RESEARCH PAPER

Cobalt Oxide Nanoparticles Synthesized from *Thalassia hemprichii* Extract: Antioxidant and Antibacterial Potentials

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ABSTRACT

This research work reports about the eco-friendly synthesis of cobalt oxide nanoparticles (Co_3O_4 NPs) with excessive reduction potential from marine seagrass, *Thalassia hemprichii*, possessing plenty of bioactive constituents. The green synthetic strategies for producing environment-friendly nanomaterials and nanomaterial-based biomedical devices is the core objective. FTIR, XRD, TEM and SEM were used to characterize the synthesized Co_3O_4 NPs for their structural and morphological properties. FTIR spectra indicated functional groups of Co_3O_4 NPs interacting with metal–oxygen bonds, validating successful nanoparticle formation. XRD confirmed the crystalline cubic spinel structure of Co_3O_4 nanoparticles with an average crystallite size of ~20–40 nm. The crystalline structure was confirmed, and the average particle size was found to be in the range of 45–52 nm. The in vitro bioactivities of the nanoparticles were evaluated, and the nanoparticles showed strongly dose-dependent free radical-scavenging activity as evidenced by DPPH and nitric oxide (NO) scavenging activities. Co_3O_4 NPs showed 89% DPPH scavenging at 100 µg/mL with IC_{50} = 42.93 µg/mL, comparable to seagrass extract (76.9%, IC_{50} = 42.40 µg/mL) and BHT (IC_{50} = 42.38 µg/mL). In nitric oxide assays, the NPs displayed dose-dependent inhibition, confirming effective free-radical scavenging capacity. The antibacterial activity of Co_3O_4 NPs was also assessed by the well diffusion method against pathogenic bacteria *Streptococcus mutans, Pseudomonas aeruginosa, Bacillus subtilis*, and *Escherichia coli*. Co_3O_4 NPs showed strong antibacterial activity with larger inhibition zones against both *E. coli* and *B. subtilis.* and strains compared to the seagrass extract. The enhanced efficacy highlights the potential of NPs as effective antimicrobial agents. This research emphasizes the novelty of marine plant extracts for the use as sustainable reducing and stabilizing agents in the synthesis of nanoparticles with respect to environmentally-friendly chemical routes. These results proposed that as-prepared green-synthesized Co_3O_4 NPs can be as potent antioxidants and antibacterial agents for biomedical and environmental uses.

Key Words: Co_3O_4 NPs, *Thalassia hemprichii*, Antioxidant activity, Antibacterial Activity, *Escherichia coli*
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INTRODUCTION

Nanotechnology is an emergent interdisciplinary field dealing with materials manipulation at the nanoscale (0.1–100 nm). This area has revolutionized a number of fields such as medicine, energy, agriculture, electronics and environmental science by modifying the properties of materials at the level of atoms and molecules [1]. The development of nanomaterials design and fabrication has had a profound impact on targeted drug delivery, regenerative medicine, biosensors, water treatment and catalysis [2]. Among several synthesized approaches, green nanotechnology has been attracting more attention as an environmental-friendly and sustainable method for the generation of nanoparticles, avoiding toxic chemicals [3]. Encapsulation of natural biological entities like plant extracts, bacteria, fungi, and algae contributes to narrow down the nanoparticle synthesis route for a safer and biocompatible approach, promising the least environmental toxicity and maximum biomedicinal potential [4].

Nanoparticles (NPs) are small particles having one dimension in the nanoscale range and display remarkable optical, electrical and chemical behaviour relative to their larger-sized counterparts. These features are owed to their large surface-to-volume ratio, quantum confinement effects, and improved catalytic activity [5]. Nanoparticles of different kinds, metals, metal oxides, were studied for their biomedical use. Metal NPs (such as Ag) and NPs (like Ag), (Au) show potential against antimicrobial, anticancer activities, and the metal oxide NPs (ZnO) NPs (like ZnO and) (TiO_2 NPs), (Co_3O_4 NPs) exhibit antioxidant, antimicrobial, and photocatalytic activity [6]. Owing to their ability to efficiently cross biological membranes at the nanometre scale, these materials represent a promising candidate for drug delivery to targeted sites such as biosensors, imaging, wound healing and antimicrobial agents [7]. Nevertheless, their cytotoxicity and their impact on the environment have made scientists to turn towards green synthesis approach that relies on the use of plant resources [8].

The seagrasses have been received great attraction for the green NP synthesis from the different natural resources on account of possessing rich bioactive compounds [9]. *Thalassia hemprichii*, marine seagrass growing in costal seawater, has received considerable attention for having high content of bioactive compounds such as flavonoids, alkaloids, phenolics, terpenoids, and antioxidants [10]. The therapeutic potential of these chemicals includes anti-inflammatory, antimicrobial and antioxidant activities. In the last few years *T. hemprichii* has been employed for reducing as equally stabilizing agent for the preparation of various metal nanoparticles [11]. Significantly, Ag NPs, Au NPs, and ZnO NPs fabricated from *T. hemprichii* extracts have shown promising antibacterial, antioxidant, and anticancer activities. Marine plant extract as a part of nanotechnology is an ecological friendly, low-cost and sustainable method for generation of biocompatible nanoparticles with improved biomedical applications [12].

Cobalt oxide nanoparticles (Co_3O_4 NPs) have attracted great interest owing to their distinctive physicochemical characteristics including magnetic properties, high catalytic activity, and redox activity [13]. These properties render them particularly attractive for biomedical applications including drug delivery, bioimaging, cancer treatment and antibacterial activity [14]. Moreover, Co_3O_4 NPs showed strong antioxidant activities indicating their possibility as an antioxidant to reduce the oxidative stress associated with several chronic disorders including cancer, cardiovascular and nervous system diseases [15]. Because of their antimicrobial potency, the nanoparticles have also been studied for application in wound dressing and infection protection, which can efficiently block the bacterial proliferation by disintegrating the microbial cell membranes while releasing the radicals of reactive oxygen species (ROS) as well into the microbials [16]. Plant-mediated green synthesis of Co_3O_4 NPs shows a prospective substitute for conventional chemical synthesis, resulting in low environmental hazards with respect to safer biomedical applications [17].

Besides *Thalassia hemprichii*, a number of other seagrass species have been investigated for cobalt oxide nanoparticle synthesis. Natural reducing and stabilizing agents such as *Halophila ovalis, Cymodocea serrulata*, and *Enhalus acoroides* were employed for the synthesis of Co_3O_4 NPs [18]. The former has indicated significant antibacterial, antioxidant, and anti-inflammatory activities [15]. The usage of seagrass extracts not only promotes greener synthesis of nanoparticles but also improves the biocompatibility and efficiency of these nanomaterials that can be used for biomedical purposes [19].

MATERIALS AND METHODS

Materials

Distilled water, Cobalt (III) Nitrate Hexahydrate ($Co(NO_3)_3 \cdot 6H_2O$) (5 mM), Ammonia Solution (NH_3), Sodium Borohydride ($NaBH_4$) (Thermo Fisher Scientific) were used in the synthesis of Co_3O_4 NPs in this study.

Sample Collection

The seagrass samples of *Thalassia hemprichii* was collected from Palk Bay and Gulf of Mannar, situated along the southeast coast of India. The regions are characterized by their large seagrass meadows, which are important habitats for a range of marine species. An initial ecological study was performed to monitor the seagrass distribution in these study regions, including the areas on the shoreward and seaward sides of the Gulf of Mannar islands and the diverse sites in the Palk Bay. The survey was performed using SCUBA and enabled direct observation as well as careful identification of healthy seagrass meadows. The information collected in this survey aided in locating the most appropriate collection sites with minimal negative impact on the marine environment.

To minimize the ecological impact of the sampling process, non-destructive techniques were employed. To collect seagrass shoots, a serrated-bladed knife (for *Serengadier*) or scissors (for *S. isoetifolium*) were used to cautiously cut plants at the base, retaining the root system and permitting natural recovery. Samples of sediment with seagrass roots, and rhizomes were collected with a sediment corer. This approach made it possible to study bioactive compounds associated with roots without substantially disturbing the habitat. The seagrass samples, once sampled, were placed in sterile plastic bag, identified and carried to laboratory into ice-cold containers to avoid bioactive compounds degradation.

Preparation of Extract

In the laboratory, *Thalassia hemprichii* samples were taken into processing to avoid any external contaminants. The seagrass was initially rinsed three times with tap water to remove sediments, debris and epiphytes, followed by three rinses with deionized water to remove any residual contaminants. Subsequently, the washed seagrass was finely cut to improve the extraction of bioactive compounds. To increase solubility, a 10% (w/v) aqueous extract of *T. hemprichii* was produced by weighing 10 g chopped seagrass to be incorporated into 100 mL distilled water in a glass beaker. The combination was then heated for 24 h at 100°C to extract the bio-active compounds. The mixture was then cooled to room temperature after boiling, and filtered through Whatman filter paper No. 1 to separate the solid residues which yielded a purified extract. This filtered extract was poured in a sterile glass container and kept in a refrigerator at 4°C to protect the bio-active compounds from degradation, until use. The resulting extract was further used as a natural reducing and stabilizing agent in eco-friendly synthesis of cobalt oxide nanoparticles.

Synthesis of Co_3O_4 NPs

Cobalt oxide nanoparticles (Co_3O_4 NPs) were synthesized through a green synthesis route based on the use of *T. hemprichii* as a natural reducing and stabilizing agent. First, *T. hemprichii* extract filtrate was made by boiling 10 g of fresh seagrass in 100 mL of distilled water at 100°C for 24 hours, with aliquots then filtered with Whatman No.1 filter paper. Cobalt (III) nitrate hexahydrate (5 mM) was dissolved in distilled water and added to the filtered plant extract during vigorous stirring at 100 °C (heated magnetic stirrer) for 24 hrs. A dropwise 1 M NaOH was used to adjust the pH to enable the reduction of cobalt ions, and gradual colour change to blackish brown confirmed the formation of cobalt oxide nanoparticles. The precipitated nanoparticles were centrifuged at 8000 rpm for 15 min and washed thoroughly with ethanol and distilled water to remove impurities from the solution. The washed nanoparticles were eventually dried at 100°C using an hot air oven as described by Safdar et al. [20].

Characterization of Co_3O_4 NPs

Fourier transform infrared (FTIR) was performed on a Bruker Alpha-II ATR spectrometer (4000–400 cm-1) to validate the existences of Co_3O_4 NPs derived from *Thalassia hemprichii* in the synthesis. X-ray diffraction (XRD) measurements were carried out for the finely powdered Co_3O_4 NPs to understand the XRD pattern (crystal structure) employing CuKα radiation and a Bruker D8 Advance X-ray diffractometer at 30 kV and 15 mA. The diffraction intensities were collected at a scanning velocity of 4°/min with 0.05° stepping size over the 2θ angle range of 5-80°. The scanning electron microscope studies using JEOL JSM IT 800 showed spherical shape of the Co_3O_4 NPs. Based on TEM analysis using a G2 20 S – Twin TEM Instrument, it can be concluded that the Co_3O_4 NPs were polydispersed.

Antioxidant activity

The preparation and characterization of cobalt oxide nanoparticles was commenced through the addition of the filtrate of *T. hemprichii* seagrass extract into a well distributed 1 M cobalt (III) nitrate hexahydrate in aqueous with 1:1 ratio by volume proposed by Govindasamy et al. [21]. The antioxidant activity of *T. hemprichii* was determined by DPPH Assay and NO Assay methods. For the evaluation different concentrations of solutions 10 - 100 µg/mL were prepared and to the various concentrations was added 1 mL of the DPPH solution (0.1 mM) and the NO solution (1 mL each). The solutions were incubated in the dark for 30 min and the absorbance readings were taken at 517 nm [22]. The percent inhibition was calculated as:

Inhibition (%) = [1 - (absorbance of control / absorbance of sample)] × 100

Antibacterial Activity

The antibacterial activity of the cobalt oxide nanoparticles (Co_3O_4 NPs) synthesised with *Thalassia hemprichii* extract were assayed by the well-diffusion method according to Clinical and Laboratory Standards Institute (CLSI). The bacterial strains such as Gram-positive strains like *Streptococcus mutans* and *Bacillus subtilis* and the Gram-negative strains *Pseudomonas aeruginosa* and *Escherichia coli* were given from Microbiology Laboratory, Saveetha Medical College. The experiment was carried out on MHA plates that are able to sustain the growth of coagulase positive and coagulase negative bacteria. The bacterial cultures were adjusted to the concentration of 1×10^8 CFU/mL with a 0.5 McFarland standard, and then the bacterial suspensions were spread uniformly on the plates with a sterile cotton swab. Values 6-8 mm diameter wells were punched in the agar and various concentration of Co_3O_4 NPs (50 µg/ml, 75 µg/ml and 100 µg/ml) were applied. In this assay, a stock suspension of the synthesized Co_3O_4 nanoparticles at a concentration of 1 mg/mL in distilled water was used. The volumes of 50 µg/ml, 75 µg/ml, and 100 µg/ml added to the wells therefore correspond to absolute doses of 50 µg/ml, 75 µg/ml and 100 µg/ml of nanoparticles respectively. This specification has been included to ensure clarity and reproducibility of the dosage-dependent effects observed.

Streptomycin sulfate (10 mg/mL) was used as the positive control and distilled water was the negative control. After 24 h of incubation at 37±2°C, the zones of inhibition (ZOI) around each well were measured by a digital Vernier caliper. The antibacterial activity of Co_3O_4 NPs was dose dependent, as higher Co_3O_4 NP concentrations resulted in larger inhibition zones. The antibacterial activity was more pronounced against Gram-positive bacteria (*S. aureus* and *B. subtilis*) than Gram-negative bacteria (*P. aeruginosa* and *E. coli*), suggesting that differences in bacterial cell wall composition influence nanoparticle susceptibility. This study highlights the potential of green-synthesized cobalt oxide nanoparticles as effective antibacterial agents for applications in biomedical and pharmaceutical fields.

Statistical analysis

The tests were performed three times in order to ensure the repeatability and reliability of the results. The accuracy of the measurements was reported in the form of mean and standard deviation for the data acquired from the experiments. The Inhibition Concentration IC_{50} was defined via the most discriminating linear regression square using response or effect at 50% level for the greatest lines coverage and best concentration quantification accuracy. Statistical analysis was conducted to validate that the IC_{50} values, which are important to study the biological activity and potential application of the tested nanoparticles.

RESULTS AND DISCUSSIONS

The present study successfully demonstrated the green synthesis of Co_3O_4 nanoparticles using Thalassia hemprichii seagrass extract, revealing significant antioxidant and antibacterial activities. The findings are consistent with and, in certain aspects, superior to previously reported results for plant-mediated Co_3O_4 NPs, underscoring the potential of marine-derived extracts in nanoparticle synthesis.

Fourier-transform infrared spectroscopy (FTIR)

Co_3O_4 NPs have been synthesized by *Thalassia hemprichii*, a seagrass species, serving as a bio-template. Different vibration modes are responsible for infrared absorption as observed in the FTIR spectrum. Fourier-transform infrared spectroscopy has been used to characterize the functional groups of seagrass extract were identified, showing unique peaks at 3354.58 cm^{-1}, 1646.18 cm^{-1}, 1349.92 cm^{-1}, 1126.74 cm^{-1}, 1000 cm^{-1}, 818.81 cm^{-1} and 685.84 cm^{-1}. The peak observed at 3354.58 cm^{-1} represents the stretching vibration of the O-H groups, suggesting water molecules and hydroxyl functionalities existed in the extract. These comprise the band located at 1646.18 cm^{-1} (C=O stretching), while the peaks at 1349.92 cm^{-1} and 1126.74 cm^{-1} are associated with C-H bending and C-N stretching (Omer et al). The low frequency peak 818.81 cm^{-1} and 685.84 cm^{-1} are ascribed to the metal-oxygen stretching vibration that symbolizes the formations of Co_3O_4 NPs. It is not a study by Thulasi Krishnan et al. 2024 [23] indicates that the observed FTIR peaks of Co_3O_4 NPs synthesized from Carica papaya leaves, at1379 cm^{-1} resulted from the O–H bending vibrations, while band at 1023 cm^{-1} was assigned to C–O stretching vibrations. In the same way, Kolahalam et al. [24] states that Co_3O_4 NPs prepared from *Lawsonia inermis* given over peaks at 1695 cm^{-1} is assignable to C-O vibrations and peaks at 662 cm^{-1} due to Co-O stretch vibration.

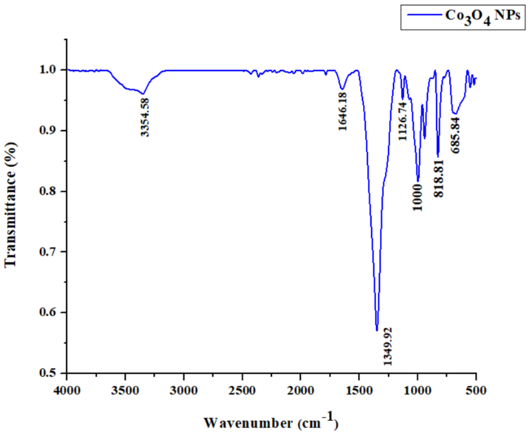

Figure 1. FTIR (Fourier-transform infrared spectroscopy) analysis of Co_3O_4 NPs

X-ray diffraction (XRD)

One of the most common equations to derive crystallite size from the broadening of X-ray diffraction (XRD) peaks is the Debye-Scherrer's formula. Scherrers formula, $D = K\lambda/\beta\cos\theta$, λ - Wavelength of the X-rays, β - full width at half maximum of the diffraction peak, and θ - Bragg angle. The XRD pattern for the *T. hemprichii* synthesised Co_3O_4 NPs showed distinct peaks at 29.61° (111), 31.99° (220), 35.62° (311), 39.14° (422), 42.66° (511), 46.70° (222), and 48.14° (101) that matched with the Co_3O_4 NPs phase crystal planes. This indicated that Co_3O_4 NPs possessed a well-defined crystalline structure in agreement with standard data on reported for Co_3O_4 NPs (JCPDS Card No. 43 – 1003). According to Akhlaghi et al., 2020 study [25] Co_3O_4 NPs synthesized from *Trigonella foenumgraceum* had angular peaks at XRD 31.51°, 37.08° and 45.06° which are corresponding to planes (111), (220) and (311) respectively. Also, one another research sputum by Anuradhu & Raji, [26] proves the XRD peaks (111), (220), (311), (222), (400), (422), (511), and (440) at surface of Co_3O_4 NPs synthesized from Arishta leaves.

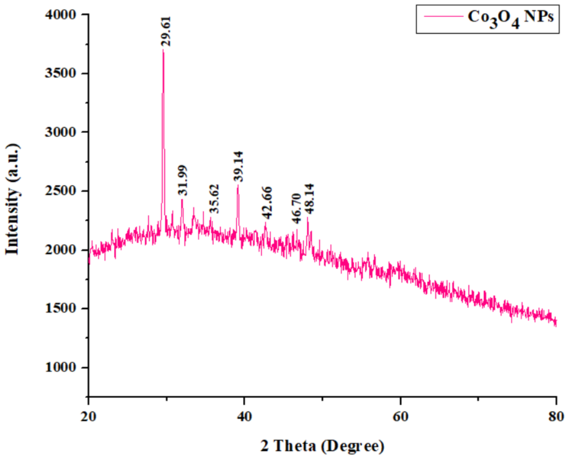

Figure 2. XRD (X – Ray Diffraction) analysis of Co_3O_4 NPs

Scanning Electron Microscopy (SEM)

The structural morphology of Co_3O_4 NPs was characterized using a Scanning Electron Microscope (SEM). FESEM image of synthesized Co_3O_4 NPs at magnification of 20 µm and 100 nm. The nanoparticles are uniform with low agglomeration, rough cubic, and spherical. The average particles size of these nanoparticles is 45.05 nm. According to the research of Din et al., 2024 [27], Cobalt nanoparticles were synthesized from *Trachyspermum ammi* have some agglomerated particles with nanorods of 325–375 nm.

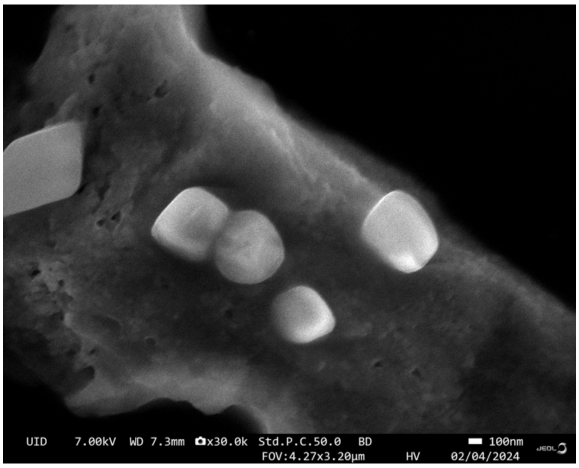

Figure 3. Scanning Electron Microscope (SEM) images of Co_3O_4 NPs

Transmission Electron Microscopy (TEM)

According to TEM, the Co_3O_4 NPs showed an irregular shape, roughly spherical and hexagonal, with minimal agglomeration, and the average size of NPs was found to be 52 ± 0.5 nm. Most of the nanoparticles are transparent in nature which indicates that thin M-O NPs were obtained. However, thick nanoparticles are also observed due to the agglomeration of nanoparticles. Almost the Co_3O_4 NPs are approximately spherical in shape, with a mean size of 22nm [23]. A study shows that TEM analysis of Yttrium doped Cobalt oxide Nanoparticles exhibits again homogeneous spheroidal distribution of NPs with sizes 37±0.49 nm [28].

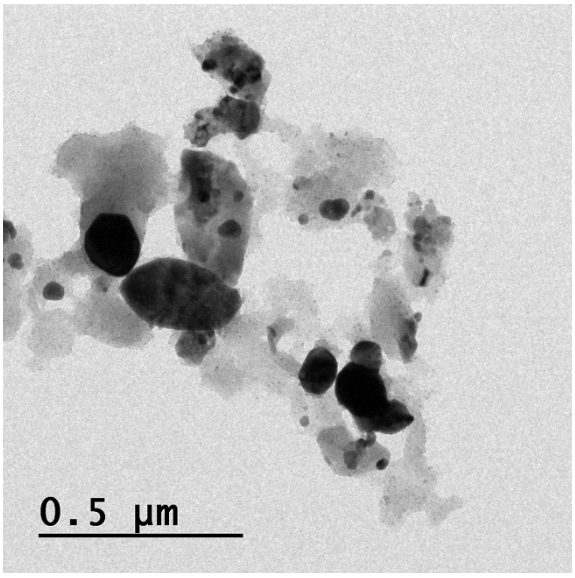

Figure 4. Transmission Electron Microscope (TEM) images of Co_3O_4 NPs

Antioxidant Activity

DPPH Assay

The radical scavenging activity of Co_3O_4 NPs was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay. In addition, the antioxidant activity of Co_3O_4 NPs was also evaluated at different concentrations (10, 20, 30, 40, 50, 75, and 100 µg/mL), while the results indicated that the antioxidant activity of Co_3O_4 NPs was enhanced in a dose-dependent manner. Notably, at highest concentration (100 µg/mL), the cobalt oxide nanoparticles showed a radical scavenging ability of 89%, higher than the seagrass extract (76.9%) and the synthetic antioxidant BHT (79.6%). Indeed, it has been shown that Co_3O_4 NPs exhibit superior antioxidant potential when injected at higher concentrations, indicating that the surface properties of nanoparticles, along with their interaction with free radicals, significantly contribute to their effectiveness. These findings corroborate prior studies showing that metal oxide nanoparticles display improved antioxidant activity because of their large surface area and strong reactivity with freeradicals [29,30].

The antioxidant activity of *Thalassia hemprichii* seagrass extract, BHT, and cobalt oxide nanoparticles is observed to be higher in case of nanoparticles (180 times) as compared to the plant extract and the synthetic antioxidant over a range of concentrations. Overall, the antioxidant activity of the seagrass extract was higher in a concentration dependent manner up to 75µg/mL but less than that of Co_3O_4 NPs (>50µg/mL). The itself unique properties of the material source of the nanoparticles synthesis also change the material properties that increase the antioxidant potential of the material. These findings are consistent with Al-Masoudi et al. (2022) [31] and Saravanan et al. (2021) [32] regarding marine-derived nanoparticles with antioxidant activity enhancement for biomedical and industrial purposes.

The radical scavenging assay data of DPPH were used to obtain the IC_{50} values of the antioxidant activity of the BHT, *T. hemprichii* seagrass extract, and cobalt oxide nanoparticles (Co_3O_4 NPs). The outcomes show that the values of IC_{50} of BHT and the seagrass extract are about 42.38 µg/mL and 42.40 µg/mL, respectively, whereas that of Co_3O_4 NPs is slightly bigger with 42.93 µg/mL. These values are indicative that all the three agents show similar antioxidant potentials in the mid-range concentration. It be mentioned, that Co_3O_4 NPs could also exhibits considerably more scavenging activity with higher concentration (89 percent at 100 µg/mL), outperforming both BHT and seagrass extract. Such a boost in efficiency at large doses demonstrates the increased antioxidant properties of Co_3O_4 NPs, which in turn can be explained by the peculiarities of its surface characteristics and free radical reactivity.

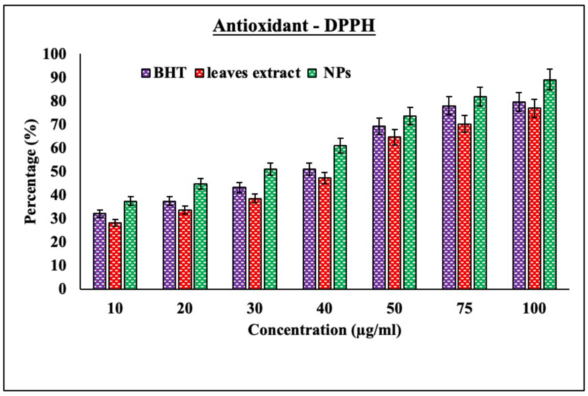

Figure 5(A). Antioxidant activity (DPPH (2,2-diphenyl-1-picrylhydrazyl) assay of Co_3O_4 NPs

NITRIC OXIDE (NO) ASSAY

The nitric oxide (NO) scavenging activity of cobalt oxide nanoparticles (Co_3O_4 NPs) synthesized from *T. hemprichii* was evaluated at different concentrations (10, 20, 30, 40, 50, 75, and 100 µg/mL). The findings were compared with the conventional antioxidant butylated hydroxytoluene (BHT), as well as *T. hemprichii* seagrass extract.

A modest NO scavenging activity of cobalt oxide nanoparticles was measured between 10 µg/mL and 30 µg/mL, which increased progressively at higher concentrations. Their total inhibition reached a high value of 87% when the concentration of cobalt oxide nanoparticles was 100 µg/mL, similar to the standard BHT at 90.9% activity. By contrast, the leaves extract of *T. hemprichii* exhibited a weaker activity, with the maximal inhibition of only 78.9% at 100 µg/mL.

Our findings are in agreement with those of previous studies of metal oxide nanoparticles, in which the increased antioxidant activity can be explained by the unique physicochemical properties of nanoparticles including a greater surface area and high reactivity. A growing number of studies have examined such a formatting effect: for instance, Almeida et al. (2020) [33]. Data obtained for the NO scavanging activity of zinc oxide nanoparticles was similar, and they demonstrated, as with silver nanoparticles, that the increase in concentration corresponds to the increase in inhibition of nitric oxide due to the catalytic behavior of the nanoparticles. Likewise a research conducted by Thirunavukkarasu in 2017 [34] explains that, cobalt oxide nanoparticles possessed strong antioxidant and anti-inflammatory activities likely due to the metal oxide's electron donation capacity to eliminate free radicals.

The IC_{50} values of the nitric oxide (NO) scavenging activity were calculated in the case of

BHT, *T. hemprichii* seagrass extract, and cobalt oxide nanoparticles (Co_3O_4 NPs). The IC 50 estimate of BHT was about 88.20 ug/mL which means that it will only be very active at higher concentration. Comparatively, the extract of the seagrass and Co_3O_4 NPs displayed IC 50 levels of approximately 61.13 µg/mL and 59.06 µg/mL, respectively. These findings point out that Co_3O_4 NPs have better scavenging activity of the NO than the seagrass extract and the BHT, especially at low concentrations. This increased action is attributed to the unusual surface activity reactivity of the metal oxide nanoparticle and high electron giving capacity as corroborated by past researches.

The lower IC_{50} of Co_3O_4 NPs (59.06 µg/mL) compared to BHT (88.20 µg/mL) reflects their enhanced nitric oxide scavenging efficiency.

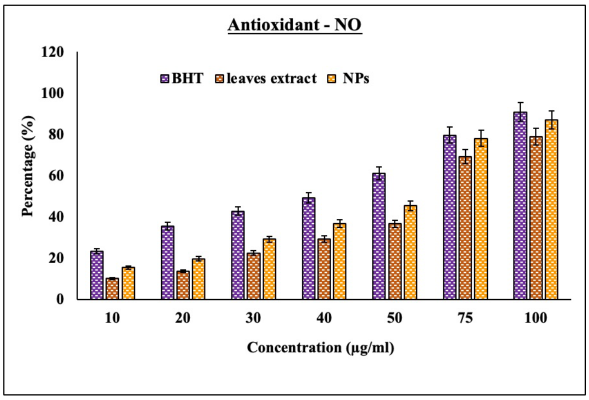

Figure 5 (B). Antioxidant activity (NO (Nitric oxide) assay of Co_3O_4 NPs

Antibacterial Activity

Co_3O_4 NPs synthesized in this study showed better antimicrobial efficacy against gram-negative bacteria than gram-positive bacteria at concentrations of 50, 75, and 100 µl. Leaf-mediated Co_3O_4 NPs nanoparticles inhibited the Gram negative bacteria *E. coli* 18±0.569 mm, 14.6± 0.159 mm 15.9±0.354 mm and 17.2±0.429 mm; *Pseudomonas aeruginosa* 11±0.326 mm, 13±0.369 mm, 12.5±0.357 mm, 13±0.146 mm with zones of inhibition at Control, 50, 75, and 100 µg/ml, respectively, followed by Gram positive bacteria *Streptococcus mutans* 17±0.486 mm, 10.6±0.258 mm, 13.3±0.984 mm, 14.7±0.218 mm; *Bacillus subtilis* 15±0.753 mm, 7.5±0.147 mm, 9.8±0.756 mm, 11±0.861 mm with zones of inhibition at Control, 5, 10, and 15 µg/ml, respectively. *E. coli* exhibited the largest inhibition zone among all tested organisms for the standard control (18±0.569 mm). The test samples showed a dose-dependent increase, with inhibition zones of 14.6±0.159 mm at 50 µg/ml, 15.9±0.354 mm at 75 µg/ml, and 17.2±0.429 mm at 100 µg/ml. The antibacterial results revealed dose-dependent activity against both Gram-positive and Gram-negative bacteria, with notable efficacy against *E. coli*. This is consistent with previous reports on biogenic Co_3O_4 NPs. For instance, Anuradha & Raji (2022) observed significant zones of inhibition against E. coli and B. subtilis using Arishta leaf-synthesized Co_3O_4 NPs. Similarly, Waris et al. (2021) reviewed that green-synthesized Co_3O_4 NPs generally exhibit stronger antibacterial action compared to chemically synthesized counterparts, due to the presence of bioactive capping agents. The enhanced activity against Gram-positive bacteria in our study may be attributed to the easier disruption of their cell wall structure by nanoparticles, as also noted by Makabenta et al. (2021) in their study on nanomaterial-based antibacterial agents.

It has been reported on the increased antibacterial activity of cobalt oxide NPs prepared from various bio-sources in several studies. For example, Co_3O_4 NPs green synthesized using marine plants were found to exhibit a wide range of antibacterial activity. Likewise, indicated that cobalt oxide NPs synthesized through marine plant possessed superior antibacterial performance compared to the chemically synthesized NPs due to the bioactive phytochemicals from the marine plant contributing synergistically with their antimicrobial activity. The antimicrobial effect differed overall between strains and concentrations, but a clear concentration effect was found for most microorganisms. The results suggest that biogenic Co_3O_4 NPs could be promising antimicrobial agents, particularly for biomedical- and environmental-related fields [35,36].

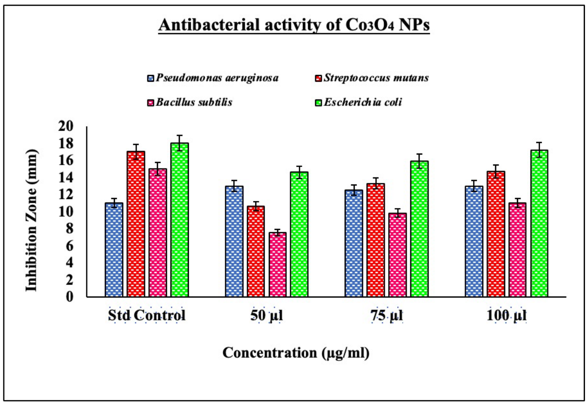

Figure 6. Antibacterial activity of Co_3O_4 NPs against *S. aureus*, *P.aeruginosa*, *E. coli, Bacillus sp.* Microorganisms

CONCLUSION

In conclusion, this study successfully demonstrated the production of cobalt oxide nanoparticles Co_3O_4 NPs through the green synthesis process by utilizing *Thalassia hemprichii* extract as an effective method that is sustainable, low cost, and ecofriendly in its production process. The biochemical pathway allowed them to turn cobalt salts into nanoparticles without the involvement of any dangerous chemical enhancing their biocompatibility. The morphology analyses using structural characterization revealed the formation of well-developed crystalline Co_3O_4 NPs with an average size of 45-52 nm. Antioxidant analysis showed that the IC_{50} of the DPPH scavenging was approximately 42.93 ug/ mL Co_3O_4 NPs, which was a little more than BHT (42.38 56 µg/ ml) and seagrass extract (42.40 µg/ ml). As far as scavenging of NO is concerned, the IC_{50} of Co_3O_4 NPs was the least at ~59.06 µg/ ml, followed by the seagrass extract (~61.13 µg/ ml) and BHT (~88.20 µg/ ml). These findings prevail that at lower doses, Co_3O_4 NPs have better antioxidant and NO scavenging activities.

Also, the Co_3O_4 NPs showed concentration dependent bactericidal activity against clinical pathogens i.e. *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans* and *Bacillus subtilis*. Among this we noted strong antibacterial action for Co_3O_4 NPs against *E. coli* and thereby warranting its antibacterial potential. These findings show that they could find application in a wide variety of areas such as biomedicine, environmental decontamination and nanocatalysis. These nanoparticles already have proven antioxidant and antibacterial effects; therefore, they can be incorporated into food, pharmaceutical, cosmetic products as well as in the purification of water. Nevertheless, additional studies are necessary to optimize their synthesis parameters and therapeutic efficiencies as well as carefully test their cytotoxicity, bio compatibility and drug delivery properties. The work represents the milestone in the enhancement of green nanotechnology in ironing out the path towards multi-functional and eco-friendly nanomaterials.

AUTHOR CONTRIBUTIONS

Shanthi Muthusamy – Writing original draft, methodology, Yogananth Nagarajan – Acquisition of Data, Statistical Analysis, Largus Shylee – Conceptualization, S. Malathy – Writing Review and Editing

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

Data availability will be made on request.

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