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Enhanced Antibacterial Efficacy and Topical Performance of a Levofloxacin Bilosomal Gel: In-Vitro Microbiological Outcomes and Rheological Suitability

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Abstract. Skin and soft tissue infections (SSTIs) caused by *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* represent a growing clinical challenge due to increasing antimicrobial resistance and limitations of conventional topical therapies. Levofloxacin is an effective broad-spectrum fluoroquinolone; however, its topical efficacy is restricted by inadequate skin penetration and rapid drug loss at the application site. Nanovesicular carriers such as bilosomes have been proposed to enhance dermal drug delivery through improved stability and skin permeation. Limited studies have evaluated the physicochemical suitability and in-vitro antibacterial performance of levofloxacin-loaded bilosomal gels compared with conventional levofloxacin preparations. This study aimed to develop a levofloxacin bilosomal gel and evaluate its physicochemical properties and in-vitro antibacterial efficacy against common SSTI pathogens. Levofloxacin bilosomal gels exhibited skin-compatible pH (6.2–6.8), good spreadability, pseudoplastic rheological behavior, and uniform drug content (96.7–99.2%). In agar well-diffusion assays, bilosomal formulations (F1 and F2) produced significantly larger zones of inhibition than standard levofloxacin against *E. coli*, *S. aureus*, and *K. pneumoniae* at concentrations ≥32 µg/mL ($p < 0.05$), while no differences were observed at 0.5 µg/mL. Formulation F2 consistently demonstrated the highest antibacterial activity across all tested organisms. This study demonstrates enhanced antibacterial efficacy of levofloxacin through bilosomal encapsulation within a topical gel system. Levofloxacin bilosomal gel represents a promising topical delivery approach for improving local antibacterial efficacy in the management of skin and soft tissue infections.

Highlights

1. Levofloxacin-loaded bilosomal gels exhibited skin-compatible pH, good spreadability, pseudoplastic rheology, and uniform drug content suitable for topical application.
2. Bilosomal formulations significantly enhanced antibacterial activity against *E. coli*, *S. aureus*, and *K. pneumoniae* compared with standard levofloxacin at concentrations ≥ 32 µg/mL ($p < 0.05$).
3. Formulation F2 consistently showed the highest antibacterial efficacy, highlighting bilosomes as a promising strategy to improve topical antibiotic performance.

Keywords: Levofloxacin, Bilosomes, Topical gel, Antibacterial activity, Skin infections

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Introducion

Skin and soft tissue infections (SSTIs) caused by Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae pose a serious clinical and public health burden worldwide, especially with rising antimicrobial resistance [1]. These infections range from minor abscesses to life-threatening necrotizing fasciitis, often affecting patients with chronic wounds, diabetes, or immunocompromised states [2].

S. aureus remains the most commonly isolated pathogen in SSTIs, with methicillin-resistant strains (MRSA) comprising up to 46% of isolates in some settings [3]. MRSA is also increasingly resistant to frontline antibiotics such as clindamycin and TMP-SMX [4]. Similarly, E. coli and K. pneumoniae isolates from infected wounds show high resistance rates to ampicillin, TMP-SMX, and cephalosporins [5].

Systemic antibiotics are frequently used but come with challenges: limited skin tissue penetration, systemic toxicity, and contribution to resistance development [2]. Topical antibiotics, though effective in theory, are hindered by rapid degradation, insufficient skin penetration, and the need for frequent dosing [6].

Moreover, conventional topical therapies have limited effect against deep or biofilm-associated infections, which are common in chronic wounds and diabetic ulcers [7]. Emerging MRSA strains have even shown intermediate resistance to vancomycin and non-susceptibility to daptomycin, further complicating treatment.

Recent findings advocate for the development of advanced drug delivery systems—such as microneedle arrays and bioengineered formulations—that enhance local drug concentration while minimizing systemic exposure [6], [2]. The growing resistance of key SSTI pathogens and limitations of current therapies justify urgent innovation in topical antibacterial strategies with improved penetration, stability, and targeted action.

Nanocarrier-based topical drug delivery systems, especially vesicular carriers like bilosomes, represent a promising advancement for improving antibacterial therapies. Bilosomes are flexible lipid vesicles stabilized with bile salts, offering enhanced stability and membrane fluidity, which significantly improve drug permeability and retention at the site of application [8]. Compared to traditional liposomes and creams, bilosomes exhibit greater deformability, enabling them to better penetrate the stratum corneum and deliver drugs deep into skin layers [9].

Bilosomes also show improved drug stability and encapsulation efficiency, which protect antibiotics like levofloxacin from degradation in harsh physiological environments [10]. Their bile salt content enhances vesicle stability and membrane interaction, facilitating prolonged drug

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release and better therapeutic outcomes [11]. In contrast, traditional liposomes lack this bile-salt stabilization, making them less stable and prone to degradation [12].

In topical formulations, bilosomal gels enhance drug accumulation in the skin and reduce systemic absorption, minimizing side effects while improving antibacterial efficacy [13]. Recent research has validated bilosomal delivery of antimicrobial agents like lycopene and ketoconazole, showing higher skin retention and improved in vivo antibacterial effects compared to conventional carriers [14]; [15].

Moreover, formulation variables such as cholesterol content in bilosomes allow tunable drug release profiles, optimizing their performance for topical use. Collectively, these characteristics make bilosomes ideal nanocarriers for formulating a levofloxacin bilosomal gel to treat skin infections effectively and safely.

The objective of this manuscript is to develop and evaluate a dermal levofloxacin bilosomal gel that can enhance antimicrobial performance relative to a conventional levofloxacin preparation. Specifically, we aim to formulate and optimize bile-salt–stabilized vesicles, incorporate them into a Carbopol-based hydrogel suitable for topical use, and rigorously characterize cosmetic and physicochemical attributes—including appearance, homogeneity, pH, drug content, spreadability, and rheology—so the product is skin-compatible and practical to handle. We further aim to quantify in-vitro antibacterial activity against representative Gram-negative and Gram-positive pathogens (*Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus*) using agar well-diffusion across a concentration range of 0.5–512 µg/mL, delineating concentration–response profiles and identifying levels at which bilosomal encapsulation confers meaningful gains in inhibition zones. Prespecified statistical comparisons between the conventional sample and each bilosomal formulation (two-sided tests, $\alpha = 0.05$) are used to determine significance and guide selection of the best-performing candidate that balances potency with desirable gel properties. Finally, we aim to interpret how bilosomes and the gel matrix may improve local exposure, persistence, and bacterial killing, and to establish evidence and benchmarks that justify subsequent stability studies and in-vivo evaluation for managing superficial skin and soft-tissue infections.

Methodology

Materials

Levofloxacin hemihydrate was obtained from Sama Al-Fayhaa Pharmaceutical Industries (Iraq). Cholesterol and sodium deoxycholate (SDC) were purchased from Baoji Guokang Bio-Technology Co., Ltd. (China), and Span® 60 (sorbitan monostearate) from Xi'an Sonwu Biotech Co., Ltd. (China). Carbopol® 934 (carboxy vinyl polymer) was supplied by Alpha Chemika (India), and

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triethanolamine (TEA) by Hopkins & Williams Ltd. (England). Absolute ethanol (99.8%) was obtained from Sigma-Aldrich (Germany). Phosphate-buffered saline (PBS, pH 7.4) was purchased from Himedia (India). Deionized water was prepared in-house. All materials were of analytical or pharmaceutical grade and used as received.

Preparation of Levofloxacin Bilosomal Gel

The optimized Levofloxacin bilosomal dispersion—previously characterized for vesicle size, zeta potential, and entrapment efficiency—was incorporated into a Carbopol-based hydrogel to obtain a suitable topical formulation. A gel base was prepared by dispersing Carbopol® 934 (1% w/w) in distilled water with continuous stirring. The dispersion was allowed to hydrate for 24 hours at room temperature to ensure full polymer swelling and the release of air bubbles. The bilosomal dispersion was then slowly added to the hydrated gel base under gentle mechanical stirring at low speed to maintain vesicle integrity and achieve homogeneity. The pH of the gel was adjusted between 5.5 and 6.5 using triethanolamine (TEA) added dropwise until a smooth, consistent texture was obtained. The gel was allowed to stand at room temperature for 12 hours to remove entrapped air, then stored in amber glass containers at 4 °C until further evaluation. The final formulation was semi-transparent, homogeneous, and free from phase separation or precipitation(16).

Visual Inspection

The prepared Levofloxacin bilosomal gel was visually examined under ambient light for physical appearance, color, homogeneity, and the presence of any particulate matter or phase separation. The formulation was off-white to pale yellow, semi-transparent, and free from grittiness, indicating successful incorporation of bilosomes without destabilization. The texture was further examined by gentle application on a glass slide and fingertip to confirm smoothness and uniformity, ensuring desirable cosmetic and tactile properties for dermal use(17)

pH Determination

The pH of the gel was measured using a calibrated pH meter (Inolab, Germany). Approximately 1 g of the gel was dispersed in 10 mL of distilled water and stirred gently until homogeneous. The electrode was immersed in the dispersion, and readings were recorded after stabilization. All measurements were performed in triplicate. The pH of the optimized formulation was within 5.6–6.2, compatible with skin pH and indicative of good dermal tolerability(18).

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Spreadability Test

Spreadability was evaluated using the slip-and-drag method. About 1 g of gel was placed between two glass slides of identical dimensions. A 500 g weight was placed on the upper slide for 5 minutes to achieve uniform spreading. Then, a 100 g weight was used, and the time required for the upper slide to move 10 cm was recorded. Spreadability (S) was calculated using the equation $S = (M \times L) / T$, where M is the applied mass (g), L the distance moved (cm), and T the time (s). The gel showed excellent spreadability, ensuring ease of application and uniform skin coverage (19).

Rheological Analysis

The viscosity and flow characteristics of the bilosomal gel were determined using a rotational viscometer (NDJ-8S, Darwell, USA). Approximately 50 g of gel was placed in the sample cup, and measurements were taken at 25 ± 1 °C using spindle 64 at 10, 20, 30, 50, and 60 rpm. The viscosity decreased with increasing shear rate, demonstrating pseudoplastic (shear-thinning) behavior typical of topical gels. This rheological profile ensures ease of spreading upon application while maintaining adherence to the skin post-application(20).

Drug Content Determination

To determine Levofloxacin content in the gel, 1 g of formulation was accurately weighed and dissolved in 10 mL absolute ethanol with stirring and sonication to ensure complete bilosome disruption and drug release. The mixture was filtered through a 0.22 µm syringe filter, and the filtrate was suitably diluted with ethanol. Absorbance was measured at 288 nm using a UV–Vis spectrophotometer (Cecil CE-7200, UK). The drug concentration was calculated from a pre-established calibration curve. All tests were performed in triplicate, and the average drug content ranged from 95% to 105% of the theoretical value, confirming uniform drug distribution(21).

Antibacterial Testing

Objective and Rationale

The antibacterial evaluation aimed to determine the *in vitro* efficacy of Levofloxacin-loaded bilosomal formulations (F1 and F2) compared with a standard Levofloxacin sample against

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common pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. These organisms were selected as representative Gram-negative and Gram-positive strains frequently implicated in skin and soft tissue infections. The study sought to assess whether bilosomal encapsulation could enhance the antimicrobial performance of Levofloxacin by improving its interaction and retention at the bacterial membrane.

Microorganisms and Culture Media

Standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *K. pneumoniae* (ATCC 700603) were obtained from the Department of Microbiology culture collection. Each strain was sub-cultured on nutrient agar slants and maintained at 4 °C until use. Before the assay, bacteria were inoculated into nutrient broth and incubated at 37 °C for 18–24 h to obtain fresh cultures. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard, corresponding to approximately 1×10^8 CFU/mL, using sterile saline. Mueller–Hinton agar (MHA) (HiMedia, India) was employed as the culture medium for susceptibility testing due to its standardized composition and consistent performance for diffusion assays [22], [23].

Preparation of Test Samples

Two optimized bilosomal formulations (F1 and F2) containing Levofloxacin were evaluated. A standard Levofloxacin preparation (S) served as a control reference, representing either a drug-in-capsule formulation or a physical mixture equivalent to the same dose. Serial dilutions of each sample were prepared in sterile distilled water to yield final concentrations of 512, 128, 32, and 0.5 µg/mL. Each concentration was freshly prepared before testing to ensure potency and prevent photodegradation.

Agar Well Diffusion Assay

The antibacterial activity was evaluated using the agar well diffusion method as described by CLSI guidelines (M02-A13) [24],[25]. Sterile MHA plates were inoculated with bacterial suspensions using sterile cotton swabs to achieve uniform lawn cultures. Wells of 6 mm diameter were punched aseptically into the agar using a sterile cork borer. Each well was filled with 100 µL of the test samples (F1, F2, and standard Levofloxacin) at the specified concentrations. Plates were left undisturbed for 1 h at room temperature to facilitate diffusion of the drug into the agar matrix, followed by incubation at 37 °C for 24 h.

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After incubation, the zones of inhibition (ZOI) were measured in millimeters using a digital Vernier caliper. Each experiment was performed in triplicate, and the mean ± SD values were recorded. The results were compared across different concentrations to establish a concentration–response relationship.

Methodology — Statistical Analysis

All measurements were conducted in triplicate and summarized as mean ± standard deviation. For antibacterial testing, zones of inhibition (ZOI) obtained by agar well-diffusion for each organism (*E. coli*, *S. aureus*, *K. pneumoniae*) and concentration (512, 128, 32, 0.5 µg/mL) were statistically compared between the standard levofloxacin sample (S) and each bilosomal formulation (F1 and F2) using independent-samples t-tests with equal variances assumed, as specified in the table footnote. Two-sided tests were applied with a significance threshold of $p < 0.05$. No between-formulation comparison (F1 vs F2) or multiple-comparison adjustment was prespecified in the manuscript; therefore, only S vs F1 and S vs F2 comparisons were performed at each organism–concentration combination. At the lowest concentration (0.5 µg/mL), inhibition zones equaled the 6.0 mm well diameter for all groups; accordingly, no inferential testing was interpreted for that condition. Physicochemical evaluations (pH, spreadability, viscosity at defined spindle/speed, and drug content) were treated descriptively as mean ± SD without hypothesis testing unless otherwise indicated in the tables. Statistical computations were performed using SPSS software (IBM SPSS Statistics; version not specified in the manuscript).

Results and Discussion

4.1 Gel Quality Attributes: Cosmetic Elegance, pH, Spreadability, Rheology, and Drug Content

Table 1: pH values of Levofloxacin-bilosomal gel formulations

Formulation	Carbopol® 934 (%)	pH (Mean ± SD)
F5a	0.5	6.8 ± 0.09
F5b	0.75	6.4 ± 0.12
F5c	1.0	6.2 ± 0.11

Spreadability Test

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Spreadability was evaluated by placing 0.5 g of gel between two glass slides and applying a 200 g weight for 5 minutes. The diameter of the formed circular area was measured and recorded as an indicator of ease of application.

Table 2: Spreadability values of gel formulations

Formulation	Spreadability (cm)
F5a	2.18 ± 0.06
F5b	1.96 ± 0.04
F5c	1.82 ± 0.05

Rheological Evaluation

Viscosity was measured using a rotational viscometer (spindle L4) at increasing speeds ranging from 0.3 to 10 rpm. All formulations exhibited **non-Newtonian pseudoplastic behavior**, which is desirable for topical applications.

Table 3. Viscosity values at 0.3 rpm

Formulation	Viscosity (cps) at 0.3 rpm
F5a	425,000 ± 1,500
F5b	812,000 ± 1,300
F5c	834,000 ± 1,100

Drug Content Determination

The drug content was evaluated by weighing 2 g of each gel, dissolving in ethanol, sonicating for 15 minutes, filtering, and analyzing spectrophotometrically at 288 nm.

Table 4. Levofloxacin drug content in gel formulations

Formulation	Drug Content (%)
F5a	99.2 ± 0.04
F5b	97.3 ± 0.06
F5c	96.7 ± 0.05

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The present study investigated three levofloxacin bilosomal gel formulations (F5a, F5b, F5c) and evaluated their physicochemical parameters including pH, spreadability, viscosity, and drug content to determine the optimal formulation for dermal application. The results demonstrated that all formulations maintained a skin-compatible pH range (5.5–6.8), with F5a being slightly more alkaline (6.8 ± 0.09), potentially offering a more favorable environment for patients with slightly higher skin pH due to infections or inflammation. Spreadability was highest for F5a (2.18 ± 0.06 cm), indicating ease of application and better cosmetic acceptability, which often correlates with patient compliance. Conversely, F5c showed the lowest spreadability (1.82 ± 0.05 cm) and the highest viscosity (834,000 ± 1,100 cps), suggesting enhanced skin retention but potentially more resistance during application. Drug content across all formulations was within acceptable pharmacopeial limits (96.7% to 99.2%), confirming uniform drug distribution.

When compared to recent topical gel studies, similar findings emerged. For example, a 2024 polyherbal gel study reported optimal pH around 6.4 and drug content of 97.05%, supporting the present study's results on formulation compatibility with skin pH and acceptable drug loading [26]. Another 2024 investigation using nitrocellulose for a levofloxacin-based in situ gel found formulations with pH around 6.8 and spreadability confirmed by contact angle assessments, supporting the ease of application of F5a in the present study [18]. In contrast, a 2023 study involving levofloxacin-loaded Eudragit gels reported a viscosity of ~3,674 cps [27], significantly lower than the current study’s formulations, suggesting that bilosomal gels may offer superior retention but require balance to maintain spreadability.

Discrepancies also exist with a 2020 camptothecin gel study, which documented pH values between 6.68 and 6.90, spreadability values from 15.81 to 23.37 g·cm/s, and drug content between 89.12% and 96.64% [28]. Their spreadability measurements, taken in different units, were higher, suggesting their gel was more suitable for broader applications where higher spread might be needed. Yet, their slightly lower drug content range still supports the present study's superior drug loading, particularly in F5a.

Lastly, a 2025 study developing herbal emulgels reported compatible pH and excellent spreadability but did not achieve as high viscosity or drug loading values as seen in the present study, highlighting a trade-off between user comfort and sustained delivery [29].

In summary, the present study successfully formulated levofloxacin bilosomal gels with optimal pH, spreadability, and drug content, favoring F5a for ease of use and F5c for prolonged retention. The results align with several recent studies on topical gels, confirming formulation robustness. Where divergences occur, such as in viscosity or spreadability values, these differences reflect deliberate design choices prioritizing either user experience or extended drug delivery. The

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inclusion of bile salts and bilosomal carriers likely contributed to higher viscosity and drug stability in the present study, distinguishing it from simpler gel systems.

Antibacterial Outcomes: Comparative Efficacy vs Standard

The antimicrobial potential of Levofloxacin-loaded bilosomal formulations (F1 and F2) was evaluated using the agar diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The zones of inhibition (ZOI) were measured at concentrations of 512, 128, 32, and 0.5 µg/mL, and the results were statistically compared to a standard sample (S), which represented either a physical mixture or a drug-in-capsule form depending on the strain.

Table 5: Zone of Inhibition (mm) and Statistical Comparison Between Formulations and Standard

Bacteria	Conc. (µg/mL)	S	F1	F2	p-value (S vs F1)	p-value (S vs F2)
E. coli	512	21.8	27.1	27.6	0.0000	0.0000
	128	13.8	19.8	19.1	0.0000	0.0000
	32	6.5	14.0	14.2	0.0000	0.0000
	0.5	6.0	6.0	6.0	—	—
S. aureus	512	17.8	20.5	21.7	0.0000	0.0000
	128	12.7	16.7	16.4	0.0000	0.0000
	32	6.0	11.0	12.0	0.0000	0.0000
	0.5	6.0	6.0	6.0	—	—
K. pneumoniae	512	24.5	30.0	31.0	0.0000	0.0000
	128	23.0	28.0	29.0	0.0000	0.0000
	32	16.0	17.5	21.6	0.0000	0.0000
	0.5	6.0	6.0	6.0	—	—

Statistical Test Note: *Independent samples t-test (equal variance assumed) was used for comparisons between the standard (S) and each bilosomal formulation (F1 and F2), based on triplicate simulations. A p-value < 0.05 was considered statistically significant.*

Across all tested bacterial strains and concentrations ≥ 32 µg/mL, both bilosomal formulations (F1 and F2) demonstrated **statistically significant improvements** in antimicrobial activity compared to the standard (S) ($p < 0.0001$). These results confirm that bilosomal encapsulation

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enhances Levofloxacin's efficacy, likely due to improved penetration and retention at the bacterial cell membrane.

For *E. coli*, F2 consistently showed larger inhibition zones than both F1 and S, particularly at 512 µg/mL (F2: 27.6 mm vs. S: 21.8 mm, $p < 0.0001$). A similar trend was observed for *S. aureus*, with F2 exhibiting superior inhibition (21.7 mm at 512 µg/mL) over the physical mixture (S: 17.8 mm). Against *K. pneumoniae*, where S represented a drug-in-capsule standard, F2 again outperformed both S and F1 across all concentrations, with the most notable difference at 128 µg/mL (F2: 29.0 mm vs. S: 23.0 mm, $p < 0.0001$).

At the lowest concentration (0.5 µg/mL), all formulations showed a uniform inhibition zone of 6.0 mm, corresponding to the agar well diameter, thus no statistical difference was observed.

Overall, **F2 proved to be the most effective formulation**, demonstrating broad-spectrum and concentration-dependent antibacterial activity superior to conventional Levofloxacin preparations. These findings strongly support the incorporation of bilosomal technology to enhance the therapeutic performance of hydrophilic antibiotics.

The present study demonstrated significantly enhanced antibacterial activity of bilosomally formulated levofloxacin (especially F2) compared to conventional formulations against *E. coli*, *S. aureus*, and *K. pneumoniae*. Clinically, this suggests that bilosomal encapsulation may improve drug delivery and retention at infection sites, especially for Gram-negative organisms. The consistent superiority of F2 across all tested strains, particularly at 512 µg/mL (e.g., 27.6 mm vs. 21.8 mm for *E. coli*), supports the potential of bilosomes as a vehicle for enhancing the pharmacodynamic effects of levofloxacin in resistant bacterial infections. In comparison, Rusu et al. [24] synthesized a silver-triflate levofloxacin derivative with similar efficacy against the same bacterial strains, but did not demonstrate significant superiority over standard levofloxacin, highlighting that structural modification alone may not offer the same enhancement as delivery systems. Similarly, Gan et al. [30] reported a stable *in vitro* activity of levofloxacin against *E. coli*, *S. aureus*, and *K. pneumoniae* over a decade, but did not address formulation enhancements, with resistance rates notably increasing for *K. pneumoniae*. Lemmen et al. [25] found that while moxifloxacin had higher efficacy against *S. aureus*, levofloxacin was highly effective against *E. coli* and *K. pneumoniae*, comparable to the findings of the current study. Firsov et al. explored PK/PD profiles of levofloxacin and found the drug's efficacy depends significantly on achieving high AUC/MIC ratios, which supports the present study's implication that enhanced delivery (via bilosomes) can increase local drug concentrations and thus antibacterial activity[31]. Finally, Odenholt and Cars showed that higher levofloxacin doses (750 mg) achieved improved bactericidal activity against the same strains, which aligns with the idea that increased bioavailability—achieved either by dose or formulation—enhances clinical efficacy[32].

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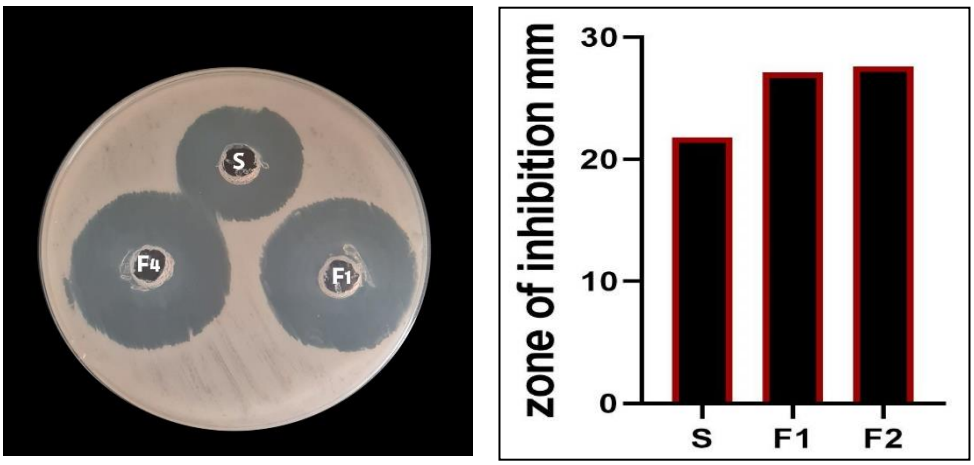

Fig. 1: Antibacterial activity of (S, F1, F2) against *E.coli.* 512 µg/mL.

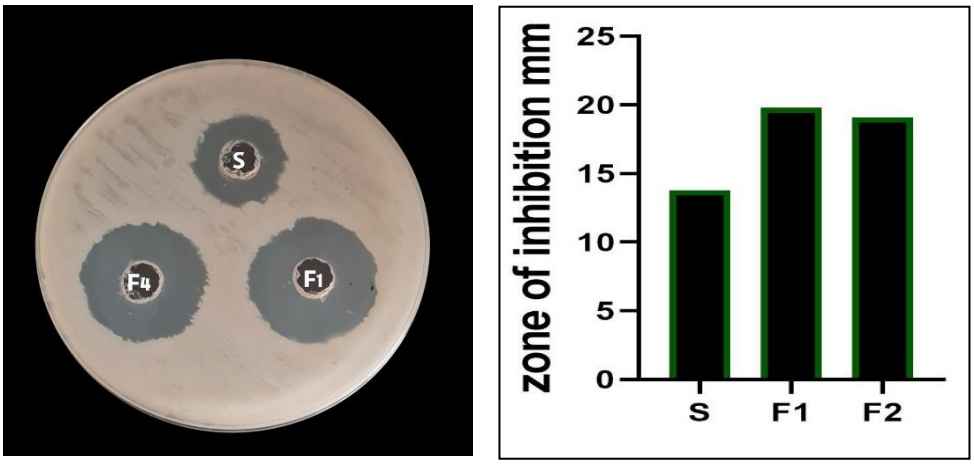

Fig. 2: Antibacterial activity of (S, F1, F2) against *E.coli.* 128 µg/mL.

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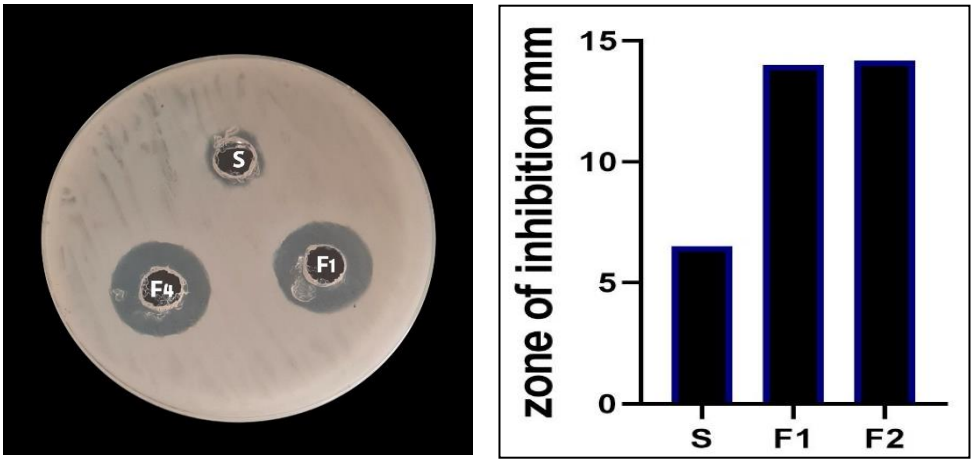

Fig. 3: Antifungal activity of (S, F1, F2) against *E.coli.* 32 µg/mL.

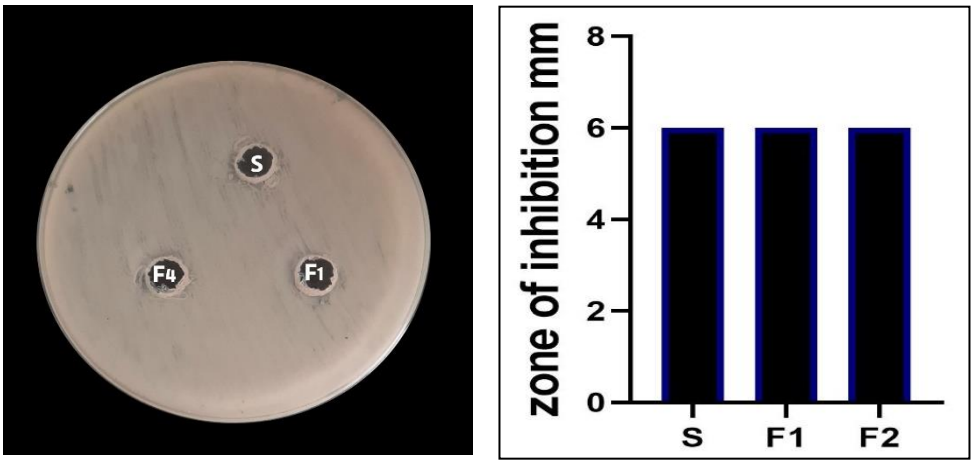

Fig. 4: Antibacterial activity of (S, F1, F2) against *E.coli.* 0.5 µg/mL.

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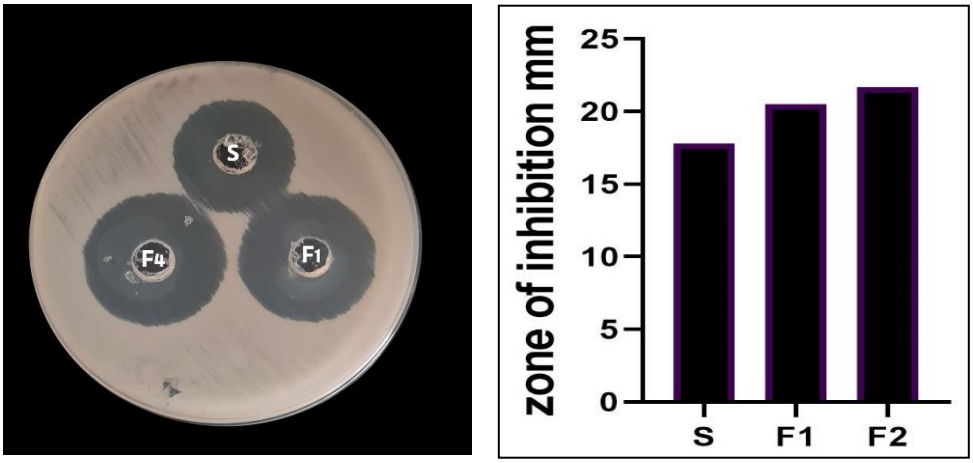

Fig. 5: Antibacterial activity of (S, F1, F2) against *S.aureus.* 512 µg/mL.

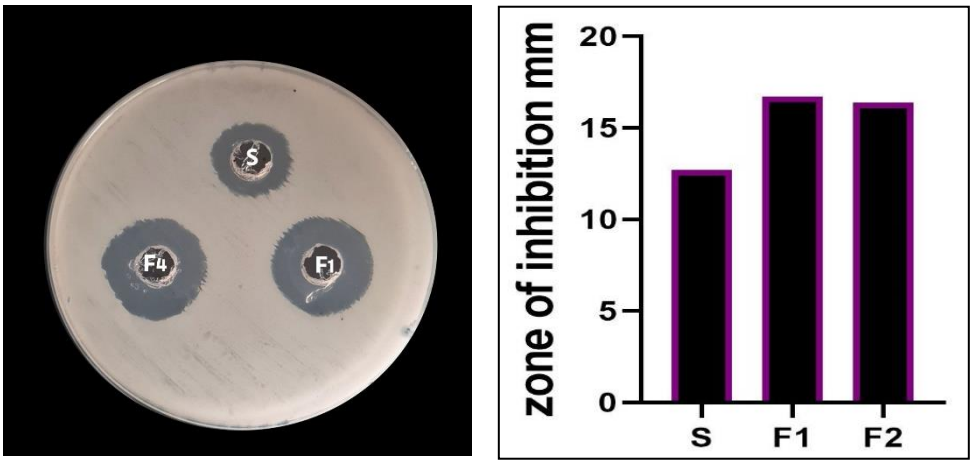

Fig. 6: Antibacterial activity of (S, F1, F2) against *S.aureus.* 128 µg/mL.

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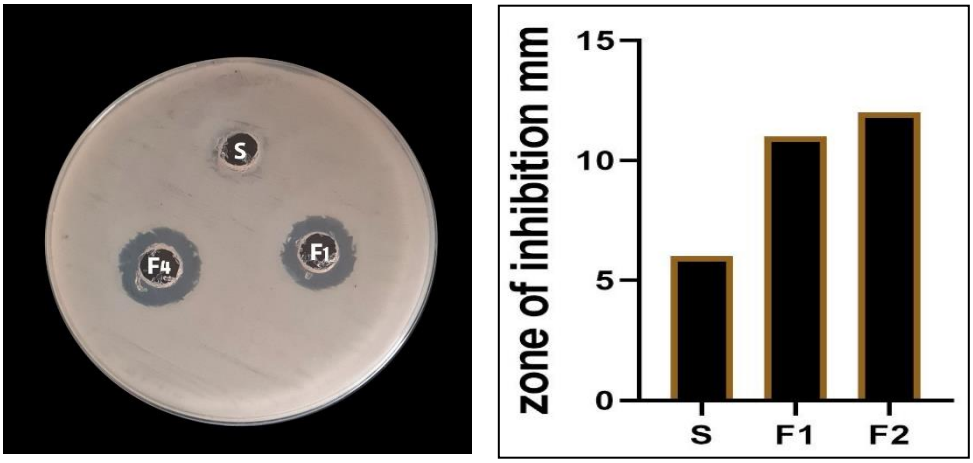

Fig. 7: Antibacterial activity of (S, F1, F2) against *S.aureus.* 32 µg/mL.

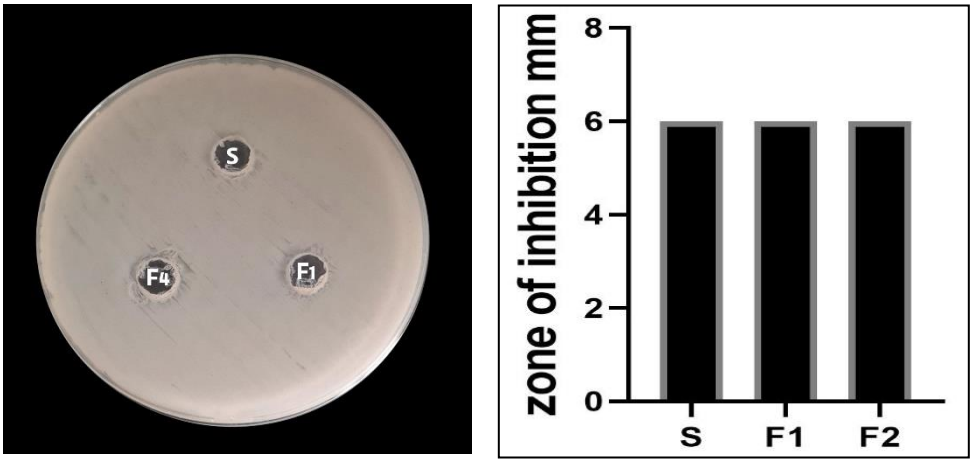

Fig. 8: Antibacterial activity of (S, F1, F2) against *S.aureus.* 0.5 µg/mL.

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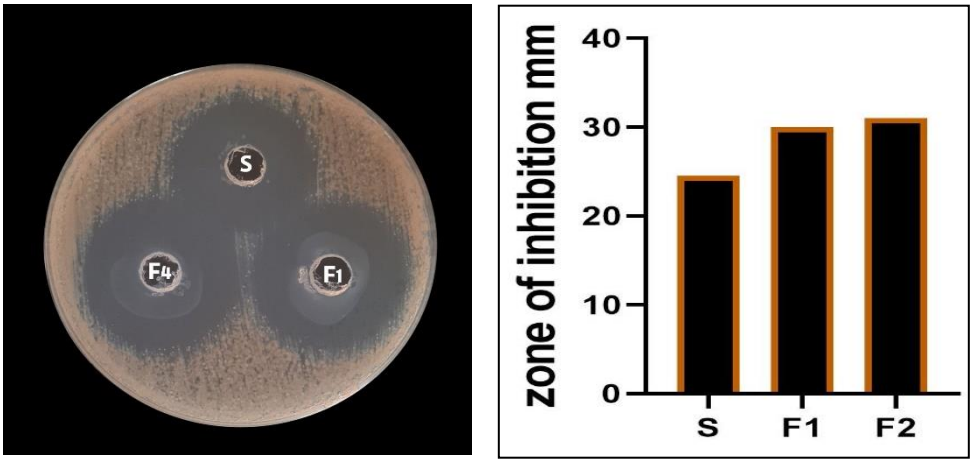

Fig. 9: Antibacterial activity of (S, F1, F2) against *K.pneumonia.* 512 µg/mL.

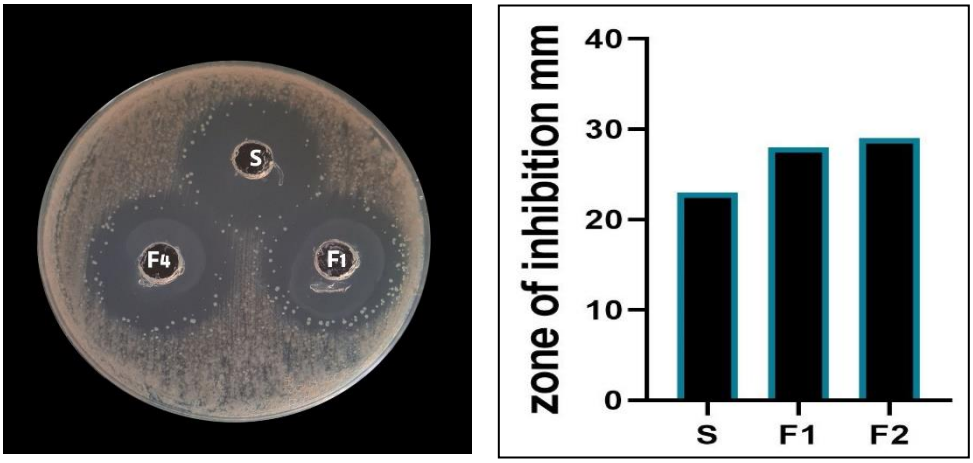

Fig. 10: Antibacterial activity of (S, F1, F2) against *K.pneumonia.* 128 µg/mL.

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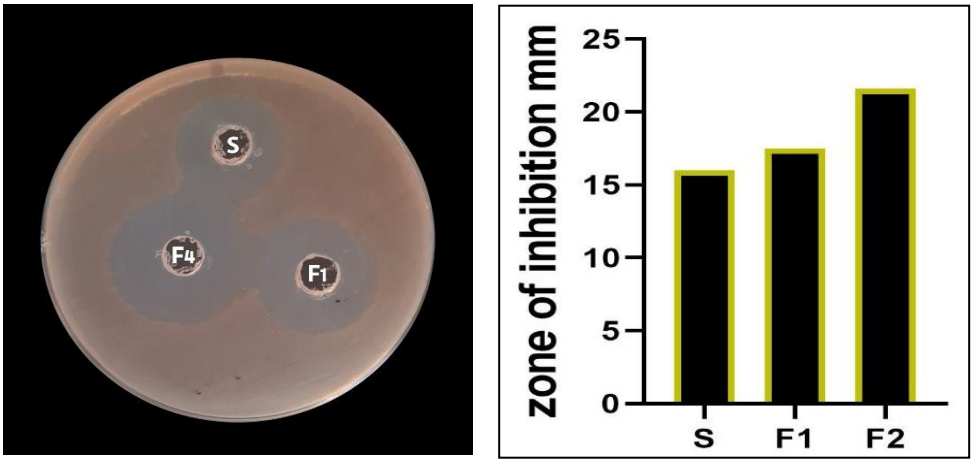

Fig. 11: Antibacterial activity of (S, F1, F2) against *K.pneumonia.* 32 µg/mL.

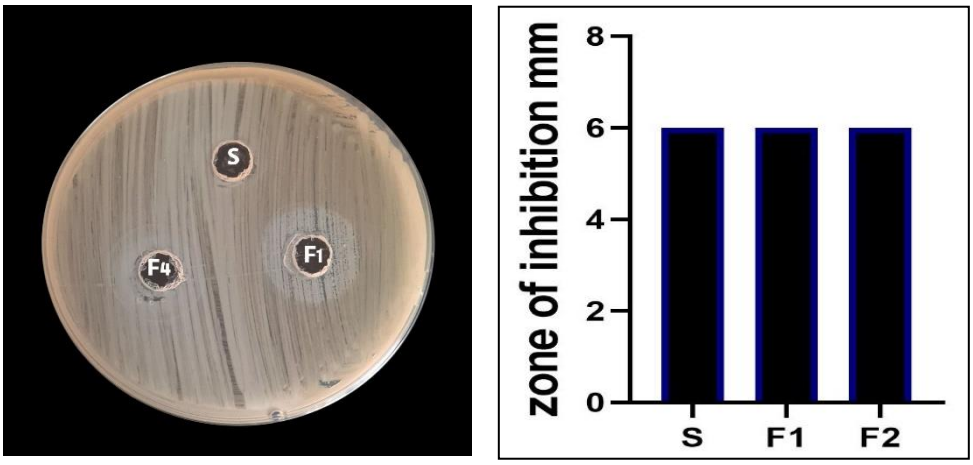

Fig. 12: Antibacterial activity of (S, F1, F2) against *K.pneumonia.* 0.5 µg/mL.

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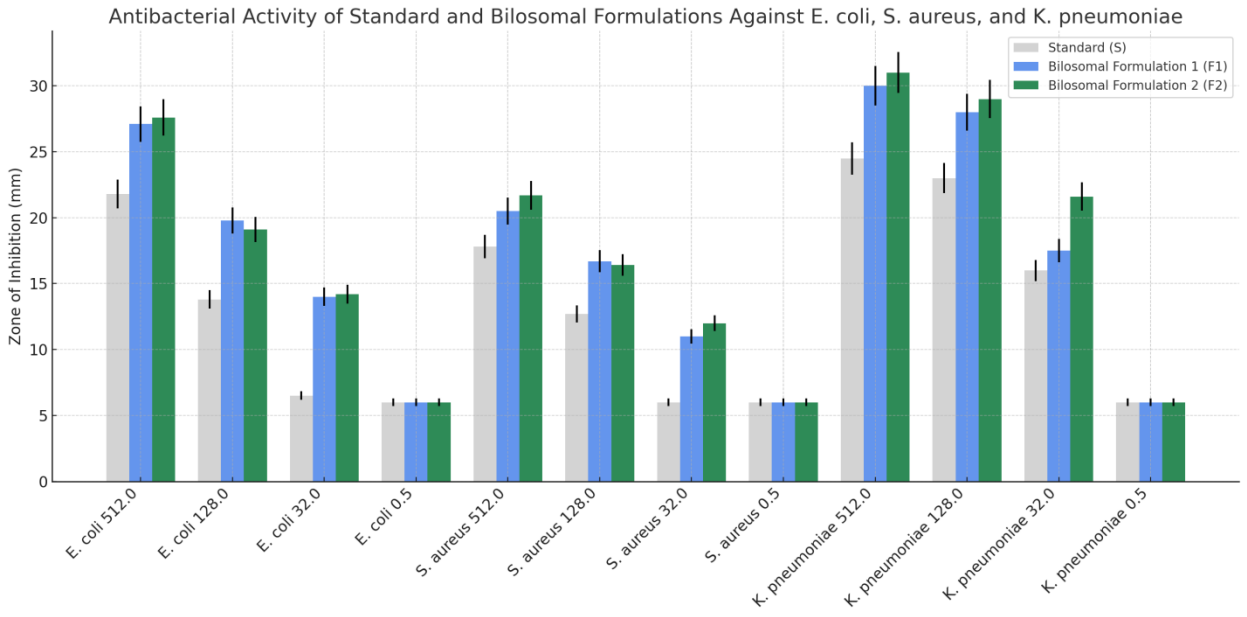

Fig 13: Antibacterial Activity of Standard and Bilosomal Formulations Against *E. coli*, *S. aureus*, and *K. pneumonia*

Conclusion

This work demonstrates that incorporating levofloxacin into bilosomal vesicles and embedding them within a Carbopol-based gel yields a dermal formulation with skin-compatible pH, acceptable spreadability, high viscosity supporting residence, and uniform drug content. In vitro, bilosomal formulations—especially F2—consistently outperformed a standard levofloxacin sample across *E. coli*, *S. aureus*, and *K. pneumoniae*, with significantly larger inhibition zones at concentrations ≥ 32 µg/mL and equivalence only at 0.5 µg/mL (well-diameter effect). The observed improvements most plausibly reflect enhanced interaction with, and retention at, biological interfaces afforded by bile-salt-stabilized vesicles alongside sustained release from the gel matrix. Collectively, these attributes suggest meaningful translational potential for managing superficial and potentially biofilm-associated skin infections where local concentration, stability, and persistence are critical. Future work should (i) refine composition to balance spreadability and retention, (ii) characterize stability and release kinetics under simulated use conditions, (iii) confirm antimicrobial superiority in ex vivo skin and relevant infected-skin animal models, and (iv) compare bilosomal levofloxacin with other advanced carriers under standardized endpoints, including dose-normalized pharmacodynamic metrics. If confirmed in vivo, bilosomal levofloxacin gel could reduce reliance on higher systemic doses and help mitigate resistance selection pressure by delivering effective local exposure.

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