

Nanoformulation of peppermint (*Mentha piperita* L.) and rosemary (*Rosmarinus officinalis* L.) Essential Oils: Antibacterial Effects

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Abstract

Essential oils are known for their natural antibacterial properties; however, their clinical and industrial applications are often limited by poor water solubility and low bioavailability. Nanoformulation techniques such as nanoemulsions, nanocomposites, nanocapsules, and liposomes have emerged as effective strategies to overcome these limitations, enhancing the stability, solubility, and antimicrobial activity of essential oils. This study investigates the antibacterial activity of peppermint (*Mentha piperita* L.) and rosemary (*Rosmarinus officinalis* L.) essential oils, delivered via various nanoformulations, against *Staphylococcus epidermidis* and *Escherichia coli*. Essential oils were extracted using a Clevenger apparatus and analyzed by GC-MS. Formulations were prepared, and particle sizes measured via electron microscopy. Antibacterial activity was also evaluated through antibiogram tests. All experiments were conducted in triplicate. The main components identified were methyl cyclohexanol (35.90%) in peppermint and camphor (23.22%) in rosemary essential oils. Particle sizes for peppermint nanoemulsions, nanocomposites, nanocapsules, and nanoliposomes were 3.34, 27.14, 19.25, and 19.01 nm, respectively; for rosemary, sizes were 2.69, 71.07, 35.02, and 25.38 nm. Both pure essential oils and their nanoformulations showed effective antimicrobial activity against Gram-positive and Gram-negative bacteria, with nanoforms exhibiting stronger effects. Gram-positive bacteria were more susceptible overall. Notably, nanoparticles and nanoliposomes had greater inhibitory effects than pure essential oils, and nanoemulsions demonstrated even higher antimicrobial activity than nanoliposomes. These results suggest that the essential oils of peppermint and rosemary, as well as their nanoformulations, may exhibit enhanced potency and could be effectively used as integrated agents against bacterial pathogens.

Introduction

Staphylococcus epidermidis and *Escherichia coli* are common bacteria associated with serious infections, particularly in hospital environments. *S. epidermidis* often causes device-related infections due to its biofilm-forming ability, while *E. coli* frequently leads to urinary tract and bloodstream infections. Effective control of these pathogens is essential to prevent infection spread, reduce antibiotic resistance, and improve patient care [1, 2]. Concerns over side effects and the rise of antimicrobial resistance linked to synthetic antibiotics have spurred growing interest in the use of medicinal plants for disease treatment [3–5]. Recent research has shown that many essential oils possess antimicrobial properties, making them eco-friendly options for eliminating pathogenic bacteria. Their antimicrobial effects have led to the widespread use of plant essential oils in the pharmaceutical, food, and plant medicine industries [4, 6]. The antimicrobial activity of essential oils is primarily due to their hydrophobic nature, which allows them to penetrate microbial cell membranes. This leads to cytoplasmic leakage, degradation of membrane proteins, coagulation of cytoplasmic contents, disruption of the proton motive force, and ultimately, cell death [7, 8]. Peppermint (*Mentha piperita* L.) and rosemary (*Rosmarinus officinalis* L.), both members of the Lamiaceae family, are valued medicinal plants known for their diverse therapeutic properties. Peppermint, in particular, exhibits antioxidant, antimicrobial, anti-allergic, anticoagulant, and analgesic effects, while rosemary has medicinal applications that include cooling effects, digestive tonic properties, antipyretic, antitussive, antiemetic activities, and disinfectant properties useful for treating lung inflammation [9–11]. A major challenge with essential oils is their low bioavailability. Converting them into nanoforms such as nanoemulsions, nanocomposites, nanocapsules, and nanoliposomes can enhance cellular penetration, protect active ingredients, control release, increase potency, and extend biological activity. These nanoformulations also help address the oils' volatility and rapid oxidation, improving their efficacy and safety. Nanoemulsions and nanocapsules are common nanotechnology approaches used in essential oil delivery [12–14]. Encapsulation protects sensitive compounds like essential oils from environmental factors such as oxidation and evaporation. Liposomes and nanoliposomes formed from phospholipids in water offer effective delivery systems. Nanocomposites, made from polymers and nanoscale fillers, are widely used in food packaging. They enhance the stability and antimicrobial effectiveness of essential oils by protecting them from moisture and oxygen [14, 15, 16]. In

this study, the effects of nanoparticle, nanocomposite, nanocapsule, and liposomal forms of peppermint (*Mentha piperita* L.) and rosemary (*Rosmarinus officinalis* L.) essential oils on *S. epidermidis* and *E. coli* were evaluated.

Materials and Methods

Plant and Essential Oil Extraction

Samples of rosemary and peppermint were obtained from the Agricultural Technology Units Growth Center of Jahad Daneshgahi Urmia and verified by botanists at Urmia University. The plant materials were fully dried in a dark location and then ground into a fine powder using a blender. Essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. A 100 g sample of powdered plant material was mixed with 700 mL of distilled water in a flask and subjected to individual distillations. The extracted oils were dried over anhydrous sodium sulfate, collected in opaque vials, sealed with aluminum foil, and stored at 4°C until needed.

Analysis of Essential Oil Compositions

The essential oil compositions were analyzed using a Hewlett-Packard 5973 gas chromatograph coupled with a mass spectrometer (GC-MS), equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The oven temperature was initially held at 80°C for 3 minutes, then increased at a rate of 8°C per minute to 180°C, followed by a further increase to 220°C where it was maintained for 5 minutes. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The mass spectrometer operated at an electron ionization energy of 70 eV. This setup enabled determination of the percentage composition of essential oil constituents, their retention indices, and corresponding mass spectra [17].

Preparation of Nanoemulsions

An aqueous phase was prepared by dissolving 10 g of Tween 10% in 90 mL of distilled water using magnetic stirring at room temperature without the application of heat. Subsequently, 3 g of essential oil was incorporated into the aqueous phase and subjected to vortex mixing for 1 minute until the mixture exhibited turbidity. The resultant turbid emulsion was then processed by ultrasonic homogenization for 30 minutes using an ultrasonicator, yielding a stable and transparent nanoemulsion [18].

Preparation of Nanocomposites

Nanocomposites were prepared using a mixture of 70% (w/v) sodium alginate solution and 30% (v/v) essential oil. To incorporate nanoclay, a 3% (w/v) nanoclay suspension was first prepared by dispersing the nanoclay in distilled water under magnetic stirring for 24 hours at room temperature. The essential oil solution (30%) was then added to the nanoclay suspension, and the mixture was homogenized for 5 minutes followed by continuous stirring for 4 hours at 40°C. Subsequently, glycerol was added as a plasticizer at 50% (w/w) relative to the polymer content, and the mixture was heated at 70°C for 20 minutes with stirring. The final nanocomposite solution was purified and dried at 40°C for 24 hours to obtain the solid nanocomposite film [19].

Preparation of Nanocapsules

Nanocapsules were prepared using the oil-in-water (O/W) emulsion polymerization technique. Essential oils served as the core material, while Tween 80 and sodium alginate were used as wall-forming pre-polymers. Initially, 5 g of

essential oil was mixed with 5 g of Tween 80 under continuous stirring to form a uniform emulsion. This emulsion was gradually added to a 1% (w/v) sodium alginate solution, prepared by dissolving 1 g of sodium alginate in 100 mL of deionized water. The resulting mixture was stirred for 2 hours at ambient temperature in a reactor equipped with a mechanical stirrer, until a turbid pre-polymer dispersion was obtained. The mixture was then subjected to ultrasonication for 10 minutes to enhance the formation of nanocapsules. Following the encapsulation process, the resulting nanocapsules were freeze-dried and the particle size was subsequently analyzed [20].

Preparation of Nanoliposomes

A mixture of 2 g lecithin and 2 g Tween 80 was combined with 38 g distilled water and stirred magnetically at room temperature for 5 hours without heating, until a homogeneous solution was achieved. Subsequently, 4 g of essential oil was incorporated into the aqueous lecithin dispersion. The mixture underwent sonication for 10 minutes, followed by an additional 2 minutes of ultrasonication to reduce particle size. The particle size distribution, surface characteristics, and morphology of the nanoliposomes were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM), respectively [21].

Measurement Method of Nanoformulation Size

The particle size of the essential oil nanoformulation was determined using two complementary techniques, Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). First, to measure the particle size distribution in the dispersed state, the samples were diluted appropriately in a compatible solvent (phosphate buffer) and filtered through a 0.45 μm filter before being analyzed by the DLS instrument. Measurements were conducted at 25°C, and parameters such as viscosity and refractive index of the medium were input into the device. The data obtained from DLS included the average hydrodynamic diameter of the particles. For direct observation of particle morphology and size, SEM was employed. The samples were dried, mounted on stubs, and coated with a thin metal layer (gold or platinum) to enhance surface conductivity. Then, the samples were imaged in the SEM device under an appropriate accelerating voltage, and particle sizes were directly determined from the images using image analysis software. The combined use of these two methods enabled more accurate determination of the size and shape of the nanoformulation particles [22, 23].

Preparation of Bacteria

Strains of *E. coli* (ATCC 25922) and *S. epidermidis* (ATCC 14990) were obtained from the Iranian Biological Resource Center (Tehran, Iran). These strains were cultured in Muller-Hinton agar medium (Merck, Germany).

Antibacterial Activity Assay

The antibacterial activity of the essential oil nanoformulation on *S. epidermidis* and *E. coli* was evaluated using the agar well diffusion method and minimum inhibitory concentration (MIC) assay against selected bacterial strains. In the agar well diffusion method, Mueller-Hinton agar plates were inoculated with bacterial suspensions (adjusted to 0.5 McFarland standard, $\sim 10^8$ CFU/mL), and wells (6 mm diameter) were punched into the agar. Each well was filled with 50–100 μL of the essential oil formulation, while controls included a standard antibiotic (gentamicin). Plates were incubated at 37°C for 24 hours, and the diameter of inhibition zones was measured. For MIC determination, the broth microdilution method was used. Serial dilutions of the essential oil were prepared in a 96-well microtiter plate using Mueller-Hinton broth. Each well was inoculated with the bacterial suspension, and the plates were incubated at 37°C

for 18–24 hours. The lowest concentration showing no visible bacterial growth was recorded as the MIC. All tests were performed in triplicate to ensure reproducibility [24].

Statistical Analysis:

Data were analyzed using one-way analysis of variance (ANOVA) to evaluate differences among treatment groups. Tukey's post hoc test was applied for multiple comparisons. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). Results were considered statistically significant at a confidence level of $P < 0.05$.

Results

Analysis of Essential Oil Components:

The composition of the essential oils was analyzed using GC–MS, and the results are presented in Table 1 and Fig. 1. According to the GC–MS analysis, a total of 50 compounds were identified in peppermint (*M. piperita*) essential oil. Among these, Methyl-Cyclohexanol (35.90%) and Menthone (23.01%) were identified as the major constituents.

Table 1
Identified components of peppermint essential oil by GC/MS
analyses

No.	Name of the compound	Time	Percent
1	Furan	4.407	% 0.04
2	Alpha-Pinene	5.111	% 1.46
3	Camphene	5.409	% 0.15
4	Sabinene	5.878	% 0.44
5	Beta-Pinene	5.964	% 1.19
6	Beta-Myrcene	6.193	% 0.58
7	3-Octanol	6.284	% 0.21
8	Sabinene	6.513	%0.05
9	Benzene	6.954	% 0.05
10	dl-Limonene	7.057	% 4.05
11	Cineole	7.120	% 0.59
12	Alpha-Ocimene	7.200	% 0.06
13	Gamma-Terpinene	7.709	% 0.07
14	Alpha-Terpinolene	8.390	% 0.08
15	Linalool	8.642	% 0.05
16	Tricyclo	9.649	% 0.07
17	Camphor	9.740	% 0.55
18	Menthone	10.021	% 23.01
19	Menthofuran	10.209	% 19.85
20	Methyl-Cyclohexanol	10.536	% 35.90
21	D-Neoisomenthol	10.679	% 0.29
22	D-Isomenthol	10.782	% 0.19
23	Bicyclo-hept2-ene-2-methano	10.959	% 0.04
24	Bicyclo-hept-3-ene-2-one	11.268	% 0.05
25	Benzoxepin	11.388	% 0.09
26	Z-3hexenyl	11.680	% 0.09
27	n-Valeric	11.760	% 0.44
28	Cyclohexanone	11.938	% 3.42
29	2-Cyclohexen	12.264	% 0.65
30	Bicyclo heptane	12.281	% 0.16

No.	Name of the compound	Time	Percent
31	Lavandulyl Acetate	12.990	% 0.13
32	Menthol acetate	13.111	% 2.48
33	Cyclohexene, 4-methyl	13.448	% 0.06
34	Car-3-en-2-one	14.249	% 0.04
35	Cyclopentane	14.335	% 0.16
36	Octyne	14.387	% 0.21
37	Beta-Bourbonene	15.170	% 0.10
38	Elemene	15.285	% 0.04
39	Trans-Caryophyllene	15.920	% 1.32
40	Trans-bete-Farnesene	16.567	% 0.16
41	Alpha-Humulene	16.630	% 0.06
42	Germacrene-D	17.196	% 0.61
43	Bicyclogermacrene	17.511	% 0.15
44	Mint Furanine	17.562	% 0.20
45	Delta-Cadinene	18.003	% 0.06
46	Eucalyptol	18.810	% 0.06
47	Caryophyllene	19.262	% 0.08
48	Naphthalene	19.428	% 0.08
49	Dodecanoic	28.548	% 0.09
50	5-Morpholino	30.557	% 0.06

The GC/MS analysis of the rosemary essential oil identified a total of 29 compounds. Among these, Camphor with 23.22% and α -Pinene with 17.99% were the major constituents. (Table 2 and Fig. 2).

Table 2
Identified components of rosemary essential oil by GC/MS analysis

No	Name of the compound	Time	Percent
1	Carene	4.972	% 0.25
2	Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl)	5.035	% 0.20
3	Alpha-Pinene	5.200	% 17.99
4	Camphene	5.487	% 6.70
5	Beta-Pinene	6.047	% 2.88
6	Octanone	6.179	% 1.08
7	Pyridineethanamine	6.282	% 4.37
8	Alpha-Phellandrene	6.602	% 1.79
9	Carene	6.866	% 0.92
10	Banzene,1-methyl	7.043	% 0.50
11	Limonene	7.146	% 5.57
12	Eucalyptol	7.215	% 12.89
13	Cyclohexadiene	7.798	% 1.52
14	Terpineol	8.010	% 0.26
15	Cyclohexene,1-methyl-4-(1-methyle thylidene)	8.479	% 1.14
16	Octadien	8.720	% 1.26
17	Cyclopenten	8.366	% 0.25
18	Camphor	8.864	% 23.22
19	Borneol	10.316	% 4.44
20	Cyclohexadie-1-ol,4-methyl-1-ethylethyl	10.573	% 1.11
21	Alpha-4-trimethyl	10.877	% 2.15
22	Cyclohexasiloxane	11.094	% 0.20
23	Santolina epoxide	11.180	% 0.74
24	Bicyclo (3.1.1) hept-3en-2-one4,6,6, trimethyl	11.334	% 3.56
25	Bicyclo (2.2.1) hepta – 2-ol	13.045	% 3.31
26	Caryophyllene	13.806	% 0.45
27	Alpha-Caryophyllene	16.742	% 0.23
28	Pentasiloxane	16.971	% 0.30
29	Alpha-Caryophyllene	17.869	% 0.24

Analysis of FESEM different formulation of rosemary and peppermint essential oils

The FESEM images of the nanoformulations are presented in Figs. 3 and 4. In the nanoemulsion images, a high degree of nanoparticle agglomeration is evident, along with indistinct boundaries between individual particles. This clustering makes it difficult to accurately calculate the average particle size and introduces potential errors in measurement. In contrast, the images of the other nanoformulations (nanocomposites, nanocapsules, and nanoliposomes) generally show well-defined, spherical, and smooth particles. The particle diameters across these nanoformulations range approximately from 0 to 100 nanometers.

Particle size determination by dynamic light scattering (DLS)

The particle size and size distribution of the colloidal nanoparticle systems including nanoemulsions, nanocomposites, nanocapsules, and nanoliposomes loaded with peppermint and rosemary essential oils are presented in Table 3 and 4. The data indicate the particle size ranges and distribution profiles for both essential oils across the different nanoformulations, providing insights into their stability and potential functional properties (Fig. 5, 6).

Table 3
Particle size of peppermint essential oil in different nanoformulations determined by DLS

Nanoformulations	Width	Vol %	Dia (nm)
Nanoparticle	1.78	100	3.34
Nanocomposite	67.09	100	71.07
Nanocapsule	21.19	100	35.02
Nanoliposome	11.71	100	25.38

(A) Nanoparticle, (B) Nanocomposite, (C) Nanocapsule, and (D) Nanoliposomeoil

Table 4
Rosemary essential oil particle size in different nanometers

Nanoformulations	Width	Vol %	Dia (nm)
Nanoparticle	1.31	100	2.69
Nanocomposite	10.43	100	27.14
Nanocapsule	7.02	100	19.25
Nanoliposome	6.08	100	19.01

Antibacterial Activity Assay:

The results of the variance analysis of the antibiogram data showed that the difference in the diameter of the growth inhibition zone is significantly affected by the different forms of peppermint and rosemary essential oils, as well as the antibiotics gentamicin on the bacteria *S. epidermidis* and *E. coli* (F (6, 14) = 88.02, P = 0.5; F (6, 14) = 52.19, P = 0.5; F (6, 14) = 164.16, P = 0.5; F (6, 14) = 56.80, P = 0.5) (Fig. 7).

The results of the Tukey post hoc test showed that the concentration of one milligram per deciliter of the nanoliposome form of rosemary essential oil and the nanoparticle form of peppermint essential oil significantly inhibited the growth of *S. epidermidis* more than the other forms, while the least growth inhibition was observed with

the pure form of rosemary essential oil and the nanocomposite form of peppermint essential oil. Furthermore, the results showed that the concentration of one milligram per deciliter of the nanoliposome form of rosemary and peppermint essential oils significantly inhibited the growth of *E. coli* more than the other forms, while the least growth inhibition of *E. coli* was observed in the nanocapsule form and the pure forms of rosemary and peppermint essential oils. Additionally, the average diameter of the growth inhibition zones of *S. epidermidis* and *E. coli* affected by the concentration of one milligram per deciliter of different forms of rosemary and peppermint essential oils was significantly smaller than that caused by the antibiotics gentamicin and penicillin (Fig. 7).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of peppermint and rosemary essential oils against *S. epidermidis* and *E. coli* are summarized in Table 6. The results indicate that rosemary exhibits a stronger bactericidal effect compared to peppermint, with this effect being more pronounced against the Gram-positive bacterium *S. epidermidis*.

Table 6
MIC and MBC of peppermint and rosmary essential oils against *Staphylococcus epidermidis* and *Escherichia coli* bacteria

Essential Oils Formulation	Bacteria	MIC (mg/ml)	MBC (mg/ml)	Essential Oils Formulation	Bacteria	MIC (mg/ml)	MBC (mg/ml)
Pur Peppermint	<i>S. epidermidis</i>	0.65	1.25	Pur Rosmary	<i>S. epidermidis</i>	2.5	5
	<i>E. coli</i>	1.25	2.5		<i>E. coli</i>	1.25	2.5
Nanoemulsions	<i>S. epidermidis</i>	0.5	1	Nanoemulsions	<i>S. epidermidis</i>	0.5	1
	<i>E. coli</i>	1	4		<i>E. coli</i>	2	4
Nanocomposites	<i>S. epidermidis</i>	0.2	0.4	Nanocomposites	<i>S. epidermidis</i>	0.4	0.8
	<i>E. coli</i>	0.5	1		<i>E. coli</i>	0.5	1
Nanocapsules	<i>S. epidermidis</i>	0.1	0.2	Nanocapsules	<i>S. epidermidis</i>	0.2	0.4
	<i>E. coli</i>	0.2	0.4		<i>E. coli</i>	0.4	0.8
Liposomes	<i>S. epidermidis</i>	0.1	0.2	Liposomes	<i>S. epidermidis</i>	0.1	0.2
	<i>E. coli</i>	0.2	0.4		<i>E. coli</i>	0.2	0.4

Discussion and Conclusion

Nanoformulation of essential oils plays a critical role in bacterial control by enhancing their stability, solubility, and bioavailability. This improvement facilitates the effective delivery and sustained release of active compounds, thereby increasing their ability to penetrate bacterial membranes and disrupt biofilms. Consequently, nanoformulated essential oils exhibit enhanced antibacterial efficacy against pathogenic bacteria, offering a promising alternative to conventional antibiotics [25–27].

In our study, two medicinal plants (peppermint and rosemary) exhibited significant antibacterial activity against *S. epidermidis* and *E. coli*. Evaluation of their efficacy in nanotechnology-based formulations revealed that both

nanocapsule and liposome forms significantly enhanced their inhibitory effects. These findings are consistent with previous research demonstrating the antibacterial effects of nanoformulations derived from rosemary and peppermint essential oils, highlighting the benefits of nanotechnology in enhancing their efficacy [28].

The phenolic compounds of rosemary, when stabilized and gradually released through nanoencapsulation, exhibited strong antibacterial activity [29]. Similarly, peppermint nanoformulations, rich in menthol and menthone, effectively disrupted bacterial membranes, particularly against the Gram-negative *E. coli* [30, 31]. Nanoparticles improve interaction with bacterial cells, increasing permeability and promoting cell death [28].

GC-MS analysis of peppermint essential oil revealed methyl cyclohexane, menthol, menthyl acetate, menthone, and menthofuran as the major constituents. These results align with previous studies [32, 33]. In rosemary essential oil, the predominant compounds were camphor, alpha-pinene, beta-pinene, camphene, and limonene, all of which contribute to its antimicrobial properties. This is consistent with the findings of Özcan & Chalchat and Jafari-Sales & Pashazadeh [34, 35].

To determine the optimal formulation for studying the antimicrobial properties of peppermint and rosemary essential oils, particle size analysis was conducted. The average sizes of nanoparticles, nanocomposites, nanocapsules, and liposomes for peppermint oil were 3.34, 71.07, 35.02, and 25.38 nm, respectively. For rosemary oil, the respective sizes were 2.69, 27.14, 19.25, and 19.01 nm. All formulations exhibited particle sizes below 100 nm, indicating their suitability for nano-based applications.

In line with our findings, Hadidi et al. reported that chitosan nanoparticles (CSNPs) loaded with clove essential oil (CEO) demonstrated enhanced antioxidant and antibacterial properties compared to free CEO, improving stability and bioefficacy [36]. Ephrem et al. prepared polycaprolactone (PCL)-based nanocapsules loaded with rosemary essential oil using the nanoprecipitation method; electron microscopy analysis revealed that nanoparticles containing rosemary and peppermint essential oils predominantly exhibited spherical, clustered structures under 100 nm, demonstrating superior antibacterial and antioxidant properties [37]. Our results corroborate these observations, confirming the enhanced efficacy of both essential oils when delivered via nanoformulations.

Osanloo et al. showed that the antibacterial activity of a nanoemulsion of *Zataria multiflora* essential oil, with a particle size of 129 ± 12 nm, was significantly higher than that of both the non-formulated and micro-formulated forms. Similarly, a nanoemulsion of *M. piperita* essential oil (160 ± 25 nm) exhibited significantly greater antibacterial efficacy compared to its free and micro-formulated counterparts; comparable results were observed in our study [38].

The antibacterial activity (MIC%) of clove essential oil nanoemulsion against target bacteria such as *Bacillus subtilis*, *Proteus vulgaris*, *S. aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* was reported as 0.080, 0.085, 0.075, 0.300, and 0.250, respectively, whereas the MIC values of the pure essential oil were significantly higher at 0.130, 0.130, 0.130, 0.500, and 0.400, respectively [39].

Similarly, da Silva Gündel et al. evaluated the antimicrobial activity of nanoemulsion and non-formulated essential oil of *Cymbopogon flexuosus* against various microorganisms; their MIC values confirmed the enhanced efficacy of nanoemulsions compared to non-nano formulations [40].

Hassanzad Azar et al. reported that both rosemary essential oil and its nanoemulsion exhibited stronger antibacterial effects against Gram-positive bacteria than Gram-negative bacteria in microdilution and disk diffusion assays, although no significant difference was found between nanoemulsion and pure oil using disk-diffusion and steam-phase diffusion methods [41].

According to Mohkami et al., mint essential oil showed stronger antibacterial activity against *Listeria monocytogenes*, *B. cereus*, *P. aeruginosa*, and *S. aureus*. Both mint and rosemary essential oils had similar, non-significant inhibitory effects on *E. coli*, whereas rosemary oil was more effective than mint oil against *Salmonella typhi* and *B. licheniformis* [42].

Finally, Cutro et al. reported that nanoformulations of *Schinus areira* essential oil, in both liposomal and silver nanoparticle (AgNP)-based forms, exhibited superior antibacterial activity compared to the crude oil. These nanodelivery systems improved the essential oil's efficacy, stability, and bioavailability, allowing for significantly stronger antimicrobial effects at lower concentrations [43].

Conclusion

Essential oils of peppermint and rosemary possess natural antibacterial properties effective against various pathogens, including *S. epidermidis* and *E. coli*. However, their practical use is often limited by poor water solubility, volatility, and low bioavailability. Nanoformulation techniques enhance the stability, solubility, and controlled release of essential oils, thereby improving their antibacterial efficacy. These studies have shown that nanoformulated essential oils of peppermint and rosemary exhibit increased penetration and disruption of bacterial cell membranes. These advancements suggest that nanoformulated essential oils could serve as effective alternatives or adjuncts to conventional antibiotics in controlling infections caused by *S. epidermidis* and *E. coli*.

Declarations

Author Contributions

S.T. and, SH. A designed all experiments, the S. T. and Sh. A. and M.PM wrote the main manuscript text and S. T, M.PM, L. J prepared figures and tables. All authors reviewed the manuscript.

Ethics Approval

Not applicable.

Availability of data and materials

The corresponding author can provide the data supporting the findings of this study upon reasonable request.

Supplementary Information

Additional supplementary material can be found in the online version of this manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Figures

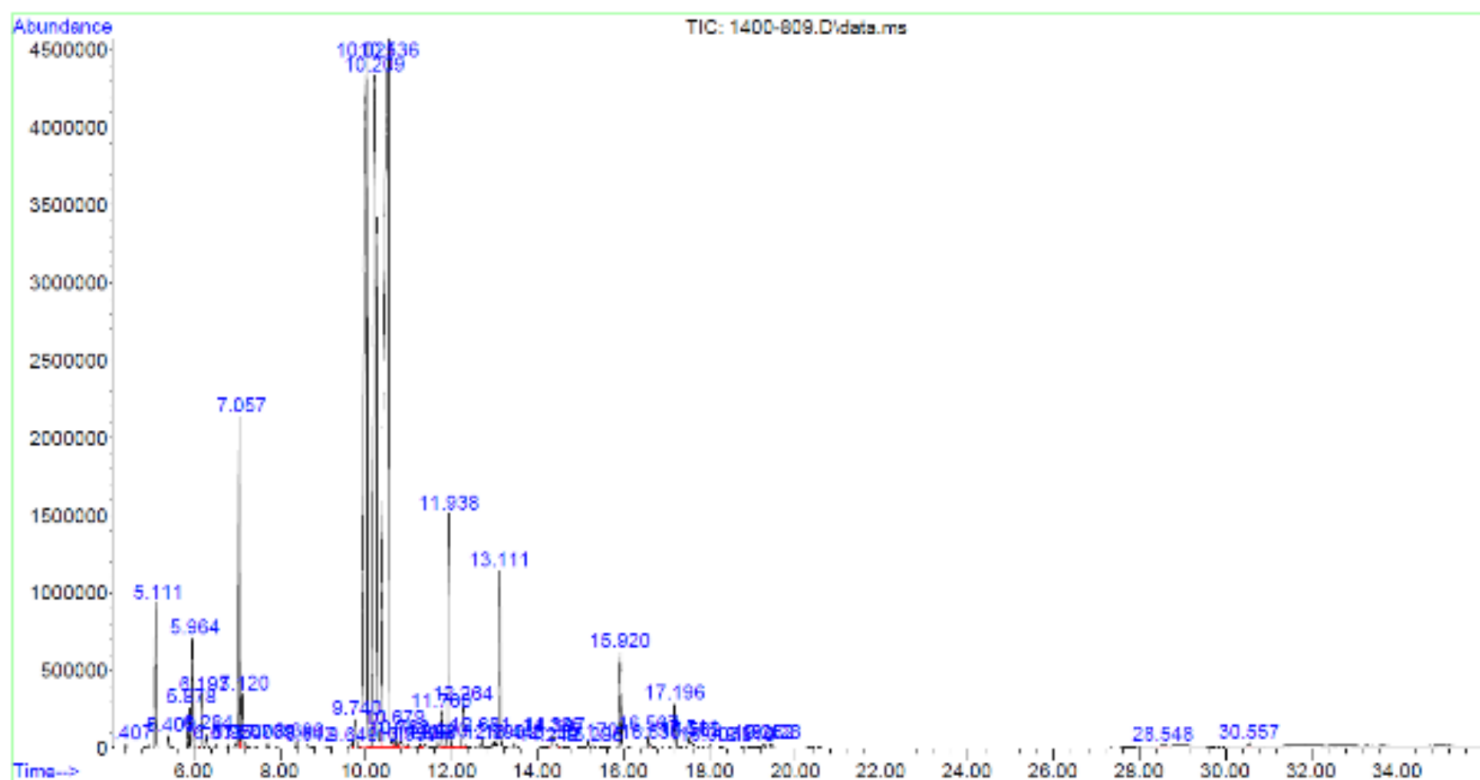


Figure 1

Total Ion Chromatogram (TIC) obtained from GC/MS analysis of the peppermint essential oil sample, showing the separation and detection of its volatile compounds.

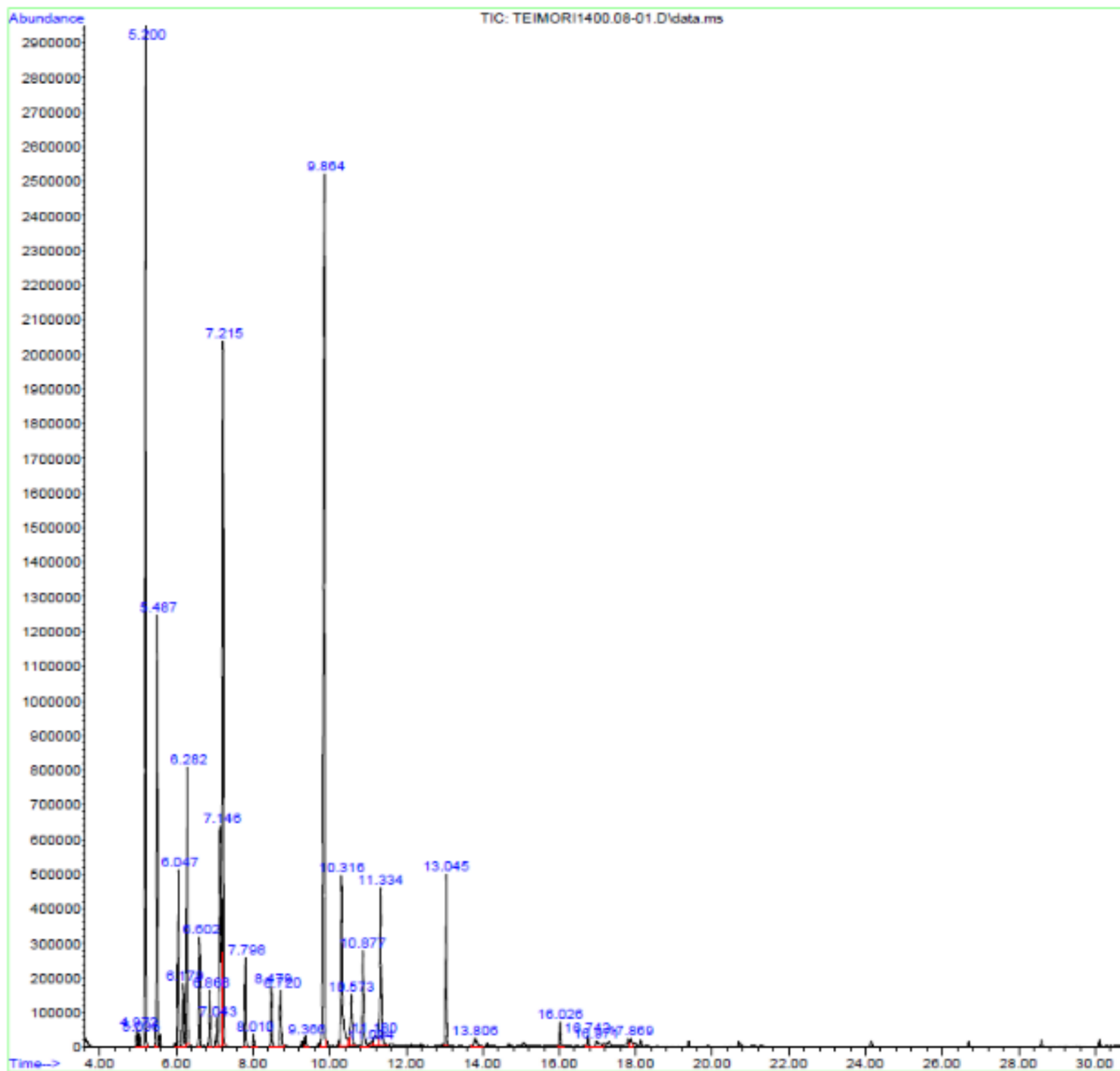


Figure 2

Total Ion Chromatogram (TIC) obtained from GC/MS analysis of the rosemary essential oil sample, illustrating the separation and detection of its volatile constituents.

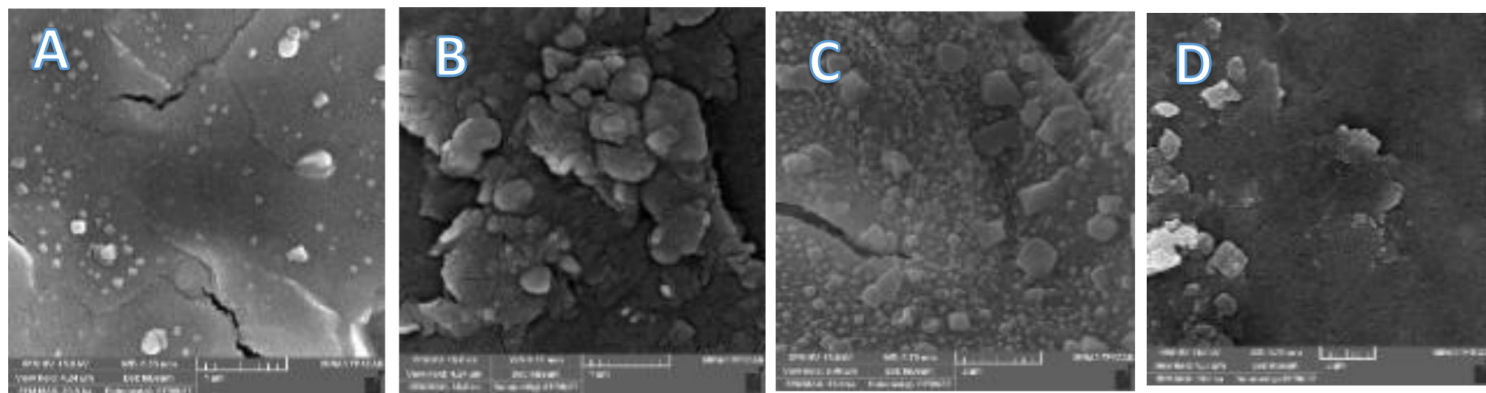


Figure 3

Scanning Electron Microscope (SEM) images of peppermint essential oil formulations: (A) Nanoparticle, (B) Nanocomposite, (C) Nanocapsule, and (D) Nanoliposome.

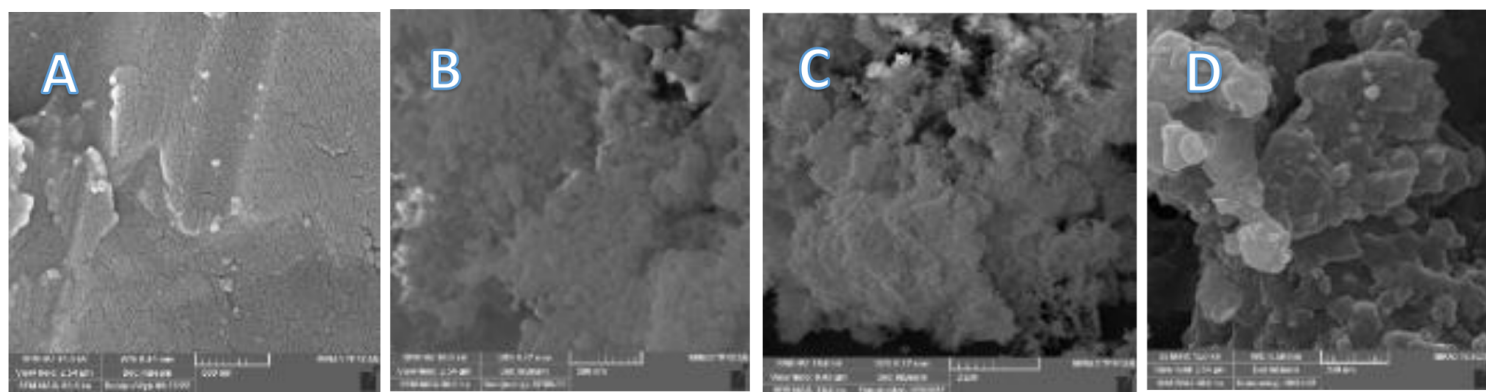


Figure 4

Scanning Electron Microscope (SEM) images of rosemary essential oil formulations: (A) Nanoparticle, (B) Nanocomposite, (C) Nanocapsule, and (D) Nanoliposome.

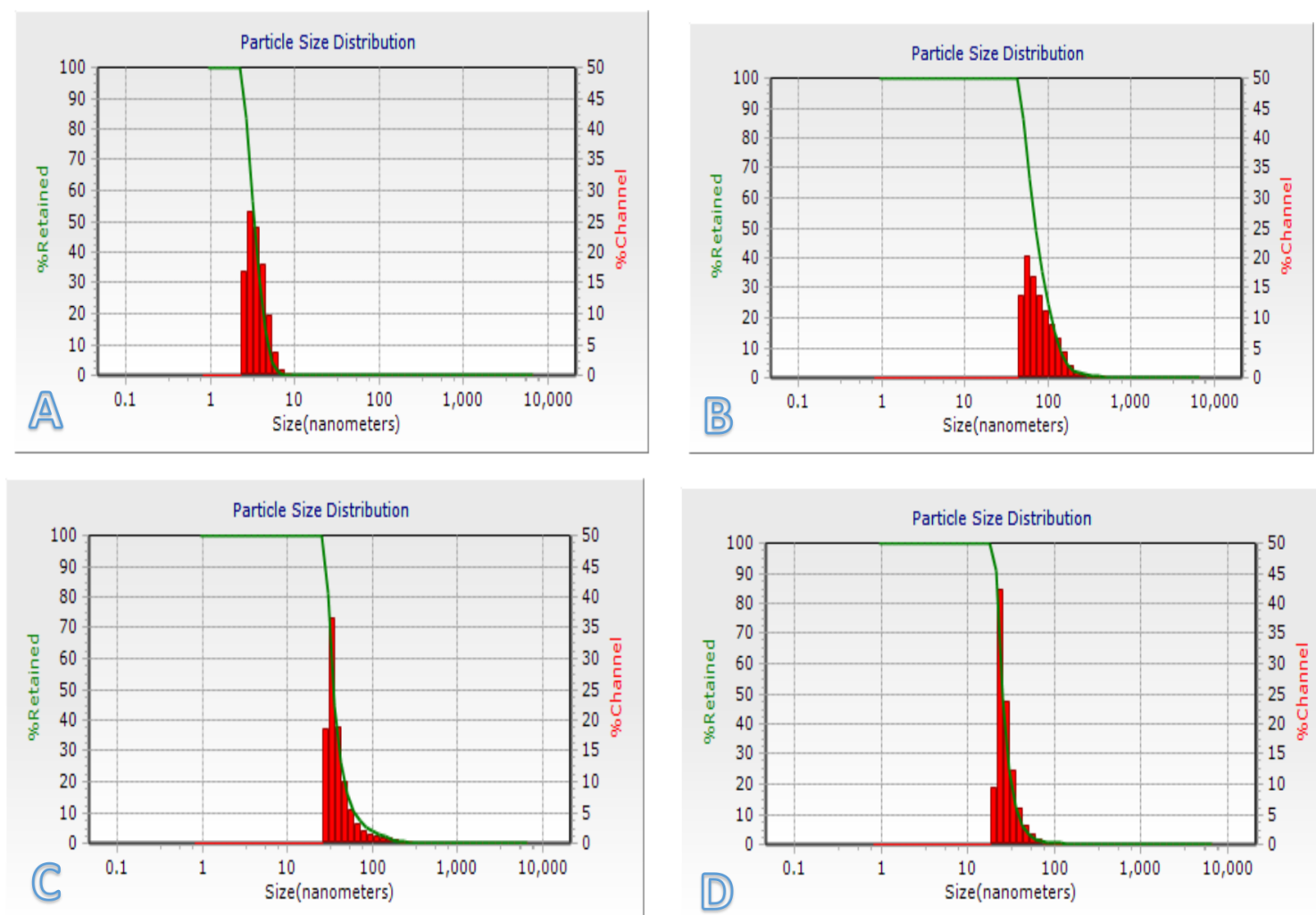


Figure 5

Dynamic Light Scattering (DLS) analysis of peppermint essential oil-based nanostructures:
 (A) Nanoparticle, (B) Nanocomposite, (C) Nanocapsule, and (D) Nanoliposomeoil

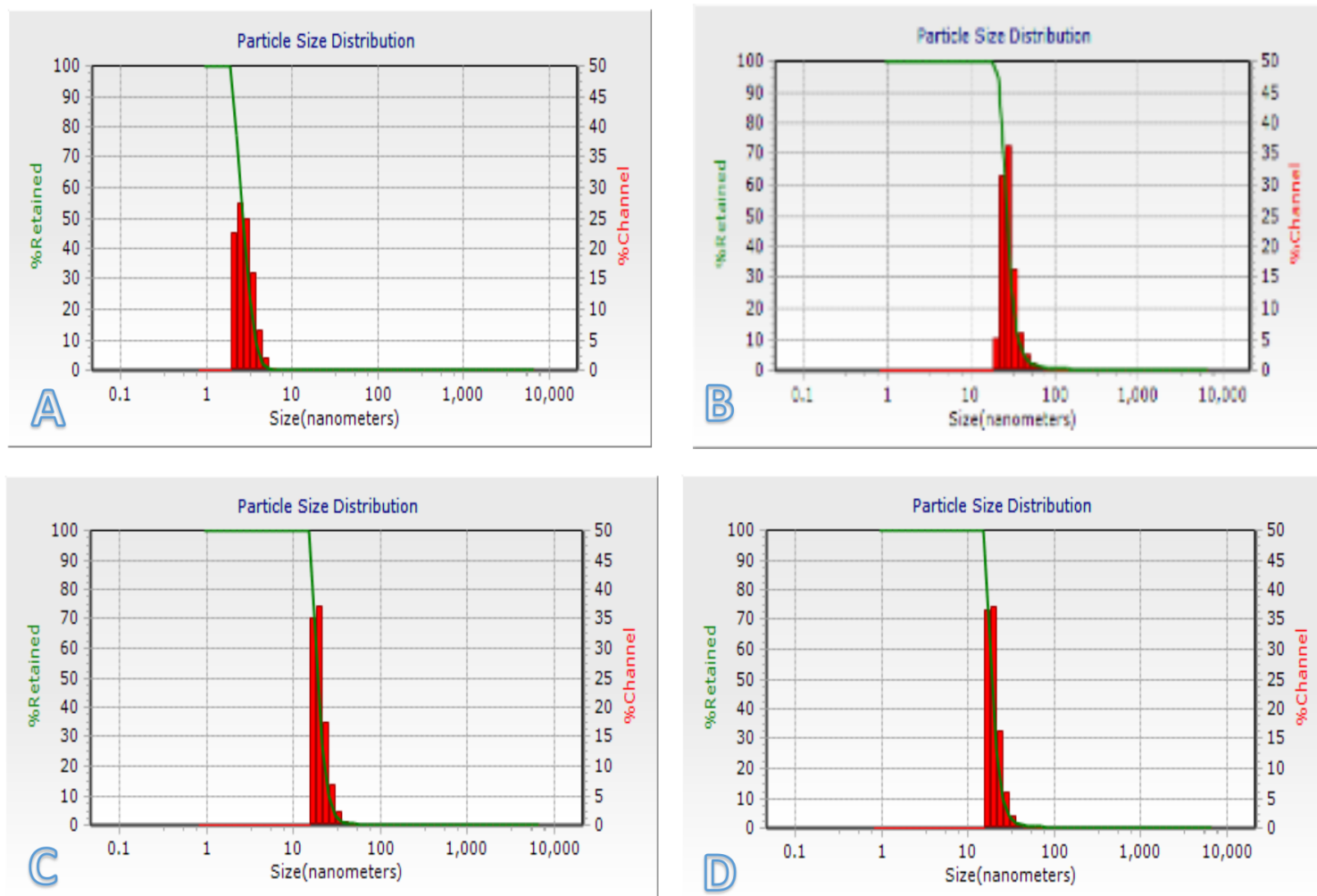


Figure 6

Dynamic Light Scattering (DLS) analysis of rosemary essential oil-based nanostructures: (A) Nanoparticle, (B) Nanocomposite, (C) Nanocapsule, and (D) Nanoliposomeoil

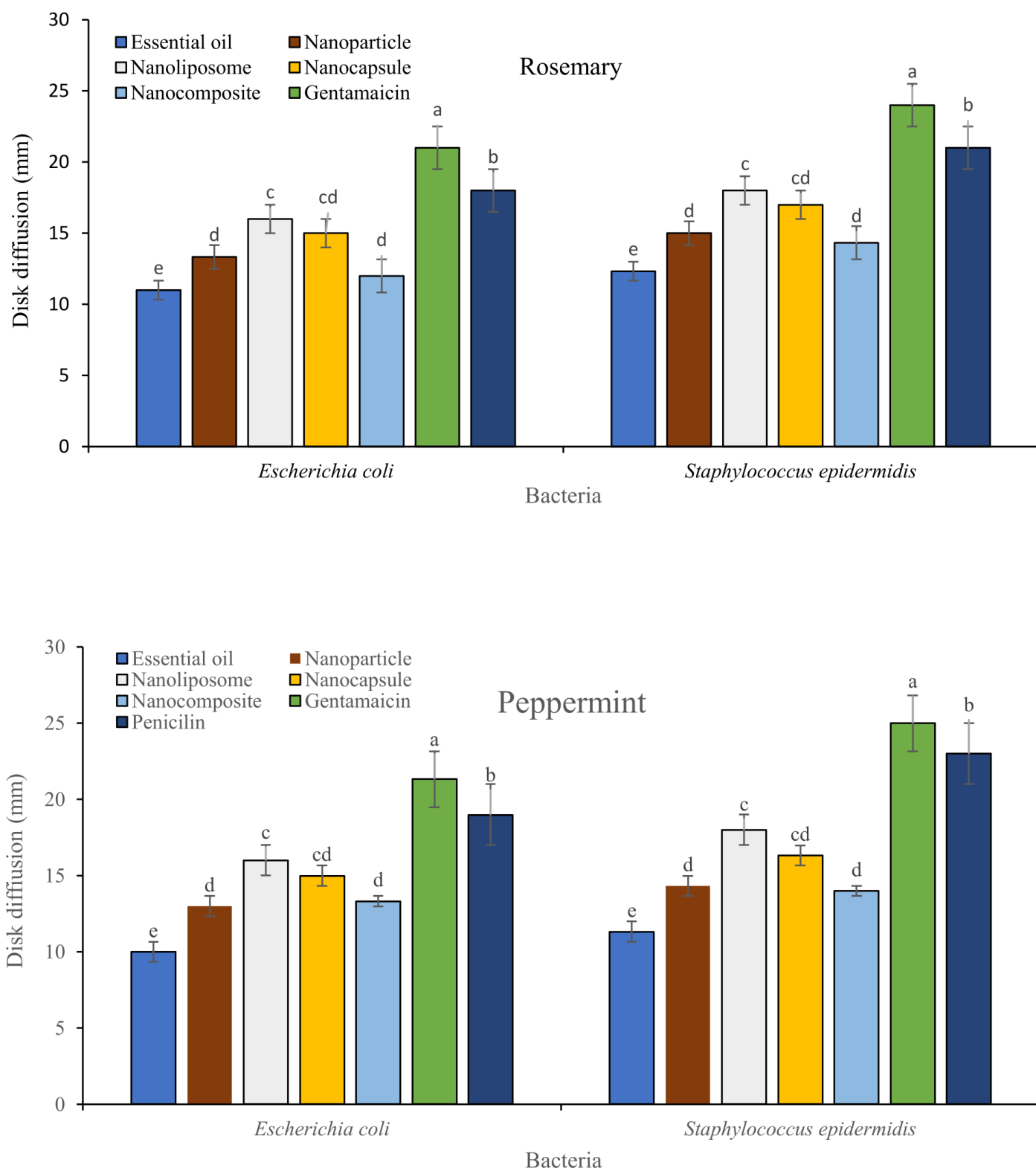


Figure 7

Comparison of the effects of a concentration of one milligram per deciliter of different forms of rosemary and peppermint essential oils, as well as the antibiotics gentamicin and penicillin, on *Staphylococcus epidermidis* and *Escherichia coli*.