

Synthesis, Characterization, and Antibacterial Activity of Cobalt Carbonate Nanoparticles

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ABSTRACT

The emergence of multidrug-resistant (MDR) bacteria is a significant challenge to contemporary medicine, which prompts the pressing necessity of new antibacterial drugs. The transition metal carbonate nanomaterials have shown great biocidal potential because of their characteristic physicochemical properties. This paper has prepared cobalt carbonate (CoCO_3) nanoparticles through a chemical reduction system at room temperature and tested the structural features and antibacterial abilities of these particles. X-ray diffraction (XRD) was used to verify that it had a pure cubic phase structure with an average size of crystallites of 35.34 nm. The presence of CO and CO_3 functional groups was confirmed by Fourier Transform Infrared Spectroscopy (FTIR), whereas Scanning Electron Microscopy (SEM) confirmed the presence of a porous network with a cubic spinel structure. The CoCO_3 nanoparticles were tested against *Staphylococcus haemolyticus* and *Staphylococcus aureus* at concentrations of 5, 10, and 15 $\mu\text{g/mL}$. The nanoparticles had strong concentration-dependent inhibitory effects. *S. haemolyticus* was more susceptible with a minimum inhibitory concentration (MIC) of 1.79 g/mL , as opposed to *S. aureus*, which had a minimum inhibitory concentration (MIC) of 2.68 g/mL . The results indicate that porous cubic architectures of CoCO_3 nanoparticles have a lot of potential in the future as antibacterial agents to resist resistant staphylococcal infections.

Keywords: Nanoparticles, synthesis, cobalt, antibacterial activity

Introduction

Nanoparticles (NPs) are generally defined as zero-dimensional nanomaterials that

measure 1-100 nm in size, and their behavior differs considerably from that of the bulk material in terms of chemical reactivity, mobility, and optical behavior (Wu et al., 2022). The formation of targeted nanoparticles needs selective attention between the top-down and bottom-up synthesis methods since the two approaches have unique strengths in regulating the size, shape, and surface area of particles (Rane et al., 2018; Huang and El-Sayed, 2010). Using different synthesis conditions, researchers have the ability to create inorganic nanoparticles, such as metals, metal oxides, and carbonates, into customized nanoparticles that have outstanding catalytic, optical, and antibacterial activities (Rahimi-Nasrabadi et al., 2017). In the last decade, shape-controlled synthesis has been put under a significant focus due to the decisive role of particle morphology in determining the performance of nanomaterials in nearly all scientific and biomedical applications (Li et al., 2006).

Cobalt (Co) and its nanomaterials have been a research subject of interest due to their bountiful occurrence on the earth and high surface-to-volume ratio (Rajesh et al., 2023). Cobalt nanoparticles, cobalt oxide (Co_3O_4), and cobalt carbonate (CoCO_3) are considered diverse magnetic nanomaterials that have the equal potential of application (Kalska-Szostko, 2012; Cong and Yu, 2009). The materials have unique electrical, optical, and magnetic characteristics that are highly dependent on their nanoscale structure, which contributes to their potential in biomedical applications, including magnetic cell purification, target drug delivery, and increased imaging resolution (Chaugule et al., 2012; Chomoucka et al., 2010). At the nanoscale, surface free energy can preferentially select the FCC phase in cobalt, and as a result, the reactivity and tunability of the FCC crystal lattice (Liang et al., 2011; Shatrova et al., 2017).

Highly pure cobalt carbonate nanostructures are usually prepared using cobalt salts in aqueous or organic solvents using controlled chemical pathways such as hydrothermal and reduction-oxidation (Duan et al., 2010; Ni et al., 2001). Physical and chemical behavior of these nanoparticles is highly reliant on the size and morphology of the particles that influence the structural stability and functional performance (Gopinath et al., 2016). Thus, it is necessary to control the synthesis conditions (temperature, precursor concentration, medium of the reaction, and stabilizing agents) to obtain crystalline CoCO_3 nanostructures with the desired features (Wu et al., 2022; Sun et al., 2011). These parameters require detailed knowledge of the optimization of the formation and stability of CoCO_3 nanoparticles to enhance their utilization in higher applications.

Currently, there is a dire need to combat a serious threat to the biomedical field as multidrug-resistant (MDR) bacteria are emerging rapidly; this has lowered the efficacy of most of the traditional antibiotics (Huh & Kwon, 2011). Here, inorganic nanoparticles like cobalt carbonate have been found to be promising alternatives due to their ability to act on bacterial cells in a different manner as compared to the action of conventional antibiotics (membrane disruption, oxidative stress induction, etc.) (Azam et al., 2012). These are alternative pathways that could be used to address the existing mechanisms of bacterial resistance and enhance the effectiveness of antimicrobials against significant pathogenic species. Hence, the current research will focus on the chemical synthesis of cobalt carbonate (CoCO_3) nanoparticles, structural characterization of the nanoparticles, determination of minimum inhibitory

concentration (MIC), and antibacterial effect of the nanoparticles against *Staphylococcus aureus* and *Staphylococcus haemolyticus*.

Materials and Methods

Preparation of Cobalt Carbonate Nanoparticles

Cobalt carbonate nanoparticles were prepared based on a modified chemical reduction protocol at room temperature. To begin with, 0.235 g of trisodium citrate trihydrate ($C_6H_5Na_3O_7 \cdot 3H_2O$) was dissolved in 10 mL of deionized water constantly stirred at 150 rpm. As the mixture was stirred, 0.1 g of sodium borohydride ($NaBH_4$) and 0.2 g of cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) were also introduced to the mixture. The decrease in reaction led to the appearance of boiling and the rapid release of hydrogen gas. After stopping the hydrogen evolution, a nanoparticle powder with a greyish-black color was obtained. The collection of this powder was performed through external magnetism, after which it was washed with absolute ethanol and deionized water several times and finally allowed to dry at room temperature in 24 hours.

Characterization Techniques

The analysis of the synthesized $CoCO_3$ nanoparticles was performed based on the structural and optical properties of the material through the application of several analytical techniques. The crystal structure and phase composition were appraised by the X-ray powder diffractometer (Rigaku D-maxIIA, Japan) at 30 kV using the $CuK\alpha$ radiations (1.54060 Å). The scans were registered between 20 and 80 2-theta. The functional groups and the chemical bonding were examined through the Fourier Transform Infrared (FTIR) spectroscopy (Schimadzu), and transmittance spectra were recorded at the wavelengths between 4000 cm^{-1} and 400 cm^{-1} by the KBr pellet technique. Also, surface morphology and particle geometry were studied by a Scanning Electron Microscope (SEM 55) of Carl Zeiss.

Antibacterial Activity Assay

Those antibacterial effects were evaluated against *Staphylococcus aureus* and *Staphylococcus haemolyticus* clinically isolated strains. To prepare the bacterial inoculums, the cultures of the strains were cultured in nutrient broth at 100 rpm for 24 hours. On Muller Hinton Agar plates, the well diffusion technique was used. $CoCO_3$ NPs 5 mg suspension in 1 mL of sterile distilled water was used to create nanoparticle suspensions. The agar plates were punctured with gel (diameter: 5 mm), and 0.10 μL of nanoparticle suspensions were placed on the plates at different concentrations (5, 10, and 15 $\mu\text{g/mL}$). The incubation of the plates was at 37°C for 24 hours. Zones of inhibition were also determined, and the Minimum Inhibitory Concentration (MIC) was calculated to find out what was the least concentration of the nanoparticle necessary to suppress the growth of the visible bacteria.

Results

Synthesis

Blending of the reducing agent ($NaBH_4$) and cobalt precursor ($CoCl_2 \cdot 6H_2O$) in the

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presence of trisodium citrate trihydrate promoted a fast reduction reaction at room temperature. The instantaneous change in hydrogen gas was being used to indicate the instant decrease and consequent development of the cobalt carbonate nanostructures. The product of the process was a stable, grayish-black magnetic powder that could be easily recovered, which means that an efficient and scalable synthesis route has been achieved.

Characterization

XRD Analysis: X-ray diffraction was used to measure the crystal structure of the CoCO_3 nanoparticles. The diffractogram had clear peaks of diffraction at values of 2θ of 10.74, 33.31, and 49.99, which corresponded to (450), (1100), and (356), respectively. These large peaks are associated with high-purity cubic phase structure. The mean size of the crystallites in the CoCO_3 nanoparticles using the Scherrer equation using the primary peaks was determined to be 35.34 nm on average.

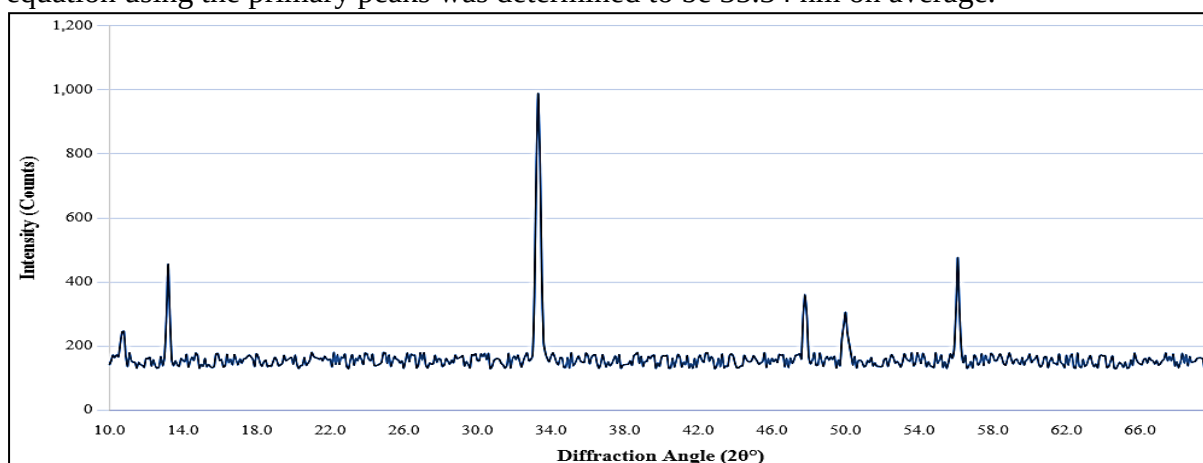


Figure 1: XRD analysis of synthesized cobalt carbonate NPs

FTIR Analysis: The chemical composition and the presence of the specific functional groups in the synthesized nanoparticles were verified by the FTIR spectroscopy. The spectra show a peak of stretching transmittance at 3.5 and 3.7 μm , respectively, these being typical of CO and CO_3 vibrations. The maximum at 3.5 μm indicates the presence of Co^{3+} ions in the octahedral position, and the maximum at 3.7 μm implies the presence of Co^{2+} in the tetrahedral position. The overall absorption band at 7.20 μm was attributed to the water present on the surface as adsorbed molecules.

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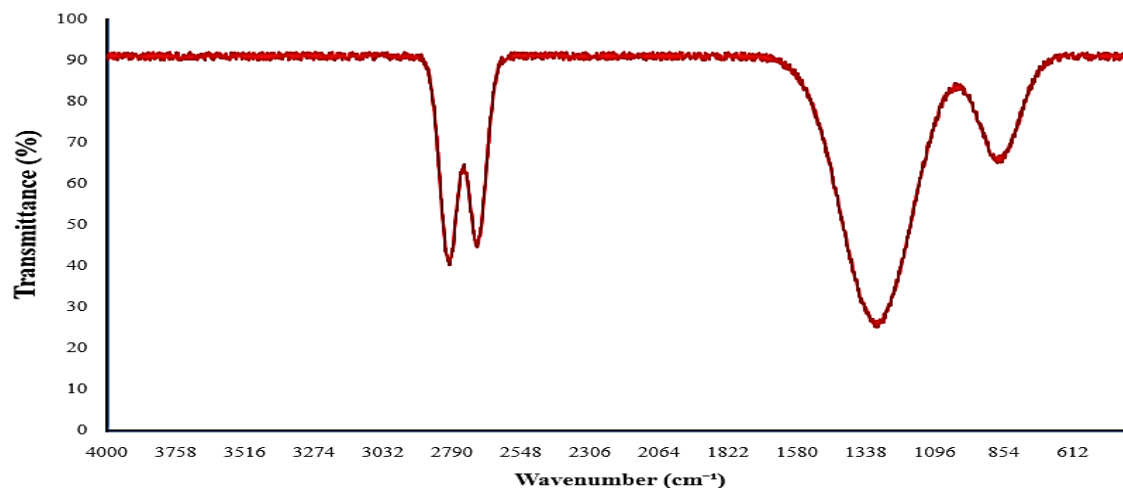


Figure 2: FTIR analysis of synthesized cobalt carbonate NPs

SEM Analysis: Micro-morphological evaluation via SEM revealed that the CoCO_3 nanoparticles possessed a porous network combined with a cubic spinel structure. The images showed that the pure, nano-sized crystals (averaging ~ 35.34 nm) agglomerate to form hierarchical architectures. The observed porous, nanosheet-like matrices suggest a high specific surface area, which is highly advantageous for surface-dependent biological interactions.

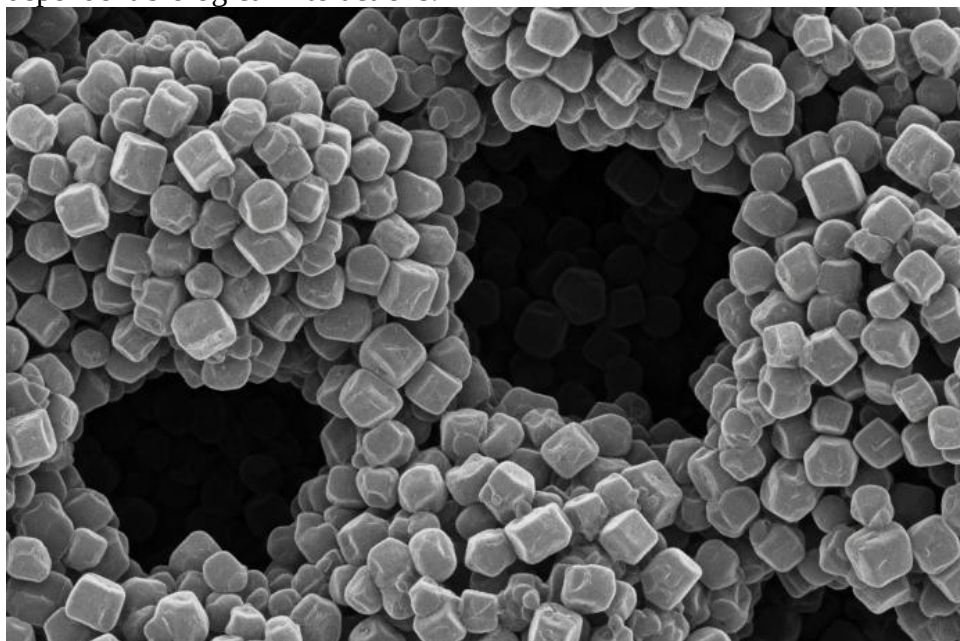


Figure 3: SEM analysis of synthesized cobalt carbonate NPs

Antibacterial Activity

The CoCO_3 nanoparticles exhibited potent, concentration-dependent antibacterial activity against both tested Gram-positive strains. The outcomes of the antibacterial assay, including mean zones of inhibition, standard deviations, and statistical significance, are summarized in

Table 1: Antimicrobial activity (Zone of Inhibition) and MIC values of synthesized CoCO₃ nanoparticles against *S. haemolyticus* and *S. aureus*

Dose (Concentration)	<i>Staphylococcus</i> <i>haemolyticus</i> (Mean $\pm$ S.D) mm	<i>Staphylococcus</i> <i>aureus</i> (Mean $\pm$ S.D) mm	T- value	P- value
Control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-	-
5 μ g/mL (MIC)	1.79 $\pm$ 0.36	2.68 $\pm$ 0.13	10.84	0.03*
10 μ g/mL	1.53 $\pm$ 0.06	1.45 $\pm$ 0.04	2.00	0.23
15 μ g/mL	5.55 $\pm$ 0.91	4.43 $\pm$ 0.83	1.64	0.26

* Statistically significant ($p < 0.05$)

As indicated in Table 1, the 5 μ g/mL dosage significantly impacted both *S. haemolyticus* and *S. aureus* ($p = 0.03$). As the concentration increased to the maximum tested dosage of 15 μ g/mL, the mean zone of inhibition expanded significantly to 5.55 mm for *S. haemolyticus* and 4.43 mm for *S. aureus*.

The Minimum Inhibitory Concentration (MIC) analysis revealed that *S. haemolyticus* was notably more susceptible to the nanoparticles, exhibiting an MIC value of 1.79 μ g/mL, while *S. aureus* demonstrated an MIC of 2.68 μ g/mL.

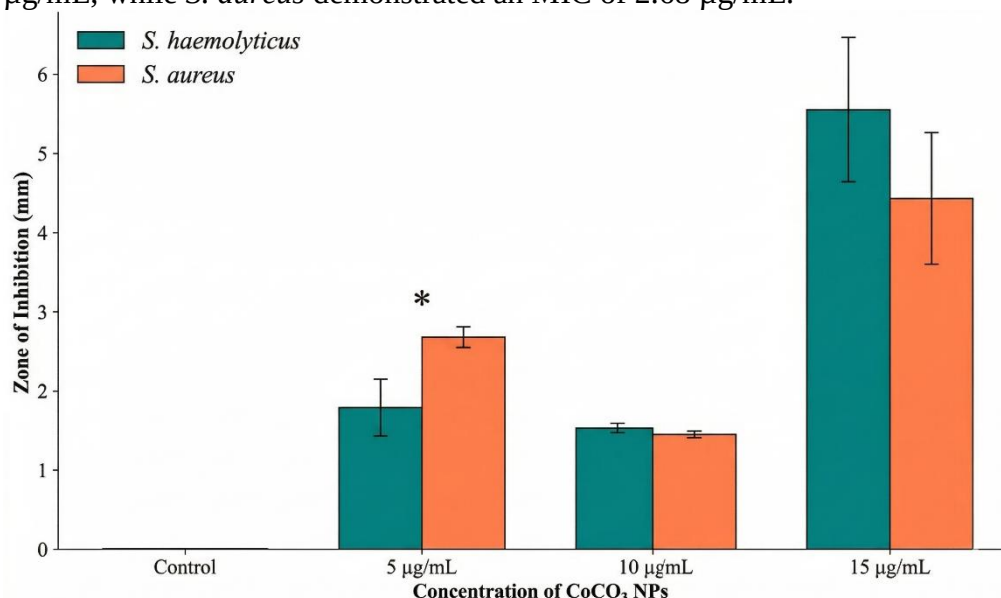


Figure 4: Graphical representation of the antimicrobial activity (Zones of Inhibition) of CoCO₃ nanoparticles at 5 μ g/mL, 10 μ g/mL, and 15 μ g/mL against (A) *S. haemolyticus* and (B) *S. aureus*.

Discussion

The structural characterization data is a clear indication that the successful synthesis of phase-pure cobalt carbonate (CoCO₃) nanoparticles through a fast chemical reduction process is valid (Chaughule et al., 2012). The X-ray diffraction (XRD) results supported the existence of a cubic structure with a well-defined and stable cobalt-based nanostructure that is a reactive and stable phase (Chomoucka et al., 2010). The measured diffraction angles of 10.74, 33.31, and 49.99 are more or less in

agreement with the earlier crystallographic patterns of the synthesis of transition-metal carbonate nanostructures in aqueous medium (Dong et al., 2010). Additionally, the average crystallite size of 35.34 nm calculated by applying the Scherrer equation with the 10-50 nm range of good nanoscale dimensions to improve catalytic and biological activity and preserve structural stability (Dwivedi et al., 2011) is in the favorable range of nanoscale. This regulated nanoscale size permits the CoCO_3 nanoparticles to have structural integrity and be functional in having a big reactive surface area, as is necessary in the functionality of nanomaterials (Leslie-Pelecky and Rieke, 1996).

The Fourier Transform Infrared (FTIR) spectroscopy served to give a further understanding of the chemical affiliation and structural disposition of the developed nanoparticles (Dumestre et al., 2002). The observation of carbonate stretching vibrations at about 3.5 μm and 3.7 μm proves the effective creation of the carbonate structure within the CoCO_3 structure (Petit et al., 2003). The peak occupying 3.5 μm by Co^{3+} ions in the octahedral coordination site and the 3.7 μm by Co^{2+} ions in the tetrahedral coordination point indicate the mixed oxidation state of the cobalt structure (Backman et al., 2001). The co-existence of Co^{2+} and Co^{3+} forms is also reported to facilitate electron transfer mechanisms that tend to be linked with better catalysis and antibacterial activities of cobalt-based nanomaterials (Salgueiriño-Maceira et al., 2006). Further, the occurrence of an absorption band at 7.2 00:00:00 of the nanoparticle means that there are adsorbed water molecules at the surface of the nanoparticles, and therefore the surface chemistry of the nanoparticle is hydrophilic and hence allows dispersion in aqueous biological systems (Ekstrand et al., 2005).

Scanning electron microscopy (SEM) was also used to provide valuable information on the morphological characteristics of the CoCO_3 nanoparticles that had been synthesized (Tzitzios et al., 2005). The porous architecture that is created as a result of cubic-like geometry greatly enhances the effective surface area as opposed to dense bulk materials (Yoshikawa et al., 2004). These nano-structures of high surface area favor a high level of interaction between the nanoparticles and bacterial cell membranes that has been largely documented as a major determinant of antibacterial efficacy (Hou et al., 2005). In addition, the porous structure contributes to the reduction of aggregation of the nanoparticles and enhances dispersibility, which means that more active cobalt sites are open to interact with the microbial cells (Chekin et al., 2016). The hierarchy of these nanoparticles is thus an effective source of release and activity of cobalt ions, which are a part of their biological functionality (Shahabuddin et al., 2016).

The concentration-dependent action of the synthesized CoCO_3 nanoparticles against multidrug-resistant bacterial strains was clearly displayed by the nature of the results of the antibacterial activity (Raman et al., 2016). It is worth noting that the minimum inhibitory concentration (MIC) of *Staphylococcus haemolyticus* was lower (1.79 $\mu\text{g/mL}$) than that of *Staphylococcus aureus* (2.68 $\mu\text{g/mL}$), which might indicate a difference in the composition of the cell wall, metabolic activity, and the defense mechanisms between the two bacterial species (Kashyap et al., 2013). Under the highest concentration tested of 15 $\mu\text{g/mL}$, we have seen two almost significant areas of inhibition of 5.55 mm and 4.43 mm, respectively, which confirms the high

antibacterial activity of the nanoparticles. The comparatively low values of MIC point to the capability of CoCO_3 nanoparticles to suppress bacterial growth at low levels, which is a significant aspect in diminishing possible cytotoxic effects in biomedical practice (Begum et al., 2016).

Antibacterial activity of these porous CoCO_3 nanoparticles can probably be explained by the synergistic effect of physical and chemical interactions of the bacteria cell with the nanoparticles (Ni et al., 2001). The particles are able to easily interact and penetrate the gram-positive bacteria peptidoglycan layer due to their nanoscale sizes (around 35.34 nm) (Makhlouf et al., 2013). When bound or incorporated into the bacterial membranes, the mixed-valence cobalt ions (Co^{2+} Co^{3+}) are able to catalyze the production of reactive oxygen species (ROS), such as hydroxyl radicals and superoxide anions (Sun et al., 2011). These ROS result in oxidative injuries to vital cellular biomolecules that include lipids, proteins, and nucleic acids and eventually cause bacterial cell death (Zhang et al., 2007). Thus, the produced cobalt carbonate nanoparticles have a complex antibacterial effect and are a good candidate of nanomaterial to fight antibiotic-resistant bacteriological infections (Gupta et al., 2020).

Conclusion

This paper showed the successful synthesis of cobalt carbonate (CoCO_3) nanoparticles at room temperature and outlined their massive potential in antibacterial use. The existence of a pure cubic phase with the best mean crystallite size of 35.34 nm was confirmed through structural analysis. Morphological analysis showed a greatly favorable porous network and cubic spinel, with the combination of Co^{2+} and Co^{3+} sites greatly enhancing the bioactivity of the material. The nanoparticles possessed a strong, concentration-dependent antibacterial activity, with *S. haemolyticus* (MIC = 1.79 $\mu\text{g/mL}$) being more sensitive compared to *S. aureus* (MIC = 2.68 $\mu\text{g/mL}$). There is evidence that porous CaCO_3 nanoparticles have strong bactericidal activity, probably through interaction of membranes and ROS formation, and therefore represent a very promising and viable alternative to traditional antibiotics in the treatment of drug-resistant bacterial infections.

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