RESEARCH PAPER

Development and Evaluation of Nanoemulsion-Based Formulation of *Madhuca Longifolia* for the Treatment of Skin Diseases

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ABSTRACT
Background: Skin diseases are commonly caused by bacterial pathogens such as *Staphylococcus aureus*, and are a global concern with limited treatment due to poor penetration of topical agents into the skin. Herbal oil gives promise to treat skin disease because of the phytoconstituents present in it. So, to overcome the poor penetration of topical agents, nanotechnological formulations based on herbal oils have been prepared to improve the topical penetration of constituents.
Objective: The study aims to prove the antibacterial activity of *Madhuca longifolia* seed oil and then compare it with a developed and optimized nanoemulsion-based formulation of *Madhuca longifolia* seed oil.
Methods: Evaluating the antibacterial activity of *Madhuca longifolia* seed oil, then preparing nanoemulsion formulations based on it using the oil-in-water emulsification method via ultrasonication. The nanoemulsion formulations are characterized by particle size, polydispersity index, zeta potential, pH, viscosity, and thermodynamic stability, and then evaluated and compared for their antibacterial activity against *Staphylococcus aureus* via zone of inhibition and the Minimum inhibitory concentration.
Result: The optimized formulation NE5 exhibits the smallest droplet size (131.53 nm), low PDI (13.9%), zeta potential of (–30.0 mV), and pH, which is suitable for topical preparations and excellent thermodynamic stability. No zone of inhibition is observed for both MLSO and NE5 due to their poor diffusion of hydrophobic oil. However, NE5 showed lower MIC (11.98 µL/mL) compared to MLSO (44.11 µg/mL), indicating enhanced antibacterial potency.
Conclusion:
Nanoemulsion effectively increased the physicochemical and antibacterial activity of *Madhuca longifolia* seed oil. Supporting its potential as an effective topical treatment for skin infections, future studies should focus on clinical evaluation and extended antimicrobial screening.
Key words: Antibacterial activity, *Madhuca longifolia*, Nanoemulsion, *Staphylococcus aureus*.
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1. INTRODUCTION

Skin is the largest organ of the body that may be affected by thousands of conditions. It plays a crucial role in protecting against bacteria, viruses, and harmful substances. Globally, people of all ages are affected by skin diseases that are caused by various factors like lifestyle, environmental exposure, and sometimes genetics[1], However, it can be categorized into nine common types, such as tumors/cancer, rashes, pigmentation disorders, fungal, viral, bacterial, and parasitic infections, among others[2]. Among all of these, bacterial skin infections are the most common that arise as a primary infection of the skin or as a secondary infection caused by scabies or insect bites[3]. Gram-positive & Gram-negative bacterial pathogens like Staphylococcus aureus (S. aureus) & Pseudomonas aeruginosa (P. aeruginosa) cause a variety of skin infections that range from minor superficial illnesses to severe life-threatening conditions[4]. Several medications, including creams, ointments, and powders, are available on the market to treat this type of skin disease. However, due to their low skin penetration rate, they can't provide a beneficial response. Skin penetration rate should be enhanced by decreasing the particle size of topical applications, which improves the absorption of the drug into the skin.

Topical drug delivery methods have gained too much interest in recent years for their non-invasiveness and suitable convenience factors that make the skin a key area for simple medications entry as in comparison with parenteral and oral routes[5]. The outermost layer of skin acts as a barrier, which is why topical permeability is being improved[6]. The topical penetration will be improved by nanoparticles that help in easy penetration into the deeper skin layer. Due to their benefits of improving skin absorption, prolonged action, and protecting the medicinal product from deterioration, nanoparticle methods for drug delivery are becoming becoming increasingly common in topical applications these days[7]. Drugs are now delivered to the target place without causing adverse effects via nanoemulsions [8,9].

Nanoemulsions are an advanced nanotechnology-based

drug delivery system with particle sizes ranging between 20nm to 200nm, which promotes skin penetration by granting access to the drug in deep layer of skin tissue[10,11]. Its remarkable solubilization ability for both hydrophilic & lipophilic active ingredients boost the loading capacity and dose administration of the formulation. Both of high-energy and low-energy approaches can be utilized to develop the NE. While the high-energy approach includes membrane emulsification, micro-fluidization, high-energy stirring and homogenization, and ultrasonication, the low-energy method includes spontaneous emulsification, phase and emulsion inversion point[12]. To increase the absorption of nanoemulsion into skin, it is needed to produce nanoemulsion with lecithin, IPM (isopropyl myristate), surfactants, and co-surfactants. Lecithin is largely used in nanoemulsions to encapsulate and transport bioactive components of herbal oil[13]. Several authors have demonstrated that the size of the particles formed during high-energy methods typically decreases with increasing pressure & lecithin content that resulting in smaller particle size and better physical stability[14]. Isopropyl myristate is a class of fatty acids that is widely used due to its effectiveness[15]. Combining IPM and surfactant can enhance viscosity & skin penetration[16]. 1% to 5% IPM exposed physic[15]al characteristics that fulfill the requirement of a NE, which prevents intergranular agglomeration during storage so that the NE becomes stable during the storage process[17,18]. Herbal oil NEs have been successfully prepared using high-energy techniques, according to contemporary NE literature, which shred off enormous oily droplets to the nanoscale [19].

Due to its natural origin and fewer side effects, herbal medications have witnessed exponential development and acceptance in recent years in both developed and developing nations. Herbal oils have been effective in treating so many bacterial skin diseases since the ancient era because of phytochemicals present in them that show notable antibacterial activity. NEs incorporated with antibacterial herbal oils could enhance the prevention of skin diseases[20,21].

The medium- to large-sized deciduous tree *Madhuca longifolia*, also called the butter nut tree, is mostly found in Nepal, India, and Sri Lanka & is belongs to the Sapotaceae family[22]. Numerous therapeutic uses for it have been documented, including antioxidant[23], anti-inflammatory, analgesic, antipyretic[24], immunosuppression[25], neuropharmacological[26], anthelminthic[27], antiulcer[28], and wound healing activities[29], etc. because of the presence of high quantity of fatty acids and essential oils in it, but its antibacterial activity is not yet been disclosed. If the presence of fatty acids, like palmitic, stearic, oleic, & linoleic acids[30,31]exhibits antioxidant and anti-inflammatory activities, then it could also treat bacterial diseases. Hence, this study firstly proves the antibacterial activity of *Madhuca longifolia* seed oil (MLSO) and then compares it with a developed and optimized nanoemulsion-based formulation of *Madhuca longifolia* seed oil by evaluating its Antibacterial activity using Zone of inhibition (ZOI) and Minimum inhibitory concentration (MIC) methods.

2. MATERIAL AND METHODS

2.1. Materials

Madhuca longifolia (Mahua) seed oil was acquired from **Salvia Cosmeceuticals Pvt. Ltd., New Delhi (India).** Tween 80, Span 80, lecithin was acquired in New Delhi, 110002 (India), from Central Drug House, Pvt. Ltd. Ethanol was obtained from Changshu Hongsheng Fine Chemicals CO., Ltd, Changshu City, Jiangsu Province. Iso propyl myristate was obtained from Loba Chemie Pvt. Ltd. Mumbai- -400005 (India). Propylene Glycol was obtained from Sisco Chem Pvt. Ltd, MH-401208. Every chemical utilized is of analytical quality. The experiment employed water that had been double-distilled.

2.2. Evaluation of Antibacterial Activity of *Madhuca longifolia* Seed Oil (MLSO)

The Antibacterial activity of marketed *Madhuca longifolia* seed oil was checked by the Zone Inhibition Method (Kirby-Bauer method) and MIC methods against *S. aureus*, a Gram-positive bacteria commonly associated with skin infections. The strain was obtained from a recognized microbial culture collection and and kept at 4°C on nutrient agar slants.

2.2.1. Zone of Inhibition (ZOI) Method:

A microbiological method that uses an agar plate to measure antibacterial activity. The inoculum was a bacterial suspension made in sterile saline that was equal to 0.5McFarland standard (~1.5×10^8CFU/mL). After using a sterile cotton swab to equally inoculate Mueller-Hinton Agar (MHA) plates with the bacterial suspension, 100µL of each test sample was impregnated into sterile filter paper discs (6mm) and put on top of the inoculated plates. A negative control was discs soaked with sterile distilled water, whereas a positive control contained ciprofloxacin (3µg/disc). Following a 24h incubation period at 37°C, the ZOI around each disc was measured in millimeters [32].

2.2.2. Minimum Inhibitory Concentration (MIC) Assay:

In 96-well microtiter plates, the broth microdilution technique was used to calculate the MIC. To get different concentrations, MLSO was serially diluted in Mueller-Hinton Broth. Next, 100µL of bacterial suspension (final concentration of 1×10^6 CFU/mL) was added to each well. The positive control was 10 µg of ciprofloxacin. Following a 24-hour incubation period at 37°C, the plates were examined visually and using a microplate reader to measure optical density at 600 nm to evaluate the bacterial growth. The minimum concentration that prevented discernible development was known as the MIC.

Additional parameters, such as the **NIC (Non-Inhibitory Concentration)** and **IC_{50}** values, were calculated to further assess antibacterial activity [33].

2.3. Formulation of NE

2.3.1. Composition of NE

Table 1: Composition of NE

Sr. No	Ingredients	NE1 (ml)	NE2 (ml)	NE3 (ml)	NE4 (ml)	NE5 (ml)
1.	MLSO	5	5	5	5	5
2.	Lecithin	0.5	0.5	1	1	1.5
3	Isopropyl Myristate	2	3	4	5	6
4.	Span 80	5	5	5	5	5
5.	Tween80	10	12	14	16	18
6.	Ethanol	3	3	3	3	3
7.	Propylene Glycol	2	2	2	2	2
8.	Distilled Water	Q. S	Q. S	Q. S	Q. S	Q. S

2.3.2. Preparation of NE

Preparation of NE is divided into two phases: Oil phase & Aqueous phase. The oil phase is ready by mixing lecithin & Span 80 with MLSO and IPM, stirring continuously at 40°C until a homogeneous mixture is generated. The aqueous phase is ready by dissolving double-distilled water with Tween 80, ethanol, and propylene glycol and agitated at 40°C. To create a pre-emulsion, emulsification is carried out by gradually adding oil phase to aqueous phase during homogenization at a high speed (10,000–12,000rpm; providing 3-4ºC temperature via ice water bath for 15 minutes). For stable NE and to minimize droplet size, the pre-emulsion is ultrasonically sonicated for 10 minutes at 20kHz and 50% amplitude. Lastly, a 0.22µm membrane filter is used to filter the NE, and then kept at 4°C for further tests.

2.4. Characterization of Nanoemulsion

2.4.1. Particle Size and Polydispersity Index (PDI)

A dynamic light scattering apparatus equipped with a Litesizer 500 Particle Analyzer (Anton Paar GmbH, Austria) was used to measure the NEs particle size distribution, mean droplet size, and polydispersity index (PDI). To prevent multiple scattering effects, the NE was diluted with suitable double-distilled water. Even with the same particle size and lower PDI values, the formulation remains more stable and dependable for drug delivery. In pharmaceutical nano-formulations, a PDI value less than 0.3 is favourable, and values up to 0.5 are also suitable for some emulsions. All measurements were performed in triplicate [34].

2.4.2. Zeta Potential

By measuring the electrical charge on the surface of the droplets, zeta potential is a crucial sign of the NE's physical stability. Particles are prevented from adhering to one another by electrostatic repulsion caused by higher positive or negative zeta potential values. The Litesizer TM 500 (Anton Paar) was used to measure the zeta potential. Zeta Potential values between -30mV and +30mV indicate strong stability. Particle size and zeta potential work together to offer important information about the NEs functionality, uniformity, and shelf life[35].

2.4.3. pH Determination

A digital pH meter (Labman, Model LMMP-07) is used for measuring the pH of NE. To measure, immerse the electrode of the digital pH meter into 2-5ml of NE and wait for 60seconds. pH ranges from 5.0 to 6.5 for topical applications indicate the minimal irritation risk.

2.4.4. Viscosity Measurement

A Brookfield viscometer using spindle No. 63 running at 100 rpm was used to measure the viscous of the MLSO NE (Model DV-E, Brookfield Engineering Labs, USA) at 25°C. Each sample was put in a clean beaker and allowed to equilibrate before being measured. Viscosity gives information on the formulation's flow behaviour and topical spreadability [36].

2.4.5. Thermodynamic Stability Studies

Multiple kinds of thermodynamic stability tests were performed on NE compositions to determine their physical stability.

- Heating-Cooling Cycles: Each formulation was kept at 40 ± 2 °C and 4 ± 2 °C for 48hours. Six cycles were completed.
- Formulations were freeze-thawed at -20 ± 2 °C and 25 ± 2 °C for three 48hrs cycles.
- Samples centrifuged at 5,000rpm for 30min using a REMI R-8C centrifuge.

Following each test, the formulations were visually examined for evidence of phase separation, cracking, turbidity, or creaming. The absence of physical changes under these settings was seen as evidence of stability [37].

2.5. Evaluation of Antibacterial Activity of Nanoemulsion

The procedure for evaluating the antibacterial activity of NE is the same as discussed above in the evaluation of antibacterial activity of MLSO.

3. RESULT

3.1. Characterization of NE

All five NE formulations: NE1, NE2, NE3, NE4, and NE5 were prepared *using Madhuca longifolia* seed oil (MLSO), as shown in Fig. 1, and were characterized for their particle size, zeta potential, polydispersity index (PDI), pH, and viscosity. The data are summarized in Table 2.

Fig.1. Final nanoemulsion preparation based on MLSO

3.1.1. Particle Size and Polydispersity Index (PDI)

From NE1 TO NE5, a progressive reduction in droplet size and PDI was observed, which indicates the enhancement in emulsification and droplet uniformity. NE5 has been found to be the smallest particle size (131.53nm) and lowest PDI value (13.9%) from all the formulations, which indicates its excellent droplet uniformity with minimal aggregation. The particle size distribution and PDI profile of NE5 are seen in Figure 2.

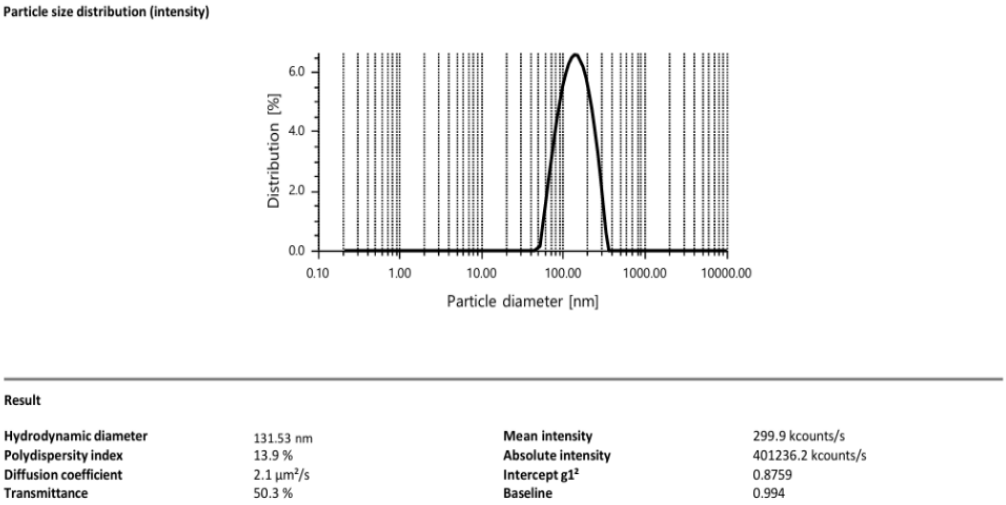

Fig. 2. Particle size distribution and PDI profile of NE5.

3.1.2. Zeta Potential

Zeta potential measurements revealed increasingly negative values across all the formulations, reflecting enhanced electrostatic repulsion and improved colloidal stability. NE5 exhibited the highest negative surface charge (–30.0 mV), indicating excellent physical stability. The Zeta Potential of NE5 is shown in Figure 3.

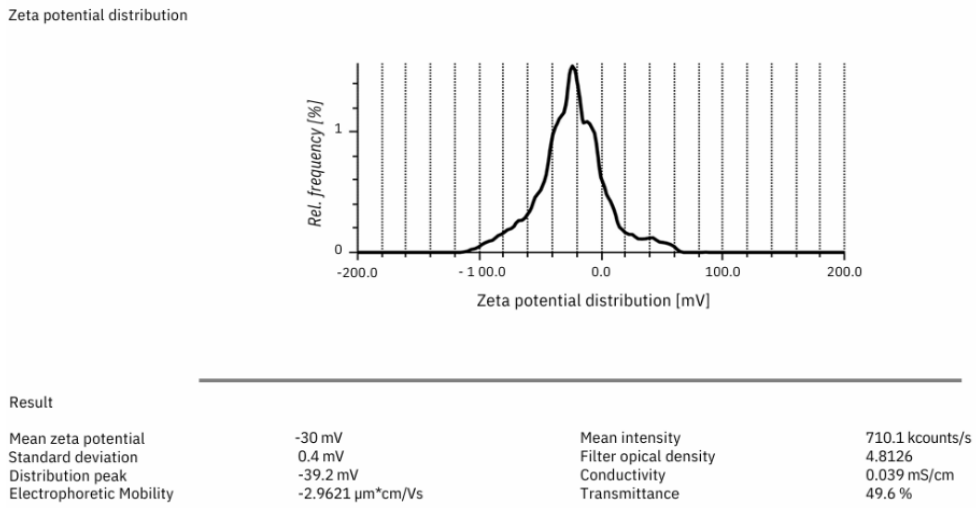

Fig.3. Zeta Potential value of NE5.

3.1.3. pH Determination

The pH values for all formulations are shown in Table 2 and are appropriate for topical applications, which range from 5.0 to 6.5.

3.1.4. Viscosity Measurement

The viscosity values for all formulations are favorable to the topical parameters, have suitable spreadability, and are easy to apply. Values are seen in Table 2.

Table 2: Physicochemical properties of all NE formulations.

Formulation	Particle Size (nm)	PDI (%)	Zeta Potential (mV)	pH	Viscosity (cP)
NE1	149.33	27.5	–19.0	6.18	98.6
NE2	148.70	16.8	–21.9	6.03	90.4
NE3	146.60	15.4	–24.4	5.88	83.1
NE4	139.53	14.3	–28.6	5.73	76.9
NE5	131.53	13.9	–30.0	5.62	72.3

3.1.5. Thermodynamic Stability Studies

To assess the thermodynamic stability of formulations, all samples were subjected to heating–cooling cycles, centrifugation stability tests, and freeze–thaw cycles. No phase separation was observed in any formulation during heating or centrifugation. NE1 and NE2 exhibited slight turbidity or visual changes during the freeze–thaw cycles, suggesting moderate sensitivity to temperature fluctuations. However, NE3, NE4, and NE5 remained completely stable, retaining their translucent appearance under all stress conditions. Notably, NE5 exhibited no signs of instability during any of the tests, confirming its excellent thermodynamic stability. These results are shown in Table 3.

Table 3. Thermodynamic Stability of all NE Formulations.

Formulation	Heating–Cooling	Freeze–Thaw	Centrifugation	Physical Appearance	Stability Outcome
NE1	No separation	Slight turbidity	No separation	Translucent	Stable
NE2	No separation	Mild change	No separation	Translucent	Stable
NE3	No change	No change	No separation	Translucent	Stable
NE4	No change	No change	No separation	Translucent	Stable
NE5	No change	No change	No separation	Translucent	Highly stable

From all NE formulations, NE5 is selected for further evaluation of antibacterial activity due to its excellent physicochemical and thermodynamic stability among all formulations.

3.2. Comparison of the antibacterial activity of both MLSO and NE5

The comparison study of the antibacterial activity of both MLSO and NE5 was evaluated against *S. aureus* by outcome in ZOI and MIC values.

3.2.1. Zone Inhibition Method

Both MLSO and NE5 show no zone of inhibition up to their 100% concentration per disc, but the positive control, ciprofloxacin (3µg), produced an inhibition zone at 27.33±0.45nm as shown in Table 4.

Table 4. Zone of Inhibition of MLSO and NE5 Against *Staphylococcus aureus*

Microorganism	Ciprofloxacin (3 µg)	MLSO (100%)	NE5 (100%)
Staphylococcus aureus	27.33 ± 0.45 mm	No zone	No zone

3.2.2. Minimum Inhibitory Concentration (MIC) Assay
Both MLSO & NE5 showed MIC against *S. aureus* via the broth microdilution method. The values MIC, NIC, and IC50 are shown in Table 5. The MIC value of NE5 was lower compared to MLSO, which indicates greater antibacterial potency.

Table 5: Comparison of the antibacterial activity of both MLSO and NE5 by MIC assay

Sample	MIC (µg/mL or µL/mL)	NIC (µg/mL or µL/mL)	IC50 (µg/mL or µL/mL)
MLSO	44.11 µg/mL	14.94 µg/mL	176.5 ± 0.139 µg/mL
NE5	11.98 µL/mL	7.375 µL/mL	8.082 ± 0.035 µL/mL

4. DISCUSSION

In this study, a stable NE-based formulation of MLSO was successfully developed and evaluated. The optimized NE5 exhibited favorable physicochemical characteristics, including a small droplet size, low PDI, tolerable zeta potential, and fair thermodynamic stability from other NE formulations, which indicates its suitability for topical use. Increase in lecithin, IPM, and surfactant content during the preparation of NE significantly helps in stabilization, smaller particle size, and skin penetration of NE, which prevents intergranular agglomeration during the storage process. The assessment of antibacterial activity revealed that MLSO and NE5, show no ZOI. This could be credited to the hydrophobic nature of oil, limiting its diffusion through the hydrophilic agar medium, thus preventing the migration of active components. The NE exhibited a lower MIC value (11.98 µL/mL) compared to the MLSO (44.11 µg/mL) due to the aqueous nature of the broth facilitated better dispersion of the NE droplets, enhancing their interaction with bacterial membranes and leading to improved antibacterial efficacy. The developed NE promises as a safe and effective topical antibacterial formulation for managing skin infections, particularly against *S. aureus*. Its natural origin and enhanced antimicrobial performance suggest its potential as an alternative therapy to synthetic drugs. Further studies are recommended to validate the preclinical and clinical trials to confirm its efficacy and therapeutic potential, and investigate its activity against other skin pathogens.

5. CONCLUSION

This research validates the potential of nanoemulsion-based delivery systems to enhance the therapeutic efficacy of Madhuca indica seed oil (MLSO) for their topical antimicrobial potential. The optimized formulation NE5 exhibited excellent physicochemical properties, including clarity, stability, and suitable pH for topical application due to the incremental increase of IPM, lecithin & Tween 80. The comparative study of both MLSO and NE5 lacked surface antibacterial activity in disc diffusion assays by showing low ZOI, NE5's significantly lower MIC and NIC values in broth microdilution tests highlight its superior antimicrobial potency. These results suggest that nanoemulsification not only stabilizes natural bioactives but also improves their penetration and bioavailability, making NE5 a viable candidate for pharmaceutical and cosmeceutical applications. The study emphasizes the importance of formulation science in overcoming the limitations of traditional herbal treatments. Future research should focus on in vivo evaluations, broader antimicrobial screening, and clinical trials to establish NE5's safety and efficacy across diverse skin pathogens. This approach could pave the way for innovative, plant-based therapies in dermatology.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

There is no conflict of interest among the authors

8. AUTHORSHIP CONTRIBUTION

Achal Tadas - Writing - original draft - Conceptualization.
Dr. Tarun Parashar – Writing - review, visualization, editing, investigation.
Ms. Chavi Mittal – Writing - review, visualization

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