Marine Algae Extract (Grateloupia Sparsa) for the Green Synthesis of Co₃O₄NPs: Antioxidant, Antibacterial, Anticancer, and Hemolytic Activities

Amira K. Hajri, Marzough A. Albalawi, Ifat Alsharif, and Bassem Jamoussi

1Department of Chemistry, Alwajh College, University of Tabuk, Tabuk, Saudi Arabia
2Department of Biology, Jamoum University College, Umm Al-Qura University, Makkah 21955, Saudi Arabia
3Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Correspondence should be addressed to Amira K. Hajri; ahejari@ut.edu.sa

Received 15 April 2022; Accepted 20 September 2022; Published 20 October 2022

Abstract

The aqueous extract of red algae was used for bio-inspired manufacturing of cobalt oxide nanoparticles (Co₃O₄NPs) and for antioxidant, antibacterial, hemolytic potency, and anticancer activity. Typical, characterization techniques include UV-Vis, SEM, EDAX, TEM, FTIR, XRD, and TGA. Using an X-ray diffraction assay, the size of the Co₃O₄NPs crystal was determined to range from 23.2 to 11.8 nm. Based on TEM and SEM pictures, biosynthesized Co₃O₄NPs had a homogeneous spherical morphology with a 28.8 to 7.6 nm average diameter. Furthermore, Co₃O₄NPs biological properties were investigated, including determining the antibacterial potency using the zone of inhibition (ZOI) method and determining the minimal inhibitory concentration (MIC). The antibacterial activity of Co₃O₄NPs was higher than that of the ciprofloxacin standard. Alternatively, scavenging of DPPH free radical investigation was carried out to test the antioxidant capacitance of Co₃O₄NPs, revealing significant antioxidant ability. The biosynthesized Co₃O₄NPs have a dose-dependent effect on erythrocyte viability, indicating that this technique is harmless. Furthermore, bioinspired Co₃O₄NPs effectively against HepG2 cancer cells (IC₅₀: 201.3 μg/ml). Co₃O₄NPs would be a therapeutic aid due to their antioxidant, antibacterial, and anticancer properties.

1. Introduction

Different methods can be employed to synthesize metal oxide nanoparticles (MNPs). Each synthesis process has benefits and drawbacks. For instance, the chemical and physical approaches have several advantages, such as producing the desired size and number of nanoparticles. Still, it is eco-toxic, consumes energy, and is expensive and time-consuming [1, 2]. Biological methods include plants, algae, microbes, and other natural substances, including starch, egg albumin, and gelatin, which are used in the biological approach to produce diverse types of MNPs. This biological method is called the “green method” [3–6].

Green synthesis of MNPs is being used to solve these issues. The green-mediated technique is more advantageous than standard approaches [7–13]. Biological substances like starch and bovine albumin have also been employed in the synthesis of green MNPs [4, 14, 15].

These natural resources comprise biomolecules and metabolites that oxidize/reduce, stabilize, and produce specific MNPs with less pollution, safer, and cheaper [16]. Current advances in marine bio nanotechnology enable and drive advancements in a wide range of industries, including nanomedicine, pharmaceuticals, environmental concerns, and agriculture. Marine organisms that can survive in extreme conditions are plants, algae, bacteria, fungi,
actinomycetes, yeast, invertebrates, and mammals. Among the phytochemicals/metabolites they can generate are peptides, polyphenols, proteins, carbohydrate polymers, polysaccharides, sulfated polysaccharides, and polysaccharide-protein complexes such as fucoidan, carrageenan, carboxymethyl cellulose, polyglutamic acid, melanin, and others. These substances have distinct characteristics that distinguish them as pioneers in the ecologically friendly production of MNPs such as Ag, Au, Ru, Cobalt Oxide, and ZnO in a single-phase system [17].

Algae are marine microorganisms that are heavily used to synthesize MNPs. Algae are bioanofactories because they produce stable nanomaterials that do not require cell upkeep [18]. Algae contain several bioactive substances like proteins, polysaccharides, and phytochemicals with -NH₂, -OH, and -COOH functional groups used in MNP production [19]. Algae are classified as microalgae or macroalgae [20]. The green macroalgae Grateloupia sparsa was used by their function group that acts as reducing and stabilizing agents to manufacture MNPs [21, 22].

Cobalt is a good transition metal for health [23]. It is a component of vitamin B12, which helps alleviate anemia by promoting the development of red blood cells [24]. Cobalt’s unusual optical, magnetic, catalytic, and electrical properties make it ideal for nanosensors and nanoelectronics fields [25–27]. Cobalt is valuable in many sectors because of its CO₂⁺, CO³⁺, and CO⁴⁺ oxidation states [28].

Dozens of studies are directed toward using metal oxide nanoparticles in many applications [12, 29–33]. The most often used metal oxide NPs are cobalt oxide (Co₃O₄NPs). These nanoparticles have recently gained popularity owing to their lower cost than noble metal nanoparticles. Their vast surface area gave a unique electrical and magnetic property [34]. Co₃O₄NPs are nontoxic at low doses, exhibit high antibacterial and antifungal activity, and have fewer adverse effects than antibiotics [34–36].

Antibiotic resistance is currently a severe global health concern. So, an antibiotic agent that can kill harmful bacteria resistant to existing antibiotics is required [37]. Because MNPs are smaller and have more surface area than larger molecules, they exhibit strong antibacterial properties. The MNPs disrupt the cell membrane and impede protein synthesis in bacteria [38]. MNPs such as cobalt oxide, iron oxide, and copper oxide all demonstrated antibacterial activity [39–41].

The Co₃O₄NPs may potentially be antimicrobial; the disc diffusion method was used to study the antibacterial activity of Co₃O₄NPs synthesized from Celosia argentea whole plant extract. These NPs were bactericidal against B. subtilis and E. coli [42]. The antibacterial efficacy of green-mediated Co₃O₄NPs was studied using Hibiscus rosa-sinensis flower extract; the results revealed a promising activity against E. coli and S. aureus [43]. Two main points have been raised. Co²⁺ and Co³⁺ interact with the negative charge sections of the bacteria and cause cell death. Second, light irradiation in the conduction and valence bands may excite electrons on the surface of cobalt oxide, and excited electrons and oxygen molecules react to generate a superoxide radical anion [44].

The cytotoxicity of human umbilical vein endothelial cells (HUVECs) was assessed in vitro using green-synthesized Co₃O₄NPs at various doses. The MTT test was performed on cells treated with varying quantities of Co₃O₄NPs; it revealed high viability up to 1,000 mg/mL of Co₃O₄NPs [45]. Also, it was found that Co₃O₄NPs are cytotoxic to HeLa carcinoma cells [46]. Besides, the biogenic Co₃O₄NPs have improved radical scavenging and reducing power [47]. According to a recent study, the scavenging capability and antioxidant properties of bio-inspired Co₃O₄NPs are dose-dependent [42].

Hemolysis occurs when disrupted erythrocyte membranes, cause hemoglobin leakage and possibly jaundice or anemia. The hemolytic potency of any newly synthesized pharmacological preparation must be tested [48]. Based on the hemolytic activity of green-synthesized Co₃O₄NPs, Shahzadi et al. results revealed that the bio-inspired Co₃O₄NPs had less hemolytic potency (2.95%) than the positive control triton-X-100 (95.25%) and less toxicity (1.02%) [42].

In this study, bioinspired Co₃O₄NPs synthesis was performed using red algae extract (Grateloupia sparsa) for Co₃O₄NPs synthesis. This metal oxide NPs characterization has been broadly done by UV, TEM, EDAX, XRD, FTIR, and TGA. Moreover, the antibacterial properties, anticancer potency, and hemolytic assay of Co₃O₄NPs have been studied in vitro.

2. Experimental

2.1. Chemicals. Cobalt (II) nitrate hexahydrate (Co(NO₃)₂·6H₂O) for analysis (MTT, 98%, and (DPPH) were purchased from Sigma–Aldrich (USA), 99.9%Methanol, and DMEM-F12 (Merck Chemicals, Germany). Otherwise, the listed compounds are analytical grade and can be used without further purification.

2.2. Collection of Red Algae. The crimson algae were collected during a trip to the Red Sea. To transport the collected algae to the laboratory, they were placed in a plastic bottle. Thus, samples are washed and cleansed with running water to remove salt, toxins, and epiphytes. Then, it was dried and ground to a powder at room temperature using an electric blender.

2.3. Bio-Inspired Synthesis of Co₃O₄NPs. The algae-dried powder was utilized to prepare the extract. 5 gm of this powder was suspended in 50 mL DD water and heated the mixture to 60°C for 4 h. Then, the extracts were cooled at room temperature (R.T), they were filtered through Whatman filter paper and kept at 4°C. After that, 10 ml of algae extracts were injected dropwise with 50 ml of cobalt nitrate at a concentration of 1 mg/ml as a source of cobalt, following which, at R.T, continual stirring was performed. Within 24 hours of incubation, the solution’s color changes from pink to brown, indicating the creation of Co₃O₄NPs.
2.4. Co₃O₄NPs Characteristics. The UV-Vis spectrophotometry (dual beam, Shimadzu, 1900, Japan) was used to determine the production of Co₃O₄NPs at wavelengths between 300 and 600 nm. The FTIR-6800 Spectrometer (JASCO, 500–4000 cm⁻¹) was used to determine the functional moieties. All samples were subjected to X-ray diffraction (XRD, Philips X Pert diffractometer, The Netherlands) to validate the crystallinity and size of the Co₃O₄NPs. Additionally, the form and size distribution of the particles were investigated by scanning electron microscopy (SEM, FEI Quanta 200 FEG, Japan). Additionally, the elemental composition was identified by an EDAX study. Further morphological images of Co₃O₄NPs were examined using 120 kV transmission electron microscopy (TEM, JEOL, and JEM 1400).

2.5. Biological Properties of Co₃O₄NPs

2.5.1. Antibacterial Potency. Antibacterial examinations against two G-negative and two G-positive bacteria (E-coli and P. aeruginosa) and (S. aureus and B. subtilis), respectively, were conducted in vitro using the zone of inhibition (ZOI) method by culturing the bacteria on Petri dish nutrient agar. Then, 6 mm filter discs containing 20 μg/ml of Co₃O₄NPs were put on bacterial streaks, and discs with ciprofloxacin (30 μg/ml) were frequently placed in the same dish as standard antibiotics. Finally, all Petri plates were incubated for 24 h at 37°C to compute the inhibitory zone [8, 49].

2.5.2. Measurement of Minimal Inhibitory Concentration (MIC). MIC values were measured using Sarker’s broth agar dilution method [50]. 100 ml of Co₃O₄NPs (2 mg/ml) were placed on the plate’s initial row, and 50 μl of nutritional broth agar was applied to the other wells. After that, serial dilutions were conducted using sterilized pipettes in 1000 to 3.90 μg. The resazurin solution was produced by mixing 260 mg in 50 μl of sterile distilled water. All wells were treated with the resazurin solution (10 μl). Also, 30 μl of nutritional broth was completed to a total capacity of 100 μl. Finally, 10 μl of culture suspensions were mixed with the contents of the wells, and then, the plate was incubated for 24 h at 37°C, and the color change was photometrically determined. The color change from colorless purple to beginning purple was a desirable outcome. The lowest MIC value in, which the solution becomes colorless [51].

2.5.3. Anticancer Potency. We evaluated the antitumor activity of bioinspired Co₃O₄NPs utilizing the hepatic cancer cell line (HepG2) using the MTT test. Streptomycin and penicillin (1%) were added to DMEM for cell development at 37°C in a 5% CO₂ incubator. Additionally, different doses of Co₃O₄NPs (50–500 μg/ml) were incubated for 48 h at 37°C in a 96-well plate. Then, each well was loaded with 20 μl MTT solution and incubated for 3 hours. Finally, 100 μl of DMSO was applied to the culture and incubated for 25 minutes; formazan production by live cells was determined using an Elisa reader set to 570 nm wavelength [52].

2.5.4. Hemolytic Activity. A standard method was used to determine the hemolytic property of Co₃O₄NPs. 3 ml of freshly prepared K₂-EDTA human blood was withdrawn and centrifuged for 5 minutes at 1500 rpm. Following that, the plasma was removed, and 2 ml of phosphate-buffered sterile saline (PBS) was added, followed by 5 minutes of centrifugation at 1500 rpm to remove any remaining PBS. The first human blood tube was filled with 100 μl of Co₃O₄NPs and incubated for 35 minutes at 37°C. The tube was then placed in a cold bath for 5 minutes before centrifuging at 1500 rpm for 5 minutes. The supernatant was diluted (1:10) with cooled PBS (4°C) [43]. The same procedure was used in the tube with PBS and 0.1% Triton X-100 as a negative and positive control, respectively. Finally, each sample’s optical density (OD) was measured using a 576 nm. The following equation was used to measure the proportion of erythrocyte lysis in each sample:

\[
\text{Hemolysis(\%)} = \frac{\text{sample (OD)} - \text{blank (OD)}}{\text{positive control (OD)}} \times 100
\]  

2.5.5. Antioxidant Property. Spectrophotometric techniques were used to evaluate the acceptor activity of the DPPH. To make a stock solution, 25 ml of methanol was mixed with 2.5 mg of DPPH as a free radical. Individually, different amounts of Co₃O₄NPs ranging from (50–500 μg/ml) were added to a microplate. Then, 100 μl of working solution were added to the microplate, covered, and incubated in the dark for 25 minutes. The activity of the radical scavenger was then evaluated by measuring the OD at 517 nm with a spectrophotometer [53]. All measurements were taken in triplicate.

The following equation is used to measure antioxidant properties:

\[
\text{DPPH Scavanging(\%)} = \frac{\text{control (OD)} - \text{sample (OD)}}{\text{control (OD)}} \times 100.
\]  

3. Results and Discussion

3.1. Co₃O₄NPs Characteristics

3.1.1. UV-Vis. One of the main structural description methods for metallic oxide nanoparticles is UV-Vis spectroscopy. Figure 1 depicts the UV-Vis spectra of benign Co₃O₄NPs synthesized by an aqueous extract of red algae. The surface plasmon resonance of Co₃O₄NPs is near 510 nm, lightly shifted from the broad to the long-wavelength area, confirming the Co₃O₄NPs formation. This tiny wavelength band was owing to transverse electrical oscillation. When Co₃O₄NPs particle size increased, the location and morphology of SPR were likewise shifted toward longer wavelengths [54].
3.1.2. FTIR. Using Fourier transform infrared spectroscopy, the functional moieties of the as-prepared Co$_3$O$_4$NPs in Figure 2 were examined (FTIR). The band at 3500 cm$^{-1}$ represents the -OH, whereas the bands at 1525 cm$^{-1}$ and 1060 cm$^{-1}$ indicate the aromatic rings and the C=O group, respectively.

3.1.3. XRD. The XRD values are depicted in Figure 3. Large diffracted intensities were recorded around 2h = 28.2°, 35.1°, 43.6°, 53.4°, 56.3°, 63.2°, and 74.2°, which correspond to (220), (311), (422), (511), (442), and (440), respectively (533). This demonstrates the formation of the Co$_3$O$_4$NPs crystalline phase according to ICDD card no. 42-1467. The crystallite size is around 23.2 to 11.8 nm, according to Scherrer’s equation. Low crystallinity has been associated with a greater susceptibility to lattice deformation; this could contribute to a greater affinity for chemical adhesion with the external environment.

3.1.4. SEM. SEM was used to scan the Co$_3$O$_4$NPs surface and size structure, demonstrating the average homogeneous formation of Co$_3$O$_4$NPs with a 28.2 nm diameter. SEM images of Co$_3$O$_4$NPs at various magnifications are shown in Figure 4(a). As explained, the nanoparticles clump together and form massive particles. The aggregation of nanoparticles has been described as an indication of metallic nanoparticle production processes [55, 56].

3.1.5. TEM and EDAX. A TEM of Co$_3$O$_4$NPs surface morphology was displayed in Figure 4(b). The particle size distribution on the TEM graphic showed that the Co$_3$O$_4$NPs were 28.8 to 7.6 nm in size. The particles seem to be spherical as well SEM and TEM investigations produced similar findings for a wide nanoparticle size range. Based on our findings, recent studies revealed that cobalt ferrite nanoparticles made from aqueous extracts of sesame ranged from 3.0 to 20.0 nm in size [57]. Nerium Indicum and Conocarpus erectus methanol extracts were also employed to biosynthesize Co$_3$O$_4$NPs ranging from 20 to 60 nm in particle sizes [58]. Furthermore, the size of Moringa oleifera extract-biosynthesized cobalt nanoparticles ranges from 20 to 50 nm [59]. According to energy dispersion analysis, the elemental contents of materials were established by high-resolution EDAX (Figure 4(c)). EDAX of Co$_3$O$_4$NPs was performed in the 0 to 20 keV range. It revealed a 7 keV Co$_3$O$_4$NPs peak [42]. The EDAX profile had a strong cobalt signal and several short peaks [60].

3.1.6. TGA. The Co$_3$O$_4$NPs’ thermal stability is essential in evaluating their ability to survive various utilities such as fuel cells and conductor-based applications. As a result, thermogravimetric analysis was performed up to 800°C. The pristine composition declines exponentially, reaching approximately 260°C to lose about 6.3% of its original weight, which could be attributable to the release of organic solvents and water, as seen in Figure 5. A brief plateau distinguishes the second phase of weight loss between 260 and 410°C, followed by a high breakdown rate and the losses, which in this case, was ~17.6%. Co$_3$O$_4$NPs appear more thermally stable than the former Co$_3$O$_4$NPs/algae, particularly in the first phase of thermal degradation due to the decomposition of organic species than reported in the literature [43, 61]. It is worth noting that Co$_3$O$_4$NPs/algae are slightly more stable below 420°C. As a result, the presence of algae may provide a good thermal scaffold for preserving stability at temperatures below 420°C.

3.2. Biological Properties

3.2.1. Antibacterial Potency. Bacterial infection is the main serious problem in infectious illnesses in terms of death and morbidity and treatment costs [62]. Antibiotic usage has also been related to many issues, including bacterial resistance, among others. As a result, researchers strive to develop novel strategies to lower the likelihood of
**Figure 2**: FTIR of Co$_3$O$_4$NPs.

**Figure 3**: XRD of the crystalline Co$_3$O$_4$NPs powder.

**Figure 4**: SEM micrograph of Co$_3$O$_4$NPs (a), TEM morphology 50 nm (b), and EDAX of bioinspired prepared Co$_3$O$_4$NPs (c).
infectious diseases starting and spreading \cite{32}. The rapid advancement of nanotechnology will provide tools for creating new substances with novel antibacterial properties \cite{63,64}. Several investigations into the antibacterial potency of biogenic metal nanoparticles have been published, with promising findings against various bacteria strains \cite{4,12}. At a dose of (30 μg/ml), bio-inspired Co$_3$O$_4$NPs were tested against various bacterial species. G-positive bacteria were (B. subtilis and S. aureus), whereas G-negative bacteria were (P. aeruginosa and E. coli) compared to the standard antibiotic ciprofloxacin disk (30 μg/ml). We found that Co$_3$O$_4$NPs were effective on candidate bacterial species but still lower than the effect of ciprofloxacin based on ZOI measurements. On the other hand, P. aeruginosa with low sensitivity at a MIC of 23.0 ± 5.3 μg/ml and B. subtilis are highly sensitive to bio-inspired Co$_3$O$_4$NPs, with MIC values of 18.6 ± 3.8 μg/ml. Table 1 depicts MIC values and the ZOI for antibacterial activity are compared to effective ciprofloxacin.

A G-positive bacteria cell wall is composed of peptido- glycan layered with (~70 nm thick), which permits Co$_3$O$_4$NPs to interface directly with the outer membrane of bacteria more readily than G-negative bacteria, which have a layer of lipopolysaccharides (1-2 mm thick) \cite{65}. This variety in bacterial cell wall structure and thickness makes G-positive bacteria’s membrane rupture faster and leads to their death \cite{66}. As a result, the antibacterial activity of Co$_3$O$_4$NPs can be compact in size, and a high surface-to-volume ratio allows them to interface with the bacterial cell membrane. Figure 6 depicts how green Co$_3$O$_4$NPs work against bacteria by attaching to the bacterial cell wall and modifying its permeability \cite{67}. The penetration of reactive oxygen species (ROS) into the cytoplasm damaged the nucleus and plasmid, causing a shift in cell signaling and, eventually, death \cite{68}.

3.2.2. Anticancer Potency of Co$_3$O$_4$NPs. Cancer remains the world’s most prominent cause of mortality. The number of cancer cases has been steadily increasing, and it is expected to reach around 21 million by 2030 \cite{69,70}. Hepatic cancer is the 2nd common cause of mortality in males and the 6th common cause of death in females. Excessive alcohol intake over a long period of time, as well as HCV and HBV infections, and other toxins, all increase the risk \cite{71}. Using the HepG2 cell line, the anticancer effects of Co$_3$O$_4$NPs were also studied. Cancer cells were applied to various doses of Co$_3$O$_4$NPs (50–500 μg/ml) over 24h. In this study, Co$_3$O$_4$NPs were discovered to have significant anticancer potential, with an IC$_{50}$ value of 201.3 μg/ml. Figure 7 revealed an estimated 80% fatality rate at 500 μg/ml. Our findings show that Co$_3$O$_4$NPs generate ROS that interacts with cells and causes cellular oxidative stress, leading to DNA destruction and cell death. This is because the microscopic nanoparticles are soluble in the internal acid medium, which has a pH of 4.5. The Co$_3$O$_4$NPs can create pores in the membrane and dissolve in the cells, and eventually, the cell dies \cite{72}. Cancer cells were also suppressed by Co$_3$O$_4$NPs, suggesting their anticancer potential. Our findings consistently show that metal nanoparticles have a significant anticancer potential \cite{73}.

Table 1: The ZOI in petri dish agar and MIC of Co$_3$O$_4$NPs for bacterial growth inhibition.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>E. coli</th>
<th>Zone of inhibition (ZOI mm)</th>
<th>P. aeruginosa</th>
<th>B. subtilis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co$_3$O$_4$NPs</td>
<td>30 μg/ml</td>
<td>11.7 ± 3.2</td>
<td></td>
<td>12.5 ± 3.9</td>
<td>14.3 ± 3.1</td>
<td>17.6 ± 4.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30 μg/ml</td>
<td>13.6 ± 3.4</td>
<td></td>
<td>12.7 ± 3.4</td>
<td>14.8 ± 2.6</td>
<td>18.1 ± 5.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MIC (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co$_3$O$_4$NPs</td>
<td>21.1 ± 6.1</td>
<td>23.0 ± 5.3</td>
<td>18.6 ± 3.8</td>
<td>20.6 ± 5.9</td>
</tr>
</tbody>
</table>

Figure 5: TGA of Co$_3$O$_4$NPs up to 800°C.
3.2.3. Hemolytic Potency. The hemolytic potency of Co$_3$O$_4$NPs was compared to that of Triton-X-100, which represents a negative control, with Co$_3$O$_4$NPs made from red algae extract being significantly less toxic (5.3%) (Figure 8(a)), Triton-X-100 having 97.3% toxicity (Figure 8(b)), and PBS having 1.01% toxicity (Figure 8(c)).

3.2.4. Antioxidant Activity. A DPPH-free radical scavenging test was used to evaluate the free radical scavenging capability of green-produced Co$_3$O$_4$NPs. These results revealed four different Co$_3$O$_4$NP concentrations; the free radical scavenging capability increased as the Co$_3$O$_4$NP concentration rose (Figure 9). The peak of DPPH radical scavenging
4. Conclusions

Red algae were employed to create eco-friendly cobalt oxide nanoparticles (Co$_3$O$_4$NPs). The Co$_3$O$_4$NPs were formed in inhomogeneous spheres with diameters ranging from 28.8 to 7.6 nm. The antibacterial activity of Co$_3$O$_4$NPs was examined, and it was revealed that nanoparticle concentrations of 30 μg/ml widened the inhibition zone against candidate species from 11.7 to 17.6 mm, still lower than standard antibiotics with a ZOI of 18.1 mm in its higher efficacy. Furthermore, the minimal inhibitory concentrations for (P. aeruginosa, E. coli, S. aureus, and B. subtilis) were adjusted to be around 23.0, 21.1, 20.6, and 18.6 for each bacterial species. Furthermore, Co$_3$O$_4$NPs were investigated for anticancer activity in vitro against the HepG2 cell line. Cell mortality for 500 μg/ml was reported to be more than 80% after 24 hours of exposure. Furthermore, the antioxidant activity was studied, and it was observed that the maximum radical scavenging of DPPH was attained at 500 mg/ml of Co$_3$O$_4$NPs (88.2%).

Data Availability

The data supporting this study’s results are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through the project number (0015-1442-S).

References


[41] M. I. Nabil and K. Kannabiran, "Biosynthesis, characterization and antibacterial activity of copper oxide


