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RESEARCH PAPER

Rosmarinus Officinalis Oil-Loaded Chitosan Nanoparticles Synthesized by the Sol-Gel Technique for Enhanced Antibacterial Efficiency

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HIGHLIGHTS

- Chitosan was prepared with rosemary oil extract using sol-gel technology in tripolyphosphate to prepare rosemary oil extract-loaded chitosan nanoparticles.
- Rosemary oil extract and nanoparticle extract showed the antimicrobial activity against Staphylococcus aureus and Sphingomyces paucimobilis bacterial isolates and nanoparticles have inhibitory effects at low concentrations.

ABSTRACT

Nanoparticles (NPs) are a promising therapeutic solution for treating serious bacterial infections that cannot be treated by traditional antibiotics, especially those that cause water contamination and result in massive deaths worldwide every year. The goal of this study was to biosynthesize Rosmarinus officinalis nanoparticles using the sol-gel technique and evaluate their efficacy against bacteria. *Staphylococcus aureus* and *Sphingomonas paucimobilis* were diagnosed by Vitek 2 Compact. Rosemary oil extract-loaded chitosan nanoparticles were synthesized via sol-gel technology in the presence of tripolyphosphate. These synthesized nanoparticles were characterized by FTIR, UV-Vis, XRD, EDX, and FESEM. Rosemary oil extract was prepared by simple hydrodistillation using a Clevenger apparatus, and phytochemical identification revealed the presence of significant amounts of phenolic and flavonoid compounds. The morphological structure and optical properties were characterized using UV-visible light, Fourier transform infrared spectroscopy, X-ray diffraction techniques, and field emission scanning electron microscopy. Energy-dispersive X-ray analysis. The maximum particle size was estimated to be 74.29 nm. The results of extract activity against bacteria using concentrations (59.5, 47.6, 35.7, 23.8 and 11.9 µg/ml(showed the killing rate 51.7, 48.3, 40.3, 33.7, and 26.7 cfu/ml for *Staphylococcus aureus* and 55.7, 43.3, 32.3, 29.3, and 23.3 cfu/ml for *Sphingomonas paucimobilis*. The nanoparticles extract at concentrations (32, 25.6, 19.2, 12.8,6.4 µg/ml(showed a killing percentage 52.3, 41.7, 39.3, 31.3, and 22.3 cfu/ml for *Staphylococcus aureus*, and 44, 44.3, 37.3, 29.7, and 27.7 cfu/ml for *Sphingomonas paucimobilis*. In conclusion, the nanoparticles revealed an antibacterial efficiency against *Staphylococcus aureus* and *Sphingomonas paucimobilis* at low concentrations.

KEYWORDS: *Nanoparticles, Staphylococcus aureus, Sphingomonas paucimobilis, Sol-Gel technology, Rosmarinus officinalis*

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INTRODUCTION

Water represent essential for life and it is important part of a vital part of humanity for numerous purposes like drinking, industry, agriculture, and household [1]. Developing countries represent a large problem due to the unavailability of safe drinking water [2]. The results of the World Health Organization (WHO) indicate that 85% of all pollution in emerging countries is caused by the consumption of polluted water. Also, about 700 million cases of diarrhea and dysentery are identified every year because of contaminated water and a lack of cleanliness [3]. Nanotechnology has enabled the production of several types of materials at the nanoscale level. Nanoparticles (NPs) are a diverse group of substances that consist of tiny particles with a size less than 100 nm in at least one dimension. The attractiveness of NPs for medical applications stems from their significant and distinctive characteristics, including their high surface-to-mass ratio, which surpasses that of other particles, and their ability to transport various substances such as medicines, probes, and proteins [4,5]. Nanoparticles are now being recognized as significant building blocks in the field of nanotechnology [6].

Nanoparticles are one-dimensional elements with a size of 1-100nm. Nanoparticles are separated into organic, which comprises carbon nanoparticles, and inorganic subdivisions, which have superior potential than bigger sizes of particular materials[7]. The significance of nanoparticles as medicine carriers lies in their outstanding stability, amazing capacity to carry medication, and their ability to accommodate both hydrophilic and hydrophobic substances [8]. Nanoencapsulation of bioactive secondary products provides physical protection to the structure and activity during storage before usage [9].

Chitin is the greatest polymer in the environment, and represents the precursor to chitosan by its chemical and enzymatic deacetylation; in addition, it may be created in a multiplicity of eukaryotic species such as fungi, crustaceans, and insects [10]. Green nanotechnology is a technique to produce nanomaterials in a cost-effective, clean, safe, and environmental friend [11].

The structure of ionic gelation of chitosan with sodium tripolyphosphate (TPP) represents a traditional method to produce nanoparticles of chitosan, and these nanoparticles are less structurally stable; it is made by changing chitosan, which is an unadulterated bio-based material, into a hybrid biopolymer synthetic chemical compound [12].

Plant extracts represent the main source of chemical structures, which are called secondary products or secondary metabolites, like alkaloids, flavonoids, saponins, terpenes, volatile oils, and steroids, which have many activities as antioxidants and antimicrobials [13]. Chitosan encapsulation of bioactive compounds in plant extracts produces structures that have more stability and the ability to function as radical scavengers, which is eco-friendly, microbicidal agents, or alternatives antibiotic, particularly that greatest of the final had occasioned in resistance of bacteria due to overemployment or misappropriation [14].

The rosemary (*R. officinalis* L.) plant belongs to the Lamiaceae (Labiatae) family, and it is commonly found in all areas of the Mediterranean Sea. The essential oil of Rosemary is employed in many ways, such as flavor, fragrance, and medicinal purposes [15].

According to Pawłowska *et al*. [16], Rosmarinus officinalis L. displayed antibacterial activity against a wide spectrum of microorganisms, including bacteria, Candida, and fungi. This action is connected to the phytochemicals contained in the plant *Rosmarinus officinalis* L. The major phytochemicals contained in this plant include phenolic metabolites, di- and triterpenes, and essential oils, such as 1,8-cineole, α-pinene, borneol, camphene, camphor, β-pinene, and limonene. The amount of these oils fluctuates depending on the physiological and ecological factors as well as the age and development stage of the vegetation [17].

Sphingomonas paucimobilis is gram negative bacteria widely distributed in wildlife and has been isolated from drinking water and the system responsible for the distribution of drinking water. As it is widespread in the environment, it may often be found in surface biofilms. Some *Sphingomonas* strains are well-recognized for metabolizing complex organic contaminants, while some are opportunistic human diseases [18]. Staphylococci are gram-positive bacteria, with widths of 0.5 – 1.5 μm and characterized by single cocci, which are divided into more than one level to produce grape-like clusters. There are more than 30 species that preferentially colonize the human body [19].

Although previous investigations have investigated the antibacterial effects of rosemary oil and chitosan individually, there is a lack of studies on their combined delivery by sol-gel-produced nanoparticles, especially against watery pathogens like *Sphingomonas paucimobilis*. This study fills the gap through the development of an environmentally friendly, efficient nano-carrier technology that exhibits improved antibacterial effectiveness at reduced doses.

This study aimed to biosynthesize nanoparticles of *Rosmarinus officinalis* using the Sol-Gel technique and detect their effectiveness on bacteria.

MATERIAL AND METHODS

Collection of *Rosmarinus officinalis* L. plant

The dried *Rosmarinus officinalis* L.plant was purchased from the local markets in Baghdad/ Iraq.

Preparation of *Rosmarinus officinalis* L. plant oil extract

The Clevenger apparatus was used to perform a basic

hydrodistillation. A circular-bottom flask was filled with about 100 g of dried leaves from the *Rosmarinus officinalis* L. plant. After blending the leaves with around 500 cc of distilled water, they were boiled at 100°C for 120 minutes. Three times, volatile oils were gathered and placed in a sanitized beaker. Using $CaCl_2$, the produced volatile oil was chemically dried (to remove the water droplets). Weighed and stored at 4°C in tightly-sealed amber-colored bottles was the refined volatile oil [20].

Determination of total phenolic compounds of *Rosmarinus officinalis* oil extract (ROOE).

Using a standard Folin-Ciocalteu reagent, the total quantity of phenolic compounds in the ethanolic extract was determined. The reaction mixture consisted of 1.5 mL of 20% sodium carbonate, 500 µl of the Folin-Ciocalteu reagent (Merck, Germany), and 100 µl of the extract. After that, the sample was combined in a vortex mixer and diluted to a final volume of 10 milliliters using distilled water. Following a two-hour reaction, the absorbance at 765 nm was measured, and the phenolic content was determined by utilizing the calibration curve made using gallic acid (Sigma-Aldrich). Gallic acid equivalent (GAE) milligrams per gram of dry weight was used to express the overall quantity of phenolic compounds[21].

Determination of flavonoid content in *Rosmarinus officinalis* oil extract (ROOE)

The aluminum chloride colorimetric technique was used to determine the total flavonoid content of the crude extract. To summarize, 50µL of crude extract (1 mg – ml ethanol) was heated to 1 mL with methanol, combined with 4 mL of distilled water, and 0.3 mL of 5% NaNO2 solution. After 5 minutes of incubation, 0.3 mL of 10% ALCL3 solution was added, and the mixture was allowed to stand for 6 minutes. After adding 2 milliliters of a 1 mol – L NaOH solution, the mixture's final volume was increased to 10 milliliters using double-distilled water. After the combination had stood for 15 minutes, the absorbance at 510 nm was determined. A calibration curve was used to determine the total flavonoid content, and the result was expressed as mg rutin equivalent g dry weight [22]. Figures 1 and 2.

Biosynthesis of Chitosan nanoparticles loaded with *Rosmarinus officinalis* oil extract using the sol –Gel technique.

Rosmarinus officinalis plant oil extract 2% (2 ml of *Rosmarinus officinalis* plant oil extract + 100 ml D.W.) and chitosan 5% (200 mg of CS + 100 ml acetic acid) were combined in equal amounts, and the procedure was carried out using a Clevenger apparatus in the presence of xylene until the theoretical amount of water was separated. Filtering was used to isolate the chitosan amide product, which was then repeatedly cleaned with methanol, hot distilled water, and ethanol. Then it was dried at 50°C in an electric oven and weighed [23].

Chitosan - *Rosmarinus officinalis* oil extract Nanoparticles using Sol- Gel technique

Using an ionic gelation approach, chitosan NPs loaded with *Rosmarinus officinalis* extract (ROOE) (CSNPs loaded ROOE) were created by combining tripolyphosphate (TPP) with CS-ROOE adduct in the following manner: 5 mg/ml of the CS-ROOE adduct was dissolved with stirring in a 1% w/v acetic acid solution, and the solution eventually became transparent. TPP was added to the CS-ROOE solution at a ratio of 1:2.5 (w/w%) for seven hours at 25 Cº while stirring continuously. The ionic-gelation method was used to create the CSNPs loaded with ROOE. Before the precipitate was dried,

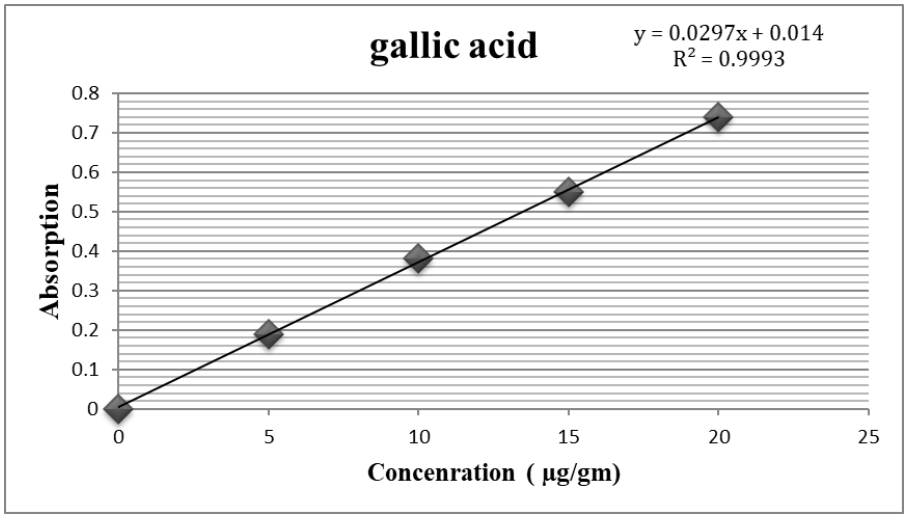


Figure 1: Standard calibration of gallic acid

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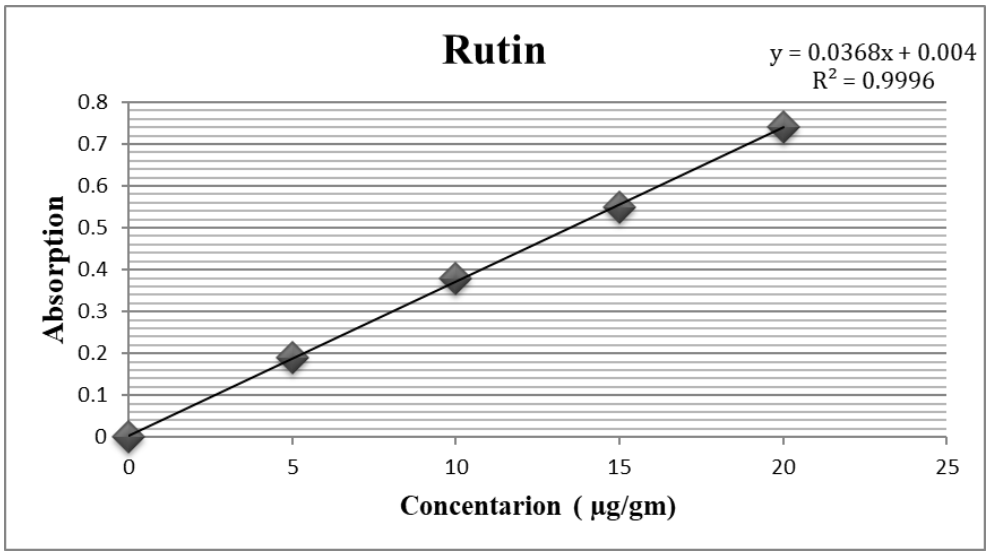


Figure 2: Standard calibration of Rutin

the nanoparticles were divided, repeatedly cleaned, and the liquid layer was eliminated [24] Figure 3).

Determination of total phenolic compounds in *Rosmarinus officinalis* oil extract and *Rosmarinus officinalis* oil extract-loaded chitosan.

Using a standard Folin-Ciocalteu reagent, the phenolic components in the ethanolic extract were measured.100 µl of the extract, 500 µl of the Folin-Ciocalteu reagent (Merck, Germany), and 1.5 ml of 20% sodium carbonate make up the reaction mixture. After that, the sample was combined in a vortex mixer and diluted to a final volume of 10 milliliters using distilled water. The absorbance at 765 nm was measured after the 2-hour treatment, and the phenolic content was determined using the calibration curve made with gallic acid (Sigma-Aldrich). Gallic acid equivalent (GAE) milligrams per gram of dry weight was used to express the overall quantity of phenolic compounds [21].

Determination of flavonoid content in *Rosmarinus officinalis* oil, *Rosmarinus officinalis* oil extract, and *Rosmarinus officinalis* oil extract-loaded chitosan ROOE-CNPs.

The aluminum chloride colorimetric method was used to determine the crude extract's total flavonoid content. In brief, 50µL of crude extract (1 mg – ml ethanol) was raised to 1 mL using methanol, mixed with 4 mL of distilled water, and 0.3 mL of 5% NaNO2 solution. After 5 minutes of incubation, 0.3 mL of 10% ALCL3 solution was added, and the combination was left to stand for 6 minutes. After adding 2 milliliters of a 1 mol – L NaOH solution, the mixture's final volume was increased to 10 milliliters using double-distilled water. After the combination had stood for 15 minutes, the absorbance at 510 nm was determined. Using a calibration curve, the total flavonoid content was calculated and expressed as mg rutin equivalent g dry weight [22].

Strains and Growth Condition

Staphylococcus aureus and *Sphingomonas paucimobilis* bacteria were isolated from the environment. Bacteria were identified by Vitek 2 compact. The bacterial growth was prepared, and a series of dilutions was made in tubes containing sterile distilled water, after which the fifth dilution of bacteria was chosen for experiments. The bacterial suspension was prepared in sterile distilled water, and the concentration of the bacterial growth was 1.5 x 10^5 CFU/ml by comparing it with the standard 0.5 McFarland solution.

Study the effect of *Rosmarinus officinalis* oil extract ROOE and *Rosmarinus officinalis* oil extract-loaded chitosan ROOE-CNPS on bacteria.

An experiment was conducted to evaluate the efficiency of the effect of ROOE and ROOE-CNPS by mixing the volume of the bacterial suspension (0.1ml) with a series of sizes of ROOE and ROOE-CNPS (0.1,0.2,0.3,0.4,0.5) mL in an Eppendorf tube for each volume, and also the control was used by adding distilled water to the bacterial suspension.

After that, each tube that contained bacterial suspension and nanoparticles was incubated for 30 minutes at 37 °C. Then, each mixture was cultured from bacterial suspension, oil extract, and nanoparticles on nutrient agar through 0.1 mL of the mixture and spread with a spreader and incubated for 24 hours at 37 °C [30].

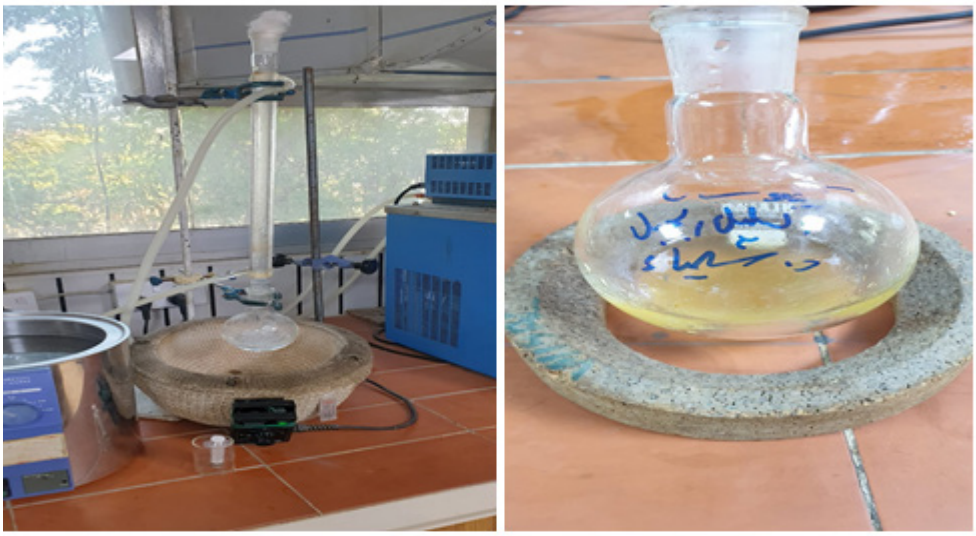


Figure 3: Chitosan - Rosmarinus officinalis oil extract Nanoparticles using the Sol-Gel technique

RESULTS AND DISCUSSION

Determination of total phenolic and flavonoid compounds in *Rosmarinus officinalis* oil extract ROOE and *Rosmarinus officinalis* oil extract-loaded chitosan ROOE-CNPS

The results showed that the total amount of phenolic compounds in ROOE was 271.89(mg Gallic/ 100mg). Also, the total amount of flavonoid compounds was 145.01 (mg Rutin/100mg). On the other hand, the total amount of phenolic compounds in ROOE- CNPS was 98.0 (mg Gallic/ 100mg), and the total amount of flavonoid compounds was 62.1(mg Rutin/ 100mg).

Biosynthesis of Chitosan nanoparticles loaded with *Rosmarinus officinalis* oil extract (CSNPs loaded ROOE)

Chitosan and ROOE were formulated, and then the TPP was added to form CSNPs loaded with ROOE. Chitosan reacts with TPP; the amine groups of the chitosan can cross-link with the phosphine groups of TPP to produce nanoparticles. Through the reaction, the chitosan's molecular structure will be changed, causing an alteration in solubility in an acid solution as well as a solution -gel form [31]. A clear color indicates the formation of nanoparticles loaded with the plant oil extract.

Characterization of CSNPs loaded with ROOE

UV-Visible spectroscopy

UV–visible spectroscopy is a first step in the approval of the synthesis of CSNPs loaded with ROOE. The absorbance of ROOE and CSNPs loaded with ROOE was measured; the results are shown in Figure 4A, B, and C. The results showed that the differences in the wavelength and absorbance of ROOE - $CSNP_s$ was 231with absorbance 1.7, which was different from chitosan, which had two wavelengths, 542.0 and 214.0, respectively, with absorbencies 0.020, 2. 856. Also, the difference that appeared between ROOE –CSNP and ROOE, which had wavelengths 273 and 2.9, indicated that approved that outcome of the connection was 20%.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

FTIR analysis (Shimadzu) was used to characterize the functional groups on the surface of chitosan and chitosan nanoparticles filled with ROOE extract. The spectra were scanned at a resolution of 4 cm^{-1} in the range of 4000 - 400 cm^{-1}. Materials were analyzed using FTIR in the 400–4000 cm^{-1} range. The peak at 2960.73 cm^{-1} in the chitosan spectrum indicates the existence of a C-H stretch, whereas the peak at 3468.01 cm-1 suggests symmetric stretching vibration of O-Stretch, H-bonded, which refers to alcohol 0 phenols. Moreover, amide stretching produced the peaks at 1080.14 cm^{-1} and 1053.13 cm^{-1}, whereas C=O stretching produced the peak at 1639.49 cm^{-1}, which is related to carboxylic acid.

The wavenumber at 881.47 cm^{-1} represents a ring extending a typical band for the β-1-4-glyosidic bond. Conversely, the spectra of CSNPs loaded with ROOE are shown in (Figure 7,8). Additionally, the peak at 1458.18 cm^{-1} is associated with the presence of an aromatic stretching ring in the C-C bond. Whereas the peak at 983.7 cm^{-1} shows the vibration of the C-O bond in the alcohol groups in the polymer, the apparent peak at 1166.93 cm^{-1} indicates the stretching vibration of the carbonyl bond, C-N stretching, and aliphatic amine.

A new spectrum that appeared at 453.27 cm^{-1} and was absent in CS was seen when comparing the spectra of CS and CSNPs loaded ROOE in the beginning. This shows that the creation

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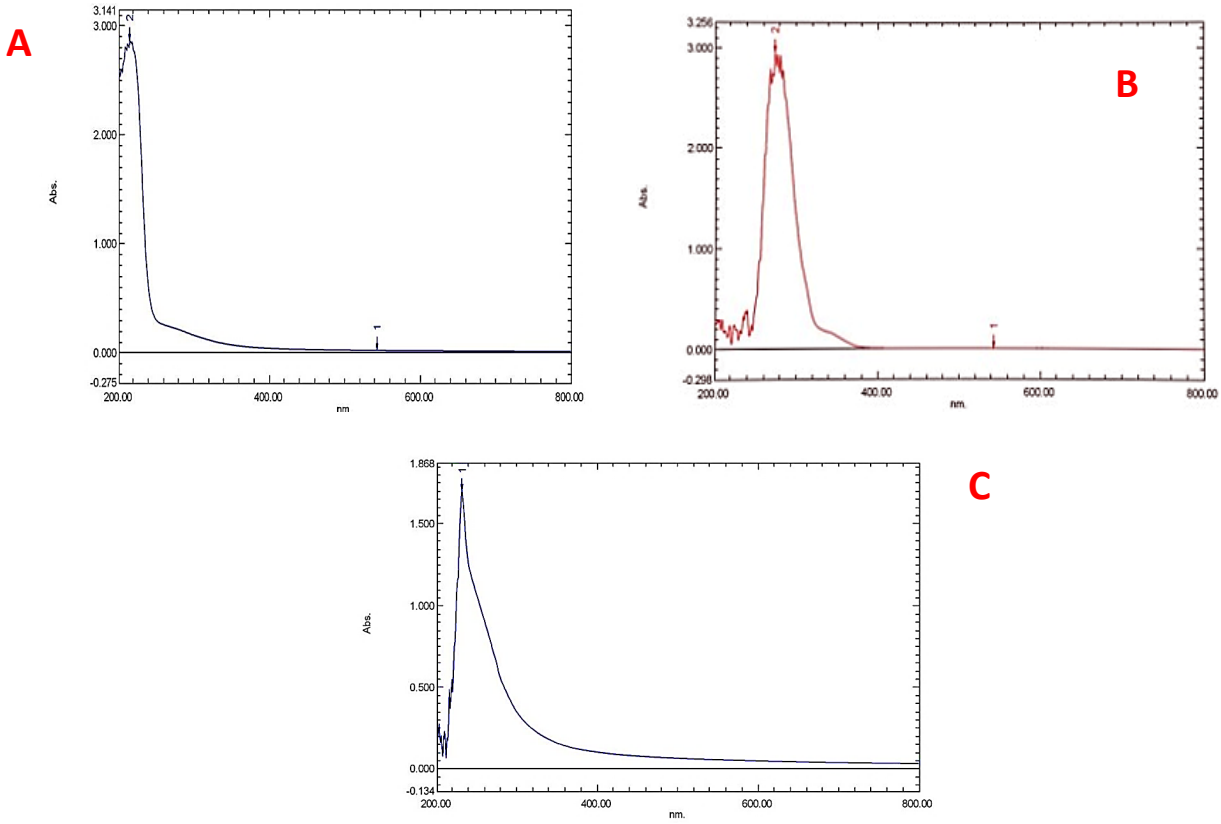


Figure 4: UV–visible spectroscopy)A(chitosan,**(B)**Rossmarinus officinals oil extract, **(C)**Chitosan nanoparticles loaded Rossmarinus officinals oil extract (CSNPs loaded ROOE). The spectra were obtained within the wavelength range of 200 to 800 nm. The alterations in absorption peaks (from 273 nm in ROOE to 231 nm in CSNPs loaded ROOE) validate the successful incorporation of the extract into the chitosan matrix and the establishment of novel interactions between chemicals.

of a new compound led to the establishment of a new bond. The OH stretch of alcohol-phenol is 3468.01 cm-1. Although the strength of the main peaks was lower for CSNPs loaded with ROOE than for CS, this was because TPP cross-linking reduced the intensity of hydrogen bonding. This suggests that the large molecules were broken up into smaller ones, and that the bond's surface area increased both before and after CS connected with TPP. Agarwal et al. [25] reported that the chemical groups of shifted peaks exhibited the successful loading of ROOE in the chitosan / TPP and the formation of CSNPs loaded with ROOE. These results were supported by [32], who demonstrated that the shifted peaks refer to the formation of a new compound. Moreover, changes in the functional groups of active biomolecules suggest that they are associated with the production of CSNPs loaded with ROOE.

X-ray diffraction (XRD)

The major peaks of the 2θ value are seen in the X-ray diffraction patterns of chitosan at 20.53°, 29.7, and 38.8, with an intensity level close to 1200 cont. The CSNPs-loaded ROOE peak is seen in Figure 9, with the principal peak of value located at 12.67°, 30.95, and 34.88. The CS diffraction peak, previously found at 20.53, has shifted to a higher value of 12.67° in this study, which may be related to the interaction of CS loaded with ROOE to form CSNPs loaded with ROOE. This change indicates the difference in the crystal structure between these two materials, with CSNPs loaded ROOE being more crystalline than CS.

The XRD data revealed that particle size effects are typically responsible for the broadening of peaks in crystalline XRD patterns. Wider peaks imply smaller particle sizes and reflect the effect of experimental settings on particle structures. Small crystals have a limited number of reflection levels with low intensity, whereas giant crystals have a vast number of these levels with great intensity. Therefore, X-ray diffraction peaks are created owing to reflection from crystal levels, and the reduction in intensity is seen in the pattern of CSNPs loaded ROOE, which shows suppressed peaks.

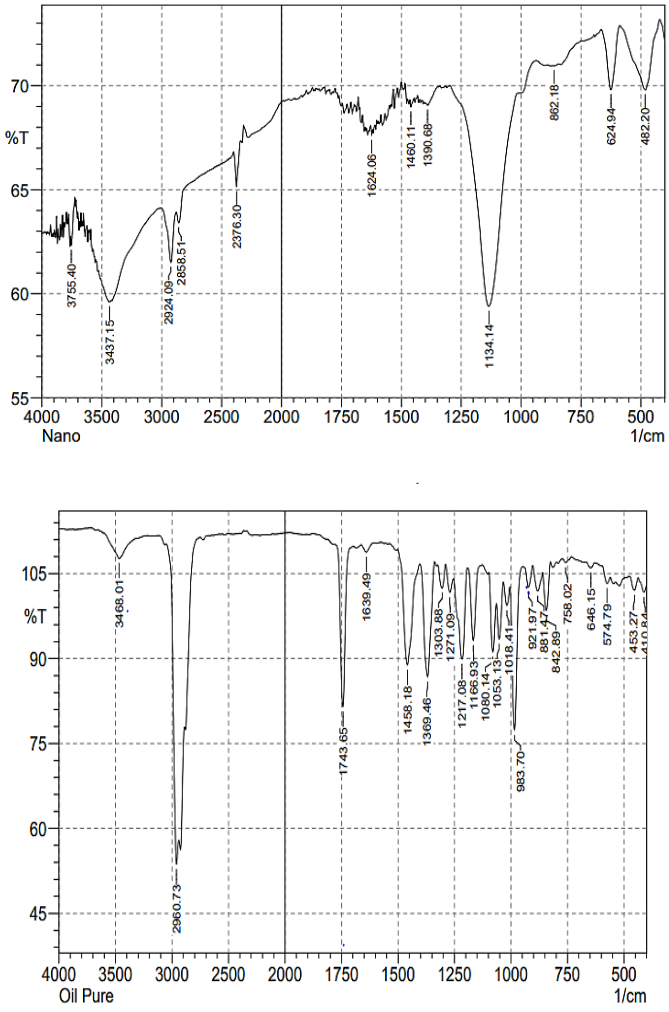


Figure 5. FTIR Spectra Pattern of **(A)**Rossmarinus officinals oil extract **(B)** Chitosan nanoparticles loaded Rossmarinus officinals oil extract (CSNPs loaded ROOE). The spectra display distinctive chitosan peaks (O-H stretch at 3468 cm^{-1}, C=O stretch at 1639 cm^{-1}) and alterations post-loading with ROOE, featuring new peaks (at 453 cm^{-1}) and shifts in pre-existing peaks, signifying effective encapsulation and chitosan interaction with plant extract components.

These findings were in excellent agreement with Anand et al.[27]who observed that the pure chitosan diffraction peak, which was previously found at 20.20°, has migrated to a lower value, 19.85°, which may be related to the interaction of CS-NPs with TPP and the crystalline structure of CS-NPs. Kahdestani et al.[31] Found that the differences among the patterns may be related to molecular arrangement changes in the crystal lattice, and the pattern of teicoplanin-loaded chitosan nanoparticles showed lower peaks at 20 22° and 25°, suggesting an intensity drop.

Energy Dispersive X-ray (EDX)

The elemental analysis of CSNPs loaded SAE via EDX shows the presence of C, O, Na, Mg, Si, S, and Ca. The EDX examination reveals a significant signal at 1.5 keV and 2.3 keV due to the presence of carbon and oxygen, respectively, confirming the presence of chitosan; the other elements are due to the presence of ROOE components. The results agree with Raza and Anwar[33], who used scanning electron microscopy equipped with EDX to confirm the presence of CSNPs on the treated fabric.

Field Emission Scanning Electron Microscopy (FESEM) Analysis

Using a field-emission scanning electron microscope (FESEM), the morphology of CSNPs loaded with ROOE was examined. The results are shown in Figure 10. The larger

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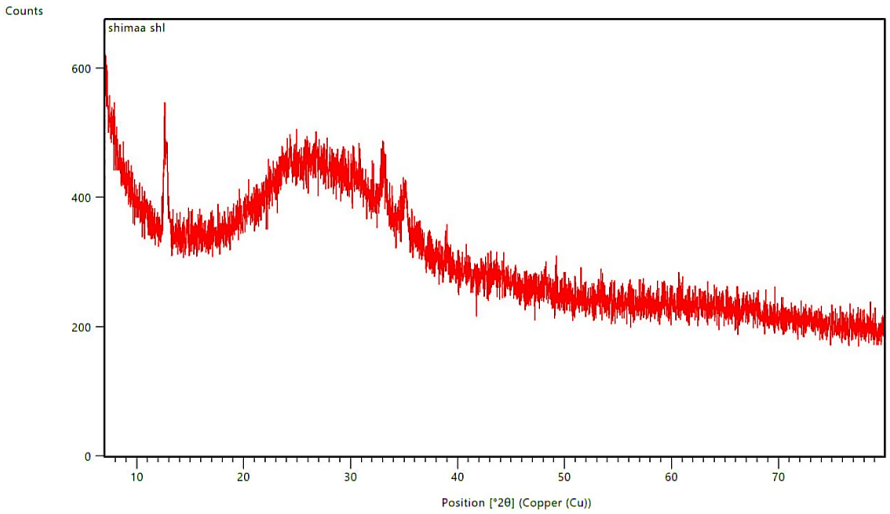


Figure 6: Diffractogram of CSNPs loaded ROOE

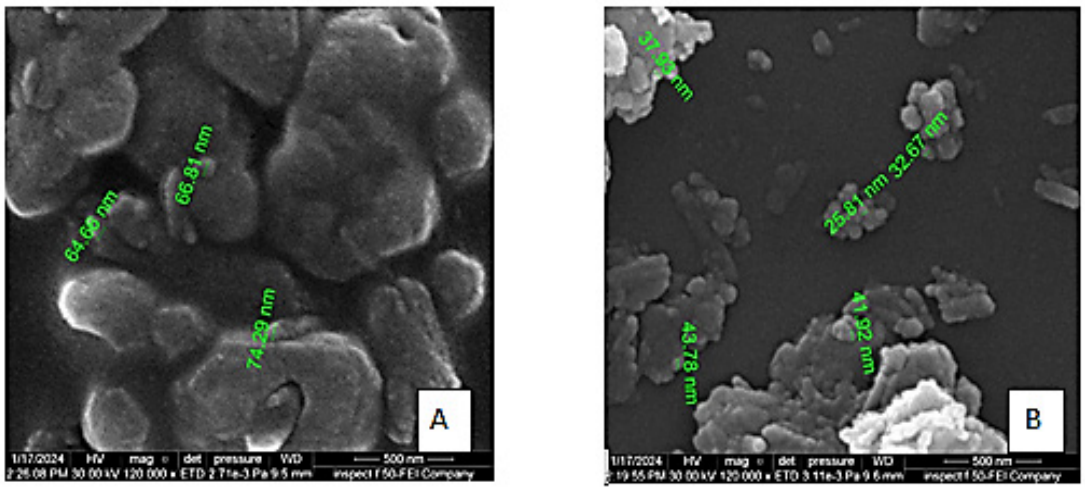


Figure 7: **(A)** and **(B)** Diameters of CSNPs loaded with ROOE using a field-emission scanning electron microscope (FESEM). Scale bar 500nm

particles may have resulted from aggregation during the drying process, giving the spherical appearance of CSNPs loaded with ROOE and a relatively homogeneous morphology. As soon as the liquid evaporates, agglomeration occurs, increasing the concentration of particles.

Study the effect of *Rosmarinus officinalis* oil extract ROOE and *Rosmarinus officinalis* oil extract-loaded chitosan ROOE-CNPS on bacteria.

The antibacterial activity of Rosmarinus officinalis oil extract (ROOE) against Staphylococcus aureus and *Sphingomonas paucimobilis* was measured using volumes of 0.5, 0.4, 0.3, 0.2, and 0.1 ml, corresponding to concentrations of 59.5, 47.6, 35.7, 23.8, and 11.9 µg/ml, respectively. The findings indicated kill ratios of 51.7, 48.3, 40.3, 33.7, and 26.7 cfu/ml for *Staphylococcus aureus* and 55.7, 43.3, 32.3, 29.3, and 23.3 cfu/ml for *Sphingomonas paucimobilis*, respectively(Table 1 and Figures 8 and 9). The chitosan ROOE-CNPS, loaded with *Rosmarinus officinalis* oil extract, was examined against *Staphylococcus aureus* and *Sphingomonas paucimobilis* using serial volumes of 0.5, 0.4, 0.3, 0.2, and 0.1 ml, which corresponded to serial concentrations of 32, 25.6, 19.2, 12.8, and 6.4 µg/ml. The results showed a kill percentage of 52.3, 41.7, 39.3, 31.3, and 22.3 cfu/ml for *Staphylococcus aureus*, and 44, 44.3, 37.3, 29.7,

Table 1: Efficiency of rosemary oil extract (ROOE) against bacteria

Concentration (µg/ml)	Volume (ml)	*S. aureus* (CFU/ml)	Standard deviation (±SD)	*S. paucimobilis* (CFU/ml)	Standard deviation (±SD)
59.5	0.5	51.7	0.58	55.7	0.58
47.6	0.4	48.3	0.6	43.3	0.58
35.7	0.3	40.3	0.6	32.3	0.6
23.8	0.2	33.7	0.5	29.3	0.6
11.9	0.1	26.7	0.57	23.3	0.6
(Control)	-	TNTC*	-	TNTC*	-

TNTC means Too numerous to count; CFU means Colony-forming unit

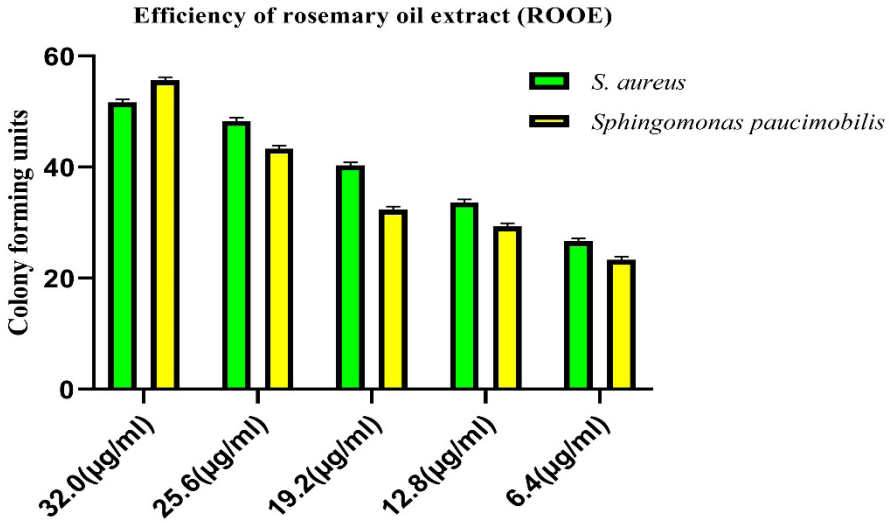


Figure 8: Efficiency of rosemary oil extract (ROOE) against bacteria

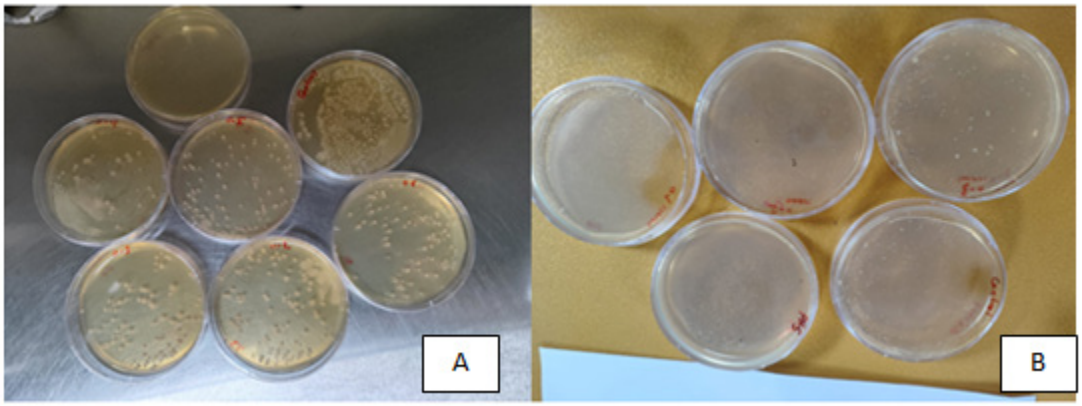


Figure 9: The effect of Rosmarinus officinalis oil extract **(A)** and Rosmarinus officinalis oil extract loaded chitosan ROOE-CNPS **(B)** on Staphylococcus aureus

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Table 2: Efficiency of extract-loaded chitosan nanoparticles (ROOE-CSNPs) against bacteria

Concentration (µg/ml)	Volume (ml)	*S.aureus* (CFU/ml)	Standard deviation (±SD)	*S. paucimobilis* (CFU/ml)	Standard deviation (±SD)
32.0	0.5	52.3	1.2	44	-
25.6	0.4	41.7	0.6	44.3	0.57
19.2	0.3	39.3	1.2	37.3	0.58
12.8	0.2	31.3	0.6	29.7	0.6
6.4	0.1	22.3	0.6	27.7	0.6
(Control)	-	TNTC	-	TNTC	-
Amoxicillin/Clavulanic acid	-	4.7	0.6	7.7	0.6

TNTC means Too numerous to count; CFU means Colony-forming unit

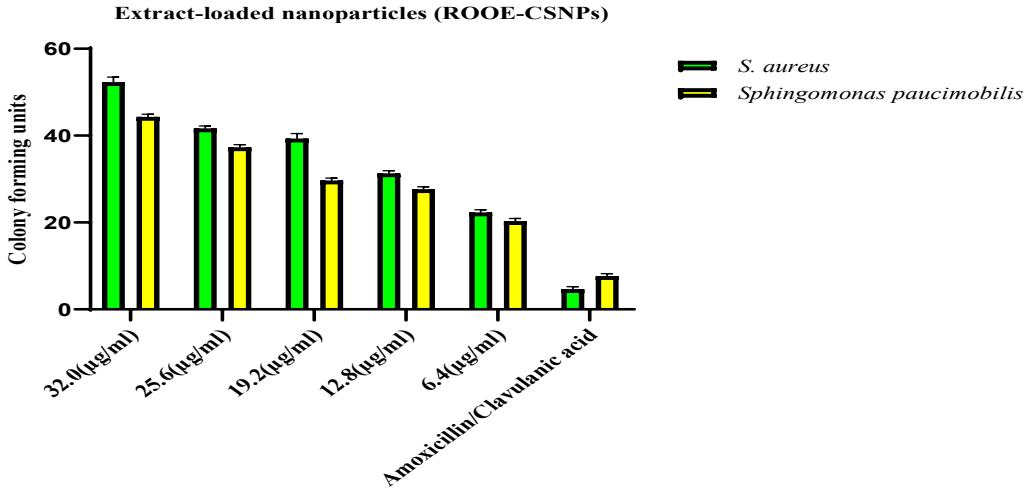


Figure 10:Efficiency of extract-loaded chitosan nanoparticles (ROOE-CSNPs) against bacteria

and 27.7 cfu/ml for *Sphingomonas paucimobilis*. All results were compared to the control, which had a total plate count that was too numerous to count (TNTC). In contrast, amoxicillin/clavulanic acid yielded 4.7 cfu/ml for *Staphylococcus* and 7.7 cfu/ml for *Sphingomonas* (Table2) (Figures 10 and 11).

In the present study, *Rosmarinus officinalis* oil extract (ROOE) was found to be an effective antibiotic against *Staphylococcus aureus* and *Sphingomonas paucimobilis* in all volumes and concentrations, and these results are similar to those reported by Bowbe *et al.* [34]. The findings of the current study are likewise consistent with previous findings of Manilal *et al.* [35] and Harfouch *et al.* [36], who referred to the effectiveness of rosemary extract against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of *Rosmarinus officinalis* oil extract-loaded chitosan (ROOE-CNPS), when applied, was found to be more effective at the same concentrations and inhibited the growth of the two bacterial isolates. This study agrees with Khaleel *et al.* (2023), who reported that *Rosmarinus officinalis* Ag NPs have more effective activities than the same extract without Ag. Table 3 shows the Comparison of the current study with other studies for the plant loaded on chitosan nanoparticles.

Antibacterial mechanism for chitosan ROOE-CNPS

ROOE-loaded chitosan nanoparticles (ROOE-CSNPs) exhibit enhanced antibacterial activity due to synergistic multi-mechanistic interactions between the matrix and the bioactive components of Rosmarinus officinalis oil extract. Chitosan nanoparticles have a positive surface charge due to protonated amine groups ($-NH_3^+$) under acidic or neutral environments. This charge interacts with negatively charged bacterial cell walls like Gram-negative *S. paucimobilis* lipopolysaccharides and Gram-positive S. aureus teichoic acids. This interaction breaks the cell membrane, increases permeability, and leaks intracellular components, killing the cell [14, 10]. Second, this study found high levels of flavonoid and phenolic compounds

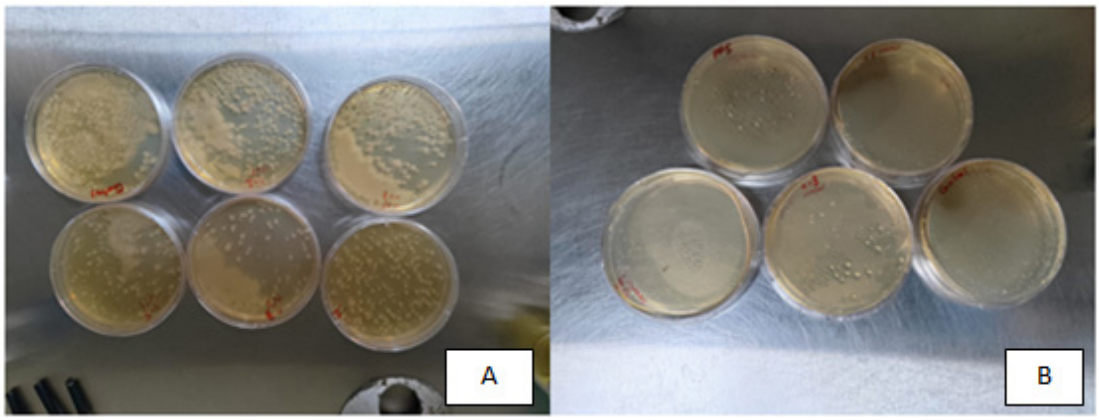


Figure 11: The effect of Rosmarinus officinalis oil extract **(A)** and Rosmarinus officinalis oil extract-loaded chitosan ROOE-CNPS **(B)** on Sphingomonas paucimobilis

Table 3: Comparison of the current study with other studies for the plant loaded on chitosan nanoparticles

Study	Nanoparticle	Plant	Bacteria	Size (nm)	Results
This current study	Plant extract-loaded chitosan nanoparticles (ROOE-CSNPs)	*R. officinalis* oil	*S. aureus*, *S. paucimobilis*	74	effective against waterborne pathogens at low concentrations
Gadkari *et al.* [12]	Chitosan-Cinnamaldehyde nanoparticles	Cinnamon oil	*E. coli*; *S. aureus*	90-120	Antioxidant and antibacterial activity
Antunes *et al.* [14]	Chitosan nanoparticles with various extracts	Thyme; rosemary	*S. aureus*; *P. aeruginosa*	50-200	Synergistic effects with antibiotics; inhibition of biofilm
Kahdestani *et al.* [31]	Chitosan-teicoplanin nanoparticles	antibiotic	Methicillin *Staphylococcus aureus* (MRSA(	100-150	Prolonged medication release; enhanced stability
Raza and Anwar [33]	Chitosan nanoparticles for textile	–	E. coli; S. aureus	40-80	Resilient antimicrobial coating on cotton
Al-Fraihat *et al.* [15]	Plant growth study	R. officinalis	–	–	Increased production of oil under stress conditions

in the concentrated rosemary oil extract, which generate reactive oxygen species (ROS), damage bacterial proteins and DNA, and inhibit critical enzymes in cellular metabolism [34, 17]. Third, chitosan nano-encapsulation improves hydrophobic bioactive chemical stability, solubility, and regulated release. Persistent contact with bacterial cells allows deeper biofilm penetration [24]. The small particle size (74 nm) increases the surface area-to-volume ratio and bacterial contact. The integrated system may hinder biofilm formation by complicating bacterial adherence to surfaces and preventing them from detecting enough population density. This final point needs more research. Antibiotic resistance is lower with this multi-target mechanism than with single-target drugs. This suggests that ROOE-CSNPs may be a sustainable solution to resistant and waterborne diseases. The present findings are corroborated by recent advancements in nanoparticle systems for microbial regulation. Hydrozincite/graphene oxide nanocomposites have exhibited considerable antiviral and antibacterial effectiveness through integrated experimental and molecular docking investigations, highlighting their interactions with membranes of bacteria and proteins[37].

Besides antibacterial uses, nanotechnology offers versatile structures for addressing various water contaminants. A study has shown that diethanolamine-functionalized polystyrene can effectively eliminate chemical pollutants, including p-toluenesulfonic acid, by adsorption techniques [38]. This highlights the adaptability of material design in water treatment, allowing for the customization of functional groups for specific contaminants. The current method, based on chitosan, refined for biological decontamination, may be incorporated with chemical elimination methods for multi-step water treatment systems, effectively tackling both microbial and chemical

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contaminants.

The present study concluded that chitosan nanoparticles loaded with *Rosmarinus officinalis* could serve as a natural alternative for combating bacteria. This conclusion is based on the unique properties of the nanoparticles, which enhance drug effectiveness and improve drug delivery efficiency.

Limitations of the study: This study presents promising results concerning the antibacterial effectiveness of ROOE-loaded chitosan nanoparticles; however, it is acknowledged that advanced characterizations, such as thermogravimetric analysis (TGA), transmission electron microscopy (TEM), surface area analysis (BET), and zeta potential tests, were not performed due to limitations in instrumentation. These studies may yield valuable insights into the thermal stability, porosity, surface morphology, and colloidal stability of the nanoparticles. Future research should combine these techniques to refine the nanoparticle system for potential biomedical or environmental applications.

CONCLUSION

Chitosan nanoparticles loaded with *Rosmarinus officinalis* oil extract can be created via the green synthesis approach by involving two biomolecules with the occurrence of TPP as a cross-linker. Both Chitosan nanoparticles loaded with *Rosmarinus officinalis* oil extract and *Rosmarinus officinalis* oil extract showed antibacterial efficacy in vitro against the studied bacteria, with high potential for Chitosan nanoparticles loaded with *Rosmarinus officinalis* oil extract.

Conflicts of interest
There is no conflict of interest associated with this article.

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Author contributions
Shaimaa Fakhri Jasim, Labeeb Ahmed Al-zubaidi, Suha Mohamed Ibrahim, Noor Nihad Baqer: Investigation; Methodology; Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Writing - original draft; Writing - review and editing.

REFERENCES

[1] Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., & Das, S. " Microbial pollution of water with special reference to coliform bacteria and their nexus with environment." Energy Nexus, 1(2021) 100008. https://doi.org/10.1016/j.nexus.2021.100008

[2] Bylund, J., Toljander, J., Lysén, M., Rasti, N., Engqvist, J., & Simonsson, M. "Measuring sporadic gastrointestinal illness associated with drinking water-an overview of methodologies." Journal of water and health, 15(3) (2017) 321-340. https://doi.org/10.2166/wh.2017.261

[3] Li, Y., Zhang, C., Wang, X., Liao, X., Zhong, Q., Zhou, T., ... & Zou, H. «Pollutant impacts on bacteria in surface water and sediment: Conventional versus emerging pollutants in Taihu Lake, China." Environmental Pollution, 323,(2023) 121334. https://doi.org/10.1016/j.envpol.2023.121334

[4] Mustafa, B. M., & Hassan, N. E. " Water Contamination and Its Effects on Human Health: A Review." Journal of Geography, Environment and Earth Science International, 28(1) (2024) 38-49. https://doi.org/10.9734/jgeesi/2024/v28i1743

[5] Khan, I.; Saeed, K. and Khan, I. " Nanoparticles: Properties, applications and toxicities." Arabian Journal of Chemistry, 12(7) (2019) 908-931. https://doi.org/10.1016/j.arabjc.2017.05.011

[6] Yusuf, M. " Silver nanoparticles: Synthesis and Applications." In Handbook of Ecomaterials, 4(2019)2343-2356. https://doi.org/10.1007/978-3-319-68255-6_16

[7] Ealia, S. A. M. and Saravanakumar, M. P. " A review on the classification, characterisation, synthesis of nanoparticles and their application." {IOP} Conference Series: Materials Science and Engineering, 263(2017) 32019. https://doi.org/10.1088/1757-899X/263/3/032019

[8] Moeinzadeh, S. and Jabbari, E. « Nanoparticles and their applications. " Springer Handbooks, 9(1) (2017) 335-361. https://doi.org/10.1007/978-3-662-54357-3_11

[9] Choudhury, S. R.; Ordaz, J.; Lo, C. L.; Damayanti, N. P.; Zhou, F. and Irudayaraj, J.» Zinc oxide nanoparticles-induced reactive oxygen species promotes multimodal cyto- and epigenetic toxicity." Toxicological Sciences, 156(1)2017)) 261-274.

[10] Elieh-Ali-Komi, D. and Hamblin, M. R. «Chitin and chitosan: production and application of versatile biomedical nanomaterials." International Journal of Advanced Research, 4(3)(2016) 411-427.

[11] Huston, M.; Debella, M.; Dibella, M. and Gupta, A. "Green synthesis of nanomaterials." Nanomaterials. 9(9)(2021)1275. https://doi.org/10.3390/nano9091275

[12] Gadkari, R. R.; Suwalka, S.; Yogi, M. R.; Ali, W.; Das, A. and Alagirusamy, R. "Green synthesis of chitosan-cinnamaldehyde cross-linked nanoparticles: characterization and antibacterial activity." Carbohydrate Polymers, 226)2019) 115298. https://doi.org/10.1016/j.carbpol.2019.115298

[13] Rai, M. and Yadav, A" Plants as potential synthesiser of precious metal nanoparticles: Progress and prospects.» IET Nanobiotechnology, 7(3)(2013)117-124. https://doi.org/10.1049/iet-nbt.2012.0031

[14] Antunes, J. C., Domingues, J. M., Miranda, C. S., Silva, A. F. G., Homem, N. C.; Amorim, M. T. et al. « Bioactivity of chitosan-based particles loaded with plant-derived extracts for biomedical applications: Emphasis on antimicrobial fiber-based systems." Marine Drugs, 19(7)(2021) 359. https://doi.org/10.3390/md19070359

[15] Al-Fraihat, A. H., Al-Dalain, S. Y., Zatimeh, A. A., & Haddad, M. A. «Enhancing Rosemary (Rosmarinus officinalis, L.) Growth and Volatile Oil Constituents Grown under Soil Salinity Stress by Some Amino Acids." Horticulturae, (2023) 9(2) 252. https://doi.org/10.3390/horticulturae9020252

[16] Pawłowska, K., Janda, K., & Jakubczyk, K. Properties and use of rosemary (L.). Pomeranian Journal of Life Sciences, 66(3)(2020) 76-82. https://doi.org/10.21164/pomjlifesci.722

[17] Swamy, M.K. and Akhtar, M.S. eds., 2019. Natural Bio-Active Compounds: Volume 2: Chemistry, Pharmacology and Health Care Practices. Springer Nature. https://doi.org/10.1007/978-

981-13-7205-6
[18] Gusman, V., Medić, D., Jelesić, Z., & Mihajlović-Ukropina, M. Sphingomonas paucimobilis as a biofilm producer.» Archives of Biological Sciences, 64(4) (2012)1327-1331. https://doi.org/10.2298/ABS1204327G
[19] Gehrke, A. K. E., Giai, C., & Gómez, M. I.» Staphylococcus aureus Adaptation to the Skin in Health and Persistent/Recurrent Infections." Antibiotics, 12(10)(2023) 1520. https://doi.org/10.3390/antibiotics12101520
[20] Jaradat N. A., Zaid, A. N., Abuzant, A. and Shawahna, R. Investigation the efficiency of various methods of volatile oil extraction from Trichodesma africanum and their impact on the antioxidant and antimicrobial activities. J. Intercult. Ethnopharmacol. 5 (3) (2016) 250-256. DOI: 10.5455/jice.20160421065949 https://doi.org/10.5455/jice.20160421065949
[21] Laouini, S. E., & Ouahrani, M. R. " Phytochemical screening, in vitro antioxidant and antibacterial activity of Rumex vesicarius L. extract. Scientific Study & Research." Chemistry & Chemical Engineering, Biotechnology, Food Industry, 18(4)(2017) 367-376.
[22] Habibatni, S., Zohra, A. F., Khalida, H., Anwar, S., Mansi, I., & Awadh Ali, N. A. «In vitro antioxidant, Xanthine oxidase-inhibitory and in vivo Anti-inflammatory, analgesic, antipyretic activity of Onopordum acanthium. "Int. J. Phytomed, 9(1)(2017) 92-100 https://doi.org/10.5138/09750185.2030
[23] Abd El-Ghaffar, M. A., & Hashem, M. S. (2013). Calcium alginate beads encapsulated PMMA-g-CS nano-particles for α-chymotrypsin immobilization. Carbohydrate polymers, 92(2), 2095-2102. https://doi.org/10.1016/j.carbpol.2012.11.084
[24] Nasti, A., Zaki, N. M.,De Leonardis, P., Ungphaiboon, S., Sansongsak, P.,Rimoli, M. G. et al. Chitosan/TPP and chitosan/TPP-hyaluronic acid nanoparticles: Systematic optimisation of the preparative process and preliminary biological evaluation. Pharmaceutical Research, 26(8)(2009) 1918-1930. https://doi.org/10.1007/s11095-009-9908-0
[25] Agarwal, M., Agarwal, M. K., Shrivastav, N., Pandey, S., Das, R. and Gaur, P. " Preparation of chitosan nanoparticles and their in-vitro characterization. " International Journal of Life-Sciences Scientific Research, 4(2) (2018) 1713-1720. https://doi.org/10.21276/ijlssr.2018.4.2.17
[26] Nandiyanto, A.B.D., Ragadhita, R. and Fiandini, M."Interpretation of Fourier transform infrared spectra (FTIR): A practical approach in the polymer/plastic thermal decomposition. "Indonesian Journal of Science and Technology, 8(1)(2023) pp.113-126. https://doi.org/10.17509/ijost.v8i1.53297
[27] Anand, M., Sathyapriya, P., Maruthupandy, M. and Hameedha Beevi, A." Synthesis of chitosan nanoparticles by TPP and their potential mosquito larvicidal application." Frontiers in Laboratory Medicine, 2(2) (2018) 72-78. https://doi.org/10.1016/j.flm.2018.07.003
[28] Hodoroaba, V. D. " Energy-dispersive X-ray spectroscopy (EDS). In Characterization of Nanoparticles: Measurement Processes for Nanoparticles.)2020(397-417. https://doi.org/10.1016/B978-0-12-814182-3.00021-3
[29] Mohammad Shafiee, M. R., Kargar, M., & Ghashang, M. " Simple synthesis of copper oxide nanoparticles in the presence of extractive Rosmarinus officinalis leaves." Journal of Nanostructures, 6(1) (2016)28-31.
[30] Alnuaimi, M. T. A., AL-Hayanni, H. S., & Aljanabi, Z. Z. «Green synthesis of gold nanoparticles from Sophora flavescens extract and their antibacterial effect against some pathogenic bacteria." Malaysian Journal of Microbiology, 19(1))2023) https://doi.org/10.21161/mjm.220060
[31] Kahdestani, S. A.; Shahriari, M. H. and Abdouss, M. Synthesis and characterization of chitosan nanoparticles containing teicoplanin using sol-gel. Polymer Bulletin, 78(2)(2021) 1133-1148. https://doi.org/10.1007/s00289-020-03134-2
[32] Jabbar Almukhtar, J. G. and Karam, F. F." Preparation characterization and application of Chitosan nanoparticles as drug carrier." Journal of Physics: Conference Series, 1664(1)(2020) 012071. https://doi.org/10.1088/1742-6596/1664/1/012071
[33] Raza, Z. A. and Anwar, F. " Fabrication of chitosan nanoparticles and multi-response optimization in their application on cotton fabric by using a Taguchi approach." Nano-Structures and Nano-Objects, 10(2017) 80-90. https://doi.org/10.1016/j.nanoso.2017.03.007
[34] Bowbe, K. H., Salah, K. B. H., Moumni, S., Ashkan, M. F., & Merghni, A. «Anti-staphylococcal activities of rosmarinus officinalis and myrtus communis essential oils through ROS-mediated oxidative stress." Antibiotics, 12(2)(2023) 266. https://doi.org/10.3390/antibiotics12020266
[35] Manilal, A., Sabu, K. R., Woldemariam, M., Aklilu, A., Biresaw, G., Yohanes, T., ... & Merdekios, B. " Antibacterial activity of Rosmarinus officinalis against multidrug-resistant clinical isolates and meat-borne pathogens". Evidence-Based Complementary and Alternative Medicine, 2021, (2021)1-10. https://doi.org/10.1155/2021/6677420
[36] Harfouch, R. M., Darwish, M., Al-Asadi, W., Mohammad, A. F., Gharib, N. M., & Haroun, M. «Antibacterial activity of essential oils of Rosmarinus officinalis, Salvia officinalis and Anthemis nobilis widespread in the Syrian coast." Research Journal of Pharmacy and Technology, 12(7) (2019) 3410-3412. https://doi.org/10.5958/0974-360X.2019.00576.6
[37] Dorazehi, J., Farrokhi, M., Bastami, T. R., Meshkat, Z., Mahmoudi, V., Tanzadehpanah, H., & Babaei, H." Exploration of the antiviral and antibacterial efficacy of hydrozincite/graphene oxide nanocomposites: Molecular docking and in vitro investigations." Journal of Environmental Chemical Engineering, (2025) 118940. https://doi.org/10.2139/ssrn.5280840
[38] Davarpanah, M., Ahmadpour, A., Rohani-Bastami, T., & Dabir, H. "Synthesis and application of diethanolamine-functionalized polystyrene as a new sorbent for the removal of p-toluenesulfonic acid from aqueous solution."Journal of Industrial and Engineering Chemistry, 30(2015) 281-288. https://doi.org/10.1016/j.jiec.2015.05.034