeutic application of phytoconstituents remains constrained by inherent physicochemical limitations, including poor aqueous solubility, instability under physiological conditions, rapid degradation, and limited dermal penetration.

Nanotechnology-based drug delivery systems have emerged as transformative tools in addressing these limitations. Nanoemulsions, characterized by droplet sizes typically ranging from 20–200 nm, offer enhanced surface area, improved kinetic stability, and superior drug solubilization capacity. The reduced droplet size facilitates improved permeation across biological membranes and enhances interaction with microbial cell walls, thereby potentiating antimicrobial activity. Additionally, nanoemulsion systems can provide controlled release, improved bioavailability, and reduced systemic toxicity, making them particularly suitable for topical therapeutic applications (Gupta *et al.,* 2016).

The Integration of phytochemicals into nanoemulsion-based delivery systems represents a promising strategy to combat resistant cutaneous pathogens. By enhancing the physicochemical stability and biological performance of plant-derived compounds, such formulations may significantly improve therapeutic outcomes against MDR organisms, including *MRSA*. Therefore, the present study is designed to develop and characterize a plantbased antimicrobial nanoemulsion, followed by comprehensive evaluation of its physicochemical properties, stability profile, and in vitro antimicrobial efficacy. This approach aims to bridge traditional herbal medicine and advanced nanotechnology, contributing toward the development of safer and more effective alternatives for the management of resistant skin infections (Turner *et al.,* 2019).

The growing limitations of synthetics antibiotics have intensified the search for alternative therapeutic agents, particularly those derived from medicinal plants. Phytochemicals are known to possess broadspectrum antimicrobial, antioxidant, antiinflammatory, and wound-healing properties. Among these, Eugenol and Curcumin have attracted considerable scientific attention due to their potent biological activities and established safety profiles.

Eugenol, a phenolic compound predominantly obtained from clove oil (*Syzygium aromaticum*), exhibits significant antimicrobial activity against Gram-positive and Gram-negative bacteria. Its mechanism of action primarily involves disruption of microbial cell membranes, leading to leakage of intracellular constituents, protein denaturation, and inhibition of enzyme activity. Studies have demonstrated that Eugenol interferes with quorum sensing and biofilm formation in Staphylococcus aureus, thereby reducing bacterial virulence and resistance potential. Eugenol possesses antiinflammatory and analgesic properties, which are advantageous in the management of infected wounds. However, its practical therapeutic application is restricted by poor aqueous solubility, volatility, and chemical instability under physiological conditions (Cowan, 1999).

Curcumin, the principal polyphenolic compound isolated from *Curcuma longa* (turmeric), is widely recognized for its antimicrobial, antioxidant, and anti-inflammatory properties. Curcumin exerts antibacterial effects by disrupting bacterial membrane integrity, inhibiting nucleic acid synthesis, suppressing FtsZ protein assembly required for cell division, and modulating inflammatory signaling pathways. Importantly, Curcumin has demonstrated inhibitory activity against MRSA strains and has shown potential to enhance antibiotic susceptibility when used synergistically. Despite these promising properties, Curcumin suffers from poor water solubility, rapid metabolic degradation, limited bioavailability, and inadequate skin penetration, which significantly hinder its clinical utility (Gupta *et al.,* 2013).

To overcome these physicochemical limitations, nanotechnology-based delivery systems have been developed to enhance the therapeutic performance of hydrophobic phytoconstituents. Among these systems, nanoemulsions have emerged as promising carriers due to their thermodynamic or kinetic stability, nanoscale droplet size (typically 20–200 nm), and enhanced surface

area. Nanoemulsions are isotropic colloidal dispersions composed of oil, water, surfactant, and co-surfactant, capable of improving drug solubilization and stability. The Incorporation of Eugenol and Curcumin into nanoemulsion systems offers. Multiple advantages. First, nanoemulsions enhance solubility and prevent premature degradation of hydrophobic compounds. Second, the small droplet size facilitates improved dermal penetration and uniform distribution across the Infected site. Third, nanoemulsions promote enhanced interaction with bacterial cell membranes due to increased surface contact, potentially amplifying antimicrobial efficacy. Moreover, nanoemulsion systems may provide sustained drug release, reduced dosing frequency, minimized systemic toxicity, and improved patient compliance (Gupta *et al.,* 2016).

In the context of resistant cutaneous infections, nanoemulsion-based topical delivery systems represent a rational and innovative approach. By combining the intrinsic antimicrobial properties of Eugenol and Curcumin with advanced nanotechnological delivery, it is possible to achieve synergistic effects against MDR pathogens such as *MRSA*. The improved permeability and retention effect at the site of infection may enhance therapeutic outcomes while limiting systemic exposure.

Therefore, the present study focuses on the formulation, optimization, and characterization of a phytochemical-based nanoemulsion containing Eugenol and Curcumin, followed by evaluation of its physicochemical stability, droplet size distribution, zeta potential, and in vitro antimicrobial activity against resistant skin pathogens. This integrative strategy aims to bridge traditional herbal medicine with modern nanotechnology, offering a promising alternative for the management of antimicrobialresistant skin infections.

Membrane-Targeted Antimicrobial Activity: One underexplored yet critical antimicrobial mechanism involves perturbation of microbial membrane biophysics. Unlike conventional antibiotics, many phytochemicals exert non-specific membranedisruptive actions that reduce the probability of resistance acquisition. Eugenol, for instance, integrates into the phospholipid bilayer, increasing membrane fluidity and permeability. This interaction disrupts proton motive force, collapses transmembrane potential, and leads to cytoplasmic leakage. Membrane destabilization enhances susceptibility to other antimicrobial agents, thereby contributing to synergistic effects.

Curcumin has similarly been shown to insert into bacterial membranes, altering lipid packing density and impairing membrane protein function. Beyond structural disruption, Curcumin interferes with bacterial cytokinesis by inhibiting FtsZ protofilament assembly, a protein critical for Z-ring formation during cell division. This dual membranecytoskeletal targeting reduces bacterial adaptability (Rai *et al.,* 2008).

Recent studies suggest that certain phytochemicals induce intracellular oxidative stress in bacteria. Curcumin can generate reactive oxygen species (ROS) under physiological conditions, leading to oxidative damage of proteins, lipids, and nucleic acids. This oxidative stress overwhelms bacterial antioxidant defense systems such as catalase and superoxide dismutase. ROS-mediated killing is considered less prone to resistance development because it affects multiple intracellular targets simultaneously.

Nanoemulsion-based delivery systems may further potentiate ROS generation due to enhanced surface reactivity and improved intracellular penetration, amplifying bactericidal activity.

Anti-Biofilm and Quorum Sensing Inhibition: Biofilm formation represents a major virulence and resistance mechanism in *MRSA* infections. Biofilms create a protective extracellular polymeric matrix that limits antibiotic diffusion and facilitates horizontal gene transfer. Phytochemicals such as Eugenol have demonstrated quorum sensing inhibitory properties, interfering with bacterial cell– cell communication pathways that regulate virulence factor production (Burt, 2004). Curcumin has been reported to downregulate biofilm-associated genes, thereby reducing adherence and colonization capacity.

Nanoemulsions enhance penetration into biofilm matrices due to nanoscale droplet size and increased surface area, improving disruption of established biofilms an area where many conventional antibiotics fail.

Nanoemulsion-Specific Antimicrobial Enhancements: Beyond simple drug delivery, Nanoemulsions themselves may contribute to antimicrobial effects. The high curvature stress of Nano-sized droplets facilitates intimate interaction with bacterial membranes. Surfactants used in nanoemulsion formulation can further destabilize lipid bilayers.

Multi-targeted agents such as Eugenol and Curcumin, especially when delivered via Nanoemulsions, exert antimicrobial effects through membrane disruption, oxidative stress induction, quorum sensing inhibition, efflux modulation, and biofilm interference simultaneously.

This polypharmacological action significantly reduces the probability of resistance evolution compared to single-target antibiotics (Daglia, 2012).

Materials and Methods

Curcumin and Eugenol were purchased from D.G sons Ayurvedic house of herbs. Neem oil and olive oil sourced from local suppliers. Tween-80 (Surfactant), PEG 400 or PEG 600 (Co-surfactant), 0.1N Citric Acid, Sodium Benzoate, 0.1N Sodium Hydroxide, DMSO (Diamethyl sulfoxide), Ethanol were also used for experimental procedure. Muller- Hinton media were obtained from HiMedia laboratories. Pre- sterilized petri plates, Pipettes, Tubes (Dilution, S-line, Suspension), Beaker, Flask, pH paper, Glass rods and transparent storage container were used for the experimental procedure. All chemicals and reagents used in the study were of analytical grade and were utilized without further purification.

Antimicrobial activity of the Phytochemicals (Eugenol & Curcumin)

The antimicrobial activity of the developed nanoemulsion against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was assessed by the agar well diffusion method. Briefly, standardized bacterial inoculum (0.5 McFarland; O.D. 0.1 at 600 nm) was seeded on Mueller– Hinton agar plates, and wells were loaded with 50 µL of the formulation. Plates were incubated at 37°C for 24 hours, and antimicrobial efficacy was determined by measuring the diameter of the zones of inhibition.

Stock solutions of Eugenol and Curcumin were prepared in DMSO (1:1 v/v) to ensure proper solubilisation. Based on the sample density (1060 mg/mL), the calculated stock concentration was 530 mg/mL. MIC was determined by two-fold serial dilution in sterile Muller-Hinton broth to obtain concentrations ranging from 132.5 to 1.035 mg/mL in a final volume of 2 mL per tube. Each tube was inoculated with 0.1 mL of bacterial suspension (O.D. 0.1 at 600 nm) and incubated at 37°C for 24 hours. Positive, negative, and media controls were included. The MIC was defined as the lowest concentration showing no visible bacterial growth.

Stable Nanoemulsion Formation Using Phytochemicals (Eugenol & Curcumin)

A stable nanoemulsion was formulated using Neem oil and olive oil (1:1) as the oil phase, in which Curcumin (5 g) or Eugenol (10 mL) was dissolved under controlled heating (40–45°C) and continuous stirring, followed by addition of Tween-80 as a non-ionic surfactant. The aqueous phase consisted of distilled water containing sodium benzoate (0.1%) as preservative, with pH adjusted to 5.5–6.0 using 0.1 N citric acid or NaOH.

The preheated aqueous phase was added dropwise to the oil phase under constant stirring (600 rpm) to form a coarse emulsion, which was further subjected to ultrasonication for 60 minutes in intermittent cycles to reduce droplet size and obtain a stable nanoemulsion. The final formulation was stored in airtight containers for further evaluation (Hassanshahian *et al.,* 2019).

Characterization of Nanoemulsion

The prepared nanoemulsion was characterized to evaluate its physicochemical stability, uniformity, and suitability for topical application.

Droplet Size: Droplet size was determined by optical microscopy (100×). Diluted samples were placed on a glass slide, and micrographs were analyzed using ImageJ software to calculate mean droplet diameter from multiple fields.

Turbidity: Turbidity was measured spectrophotometrically at 600 nm using distilled water as blank. Absorbance values indicated dispersion characteristics of the system.

Conductivity: Electrical conductivity was measured using a digital conductivity meter. Higher conductivity confirmed the oil-in-water (O/W) nature of the nanoemulsion.

Centrifugation Test: Samples were centrifuged at 2500 rpm for 30 minutes and observed for phase separation or

instability. Absence of separation indicated good kinetic stability.

pH: pH was measured using a calibrated digital pH meter or pH paper at room temperature and maintained between 5.5–6.0 for skin compatibility.

Polydispersity Index (PDI): PDI was determined by Dynamic Light Scattering (DLS) at 25 ± 1 °C. Measurements were performed in triplicate, and lower PDI values indicated uniform droplet distribution and enhanced stability.

Antimicrobial Activity of Nanoemulsion

Test organisms: *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions. Wells were punched aseptically, and 50 µL of Eugenol/Curcumin nanoemulsion was added. Plates were incubated at 37°C for 24 h, and zones of inhibition (mm) were measured.

A stock solution (530 mg/mL) was prepared using nanoemulsion and DMSO (1:1). Serial two-fold dilutions were prepared in nutrient broth (total volume 2 mL) and inoculated with 0.1 mL culture (0.1 OD at 600 nm). After incubation at 37°C for 24 h, the lowest concentration showing no visible growth was recorded as MIC.

Bacterial suspensions (~10^6 CFU/mL) were exposed to selected nanoemulsion concentrations and incubated at 37°C (120–150 rpm).

Samples were collected at 0–6 h, serially diluted, plated on nutrient agar, and incubated for 24 h. CFU/mL were calculated, and $\log_{10}$ CFU/mL was plotted against time to evaluate bactericidal activity.

Comparative Study

The plant-derived nanoemulsion was compared with Heal-O-Kind Nanofine Gel (nanocrystalline silver 0.002%, Mankind Pharma Ltd).

Agar well diffusion and broth dilution methods were performed under identical conditions. Zones of inhibition and MIC values were recorded and compared to assess relative antimicrobial efficacy.

Results and Discussion

Antimicrobial activity of the Phytochemicals (Eugenol & Curcumin)

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of Eugenol and Curcumin was determined by the broth dilution method against Staphylococcus aureus and Pseudomonas aeruginosa. Eugenol exhibited an MIC of 33.125 mg/mL against S. aureus and 132.5 mg/mL against P. aeruginosa. Curcumin showed an MIC of 66.25 mg/mL against both test organisms.

The results indicate comparatively stronger antibacterial activity of Eugenol against the Gram-positive strain, whereas both phytochemicals required higher concentrations to inhibit the Gram-negative organism.

Stable nanoemulsion Formation using Phytochemicals (Eugenol & Curcumin)

The Nanoemulsions formulated with phytochemicals (Eugenol and Curcumin) were successfully prepared and showed homogeneous, uniformly dispersed systems without phase separation, creaming, or cracking.

The Eugenol nanoemulsion appeared milky white, while the Curcumin nanoemulsion exhibited a characteristic orange colour, confirming successful incorporation. Both formulations demonstrated smooth consistency and good physical stability with no visible sedimentation, indicating the formation of stable oil-in-water Nanoemulsions.

Characterization of Nanoemulsion

The physicochemical characterization of the developed phytochemical nanoemulsions demonstrated satisfactory formulation properties within acceptable standard limits. The pH of the Eugenol nanoemulsion was recorded at 5.5, while the Curcumin nanoemulsion exhibited a pH of 6.0, both falling within the standard skin-compatible range of 5.0–6.5, indicating suitability for topical application. Centrifugation stability testing at 2500 rpm for 15 minutes showed no phase separation in either formulation, confirming good physical stability and proper dispersion of the oil phase within the aqueous medium.

Table.1 Agar well diffusion method

Culture	Sample	Diameter of Zone of inhibition(mm)
S.aureus	Eugenol	19
	Curcumin	12
	Control (DMSO)	6
P.aeruginosa	Eugenol	18
	Curcumin	11
	Control (DMSO)	7

Table.2 Agar well diffusion method

Culture	Sample	Diameter of of inhibition (mm)
S.aureus	Eugenol	19
	Curcumin	13
	Neem oil	-
	Eugenol (1:1)	-
P.aeruginosa	Eugenol	17
	Curcumin	10
	Neem oil	-
	Curcumin (1:1)	-

Table.3 Time-Kill curve Determination

Time (Hrs)	Avg. Cfu/ml
0 hrs	**2.450×10^{7} cfu/ml**
1 hrs	**6.72×10^{6} Cfu/ml**
2 hrs	**2.975×10^{6} Cfu/ml**
3 hrs	**2.925×10^{5} Cfu/ml**
4 hrs	**1.98×10^{5} Cfu/ml**
5 hrs	**3.9×10^{4} Cfu/ml**
6 hrs	**--**

Graph.1 Time- Kill curve Shows the rate and extent of antimicrobial activity of the nanoemulsion (log10 avg.cfu/ml vs time)

Fig.1 Agar well diffusion method plates 1st plate of *S.aureus* and 2nd plate of *P.aeruginosa*

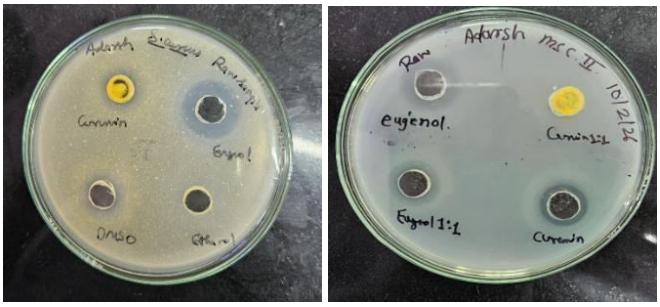

Fig.2 Stable nanoemulsion formulation White colour is of Eugenol based Nanoemulsion and Orange-Yellow is of Curcumin based nanoemulsion

Fig.3 Agar well diffusion method of Eugenol & Curcumin against *S.aureus* and *P.aeruginosa*

Turbidity analysis at 600 nm revealed optical density values of 1.2 for the Eugenol nanoemulsion and 1.9 for the Curcumin nanoemulsion, suggesting successful nano-scale dispersion with moderate light scattering properties. The droplet size of the Eugenol nanoemulsion ranged between 200–215 nm, while the Curcumin nanoemulsion exhibited slightly smaller droplets ranging from 185–205 nm, approaching the standard nanoemulsion size range of 20–200 nm. Conductivity measurements were 235 µS/cm and 253 µS/cm for Eugenol and Curcumin nanoemulsions, respectively, which lie within the acceptable range of 100–300 µS/cm, indicating the formation of oil-in-water type Nanoemulsions. The polydispersity index (PDI) was 0.25 for the Eugenol nanoemulsion, reflecting a uniform droplet distribution within the acceptable limit (≤0.3), whereas the Curcumin nanoemulsion showed a slightly higher PDI of 0.38, indicating moderate polydispersity. Both formulations exhibited moderate optical clarity, consistent with the expected transparent to slightly opalescent appearance of stable Nanoemulsions. Overall, the results confirm the successful formulation of stable and pharmaceutically acceptable phytochemical Nanoemulsions.

Antimicrobial Activity of Nanoemulsion Prepared from Phytochemicals (Eugenol & Curcumin)

Minimum Inhibitory Concentration

The MIC results demonstrated that Eugenol exhibited stronger antibacterial activity than Curcumin against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For *S. aureus,* Eugenol showed inhibition up to 8.28125 mg/ml, whereas Curcumin inhibited growth up to 16.5625 mg/ml. Similarly, against *P. aeruginosa,* Eugenol was effective up to 16.5625 mg/ml, while Curcumin required higher concentrations for inhibition. These findings indicate that Eugenol possesses comparatively lower MIC values and greater antimicrobial potency than Curcumin.

Result comparison between the Raw phytochemical and the nanoemulsion that are formulated from the Phytochemical

The comparative evaluation of raw phytochemicals (Eugenol and Curcumin) and their nanoemulsion formulations against *Staphylococcus aureus* and *Pseudomonas aeruginosa* demonstrated significantly enhanced antibacterial activity in the nanoemulsion form. In the agar well diffusion assay, raw samples showed no detectable zones of inhibition, whereas Eugenol nanoemulsion produced zones of 19 mm *(S. aureus*) and 17 mm *(P. aeruginosa)*, and Curcumin nanoemulsion showed 13 mm and 10 mm respectively.

Similarly, MIC values were markedly reduced in nanoemulsions. Raw Eugenol exhibited MIC at Overall, nanoemulsion formulations significantly improved antimicrobial efficacy due to enhanced solubility, better diffusion, increased surface area, and improved penetration of active compounds, with Eugenol nanoemulsion showing superior activity compared to Curcumin. 33.125 mg/ml (*S. aureus*) and 132.5 mg/ml. All experimental results of the formulated plant-

Comparative study Between the Plant- derived Nanoemulsion and conventional Nanoemulsion

The comparative antimicrobial activity of the plantderived nanoemulsion and the conventional formulation was evaluated using the agar well diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results demonstrated that the Eugenol-based nanoemulsion exhibited the highest antibacterial activity, producing a zone of inhibition of 14 mm against *S. aureus* and 12 mm against *P. aeruginosa.* The Curcumin nanoemulsion also showed

considerable activity, with inhibition zones of 11 mm and 10 mm, respectively. In comparison, the conventional formulation, Heal-OKind Nanofine Gel, showed lower antimicrobial efficacy, with zones of 9 mm against *S. aureus* and 6 mm against *P. aeruginosa.* The solvent control, DMSO, exhibited minimal inhibition (7 mm against *S. aureus*) and no activity against *P. aeruginosa.* The plant-derived nanoemulsions demonstrated superior antimicrobial potential compared to the conventional Nanoformulation, particularly against Gram-positive bacteria.

Biostatistical Analysis

(*P. aeruginosa*), while Curcumin showed 66.25 mg/ml against both organisms. In contrast, Eugenol nanoemulsion showed MIC at 8.28125 mg/ml and Curcumin nanoemulsion at 16.5625 mg/ml against both bacteria. Derived nanoemulsions were systematically analysed and confirmed statistically. The prepared Eugenol and Curcumin nanoemulsions showed good physical stability, homogeneity, and appropriate physicochemical characteristics. Antimicrobial evaluation using the agar well diffusion method demonstrated higher zones of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biostatistical analysis using two-way ANOVA indicated that the observed differences were statistically significant ($p < 0.05$), confirming that the plant-derived nanoemulsions possess superior and scientifically validated antimicrobial efficacy.

In conclusion, the present study highlights the potential of phytochemical-based nanotherapeutics as a promising alternative strategy to combat antimicrobial-resistant skin pathogens. Eugenol and Curcumin demonstrated significant antimicrobial efficacy through multi-targeted mechanisms, including membrane disruption, oxidative stress induction, biofilm inhibition, and modulation of resistance pathways. However, their intrinsic physicochemical limitations such as poor solubility and limited bioavailability restrict direct therapeutic application. The development of a nanoemulsion delivery system effectively enhanced the stability, dispersion, and antimicrobial performance of these bioactive compounds. Improved droplet size distribution and increased surface interaction contributed to enhanced penetration and bacterial inhibition, particularly against resistant strains such as *MRSA*. The integration of plant-derived antimicrobials with nanotechnology represents a rational, safe, and effective approach for managing drug-resistant cutaneous infections. This strategy not only improves therapeutic efficacy but also reduces the likelihood of resistance development, offering a sustainable direction for future antimicrobial research.

Author Contributions

Adarsh Satish Singh: Investigation, formal analysis, writing—original draft. S. V. Raut: Validation, methodology, writing—reviewing. Bony Shashikant Dasari:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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