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Characterization, Antioxidant and Antibacterial Activity of Fennel Extract Loaded Phytosome

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ABSTRACT

Background: This study investigates the characterization, antioxidants, and antibacterial properties of fennel extract-loaded phytosomes. **Materials and Methods**: Fennel (*Foeniculum vulgare*) extract was obtained through hydroalcoholic maceration and encapsulated using phosphatidylcholine to improve stability and bioavailability. **Results:** Transmission Electron Microscopy (TEM) confirmed the formation of spherical nanoparticles with a uniform size distribution (44-98 nm), while zeta potential analysis (-25.3 mV) indicated good colloidal stability. Phytochemical analysis demonstrated high phenolic (152.3±0.63 mg GAE/g), flavonoid (89.2±0.71 mg QE/g), and tannin (28±1.00 mg TAE/g) content, supporting its antioxidant potential. The DPPH assay revealed strong radical scavenging activity, with an IC50 value of 0.067 mg/mL, suggesting its suitability as a natural antioxidant. Antibacterial assessment using the agar well diffusion method showed broad-spectrum efficacy, with significant inhibition zones against *Salmonella Typhi* (22.18±0.05 mm) and *Bacillus cereus* (23.18±0.06 mm), in some cases outperforming azithromycin. **Conclusion:** These findings highlight the formulation's potential as an antimicrobial and antioxidant agent with applications in drug delivery, food preservation, and therapeutic interventions. Further studies are required to optimize stability, investigate mechanisms of action, and evaluate clinical applicability.

INTRODUCTION

Plants serve as a valuable source of therapy due to their versatility and easy accessibility (Newman and Cragg, 2020). Numerous plants, including olives, ginger, turmeric, garlic, green tea, and fennel, are well-known for their medicinal applications (Desideri *et al.,* 2010; Winkler *et al.,* 2020). Their biological functions can be attributed to secondary metabolites such as flavonoids, alkaloids, phenolic acids, saponins, triterpenes, and tannins (Samuel and Adekunle, 2021; Twaij and Hasan, 2022).

Fennel (*Foeniculum vulgare* Mill.) is a popular aromatic and medicinal herb, valued for its diverse pharmacological properties, including anticancer, hepatoprotective, antiplatelet, neuroprotective (antidementia), lipid-lowering activity, and anti-hirsutism, attributed to its unique phytochemical profile (Kalleli *et al.,* 2019; Noreen *et al.,* 2023).

The leaves of fennel are rich in essential nutrients, including vitamins (A, C, niacin, riboflavin, and thiamine), minerals (manganese, calcium, sodium, and potassium) (Saddiqi and Iqbal, 2011), and omega-3 fatty acids (Barros *et al.,* 2010). In addition, fennel extracts containing bioactive substances like carotenoids, flavonoids, tocopherols, terpenoids, and phenolic acids (Badgujar et al., 2014; Choi and Hwang, 2004; Agarwal *et al.,* 2017). Its essential oils also show promise as natural preservatives in food processing due to their antioxidant and antimicrobial activities (Abdellaoui *et al.,* 2020; Diao *et al.,* 2014; Sun *et al.,* 2021).

Many bioactive compounds found in medicinal plants, vegetables, and fruits often suffer from chemical instability and poor absorption characteristics. These phytochemicals often degrade during food processing, storage, or gastrointestinal transit, significantly reducing their bioavailability (Desai and Park, 2005). To address these challenges, innovative delivery systems are needed. Phytosome technology has emerged as a promising solution this method involves complexing plant-derived compounds with phospholipids to create lipid-compatible molecular structures that enhance both stability and absorption (Patel *et al.,* 2009; Fang and Bhandari, 2010; Gandhi *et al.,* 2012; Gibbs *et al.,* 1999). By shielding sensitive phytochemicals from environmental degradation, this approach helps preserve their biological activity (Goyal *et al.,* 2011; Kalita *et al.,* 2013). Given the demonstrated efficacy of fennel extract's bioactive components and the proven benefits of phytosome technology for improving compound stability and bioavailability, we propose that formulating fennel extract into phytosomes may significantly enhance its antimicrobial potency.

MATERIALS AND METHODS

Fennel Preparation, Extraction and Phytosome Formulation:

Fresh fennel plant samples were thoroughly washed and oven-dried (Memmert GmbH, USA) at 60°C for Three days. The dried material was then pulverized into a fine powder using a mechanical grinder. A hydroalcoholic extraction (95% ethanol) was performed using maceration, where the powdered fennel was soaked in the solvent for optimal compound extraction. The resulting mixture was centrifuged at 3,000 rpm for 10 minutes to separate solid residues, followed by filtration through Whatman No. 1 filter paper. The liquid extract was concentrated using rotary evaporation and subsequently freeze-dried to obtain a stable powdered form. Phosphatidylcholine was chosen as the primary encapsulation material due to its phospholipid properties. A 1:1 (w/w) ratio of fennel extract to phosphatidylcholine was used for complex formation. The fennel extract was first dissolved in 100 mL of 50% ethanol, while phosphatidylcholine was separately dissolved in 50 mL of dichloromethane. The two solutions were combined and magnetically stirred at 25°C for 8 hours to facilitate interaction.

Following this, residual solvents (ethanol and dichloromethane) were removed using a rotary evaporator at 45°C for 3 hours. The concentrated solution was then frozen at -80°C overnight and subjected to lyophilization for 48 hours under controlled conditions (-86°C, 0.008 mbar). The final dried phytosome product was carefully packaged and stored in a desiccator containing silica gel at 4°C to prevent moisture absorption and degradation (Palachai *et al.,* 2019).

Characterization of Fennel Extract Loaded Phytosome:

The morphology of the freshly

Prepared fennel extract phytosomes was examined using a Transmission Electron Microscope (TEM) (JEOL JEM-2100, Tokyo, Japan). The sample was adequately diluted with deionized water (DW) and subjected to sonication to ensure uniform dispersion. A carbon-coated copper grid was then evenly coated with the phytosome suspension and air-dried at room temperature. The samples were directly analyzed under TEM at an accelerating voltage of 160 kV without staining. Image acquisition and analysis were performed using Digital Micrograph (v2.11.1404.0) and Soft Imaging Spectator software, which facilitated particle sizing and morphological characterization.

Agar Well Diffusion Assay:

The microbial inoculum was uniformly spread across the surface of the agar plate using a sterile spreader. A 9 mm diameter well was then aseptically created in the agar using a sterile cork borer or pipette tip. Subsequently, 100 µL of the test sample (at the desired concentration) was carefully pipetted into the well. The inoculated plates were incubated under optimal growth conditions specific to the target microorganism. During incubation, the antimicrobial compounds diffused radially from the well into the surrounding agar, creating a concentration gradient that inhibited microbial growth in proportion to the sample's potency. The resulting zone of inhibition around the well was measured to evaluate antimicrobial activity (Magaldi *et al.,* 2004; Valgas *et al.,* 2007).

Antioxidant Activity Assessment Using DPPH Assay:

The antioxidant activity of *fennel* extracts was evaluated using the DPPH radical scavenging assay (Alanazi, et al., 2025). Briefly, serial dilutions of the samples in methanol were mixed with an equal volume of 0.135 mM DPPH solution. After 30 min of incubation in the dark, absorbance was measured at 517 nm.

The % DPPH• remaining was calculated as: % DPPH• remaining = $[DPPH^{\bullet}]_T / [DPPH^{\bullet}]_{T=0} \times 100$ Eq. (1)

The IC50 (concentration required to scavenge 50% of DPPH• radicals) was determined from a dose-response curve. Lower IC50 values indicate stronger antioxidant activity (Parejo et al., 2000).

Phytochemical Analysis:

The total phenolic content was determined using the Folin-Ciocalteu method (Sánchez-Rangel et al., 2013), where samples reacted with the reagent and sodium carbonate solution, with absorbance measured at 765 nm and quantified against a gallic acid standard ($y = 0.0062x$, $r^2 = 0.987$). Flavonoid content was assessed via aluminum chloride colorimetry (Zhishen et al., 1999), involving sequential addition of sodium nitrite, aluminum chloride, and sodium hydroxide, with absorbance read at 510 nm and calculated using a quercetin standard curve ($y = 0.0028x$, $r^2 = 0.988$). Tannin content was evaluated using a vanillin-hydrochloride assay (Aberoumand, 2009), where samples reacted with the acidic vanillin reagent, and absorbance at 510 nm was compared to a tannic acid standard curve, with results expressed as tannic acid equivalents. All measurements were performed spectrophotometrically and expressed relative to dry weight.

Statistical Analysis:

All experiments were performed in triplicate, with results expressed as mean ± standard deviation (M±SD). Statistical significance was assessed using one-way ANOVA, with post-hoc comparisons where applicable. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of Fennel Extract Phytosome:

Morphological Characterization of Fennel-Loaded Phytosomes:

Transmission electron microscopy (TEM) analysis revealed the successful

formation of fennel-loaded phytosomes with distinct morphological characteristics (Fig. 1A). The phytosomes appeared as discrete, nearly spherical nanoparticles with uniform shape distribution.

Particle size analysis demonstrated a narrow size distribution range, with the majority of particles (approximately 85%) measuring between 44-98 nm in diameter (Fig. 1B). The histogram showed a monodisperse population with minimal aggregation, indicating good stability of the formulation. The spherical morphology and nanoscale size distribution suggest optimal characteristics for enhanced bioavailability and cellular uptake.

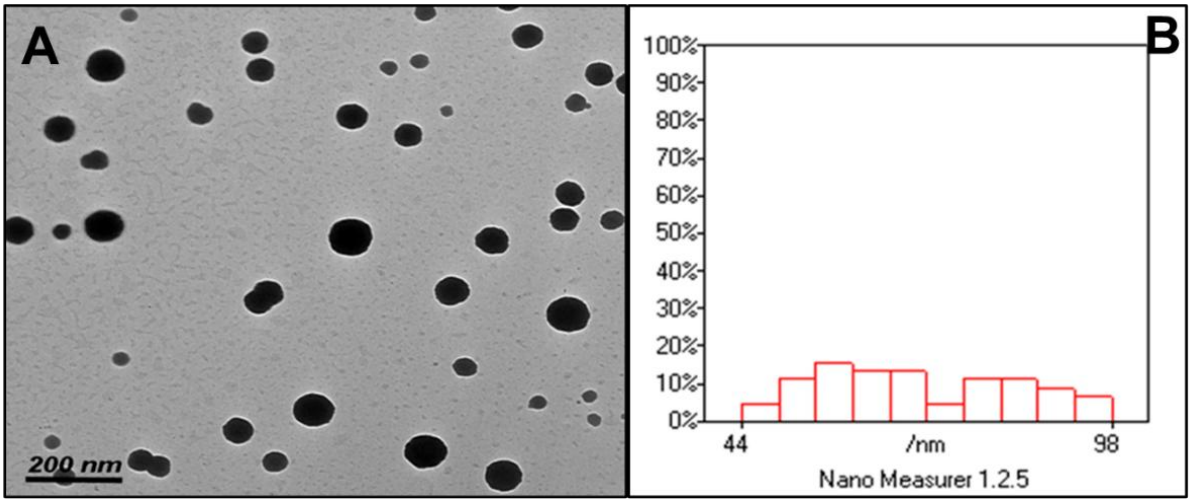

Fig. 1. (A)The morphology of fennel-Loaded Phytosomes by TEM showing nearly spherical particles, (B) Histogram shows that most particles cluster around 44 to 98 nm, with a good distribution.

Zeta Potential Analysis:

The physicochemical characterization of the fennel extract phytosome revealed favorable properties for drug delivery applications. Dynamic light scattering analysis showed a Z-average particle size of 75.5 nm with a polydispersity index (PDI) of 0.612, indicating the formation of nanoparticles with moderate size distribution (Fig. 2A). Zeta potential measurements demonstrated a surface charge of -25.3 mV, suggesting good colloidal stability due to sufficient electrostatic repulsion between particles (Fig 2B).

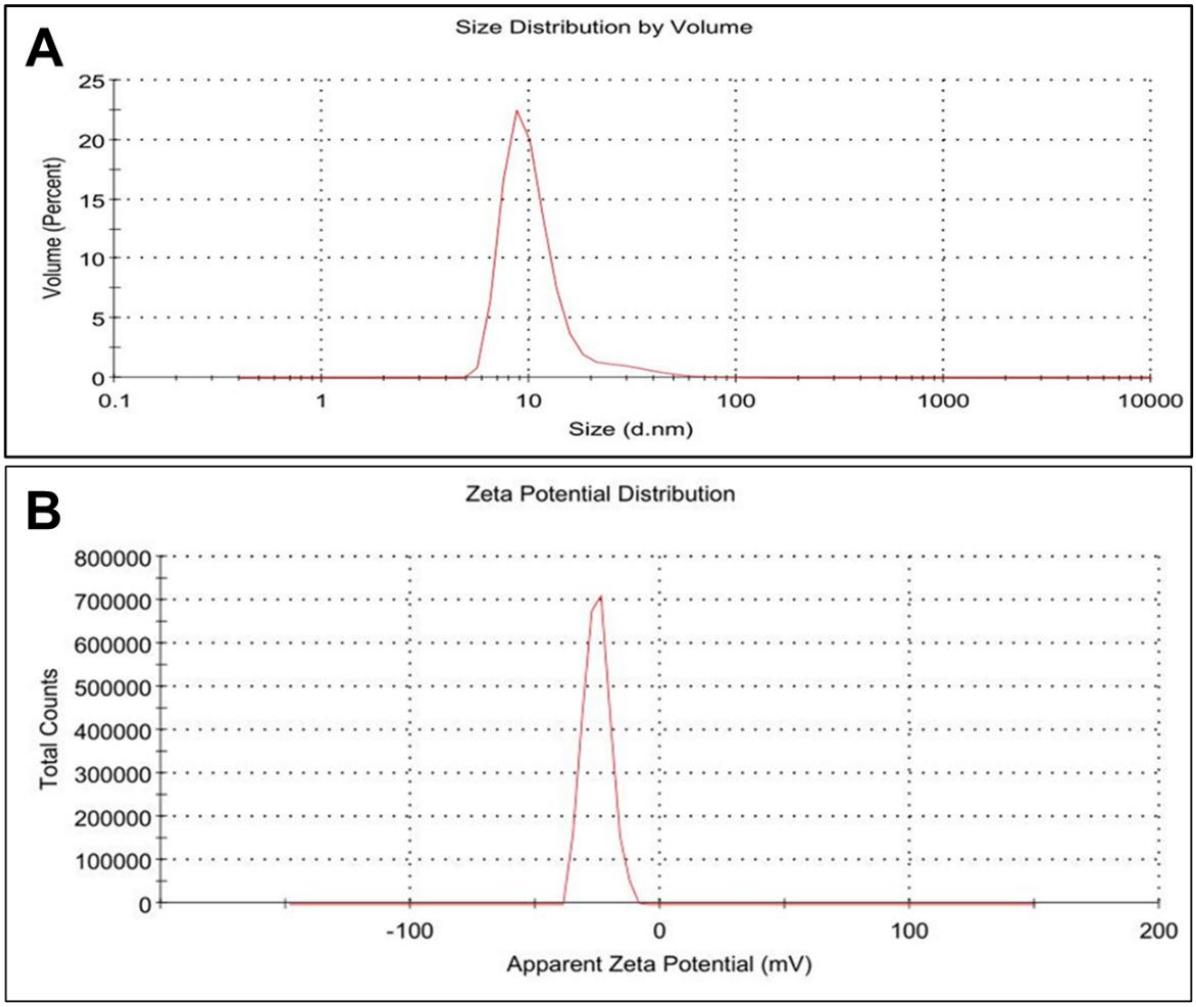

Fig. 2. (A) Zeta size distribution by volume. (B) Zeta potential distribution. The values of Z-average size, PDI, and ZP of fennel extract loaded phytosome were found to be 75.5 nm, 0.612, and -25.3mV respectively.

The fennel-loaded phytosomes (75.5 nm) developed in our study demonstrated superior nanoscale characteristics compared to similar formulations. For instance, rutin phytosomes reported by Kumbhar *et al.* (2024), exhibited larger particle sizes (112–391 nm) while maintaining comparable polydispersity indices (PDI; 0.3–0.7).

Zeta potential analysis revealed a value of −25.3 mV for our formulation, indicating excellent colloidal stability due to strong electrostatic repulsion between particles. In contrast, Kapse and Mulla (2024), reported a lower zeta potential (−8 mV) for polyherbal phytosomal gels, suggesting that our system may offer enhanced stability and reduced aggregation tendencies.

Phytochemical Analysis:

As shown in Table 1**,** fennel phytosomes exhibit a rich phytochemical profile, containing 152.3±0.63 mg GAE/g phenolics, 89.2±0.71 mg QE/g flavonoids, and 28±1.00 mg TAE/g tannins. These findings confirm the successful encapsulation of fennel's key bioactive compounds, with the high phenolic content indicating strong antioxidant potential. The formulation effectively preserves fennel's characteristic phytochemical composition while enhancing its delivery efficiency through nanoencapsulation. The retention of flavonoids and tannins further supports the phytosomes' ability to maintain

pharmacologically active components, reinforcing their potential therapeutic efficacy.

These results highlight the robust phytochemical profile of fennel phytosomes, characterized by high levels of phenolics, flavonoids, and tannins. This composition ensures effective bioactive compound encapsulation while preserving antioxidant capacity.

Previous studies on conventional fennel extracts (Rather *et al.,* 2016; Faudale *et al.,* 2008) reported similar phytochemical compositions. However, non-encapsulated formulations often experience reduced stability and bioavailability due to digestive and metabolic degradation. The phytosomal delivery system significantly improves compound protection and absorption, as demonstrated by sustained flavonoid and tannin concentrations—an observation consistent with findings from other phospholipid-complexed herbal extracts (Semalty *et al.,* 2010).

Table 1. The results of the phytochemical analysis of the fennel phytosome.

Samples	Phenolics Content [a]	Flavonoids Content [b]	Tannins Content [c]
Fennel phytosome	152.3±0.63	89.2±0.71	28±1.00

Antioxidant Activity by DPPH Assay:

The DPPH assay results, presented in Table 2, demonstrate that fennel phytosomes exhibit significant concentration-dependent antioxidant activity, with scavenging rates ranging from 23.55% to 65.11% at concentrations of 0.017-0.136 mg/mL and an IC50 value of 0.067 mg/mL. Although the formulation is less potent than ascorbic acid (IC50 0.022 mg/mL, 25.19-84.73% activity), it retains substantial radical scavenging capacity, reaching approximately 65% of ascorbic acid's maximum activity. These findings confirm the successful preservation of antioxidant compounds within the phytosomal system, supporting its potential as an effective natural antioxidant delivery mechanism. Despite being roughly threefold less potent than pure ascorbic acid on a concentration basis, the formulation exhibits a measurable IC50 and a well-defined dose-response relationship, validating its bioactive potential.

These findings are consistent with previous research on fennel's antioxidant properties, demonstrating that crude extracts and essential oils of *Foeniculum vulgare* exhibit significant DPPH radical scavenging activity, largely due to phenolic compounds such as flavonoids and polyphenols. Prior studies, including those by Rather *et al.* (2016), have reported strong DPPH inhibition by fennel seed extracts, highlighting its potential as a natural antioxidant. Similarly, Oktay *et al.* (2003), identified a direct correlation between fennel's phenolic content and its free radical scavenging capacity. The slightly lower potency of phytosomes compared to pure ascorbic acid may result from the encapsulation process, which could influence release kinetics; however, the dose-dependent efficacy indicates that bioactivity is effectively preserved. Overall, these findings reinforce fennel's role as a natural antioxidant and validate phytosomes as an efficient delivery system for optimizing its therapeutic applications.

Table 2. The Antioxidant Results by DPPH Assay.

Samples	Concentrations (mg/mL)	% Remaining DPPH	% Scavenging Activity	IC_{50} (mg/mL)
Fennel phytosome	0.136	34.89	65.11	0.067
	0.068	42.98	57.02	
	0.034	59.57	40.43	
	0.017	76.45	23.55	
Ascorbic acid	0.06	15.27	84.73	0.022
	0.03	39.08	60.92	
	0.02	61.07	38.93	
	0.01	74.81	25.19	

Antibacterial Activity Results:

Fennel phytosome preparation was found to possess broad-spectrum antibacterial activity against Gram-negative and Gram-positive bacteria, as evidenced by large inhibition diameters (Table 3, & Fig. 3). Among Gram-negative bacteria, the preparation exhibited significant inhibitory activity against Salmonella Typhi (22.18±0.05 mm) and E. coli (21.17±0.06 mm) and was found to exhibit comparable inhibitory activity to that of azithromycin (14.19±0.12 mm) against Klebsiella pneumoniae. It was also inhibitory against Gram-positive, more so against Bacillus cereus (23.18±0.06 mm) and Staphylococcus aureus (22.18±0.05 mm), and even outperforming azithromycin against S. epidermidis (21.17±0.06 mm vs. 16.30±0.14 mm). Water control lack of activity ensured antibacterial activity was due to phytosome components.

These findings highlight the intense antimicrobial efficacy of fennel phytosomes against clinically relevant pathogens, particularly Bacillus cereus and Salmonella Typhi, suggesting potential applications in treating enteric and systemic bacterial infections. The wide spectrum of activity against both types of bacteria suggest versatility as an antimicrobial agent.Previous research supports the antimicrobial action of fennel; Foeniculum vulgare essential oils and extracts inhibited growth in S. aureus, E. coli, and Salmonella typhimurium, Bacillus subtilis according to Rather *et al.* (2-16) and Diao *et al.* (2014). These activities are primarily due to bioactive constituents such as anethole (68-80% of the essential oil), fenchone (10-15%), and phenolic acids (e.g., gallic acid, caffeic acid), which disrupt microbial cell membrane structure and metabolism.

The enhanced antibacterial action of phytosomal preparation compared to crude extracts is likely due to greater solubility, bioavailability, and cell membrane uptake offered by the phospholipid complex. According to Semalty *et al.* (2010), phytosomes enhance the delivery of hydrophobic bioactive compounds by increasing membrane permeability as well as site-specific release.

Due to its broad-spectrum efficacy, fennel phytosomes find a wide range of application in combating: 1. Foodborne microorganisms (Salmonella spp., *E. coli*). 2. Skin infections (S. aureus, S. epidermidis), and 3. Multidrug-resistant bacteria.

Additionally, Kwiatkowski *et al.* (2017), reported synergy between fennel essential oil and conventional antibiotics against multidrug-resistant Klebsiella pneumoniae, suggesting phytosomal formulations can augment antimicrobial therapy while possibly reducing dosages required.

Table 3. Antibacterial activity of tested samples against pathogenic bacterial strains, expressed as inhibition zone diameters (mm). Larger values indicate greater antimicrobial efficacy.

Bacterial strains	*Samples*		
	Fennel phytosome	**Control (water)**	**Antibiotic (azithromycin 2mg/ml)**
Gram -ve			
***E.coli* (ATCC 10536)**	21.17±0.06 mm	-VE	-VE
Sal. Typhi (ATCC25566)	22.18±0.05mm	-VE	-VE
Klebsiella (ATCC10031)	21.17±0.06 mm	-VE	14.19±0.12mm
***Enterobacter cloacae* (DMS 30054)**	19.15±0.06mm	-VE	-VE
Gram +ve			
***S.aureus* (ATCC 6538)**	22.18±0.05mm	-VE	21.17±0.06 mm
s.epidermidis (EMCC1353t)	21.17±0.06 mm	-VE	16.30±0.14mm
Bacillus cereus (EMCC1080)	23.18±0.06mm	-VE	14.19±0.12mm
***Bacillus subtilis* (DMS 1088)**	20.14±0.14mm	-VE	20.14±0.14mm

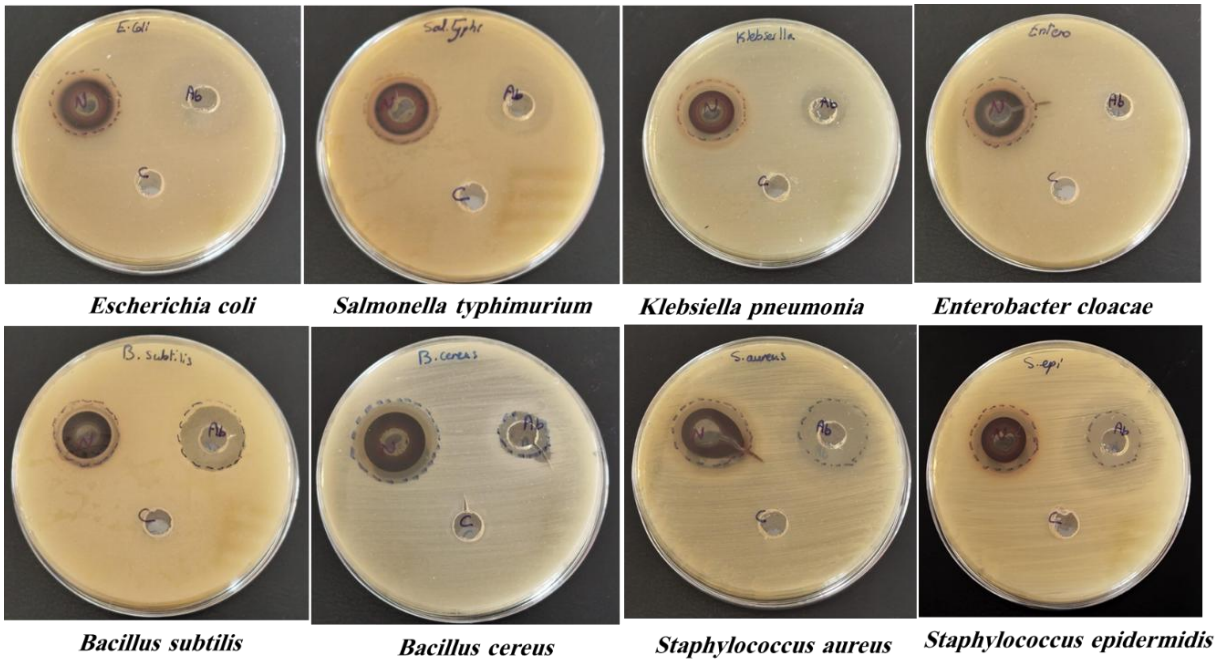

Fig. 3. Antibacterial activity assessment of fennel phytosome. The Petri dish images demonstrate the zones of inhibition, indicating the antimicrobial efficacy against the target bacterial strains. Clear zones surrounding the sample application sites represent bacterial growth suppression, with larger diameters corresponding to stronger antibacterial effects.

CONCLUSION

The fennel extract-loaded phytosome formulation exhibited promising physicochemical properties, characterized by its nanoscale size, uniform morphology, and good colloidal stability. The phytochemical analysis confirmed the successful encapsulation of bioactive compounds, preserving high phenolic, flavonoid, and tannin content, which contributed to its strong antioxidant activity. The formulation demonstrated notable antibacterial effects against both Gram-negative and Gram-positive pathogens, with superior efficacy against *Salmonella Typhi*, *Bacillus cereus*, and *Staphylococcus aureus*. These findings highlight the potential of fennel phytosomes as effective antimicrobial and antioxidant agents, with possible applications in drug delivery, food preservation, and natural therapeutic interventions. Further studies are necessary to optimize the formulation and explore its clinical relevance.

List of Abbreviations

TEM – Transmission Electron Microscopy
PDI – Polydispersity Index
ZP – Zeta Potential
DW – Deionized Water
IC50 – Half-Maximal Inhibitory Concentration
GAE – Gallic Acid Equivalent
QE – Quercetin Equivalent
TAE – Tannic Acid Equivalent
DPPH – 2,2-Diphenyl-1-Picrylhydrazyl
ANOVA – Analysis of Variance
rpm – Revolutions per Minute
°C – Degrees Celsius
µL – Microliter
mV – Millivolts
nm – Nanometer
CFU – Colony Forming Units
MIC – Minimum Inhibitory Concentration
µg/mL – Micrograms per Millilite

DECLARATIONS:

Ethical Approval: Not applicable.

Authors' Contributions: LAN conceptualized, designed the research protocol, participated in the entire research work and wrote the first and final draft of the manuscript.

Declaration of Competing Interest: The author declares no competing interests.

Data availability Statement: All data used for the study are available in the manuscript.

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