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Rosemary-loaded nanolipid carriers for multifunctional antioxidant, anti-inflammatory, and antifungal therapy enhancement

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Abstract. *Padmanabhan S, Dharshini BP, Vasugi S, Dilipan E. 2025. Rosemary-loaded nanolipid carriers for multifunctional antioxidant, anti-inflammatory, and antifungal therapy enhancement. Asian J Nat Prod Biochem 23: 146-152.* We developed and characterized rosemary-loaded Nanostructured Lipid Carriers (NLCs) and evaluated their multifunctional bioactivity. Rosemary extract (Soxhlet, 80% methanol) was incorporated into a lipid matrix via a microemulsion route. Physicochemical analyses confirmed successful encapsulation and stability: X-ray diffraction showed a semi-crystalline/disordered matrix consistent with efficient loading, and FTIR revealed characteristic bands (O–H, C–H, C=O/C–O–C) indicating compatible interactions between extract and lipids. In vitro bioassays demonstrated strong, concentration-dependent effects. Anti-inflammatory activity (protein-denaturation model) rose from 44% inhibition at 50 µg to 94% at 200 µg. Antioxidant capacity (DPPH) increased from 52.32% at 50 µg to 89.47% at 200 µg. Antifungal testing against *Candida albicans* showed dose-dependent zones of inhibition, with maximal effects at the highest tested volume (100 µL). Collectively, these data indicate that NLC encapsulation enhances the functional performance of rosemary bioactive across complementary therapeutic axes anti-inflammatory, antioxidant, and antifungal while maintaining structural integrity of the carrier system. The eco-friendly formulation strategy, relying on biocompatible lipids and surfactants, supports improved bioavailability and stability and is well-suited for translation to topical wound care, infection control, and targeted drug-delivery applications. Future work will optimize particle attributes and release kinetics and benchmark efficacy against standard comparators in relevant preclinical models.

Keywords: Bioactive compounds, drug delivery system, lipid-based nanocarriers, rosemary extract, therapeutic potential

INTRODUCTION

A Nanostructured Lipid Carrier (NLC) is a solid matrix–based drug delivery system at room temperature, composed of physiological, biodegradable, and biocompatible lipids with surfactants. Approved by regulatory bodies for various drug delivery modalities, NLCs are considered a superior alternative to Solid Lipid Nanoparticles (SLNs) because the inclusion of liquid oil droplets within the solid matrix increases drug loading capacity and minimizes drug mobility. Compared with polymeric nanoparticles, NLCs offer distinct advantages such as reduced toxicity, biodegradability, drug protection, controlled release, and solvent-free production. They have been extensively explored for delivering both hydrophilic and hydrophobic compounds (Iqbal et al. 2012) and are regarded as highly promising solubilizing systems (Chinsriwongkul et al. 2012). Key therapeutic benefits include enhanced solubility, improved stability, higher permeability and bioavailability, reduced side effects, prolonged half-life, and targeted tissue distribution (Fang et al. 2013; Afeeza et al. 2025).

Several preparation methods have been established, with solvent diffusion and hot high-pressure homogenization (≥80°C) being the most effective for scalable synthesis (Selvamuthukumar and Velmurugan 2012). Stability during storage remains critical to ensure chemical, physical, and microbiological integrity (Obeidat et al. 2010). The nanoscale size and lipid matrix also allow NLCs to penetrate biological barriers, including the brain, offering controlled release, high drug loading, biocompatibility, and scalability (Agrawal et al. 2020). Their clinical utility has been demonstrated in wound healing, where reduced particle size, enhanced solubility, and prolonged release improve therapeutic efficacy and sustain dosing at the wound site (Wathoni et al. 2024). By modulating lipid composition, NLCs can maintain their solid state while incorporating both solid and liquid lipids, ensuring effective drug immobilization and preventing particle agglomeration (Jaiswal et al. 2016). Beyond conventional therapy, NLCs have gained attention in cancer treatment due to their precision delivery and reduced side effects (Naseem et al. 2024), as well as in nutraceuticals to improve the stability and distribution of antioxidants (Gunawan and Boonkanokwong 2024).

Plant-derived bioactive compounds are increasingly explored as alternatives or complements to synthetic drugs due to their broad pharmacological activities and relatively low side-effect profiles. However, many phytochemicals suffer from poor aqueous solubility, low chemical stability, and limited bioavailability, which restrict their clinical translation (Aziz et al. 2021). Nanoencapsulation using lipid-based carriers such as NLCs has proven effective in addressing these limitations by shielding bioactives from

degradation, improving solubilization, and enabling sustained release (Lacatusu et al. 2010; Soleimanifard et al. 2019).

Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is a medicinal plant rich in flavonoids, recognized for its broad-spectrum bioactivities including antioxidant, antibacterial, and antitumoral effects (Lacatusu et al. 2010). Widely used as a food additive in Europe, America, and Asia, rosemary possesses therapeutic potential against infections, diabetes, obesity, and cancer (Khatoon et al. 2020), and has been studied for dermatological applications such as preventing age-related skin damage (Montenegro et al. 2017). It is also applied as a natural preservative in food systems, reducing oxidative spoilage (Kaur et al. 2024), and exhibits antifungal, anti-aflatoxigenic, and herbicidal activities (Hendel et al. 2024).

Inflammation and oxidative stress are closely interrelated pathological processes implicated in the progression of chronic diseases, infections, and tissue damage (Afeeza et al. 2025). Protein denaturation is a widely accepted in vitro model for assessing anti-inflammatory activity, while the DPPH radical scavenging assay remains a standard method for evaluating antioxidant potential (Aksoy et al. 2013; Gunathilake et al. 2018). Furthermore, fungal infections caused by *Candida albicans* pose a significant clinical challenge, particularly due to rising antifungal resistance and limited efficacy of existing therapies (Ross et al. 2008). Plant-based antifungal agents delivered through nanocarrier systems represent a promising alternative approach to address these challenges.

In this context, the present study aims to develop rosemary extract-loaded nanostructured lipid carriers using an eco-friendly microemulsion technique and to evaluate their multifunctional biological activities. Structural characterization of the formulated NLCs was performed using X-Ray Diffraction (XRD) and Fourier-Transform Infrared Spectroscopy (FTIR) to confirm successful encapsulation and molecular interactions. The anti-inflammatory, antioxidant, and antifungal properties of the rosemary-loaded NLCs were systematically investigated through in vitro assays. This study seeks to establish rosemary-loaded NLCs as a promising nanotechnology-based platform for enhanced therapeutic applications in inflammation control, oxidative stress management, and antifungal therapy.

MATERIALS AND METHODS

Collection of samples

The Rosemary plant (*R. officinalis*) was collected from Salem District, Tamil Nadu, India and transported to laboratory. After being washed well the plant material was shade dried for 10-16 days. In shade drying, the sample was dried in air at normal room temperature without sunlight to avoid abiotic stress. After drying, the plant material was continuously monitored and maintained to prevent unwanted things and moisture content.

Preparation of extraction

Approximately 10 grams of powdered rosemary were accurately weighed and placed into a Soxhlet extraction thimble. The round-bottom flask of the Soxhlet apparatus was filled with 80% methanol as the extraction solvent for 4 hrs at 80°C. Following the extraction process, the resulting solution was filtered through Whatman No. 1 filter paper to eliminate insoluble plant debris and particulate matter. The concentrated extract was subsequently transferred to a clean, sterile vial, and stored at 4°C for storage prior to further analysis.

Synthesis of Nanostructured Lipid Carriers (NLCs)

Nanostructured Lipid Carriers (NLCs) were synthesized utilizing the microemulsion method. Initially, a Tris buffer solution (pH 6.8) was prepared, to which a few drops of coconut oil and 16 mg of beeswax were added to 10 mL of the buffer. The mixture was heated to 70°C with continuous stirring until the beeswax completely melted, resulting in a uniform lipid phase. Concurrently, in a separate beaker, 100 mg of Polyvinyl Alcohol (PVA) was dissolved in 10 mL of Tris buffer, and 0.5% w/v Sodium Dodecyl Sulfate (SDS) was incorporated, stirring until fully dissolved. The lipid phase was subsequently and gradually introduced into the aqueous phase while maintaining continuous stirring at 70°C for two hours, ensuring the formation of a stable emulsion. Following this, 100 mg of rosemary extract was introduced to the emulsion, and thorough mixing was conducted to ensure the even distribution of the bioactive compounds. The emulsion was then allowed to cool to room temperature and was stored at -20°C overnight to stabilize the NLCs. After 24 hours, the container was transferred to -80°C for additional freezing, ensuring complete solidification of the lipid particles. Ultimately, the emulsion was subjected to lyophilization, resulting in stable NLCs that are suitable for further characterization and analysis (Garg et al. 2022).

X-Ray Diffraction (XRD)

The dried mixture of nanolipid carriers containing rosemary extract was subjected to analysis using an X'Pert Pro X-ray diffractometer (PANalytical BV, The Netherlands) to confirm the incorporation of the extract within the carriers. The instrument was operated at a voltage of 40 kV and a current of 30 mA, employing Cu Kα radiation to ensure precise measurements. The analysis was conducted in a standard configuration, facilitating the identification of crystalline phases and structural patterns. This method provides valuable insights into the molecular arrangement and stability of the rosemary extract integrated within the nanolipid carrier matrix (Sarvesh et al. 2024).

FTIR analysis

The dried mixture of nanolipid carriers containing rosemary extract was subjected to analysis via a Fourier-Transform Infrared (FTIR) spectrophotometer (Nicolet b6700 FTIR, Thermo Scientific) to identify the functional groups present within the sample. The instrument operated with a resolution of 4 cm^{-1}, encompassing a spectral range of 4000-400 cm^{-1}, thereby ensuring high precision in the

detection of molecular vibrations and chemical bonds. This analysis yielded comprehensive information regarding the interactions between the rosemary extract and the nanolipid carrier matrix, thereby confirming the incorporation and stability of the extract. The FTIR spectra facilitated the identification of characteristic peaks corresponding to the functional groups present in the sample (Dilipan et al. 2023).

Protein denaturation assay

The protein denaturation experiment was conducted with slight modifications to a previously reported method (Gunathilake et al. 2018). A 5 mL reaction mixture was prepared, consisting of 0.2 µL of 1% bovine serum albumin, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and the lyophilized sample at a concentration of 10 mg/mL. The mixture was incubated in a water bath at 37°C for 15 minutes and then heated to 70°C for 5 minutes to induce denaturation. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Optima SP-3000, Tokyo, Japan).

A phosphate buffer served as the control. The inhibition percentage of protein denaturation was calculated using the formula: % inhibition = 100 × (1 - A2/A1), where A1 is the control sample absorption and A2 is the test sample absorption.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The radical scavenging activity of the test sample was assessed utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Aksoy et al. 2013). The procedure entailed the combination of the sample with DPPH solution, followed by incubation in the dark at 37°C for 30 minutes to facilitate the reaction. Subsequent to the incubation period, the absorbance of the DPPH solution was measured at 517 nm employing a UV-VIS spectrophotometer, with ascorbic acid employed as a positive control (Ramachandran et al. 2021).

$$\text{DPPH scavenging effect} = ((A0 - A1)/A0) \times 100,$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Antifungal activity

Antifungal activity was assessed using the agar well diffusion method. The inoculum of *C. albicans* was prepared by culturing the strain in Sabouraud Dextrose Broth (SDB) at 37°C for 24 hours, with turbidity standardized to the 0.5 McFarland standard. Subsequently, Sabouraud Dextrose Agar (SDA) plates were uniformly inoculated with the prepared suspension, and wells measuring 6 mm in diameter were created using a sterile gel punch. Test samples were introduced into the wells in varying volumes (50 µL, 75 µL, and 100 µL). The plates were then incubated at room temperature for a duration of 24 to 48 hours, after which the zones of inhibition surrounding the wells were measured to evaluate antifungal activity (Ross et al. 2008).

Statistically analysis

All experiments were performed in triplicate, and results were expressed as mean ± Standard Error of the Mean (SEM). Statistical analyses were conducted using OriginPro software. Pearson correlations and one-way ANOVA were applied to evaluate the significance of parameters and treatments. Additional analyses were performed using SPSS version 21.0, with $p<0.05$ considered statistically significant.

RESULTS AND DISCUSSION

X-Ray Diffraction

The X-Ray Diffraction (XRD) analysis of the Nanostructured Lipid Carrier (NLC) infused with rosemary extract revealed distinct peaks that substantiate its semi-crystalline nature (Figure 1). Notable diffraction peaks were observed at 2θ values of 11.21°, 16.54°, 22.03°, 22.59°, 26.24°, 31.14°, and 41.84°. The sharp and intense peak at 22.59° indicates a highly ordered crystalline region, suggesting an alignment of lipid molecules and effective encapsulation of rosemary extract within the carrier. In contrast, the broader peaks with lower intensities, such as those at 11.21° and 16.54°, indicate amorphous regions, which are essential for enhancing drug-loading efficiency and promoting sustained release properties of the carrier. These findings confirm that the rosemary extract was integrated into the lipid matrix without compromising its structural integrity.

Fourier-Transform Infrared spectroscopy

The FTIR spectrum of the Nanostructured Lipid Carrier (NLC) infused with rosemary extract reveals essential functional groups and molecular interactions, thereby confirming the successful incorporation of the extract into the lipid matrix (Figure 2). The broad peaks at 3348 cm^{-1} and 3289 cm^{-1} are indicative of O–H stretching vibrations, signifying the presence of hydroxyl groups from both the lipid carrier and the rosemary extract. These bands suggest the occurrence of hydrogen bonding, which contributes to the stabilization of the system. The peaks at 2916 cm^{-1} and 2848 cm^{-1} are attributed to C–H stretching vibrations from the alkyl chains of the lipids, further affirming the lipid nature of the carrier. The sharp peak at 1589 cm^{-1} corresponds to C=C stretching vibrations characteristic of the aromatic rings present in the phenolic compounds of rosemary extract. Additionally, the peaks at 1402 cm^{-1} and 1287 cm^{-1} indicate C–H bending and aromatic interactions, thereby further substantiating the presence of bioactive compounds in rosemary. A strong band at 1218 cm^{-1} represents C–O stretching, which is associated with esters or phenolic ethers in either the carrier or the extract. Notable absorption bands at 1082 cm^{-1} and 1024 cm^{-1} are attributed to C–O–C stretching vibrations, thereby confirming the existence of ester linkages within the lipid matrix. The peaks at 789 cm^{-1}, 627 cm^{-1}, and 526 cm^{-1} suggest out-of-plane bending vibrations associated with the aromatic structures and lipid functional groups. The FTIR spectrum demonstrates the successful encapsulation of

rosemary extract within the nanolipid carrier, revealing stable molecular interactions that preserve the functional integrity of both components. This confirms the system's potential for pharmaceutical and therapeutic applications.

Anti-inflammatory activity: Protein denaturation assay

The protein denaturation assay for the NLC infused with rosemary extract demonstrates its substantial anti-inflammatory activity, as evidenced by the percentage of inhibition observed at various concentrations (Figure 3). The data presented in the graph illustrate a concentration-dependent inhibition of protein denaturation, which serves as a critical marker of anti-inflammatory properties. At a concentration of 50 µg, the NLC exhibited a 44% inhibition, indicating moderate anti-inflammatory activity. This effect significantly increased at higher concentrations, reaching 56.5% inhibition at 100 µg and 81.5% at 150 µg. The highest concentration tested, 200 µg, exhibited a remarkable inhibition of 94%, thereby confirming the formulation's considerable anti-inflammatory potential.

Antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The results of the DPPH assay for the NLC infused with rosemary extract indicate substantial antioxidant activity, as demonstrated by the percentage of inhibition observed at various concentrations (Figure 4). The data reveal a distinct concentration-dependent enhancement in the scavenging activity of the NLC. At a lower concentration of 50 µg, the system exhibited moderate inhibition of 52.32%, suggesting the presence of bioactive antioxidant compounds. As the concentration increased to 100 µg, the inhibition rose to 67.05%, reflecting an improved capacity for free radical scavenging. At 150 µg, the NLC achieved significant inhibition of 78.84%, underscoring its potent antioxidant potential. The highest concentration tested, 200 µg, recorded an impressive 89.47% inhibition, nearing complete neutralization of free radicals. This progressive increase in antioxidant activity with concentration is attributed to the bioactive compounds present in rosemary extract, including phenolic acids, flavonoids, and terpenoids, which are well-documented for their free radical scavenging properties.

Antifungal activity

The antifungal activity of the NLC infused with rosemary extract was evaluated using the agar well diffusion method (Figure 5). The results indicated a strong efficacy against *C. albicans*. The zones of inhibition surrounding the wells increased in size with escalating concentrations of the NLC, demonstrating a concentration-dependent antifungal effect. At a lower concentration of 50 µL, the zone of inhibition was moderate, signifying initial antifungal activity. Conversely, as the concentration increased to 75 µL and 100 µL, the zones of inhibition expanded significantly, illustrating enhanced efficacy at higher doses.

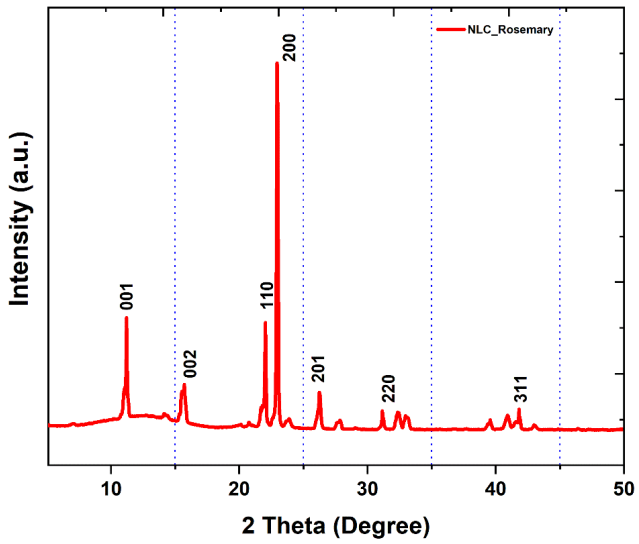

Figure 1. X-Ray Diffraction (XRD) pattern of Rosemary extract-loaded Nanostructured Lipid Carriers (NLCs). The diffractogram shows reduced crystalline peaks of rosemary extract within the lipid matrix, indicating successful encapsulation and transformation to a more amorphous state

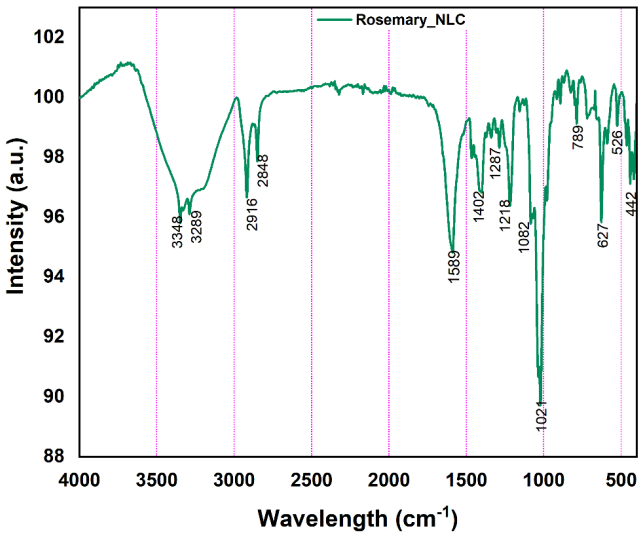

Figure 2. Fourier-Transform Infrared Spectroscopy (FTIR) spectral analysis of rosemary extract-loaded Nanostructured Lipid Carriers (NLCs)

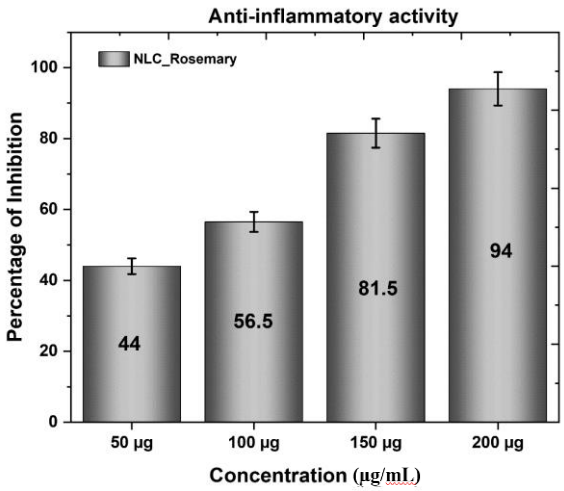

Figure 3. Anti-inflammatory activity of rosemary extract-loaded Nanostructured Lipid Carriers (NLCs)

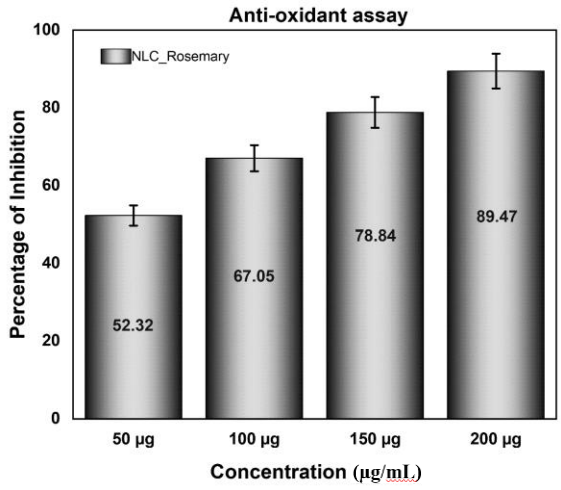

Figure 4. Antioxidant activity of rosemary extract-loaded Nanostructured Lipid Carriers (NLCs)

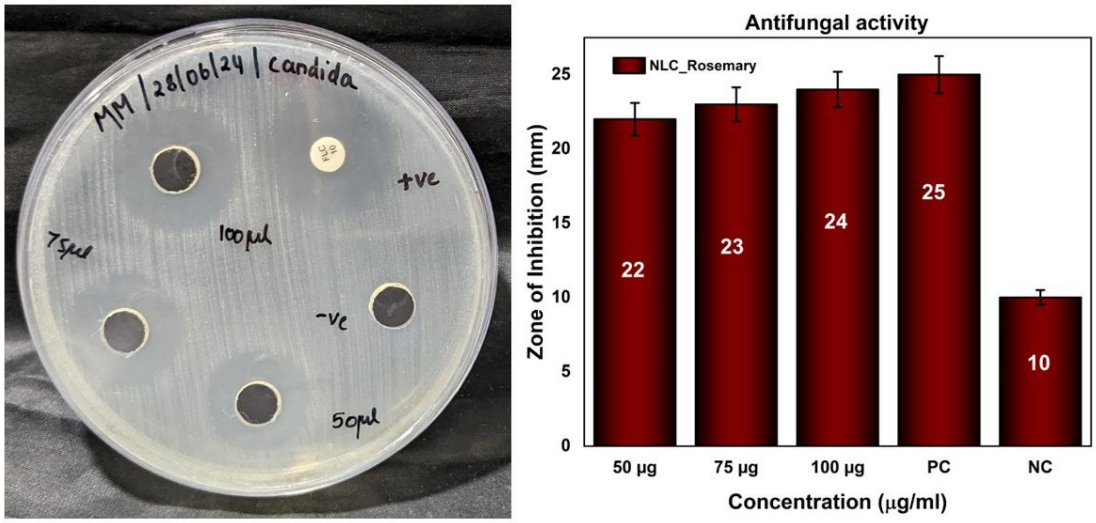

Figure 5. Antifungal activity of rosemary extract-loaded Nanostructured Lipid Carriers (NLCs)

Discussion

The present study demonstrates that Nanostructured Lipid Carriers (NLCs) markedly enhance the therapeutic potential of rosemary extract compared to its free form or conventional delivery systems. Encapsulation improved the stability of bioactive compounds, sustained their release, and enhanced their bioavailability, thereby maximizing antioxidant, anti-inflammatory, and antifungal effects (Javed et al. 2024). These findings are particularly significant given that free rosemary extract is often limited by rapid degradation, low solubility, and reduced bioactivity under physiological conditions (Aziz et al. 2021). By contrast, the rosemary-NLC formulation provided a protective lipid matrix that preserved phenolic compounds such as rosmarinic and carnosic acid, while simultaneously improving their therapeutic delivery.

The XRD and FTIR analyses confirmed the semi-crystalline structure and stable encapsulation of rosemary bioactives, features that explain the superior drug-loading capacity and release kinetics observed in this study. The coexistence of crystalline and amorphous lipid domains has been shown to enhance solubility and facilitate controlled release, consistent with previous reports on lipid-based systems (Soleimanifard et al. 2019; Malekmohammadi et al. 2023). Furthermore, the absence of significant spectral shifts in FTIR suggests that the encapsulation process preserved the chemical integrity of bioactive compounds, preventing degradation (Musuc et al. 2013). Together, these structural features provide mechanistic insight into the observed pharmacological efficacy.

The biological assays further underscore the advantages of rosemary-NLCs. The formulation achieved strong anti-inflammatory activity, likely through improved

stabilization and delivery of rosmarinic and carnosic acid, which are known to suppress pro-inflammatory mediators (Al-Sereiti et al. 1999). Likewise, the enhanced antioxidant activity can be attributed to the sustained release of phenolics such as caffeic acid and flavonoids, which effectively neutralize free radicals (Rašković et al. 2014). The antifungal efficacy against *C. albicans* was also potentiated, most likely due to improved penetration of terpenoids such as cineole and camphor across fungal membranes, an effect facilitated by the lipid carrier system (Agrawal et al. 2020). Collectively, these results highlight the multifaceted therapeutic advantages of NLC encapsulation over free rosemary extract and other conventional carriers.

Despite these promising outcomes, certain limitations must be acknowledged. The present work was confined to in vitro evaluations, and the absence of in vivo or clinical validation restricts the generalizability of findings. Moreover, issues such as large-scale manufacturing, batch-to-batch consistency, and long-term stability remain unresolved, and these represent key barriers to clinical translation. Regulatory challenges surrounding the safety assessment of nanocarrier systems must also be addressed before rosemary-NLCs can be adopted for therapeutic use.

In this study, rosemary-loaded NLCs offer a superior delivery platform that enhances the stability, bioavailability, and therapeutic performance of rosemary bioactives. While these findings provide a strong proof of concept, future work should focus on in vivo validation, pharmacokinetic profiling, and clinical evaluation to bridge the gap between experimental outcomes and practical biomedical applications.

In conclusion, rosemary extract-loaded Nanostructured Lipid Carriers (NLCs) demonstrate considerable biomedical potential, highlighting their multifunctional therapeutic properties. X-Ray Diffraction (XRD) analysis elucidates the structural complexity and disordered polymeric nature of the NLCs, while Fourier-Transform Infrared Spectroscopy (FTIR) confirms the presence of functional groups such as alcohols, amines, and esters, which contribute to their bioactivity. The NLCs exhibit significant anti-inflammatory activity, achieving 94% inhibition of protein denaturation at a concentration of 200 µg/mL, alongside remarkable antioxidant efficacy, scavenging 89.47% of DPPH radicals at the same concentration. Additionally, the NLCs manifest antifungal activity against *C. albicans*, with effective inhibition further enhanced by the encapsulation of rosemary extract. These findings suggest that the incorporation of rosemary extract into NLCs enhances bioavailability, stability, and therapeutic efficiency. This study underscores the potential of rosemary-loaded NLCs as a cost-effective and environmentally friendly nanotechnology platform for various biomedical applications, including drug delivery, wound healing, antioxidant therapy, and disease management. This innovative approach paves the way for further exploration and clinical applications of plant-based nanocarriers.

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