

Original Article



Antibacterial Properties of a *Boswellia serrata* Nanoemulsion Gel in Comparison to Its Hydroalcoholic Extra

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Abstract

Introduction: Bacterial infections are a major health concern worldwide. Although antibiotics are widely used as cost-effective treatments, the rise of multidrug-resistant bacteria has limited their efficacy, increasing the search for alternative antimicrobial agents, particularly plant-derived products. *Boswellia serrata*, rich in bioactive compounds, has recently been investigated for antibacterial potential. This study evaluated the antibacterial activity of a *B. serrata* nanoemulsion-based gel compared with its hydroalcoholic extract.

Methods: Bacterial strains, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 9997), *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* (ATCC 29212), were tested. Nanoemulsions were characterized using dynamic light scattering and transmission electron microscopy. Moreover, the functional groups of the extract and gel were examined by Fourier-transform infrared spectroscopy. Antibacterial activity was assessed by the microbroth dilution method to determine the minimum inhibitory concentration (MIC).

Results: The hydroalcoholic extract demonstrated MICs of 20 mg/mL for *S. aureus* and *P. aeruginosa*, >20 mg/mL for *E. coli* and *K. pneumoniae*, and 2.5 mg/mL for *E. faecalis*. Furthermore, the nanoemulsion showed MICs of <50 mg/mL for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, as well as 25 mg/mL and 6.5 mg/mL for *S. aureus* and *E. faecalis*. The gel formulation also displayed MICs of <50 mg/mL for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, as well as 12.5 mg/mL and 6.25 mg/mL for *S. aureus* and *E. faecalis*.

Conclusion: Both formulations displayed antibacterial activity, particularly against Gram-positive bacteria. The hydroalcoholic extract was more potent, suggesting promise as a natural antimicrobial agent.

Keywords: *Boswellia serrata*, Nanoemulsion gel, Hydroalcoholic extract, Antibacterial activity, Multidrug-resistant bacteria, Gram-positive bacteria



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Introduction

Microorganisms, including bacteria and fungi, are well-established as the primary etiological agents of infectious diseases. Synthetic and semi-synthetic antibiotics and antifungals continue to play a vital role in the effective and cost-efficient control of pathogenic microorganisms in both therapeutic settings and food preservatives (1).

The oral mucosal surfaces of healthy individuals are frequently colonized by transient bacterial flora. Under specific conditions, these bacteria can precipitate various endogenous infections in the orofacial and cervical regions; in some instances, they may even lead to systemic infections, posing serious concerns, particularly in dental practice (2). The discovery and subsequent development



of antibiotics in the early 20th century marked a pivotal milestone in modern medicine, dramatically reducing mortality rates and improving life expectancy (3). This progress fostered widespread optimism among clinicians and researchers regarding the potential eradication of infectious diseases (4). However, the widespread and often indiscriminate use of these drugs has led to the emergence of antimicrobial resistance among numerous pathogenic microorganisms, posing serious global health, therapeutic, and economic challenges (5). In addition, infections caused by antibiotic-resistant bacteria are currently estimated to be responsible for approximately 700,000 deaths annually, a figure projected to exceed 10 million per year in the coming decades (6, 7). The limited pipeline of new antimicrobial agents, coupled with the growing prevalence of multidrug-resistant bacteria, has further contributed to treatment failures, thereby making these pathogens a critical public health threat (4). Consequently, there is an urgent and widely recognized need to discover novel, effective antibacterial agents and alternative therapeutic strategies (3). In this context, natural antibacterial agents have attracted considerable attention as alternatives to synthetic drugs. The appeal of such agents stems from their remarkable structural diversity, potent bioactivity, and unique mechanisms of action, which can help circumvent existing resistance pathways (8). It is noteworthy that the dual pressures of rising antibiotic resistance and the adverse effects associated with many chemical drugs have motivated extensive research into plant-based alternatives (9). In this context, herbal compounds offer distinct advantages, including demonstrated efficacy against resistant strains, fewer side effects, cost-effectiveness, the potential for rapid production, and favorable safety profiles (10, 11). One notable example is *Boswellia*, a medicinal plant with a long-standing history of therapeutic use. The resin, obtained from the trees of the *Boswellia* genus, is renowned for its rich phytochemical composition and diverse medicinal properties (12). Key species such as *B. serrata*, *B. carterii*, *B. papyrifera*, and *B. frereana* contain active constituents, including boswellic acids, incensole, and various terpenes (13, 14). These bioactive compounds are responsible for *Boswellia*'s documented anti-inflammatory, antioxidant, and antimicrobial properties, which support its traditional use in treating conditions, such as asthma, arthritis, bronchitis, and wounds (15–18). The antibacterial and antifungal activities of this medicinal plant, in particular, underscore its potential as a source of novel antimicrobial agents (19). Parallel to the exploration of natural products, nanoemulsions have emerged as highly promising delivery systems for lipophilic bioactive compounds. Some of their advantages include ease of preparation, small droplet size, enhanced bioavailability, and improved stability for encapsulated drugs (20). Furthermore, nanoemulsions are utilized for controlled drug release, transdermal delivery, and site-specific targeting, as well as in formulations for vaccines, gene therapy, and intravenous administration (21). Moreover, they can reduce the toxicity of cytotoxic drugs and protect sensitive compounds from degradation

under physiological conditions (22). Similarly, some studies have suggested that nanoemulsions may possess intrinsic antibacterial properties by mechanisms such as reducing water availability to microorganisms (23). Accordingly, the present study has been designed to investigate the antibacterial effects of *B. serrata* nanoemulsion gel and compare its efficacy with that of the plant's hydroalcoholic extract.

Materials and Methods

Following receiving approval from the Research Council and Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1402.425), this study was initiated by obtaining standard bacterial strains, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 9997), *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* (ATCC 29212), from the Pasteur Institute of Iran (Tehran). Subsequently, the hydroalcoholic extract of *B. serrata* was prepared in the Comprehensive Research Laboratory according to standardized protocols.

Preparation of Hydroalcoholic Extract

The hydroalcoholic extract was prepared from high-quality raw yellow *B. serrata* resin. The botanical identity of the resin was confirmed by morphological comparison with authenticated specimens deposited at the Herbarium of the School of Pharmacy, Birjand University of Medical Sciences. In addition, visible impurities and darkened resin fragments were manually removed prior to extraction. Next, 100 g of the purified resin was macerated in 1000 mL of 70% ethanol for 24 hours under continuous gentle stirring at room temperature. The resulting mixture was then filtered to separate the liquid extract from the residual solids. Subsequently, the filtrate was concentrated using a rotary evaporator at 80°C for 1 hour in order to remove the solvent and obtain a semi-solid extract. Finally, the extract was transferred to amber glass containers and stored at 4°C until further use. The extraction yield was 20.5% (w/w).

Preparation of Nanoemulsion Gel

The *B. serrata* nanoemulsion was prepared using a combination of self-emulsifying and ultrasonic-assisted emulsification techniques based on a previously optimized formulation (24). Briefly, the oil phase consisted of *B. serrata* extract dissolved in isopropyl myristate (2% w/v). Additionally, the surfactant system included Tween 80 (36% w/v), while ethanol (9% w/v) was used as a co-surfactant. Further, the surfactant-to-co-surfactant ratio (*Smix*) was optimized prior to formulation development.

The oil phase was mixed with the *Smix* under gentle stirring, followed by the gradual addition of distilled water under continuous homogenization in order to form a coarse emulsion. The mixture was further subjected to ultrasonic treatment at 150 W for 3 minutes to obtain a clear and stable nanoemulsion.

For gel preparation, Carbopol 934 (1% w/v) was dispersed in distilled water and allowed to hydrate

overnight. Furthermore, the pH was adjusted to 7.0 using 1N sodium hydroxide under gentle stirring. The prepared nanoemulsion was then incorporated into the hydrated gel base under continuous mixing in order to obtain a homogeneous nanoemulsion-based gel.

The final formulation was allowed to stabilize at room temperature and then stored under appropriate conditions until further analysis. It should be noted that the physicochemical characteristics of the optimized nanoemulsion were thoroughly evaluated in our earlier study (25).

Physicochemical Characterization

The physicochemical properties of the *B. serrata* nanoemulsion were evaluated in terms of particle size, morphology and chemical compatibility. Moreover, particle size was analyzed using dynamic light scattering (DLS; Brookhaven instrument, Faculty of Pharmacy, Mashhad University of Medical Sciences). Additionally, the mean droplet size and polydispersity index were recorded to assess size distribution and homogeneity of the formulation. Likewise, morphological evaluation was performed using transmission electron microscopy (TEM; Philips CM120, Partavous Company, Mashhad, Iran). In addition, a diluted sample of the nanoemulsion was placed on a carbon-coated grid, air-dried, and examined to determine droplet shape and dispersion. Fourier transform infrared (FTIR) spectroscopy was also used to investigate possible chemical interactions between the extract and excipients after formulation. Then, the FTIR spectra of the nanoemulsion gel were compared with those of the crude extract in order to evaluate structural integrity and compatibility. It should be noted that the physicochemical characterization and detailed optimization of this nanoemulsion system (i.e., particle size distribution and stability profile) have been comprehensively reported in our previous study (24). Accordingly, only the essential characterization data relevant to the present antibacterial investigation are presented in this manuscript.

Preparation of Bacterial Inoculum

A standardized bacterial inoculum was prepared from freshly subcultured bacterial colonies. Briefly, several well-isolated colonies were transferred into 10 mL of peptone-sodium chloride diluent containing sterile glass beads and then thoroughly mixed for 3 minutes using a vortex mixer to obtain a homogeneous suspension. Further, the turbidity of the suspension was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, and spectrophotometrically verified at 625 nm. The standardized suspension was subsequently diluted to obtain the desired inoculum concentration for antimicrobial testing. Viable bacterial counts were also confirmed by serial dilution and plating on culture media in triplicate. Ultimately, the inoculum was maintained at room temperature and used within 2 hours of preparation.

Determination of Minimum Inhibitory Concentration

The antibacterial activity of the *B. serrata* hydroalcoholic extract and nanoemulsion gel was evaluated using the broth microdilution method in sterile 96-well microplates. Furthermore, Mueller–Hinton broth (MHB) was used as the culture medium throughout the assay. Briefly, 100 μ L of sterile MHB was dispensed into wells 1–10 of each row. Subsequently, 100 μ L of the test sample was added to the first well, and serial two-fold dilutions were prepared across the plate. Following dilution, 100 μ L of the bacterial suspension, previously adjusted to a 0.5 McFarland standard and diluted to achieve a final inoculum concentration of approximately 1×10^6 CFU/mL, was added to each test well. Wells containing only MHB served as negative controls, whereas those consisting of broth and bacterial inoculum without test samples functioned as positive controls. Tetracycline was also included as a reference antibacterial agent. The microplates were then incubated at 37°C for 24 hours. For *E. faecalis*, incubation was performed under candle jar conditions to provide an appropriate atmosphere for growth. After incubation, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the tested sample that completely inhibited visible bacterial growth. All experiments were performed in triplicate, and the results were expressed as the mean of three independent determinations.

Results

Nanoemulsion Morphology Analysis Using Transmission Electron Microscopy, Fourier Transform Infrared, and Dynamic Light Scattering

Transmission Electron Microscopy Analysis

Figure 1 shows the TEM image of the prepared *B. serrata* nanoemulsion. The droplets appeared predominantly spherical with a relatively uniform size distribution. Likewise, they were well dispersed with minimal aggregation, indicating effective stabilization by the surfactant system. Overall, the observed morphology was consistent with typical nanoemulsion structures, confirming the successful formation of a stable colloidal system.

Fourier Transform Infrared Spectroscopy Analysis

The FTIR spectrum of the *B. serrata* nanoemulsion exhibited characteristic absorption bands corresponding

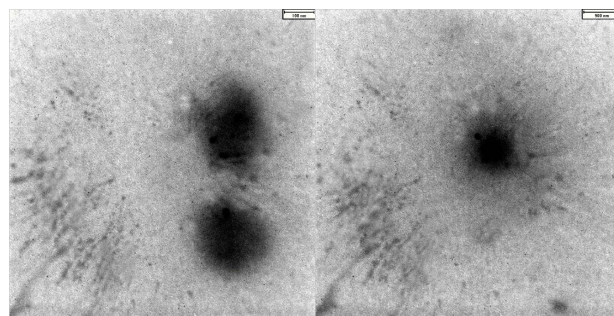


Figure 1. TEM Image of the Synthesized Nanoemulsion
Note. TEM: Transmission electron microscopy

to the main functional groups of the formulation. A broad band around 3500 cm^{-1} was attributed to O–H stretching vibrations, while the peak near 2900 cm^{-1} corresponded to C–H stretching. Additionally, the band observed at approximately 1700 cm^{-1} was assigned to C=O stretching vibrations. Additional peaks in the fingerprint region ($1240\text{--}1045\text{ cm}^{-1}$) were associated with C–O stretching and other phytochemical-related vibrations. Based on the results (Figures 2 and 3), the FTIR profile of the nanoemulsion gel remained comparable to that of the crude extract, implying that the formulation process

did not induce major structural changes in the active constituents (26–28).

Determination of Nanoemulsion Size

DLS analysis revealed that the mean hydrodynamic diameter of the *B. serrata* nanoemulsion was 35.07 nm, confirming the formation of nanosized droplets. The polydispersity index was below 0.3, representing a relatively narrow size distribution and acceptable homogeneity of the system. Moreover, the zeta potential was measured at

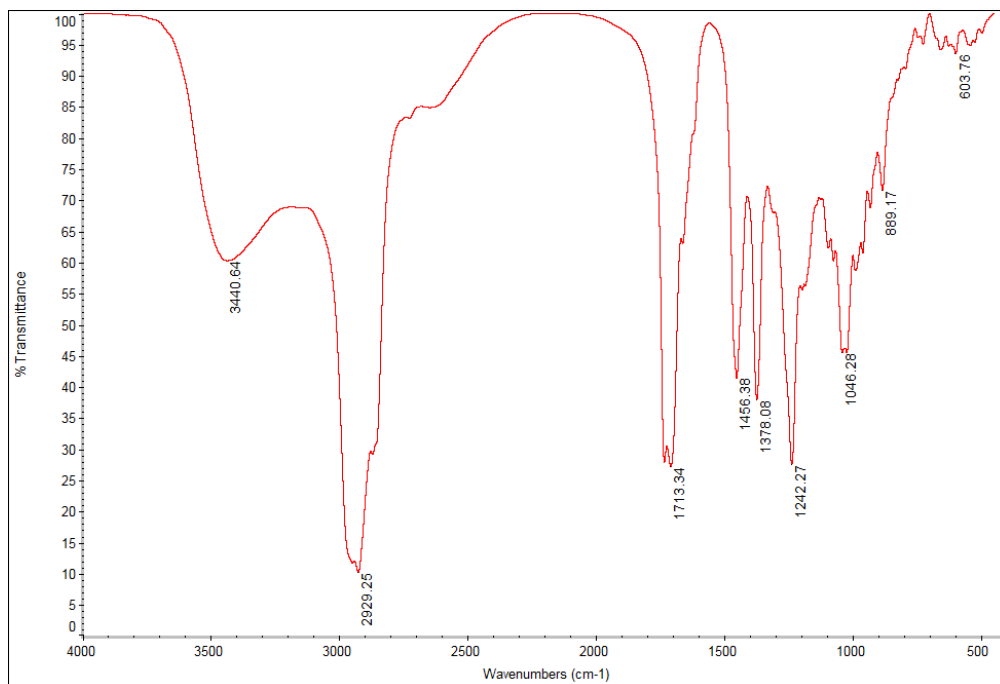


Figure 2. FTIR Spectroscopy Analysis Chart of *Boswellia* Extract
Note. FTIR: Fourier transform infrared

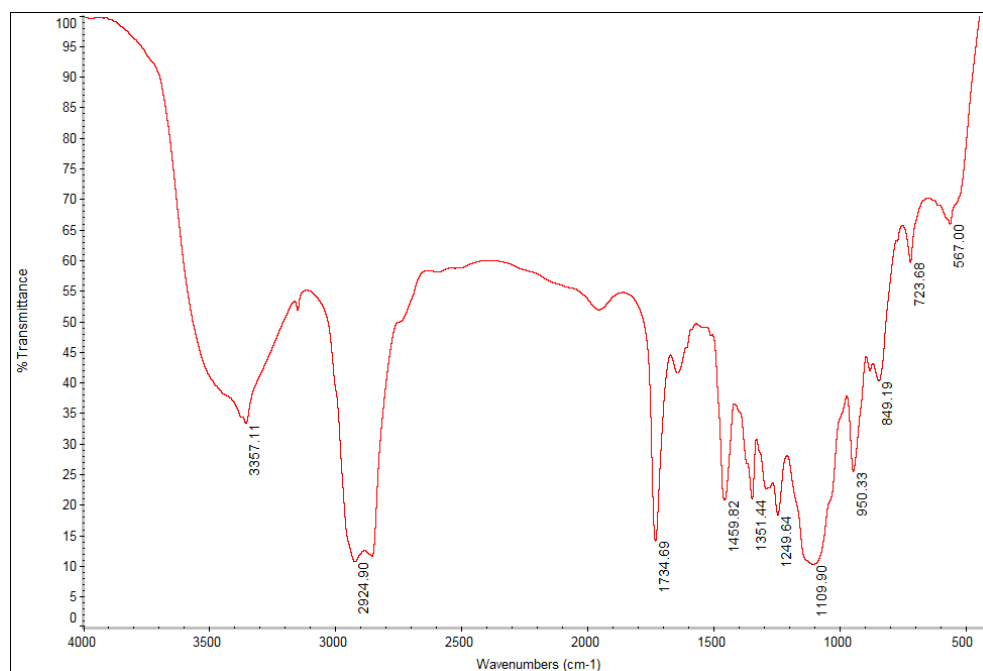


Figure 3. FTIR Spectroscopy Analysis Chart of the Synthesized *Boswellia* Extract Nanoemulsion
Note. FTIR: Fourier transform infrared

−30.68 mV, suggesting moderate physical stability, most likely supported by steric stabilization provided by the surfactant layer. Overall, the formulation demonstrated suitable physicochemical properties for topical application (Figure 4).

Summary of Physicochemical Characterization

The physicochemical properties of the nanoemulsion were previously optimized and thoroughly characterized in our earlier study (25). In the present work, only key representative data (TEM, FTIR, and DLS) have been presented to confirm reproducibility and support the antibacterial evaluation.

Examination of Antibacterial Properties

Antibacterial activity was evaluated using the broth

Table 1. Minimum Inhibitory Concentration of *Boswellia* Extract, Gel, and Tween Emulsion Against Tested Microorganisms

Microorganism	Extract (mg/mL)	Gel (mg/mL)	Tween Emulsion (mg/mL)	Nystatin (µg/mL)
<i>Aspergillus flavus</i>	<20	>50	>50	0.78
<i>Bacillus subtilis</i>	<20	>50	>50	6.25
<i>Staphylococcus aureus</i>	20	12.5	25	0.78
<i>Salmonella typhi</i>	20	>50	>50	3.12
<i>Escherichia coli</i>	2.5	6.25	6.25	6.25

microdilution method against five clinically relevant bacterial strains, the results of which are summarized in Table 1. The hydroalcoholic extract showed MIC values of 20 mg/mL for *S. aureus* and *P. aeruginosa*, > 20 mg/mL for *E. coli* and *K. pneumoniae*, and 2.5 mg/mL for *E. faecalis*. Similarly, the nanoemulsion formulation demonstrated MIC values of 25 mg/mL and 6.5 mg/mL for *S. aureus* and *E. faecalis*, respectively, and < 50 mg/mL for the remaining Gram-negative strains. Likewise, the gel formulation displayed MIC values of 12.5 mg/mL and 6.25 mg/mL for *S. aureus* and *E. faecalis*, respectively, while exhibiting MIC values < 50 mg/mL against the Gram-negative bacteria.

It should be noted that, in some cases, complete inhibition was not achieved within the tested concentration range; therefore, MIC values are reported as ranges. This limitation should be considered when interpreting direct comparisons between formulations. Overall, both formulations depicted measurable antibacterial activity, particularly against Gram-positive bacteria, with a relatively improved effect observed for the nanoemulsion-based system.

Discussion

The present study evaluated the antibacterial activity of *B. serrata* nanoemulsion gel in comparison with its hydroalcoholic extract, providing insights into how formulation may influence the biological performance

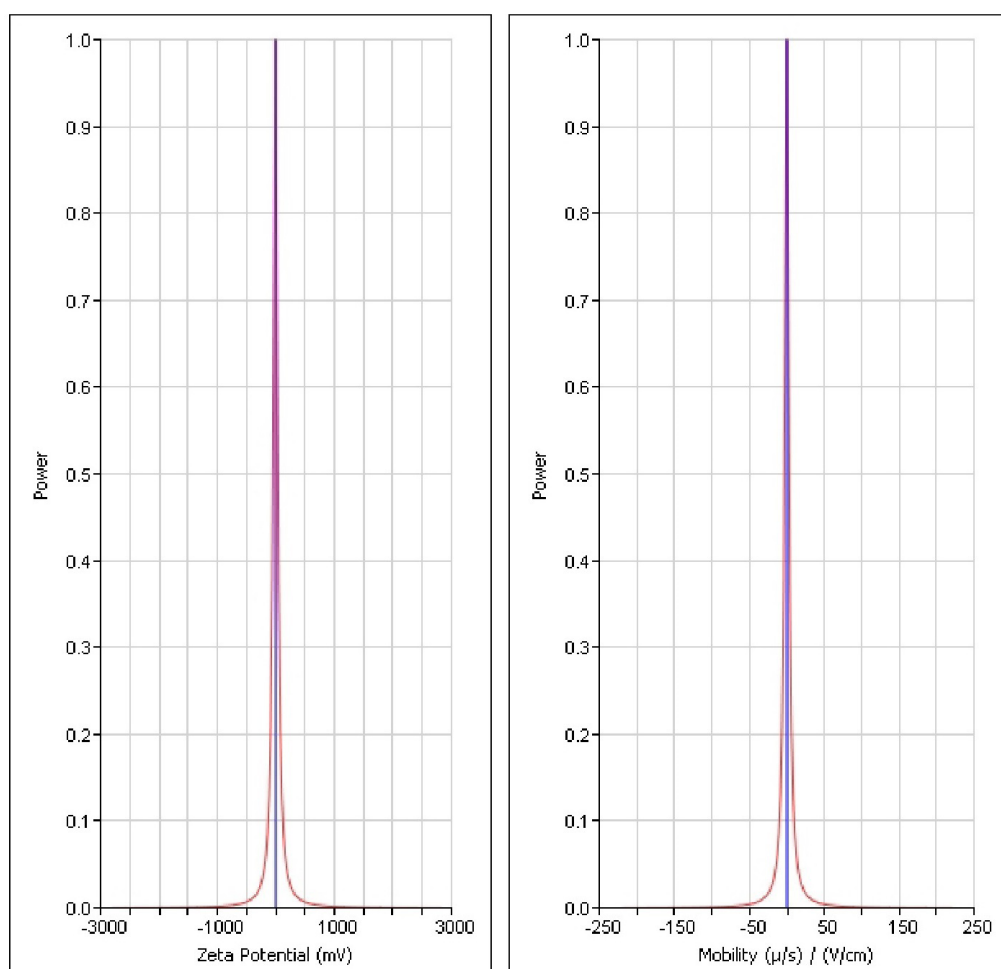


Figure 4. Particle Size Distribution and Zeta Potential of the *Boswellia serrata* Nanoemulsion

of plant-derived compounds. Based on the results, both formulations exhibited antibacterial activity, especially against Gram-positive bacteria. However, the nanoemulsion gel generally showed comparatively higher inhibitory effects in some of the tested conditions. Among the investigated strains, *E. faecalis* appeared to be more susceptible, with lower MIC values observed for both formulations. These findings are consistent with previous reports, indicating that *Boswellia* species tend to exhibit stronger activity against Gram-positive bacteria. This may be related to differences in cell wall structure, particularly the thick peptidoglycan layer, which may allow better interaction with lipophilic phytochemicals (29, 30).

Furthermore, the relatively improved antibacterial performance of the nanoemulsion-based formulation may be attributed to the physicochemical properties of the system. It is noteworthy that nanoemulsion systems can enhance the dispersion of hydrophobic compounds and improve their interaction with microbial cells, potentially increasing the local availability of active constituents. Similar trends have been reported for other plant-derived compounds (e.g., curcumin and tea tree oil), where nanoemulsion-based formulations improved antimicrobial outcomes compared to crude extracts (31). In addition, the gel matrix may contribute to prolonged retention at the application site and sustained release of active compounds, which can further support antibacterial effects over time. Additionally, this issue has been reported in previous studies involving herbal gel systems, such as propolis and aloe vera formulations, where extended contact time was associated with improved antimicrobial performance (32). It is worth noting that both formulations showed relatively limited activity against Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. This observation is in line with the findings of previous studies, indicating that the outer membrane of Gram-negative bacteria can act as a permeability barrier, limiting the penetration of many phytochemicals (33). Nonetheless, further optimization or combination strategies may be required to enhance activity against these strains. Overall, the findings revealed that *B. serrata* nanoemulsion gel may provide improved antibacterial performance compared to the hydroalcoholic extract, particularly against Gram-positive bacteria. Nevertheless, the extent of this improvement should be interpreted with caution due to variability in MIC ranges across different strains. Further, the hydroalcoholic extract itself demonstrated measurable antibacterial activity, highlighting the intrinsic bioactivity of the plant. Thus, it is recommended that further in vivo studies be conducted to better evaluate the therapeutic potential and clinical relevance of this formulation. Finally, the present findings should be interpreted in light of the limitations inherent to in vitro antimicrobial assays and concentration-dependent variability among bacterial strains.

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Authors' Contribution

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

Research Ethics Committees of Birjand University of Medical Sciences Investigating the antibacterial effect of *Boswellia serrata* nanoemulsion gel and comparing it with the hydroalcoholic extract of the plant (IR.BUMS.REC.1402.425).

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