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Nanoencapsulation of Thyme Essential Oil by Electrospinning and Evaluation of Its Antimicrobial Properties Against Antibiotic-resistant *Salmonella typhimurium*

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Abstract

Background: Controlling *Salmonella* in poultry is a public health priority. Essential oils have attracted interest as natural food preservatives because of their antioxidant and antimicrobial properties.

Objectives: This study aimed to nanoencapsulate thyme essential oil using electrospinning and to characterize the resulting nanofibers and their antibacterial properties.

Methods: Nanofibers containing different percentages of essential oil were prepared, and their physicochemical and antimicrobial properties were investigated.

Results: Fourier transform infrared spectroscopy was used to evaluate molecular interactions in zein-acetic acid nanofibers containing thyme essential oil. The broad band at 3200 - 3400 cm^{-1}, corresponding to O-H and N-H stretching vibrations, increased in intensity and broadened after the addition of essential oil, indicating the formation of hydrogen bonds between the phenolic compounds in the essential oil and zein. The characteristic amide I and amide II bands, at approximately 1650 cm^{-1} and 1540 cm^{-1}, respectively, remained largely unchanged, indicating that the zein protein structure remained stable during electrospinning. Increased band intensity in the 1000 - 1250 cm^{-1} region, corresponding to C-O vibrations, confirmed the presence of essential oil in the fiber matrix.

Conclusions: The fibrous nanoparticles exhibited good antimicrobial properties and could be used as food coatings.

Keywords: Nanoencapsulation, Thyme Essential Oil, Antimicrobial Properties, *Salmonella typhimurium*

1. Background

The United States Department of Agriculture has made the control of *Salmonella* in poultry a high priority because of its serious public health implications (1). *Salmonella* forms biofilms, resists disinfection, and can detect, adapt to, and survive stressful environmental conditions (2). *Salmonella* is responsible for common foodborne illnesses associated with poultry and poultry products, other meats such as pork, fish, and beef, vegetables, milk, eggs, fresh fruits, and cheese. It is a major cause of diarrheal disease and human salmonellosis (2). Salmonellosis is a zoonotic disease that can cause severe complications (3). Essential oils have therefore attracted interest as natural food preservatives because of their combined antioxidant and antimicrobial properties (4). Many plant extracts have low water solubility, chemical instability, high volatility, and rapid degradation upon exposure to light or oxygen, which reduce their bioavailability and therapeutic efficacy (5, 6).

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These disadvantages necessitate technological interventions to improve formulation and delivery. Encapsulation in nanocarriers protects plant extracts from environmental degradation and enhances their solubility, bioavailability, targeted delivery, and sustained release, thereby optimizing their therapeutic effects (7, 8). Advances in pharmaceutical nanotechnology, including solid lipid nanoparticles, nanoemulsions, liposomes, polymer nanoparticles, and mesoporous silica systems, have been applied to encapsulate extracts of Iranian medicinal plants, with promising antibacterial and antifungal results (9).

Thymus vulgaris Linn., a herbaceous member of the mint family, occurs in southern Europe and the Mediterranean region (10). Chitosan nanoparticles containing thyme and oregano oils have shown increased bactericidal activity against foodborne pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* (11). Chitosan nanocapsules containing thyme oil have also shown inhibitory activity against six foodborne bacteria (12).

2. Objectives

This study aimed to nanoencapsulate thyme essential oil using electrospinning and to characterize the resulting nanofibers and their antibacterial properties against *Salmonella typhimurium*.

3. Methods

3.1. Essential Oil Extraction and Polymer Solution Preparation

Thyme essential oil was extracted from thyme leaves by steam distillation. Zein was dissolved at concentrations of 20%, 25%, and 30% (w/v) in a mixture of ethanol (80%) and acetic acid (20%). To prepare the nanofibers, thyme essential oil was incorporated into the polymer solution at 0%, 5%, and 10% by weight.

3.2. Electrospinning

The polymer solution was loaded into a 10-mL syringe and placed in an electrospinning device. The electrospinning parameters were adjusted, and the nanofibers were collected on an aluminum collector and dried in a room.

3.3. Nanofiber Morphology and Diameter

Nanofiber morphology and diameter were examined using scanning electron microscopy (SEM). Images were obtained at magnifications of 1000 - 5000× to analyze morphology and diameter distribution.

3.4. Chemical Analysis

Fourier transform infrared spectroscopy (FTIR) spectra were recorded over the range of 400 - 4000 cm^{-1} to confirm the presence of thyme essential oil in the nanofiber structure.

3.5. Thermal Stability

Thermogravimetric analysis (TGA) was performed to investigate the thermal stability of the nanofibers over a temperature range of 25 - 600 °C at a heating rate of 10 °C/min.

3.6. Bacterial Isolation

Poultry fecal samples were collected from Zabol County over a two-month period in 2025. The samples were transferred to selective *Salmonella-Shigella* agar and Simon sulfite agar and incubated for 24 hours at 37 °C. Bacteria that grew during this step were subsequently cultured in the following differential media: TSI-MRVP-lysine-lysine iron agar, Simon citrate, and urea.

3.7. Disk Diffusion Assay

The nanofibers were cut into 6-mm disks and placed on agar plates containing bacteria. The total inhibition-zone diameter, including the disk, was measured after 24 hours of incubation at 37 °C.

4. Results

4.1. Antimicrobial Activity

At an essential oil concentration of 10%, the largest inhibition-zone diameter was 6 mm and was observed for two *Salmonella typhimurium* strains. At 5%, the largest inhibition-zone diameter was 4 mm and was also observed for two strains (Table 1).

4.2. Fourier Transform Infrared Spectroscopy

The FTIR spectrum of zein-acetic acid nanofibers containing thyme essential oil was evaluated to investigate molecular interactions. The broad band at 3200 - 3400 cm^{-1}, corresponding to O-H and N-H stretching vibrations, increased in intensity and broadened after the addition of essential oil, indicating hydrogen-bond formation between the phenolic compounds in the essential oil and zein. The characteristic amide I and amide II bands, at approximately 1650 cm^{-1} and 1540 cm^{-1}, respectively, remained largely unchanged, indicating that the zein protein structure remained stable during electrospinning.

Table 1. Inhibition-Zone Diameters of Nanoencapsulated Thyme Essential Oil Against *Salmonella* Typhimurium

Bacterial Strain	Zone Diameter at 10% Essential Oil (mm)	Zone Diameter at 5% Essential Oil (mm)
1	4	1
2	5	3
3	4	2
4	5	2
5	5	2
6	6	4
7	6	4
8	5	3
9	5	3
10	4	3

Increased band intensity in the 1000 - 1250 cm^{-1} region, corresponding to C-O vibrations, confirmed the presence of essential oil in the fiber matrix. Overall, the FTIR results indicated that thyme essential oil was encapsulated mainly through physical interactions and hydrogen bonding.

4.3. Scanning Electron Microscopy

Scanning electron microscopy was used to investigate fiber morphology and size distribution in zein-acetic acid samples, both with and without thyme essential oil. Images showed a uniform fibrous structure arranged in a disordered network in both samples. Fibers in the pure zein sample had smooth surfaces without bead formation. The average fiber diameter ranged from 150 - 250 nm in the pure zein sample and increased slightly to 200 - 300 nm after the addition of thyme essential oil. This increase was probably attributable to changes in the viscosity and conductivity of the electrospinning solution caused by essential oil components. Some fibers containing essential oil showed slight surface roughness, which could be related to the distribution of essential oil molecules in the zein matrix. Overall, SEM showed uniform fiber formation without cracks or abnormal aggregation, confirming the successful encapsulation of thyme essential oil in the zein nanofiber structure.

4.4. Thermogravimetric Analysis

The thermogravimetric analysis curves of zein samples with and without thyme essential oil showed three distinct weight-loss regions. The initial weight loss at 50 - 150 °C was associated with the evaporation of moisture and residual solvent. The main degradation stage at 220 - 320 °C was associated with degradation of the zein protein structure and cleavage of peptide bonds. The sample containing essential oil showed a mild additional stage at 150 - 220 °C, attributable to the decomposition of volatile essential oil components, including thymol and carvacrol. The carbon residue above 450 °C was slightly higher in the sample containing essential oil than in the pure zein sample, indicating the presence of more stable essential oil components. These results confirmed the effective encapsulation of thyme essential oil in the zein matrix without a significant effect on overall thermal stability.

4.5. Dynamic Light Scattering

Dynamic light scattering showed a uniform particle size distribution in the nanostructures obtained from zein with and without thyme essential oil. The average particle size was approximately 180 - 220 nm in the pure zein sample and 230 - 280 nm in the sample containing thyme essential oil. The slight increase in particle size in the presence of essential oil was attributed to increased viscosity and surface tension of the electrospinning solution. The size distribution index (PDI) was less than 0.3 in both samples, indicating a uniform size distribution and good stability in the dispersion medium.

5. Discussion

The results indicated that antimicrobial activity increased with essential oil concentration.

A study investigated the nanoencapsulation of thyme essential oil in chitosan-gelatin nanofibers using nozzleless electrospinning and the application of these nanofibers to reduce nitrite in sausages. Nanofibers prepared with a gelatin/chitosan ratio of 1:6 and containing 40% essential oil were the best-performing samples, with diameters of 97 - 343 nm (13).

Ozogul et al. investigated the antimicrobial activity of thyme essential oil nanoemulsions against fish spoilage bacteria and foodborne pathogens. The nanoemulsions showed significantly greater antibacterial activity against *S. aureus*, with an inhibition-zone diameter of 26.1 mm, than thyme essential oil, which had an inhibition-zone diameter of 25.0 mm ($P < 0.05$) (14).

Another study investigated the use of thyme oil-loaded solid lipid and chitosan nanocarriers as food preservatives and their antibacterial effects against *Salmonella*

typhimurium and *E. coli*. The minimum inhibitory concentrations (MICs) against *E. coli* and *Salmonella typhimurium* were 4 and 1.5 µg/mL for thyme oil-loaded chitosan nanocarriers, 60 and 40 µg/mL for thyme oil-loaded solid lipid nanocarriers, and 180 - 150 µg/mL for pure thyme oil, respectively (15).

In a study of the antimicrobial activity of medicinal plants collected from the Sistan region against foodborne bacteria, the lowest and highest inhibitory concentrations were observed for *Peganum harmal* against *Vibrio cholerae*.

Saeidi et al. investigated the antimicrobial activity of *Mentha piperita* and *Zataria multiflora* essential oils and found that both exhibited strong inhibitory effects against *Pseudomonas aeruginosa* (16).

In another study, thyme essential oil inhibited *S. aureus*, *Salmonella typhimurium*, *E. coli*, and *P. aeruginosa* (17).

One study reported thyme essential oil MICs of 31, 250, 500, and 500 µg/mL against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, and *P. aeruginosa*, respectively. The MICs against the Gram-negative bacteria *E. coli* and *Salmonella typhimurium* were 250 and 500 µg/mL, respectively (18).

5.1. Conclusions

Nanofibers containing essential oil exhibited good antimicrobial activity, which increased with the amount of essential oil in the nanofibers.

Footnotes

Artificial Intelligence Use Disclosure: The authors declare that no generative artificial intelligence tools were used in the creation of this article.

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