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Antibacterial Assessment of Biofabricated Magnesium Oxide Nanoparticles (MgO NPs) using *Conocarpus erectus* Leaf Extract

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Abstract

Nanobiotechnology is an advanced discipline of science that deals with nanoscale materials in areas including nanotechnology, chemistry, medicine, and biotechnology. The current trend is to use sustainable and biological methods utilizing natural sources to quickly synthesize metal oxide nanoparticles rather than toxic, dangerous biochemical ones. In this paper, the biogenesis of magnesium oxide nanoparticles (MgO NPs) utilising *Conocarpus erectus* as a natural source is described for the first time. In producing MgO NPs, *C. erectus* leaf extract was a bioreductor and stabilising agent. Several characterisation techniques, including XRD, FTIR, HRTEM, SEM, and EDX studies, were employed to confirm the formation, crystalline structure, and surface morphology of the synthesized nanoparticles. XRD measurement confirmed the crystalline f.c.c structure of MgO NPs.The production and stabilisation of MgO NPs by *C. erectus* leaf extract were attributed by FTIR analysis to the active functional groups. Studies using HRTEM and SEM revealed that the magnesium oxide nanoparticles were small-sized, spherical, and uniformly dispersed. The EDX profile also approved the elemental composition of the produced nanoparticles. The bacterial inhibition by the synthesized MgO NPs was evaluated against S. aureus and *E. coli* that plant-mediated MgO NPs effectively inhibited, with inhibition zones of 17(±0.4) mm and 16(±0.5) mm, respectively.

Keywords Biofabrication, *Conocarpus erectus*, MgO NPs, Antibacterial activity

1. Introduction

Nanotechnology is a sponsoring area of material science that is proficient in fabricating nano-scale materials. A broad category of materials known as "nanomaterials" consists of particles with sizes between 1-100 nm (1). Nanotechnology has broadly produced efficient catalysts that are biochemically active in regulating various infections caused by pathogenic microbes. In current eras, metal and metal oxide NPs have attained much more interest among researchers due to their infrequent possessions as opposed to other nanoparticles (2). MONPs have extensive biological and therapeutic applications as alternate anti-microbial mediators ascribed to the reoccurrence of contagious infections and the arrival of antibiotic-resistant strains. MgO NPs have been used in contaminated waste remediation, antiseptic, electronics, paints, optical, antibacterial agents, semiconductors, catalysis, and catalytic devices (3-11).

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Typically, the photodegradation of MB dye and bacterial obstruction is accomplished in the attendance of MONPs, which impede the growth of bacteria and deprive toxic dye by generating reactive oxygen species (ROS) (12, 13).

Generally, metal and MONPs have been fabricated using conventional chemical and physical procedures that are toxic, costly, and cause several ecological and health problems (14, 15). The preparation of metal and MONPs using plant extract is an appealing approach compared to other routes (16-21). The biochemical preparation of MONPs by plant arbitrated route has the aid of being facile, innocuous, and biodegradable (22-24). Phytochemicals of plant sources performed an imperative role in the development and stabilization of nanomaterials. Accordingly, assets of the created NPs, i.e., antibacterial activity and biocompatibility based on the possessions of biomolecules of the plant source from which they were prepared. Several reports have been available regarding the fabrication of MgO NPs via green synthetic procedures. Several plant extracts, i.e., white button mushroom (25), *Rosmarinus officinalis* (26), *Trigonella foenumgraceum* (27), *Emblica officinalis* (28), *Trigonella foenum-graecum* (29), *Matricaria chamomilla* (30), etc., were reported for the green synthesis of MgO NPs. In this study, MgO NPs were biochemically prepared using leaf extract of *C. erectus*. The mangrove shrub *C. erectus*, also referred to as green buttonwood or button mangrove, belongs to the *Combretaceae* family (31), cultivated on coastlines in sultry and sub-tropical areas of the biosphere (32). This plant is also widely grown as ornamental furniture, for land reclamation, as a hedge, and as a very hard and strong wood (33). This plant species has been used traditionally by local people for the treatment of various such as headache, anemia, orchitis, bleedings, prickly heat, diabetes, catarrh, syphilis, gonorrhea, tumors, conjunctivitis, swellings, antipyretic and anti-inflammatory, etc. (34-37). *C. erectus* has shown strong antioxidant, antibacterial, anticancer, hepatoprotective, DPPH assay, and antimicrobial activities (38-41). A number of phytochemicals, such as gallic acid, tannins, phenolics, terpenoids, flavonoids, etc., were found in the leaf extract of *C. erectus* (42-43). Due to such phyto constituents, *C. erectus* intensely played a crucial role in developing magnesium oxide NPs.

Thus in this report, we have successfully synthesized MgO nanoparticles by an eco-friendly, nontoxic, green deposition method using *C. erectus* leaf extract as stabilizing and reducing mediator. Additionally, MgO NPs were tested for the inhibition of bacteria. The naturally accessible and harmless plant source was used to manufacture MgO NPs.

2. Experimental

2.1 Materials

Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), methylene blue, sodium hydroxide, nutrient agar, nutrient broth, and all other chemicals used in this report were procured from Sigma Aldrich (Pakistan). All the chemicals and reagents were of optimal purity and used without further sanitization.

2.2 Leaf Extract Preparation

Fresh and unspoiled leaves of *C. erectus* were calm from D. I. Khan, KPK, Pakistan. The leaves were eroded with deionized water, allowed to desiccate at room temperature, and then mechanically ground into powder. 5g leaf powder was added to 120 mL of water, and mixture was agitated for 5 hrs at 40 °C (see Figure 1A). Finally, the supernatant was filtered and kept at 4 ^oC.

2.3 Biosynthesis of MgO NPs

To synthesize MgO NPs, 1.2 g $MgSO_4.7H_2O$ (0.1 M) was added to 100 mL of water. The mixture was agitated at 70 ^oC for 3 hrs to form a homogeneous solution. NaOH (1M) solution was added dropwise with the help of a burette, and the mixture was stirred again for the next 1 hr. Green extract of *C. erectus* was added drop wise at continuous stirring. The appearance of brown color confirms the creation of MgO nanoparticles, as illustrated in Figure 1(B). The color change prompted the reduction of Mg to MgO NPs. The solution was centrifuged (3500 rpm, 10 mints), and the precipitates were cleansed with acetone and then deionized water to eradicate the unwanted impurities. The nanoparticles were dried for 12 hrs at 80 °C in an electric oven. Finally, after being calcined for three hours at 400 °C in a Muffle furnace, the well-dried MgO NPs were produced as pale yellow MgO nanoparticles.

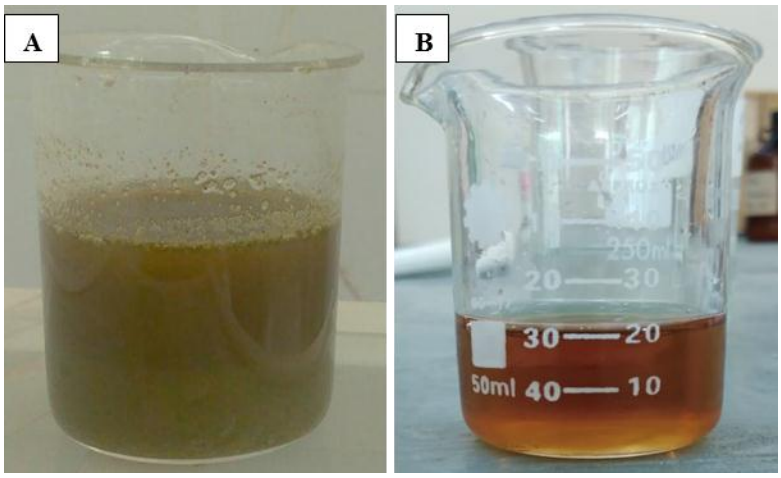

Figure 1: (A) Leaf extract and **(B)** MgO NPs

2.4 Antibacterial Assessment of MgO Nanoparticles

The antibacterial activity of biogenic MgO nanoparticles was tested by using *S. aureus* and *E. coli* bacteria. These strains were obtained from the Department of Biotechnology, Gomal University, Dera Ismail Khan. These strains were kept on agar slants at 4 °C for the antibacterial assay. The bacteria were incubated for 24 hrs at 37 °C in Muller Hinton Broth (Oxoid) with a pH of 7.2. An agar well diffusion procedure was applied to scrutinize the antibacterial assessment of biosynthesized magnesium oxide nanoparticles (44). The bacterial strains were cultured in nutrient broth for 24 hrs at 37 °C in an incubator. To ensure a consistent, dense sheet of growth after incubation, the inocula of the specific bacteria were patterned onto the Muller Hinton agar platters using a sterile swab. Borers (6 mm) were designed using disinfected cork bradawl onto the nutrient agar plates. In the agar, well diffusion method, 1mg MgO NPs was added with 1 mL of deionized water. The sterile bores were soaked with 50 mL of MgO nanoparticles with a high concentration of respective agar bioassay. The MgO NPs integrated wells were sited onto petri dishes and incubated at 37 ^{o}C for 24 hrs. The embarrassment regions were measured very cautiously.

2.4.1 Determination of MIC

MIC is the least quantity of material that stop bacteria from growing. The MIC of biodirectedMgO NPs can be examined by serial dilution strategy. Different concentrations of greener MgO NPs were treated with 1 mL of bacterial solution. The suspension turbidity was adjusted to 0.5 Mc-Farland turbidity standards. 1 mgmL^{-1} to 0.125 mgmL^{-1} concentration of biomediated MgO NPs were added to the test tubes. These test tubes were then kept at 37 °C for an overnight incubation period. The control was a test tube containing growth media and a bacterial solution. The assay was repeated thrice.

2.5 Characterization

The wide angle XRD pattern of MgO NPs was recorded by using Rigaku D/Max2500 VBZ+/PC Diffractometer. An ABB MB 3000 Spectrophotometer was applied for recording FTIR spectra of plant extract and biosynthesized MgO NPs. The size and dispersion of MgO NPs were analyzed using HRTEM (JEM-3010 Microscope). The surface morphology and elemental composition of the sample material were examined using a Hitachi S-4700 SEM.

3. Results and Discussions

3.1 XRD Analysis

Structural exploration of greener magnesium oxide NPs was done using an X-ray diffractometer. Figure 2 symbolizes the characteristic XRD pattern of biodirected magnesium oxide NPs. The angle (2θ) range was adjusted between 30-80^{o}. The result shows that five well-observed peaks have appeared 38.34^{o}, 44.58^{o}, 65.35^{o}, 74.6^{o,} and79.3^{o,} which can be indexed to (111), (200), (220), (311), and (222) Braggs reflections of the f.c.c structure of MgO NPs. The result matched with JCPDS-21-1272. The most prominent peak at 38.34^{o} (111) can be attributed to the f.c.c crystal structure of MgO NPs the results matched the previously reported work (45). The diffractogram exhibits no ancillary phase or impurity peaks.

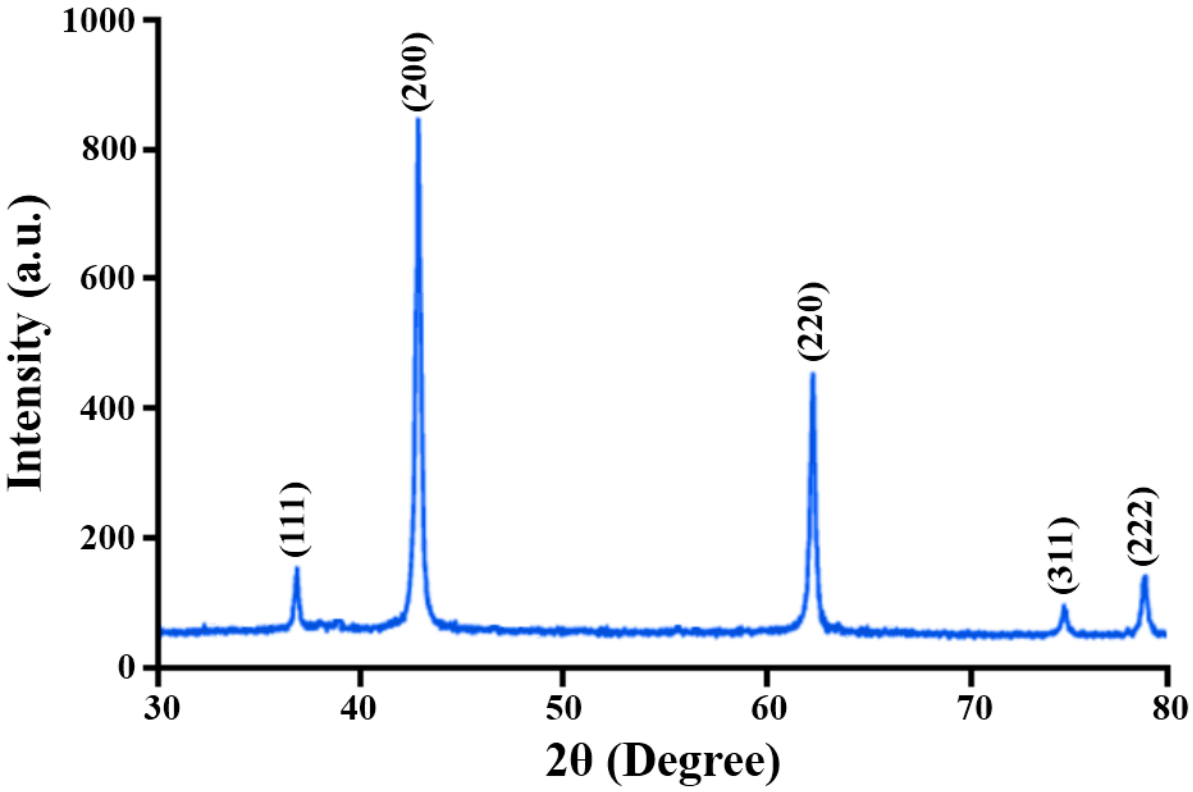

Figure 2: XRD pattern of biosynthesized MgO NPs

3.2 FT-IR Spectral Analysis

FT-IR study was performed to specify the phyto constituents of *C. erectus* liable for the formation and stabilization of MgO NPs. FT-IR spectra of leaf extract and biomediated MgO NPs are illustrated in Figure 3. The results confirmed that the plant precursors act as stabilizing and reducing mediators. The major peaks at 3332 cm^{-1} confirm the stretching vibrations of O—H bonds, which confirm the phenolic character of plant extract. The peaks at 2932 cm^{-1} are associated with the C—H stretching vibrations by confirming the aldehydic character of plant extract. The bands at 1613.4 cm^{-1} reflect the C=C stretching frequencies, whereas the arrival of the bands at 1500 cm^{-1} are due to N—N bending vibrations. The peaks at 1064.48 cm^{-1} are related to the bending frequencies of absorbed water molecules and surface O—H radicals. A well-observed peak at 534.7cm^{-1} indicates a C—O diagnostic bond confirmed the development of MgO NPs. The results clear that most of the biomolecules found in plant extract were intricate in the formation of NPs (46).

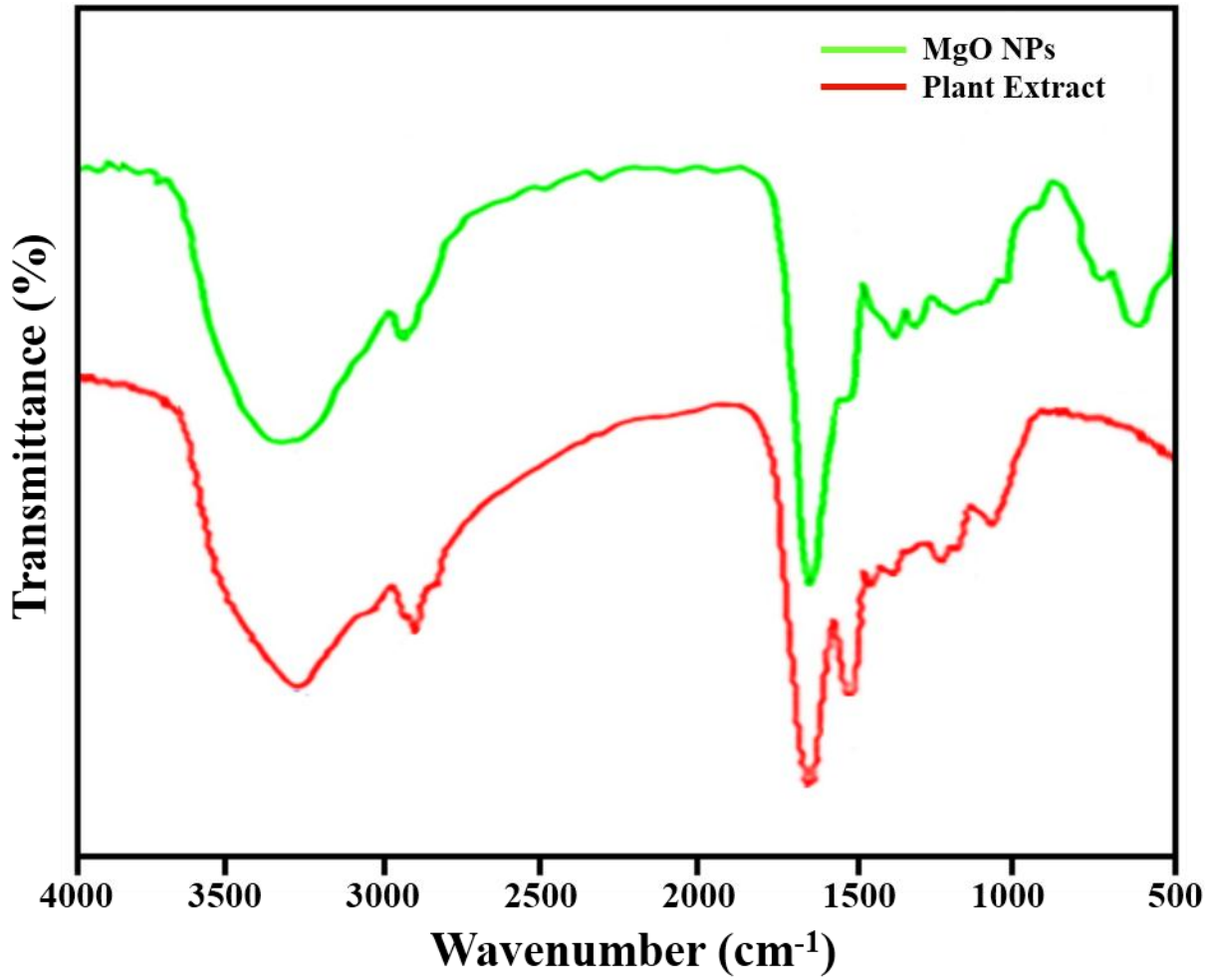

Figure 3: FTIR analysis of plant extract and biosynthesized MgO NPs

3.3 HRTEM Analysis

HRTEM is an important research technique for the direct imaging of NMs to achieve quantifiable measures of particle size, shape, and dispersion. Figure 4(A) signifies the HRTEM image of plant-mediated magnesium oxide nanoparticles. The microscopic image indicates that MgO nanoparticles are of small size (average size =27 nm), spherical shape with the high distribution. The results are fairly well matched with previously reported work. Due to such features, MgO NPs exhibit outstanding catalytic and biomedical applications.

3.4 SEM and EDS analysis

SEM study was accomplished to scrutinize the surface morphology of greener magnesium oxide nanoparticles. The biogenic nano-scale magnesium oxide particles were perceived in sphere-shaped morphology. The SEM microscopic image of biodirected MgO nanoparticles was taken at a magnification of 800 x, as illustrated in Figure 4(B). The particle size of MgO NPs was observed to be 27 nm. The microscopic image shown that the synthesized MgO NPs aggregated, mostly sphere-shaped and occasional in cubes structures.

EDS study provided qualitative and quantitative analysis of the elements that might be involved in the stabilization and formation of nanoparticles. Figure 4(C) indicates the EDS profile of biogenic magnesium oxide NPs. The peaks in the region of "Mg" and "O" confirm the establishment of MgO nanoparticles.

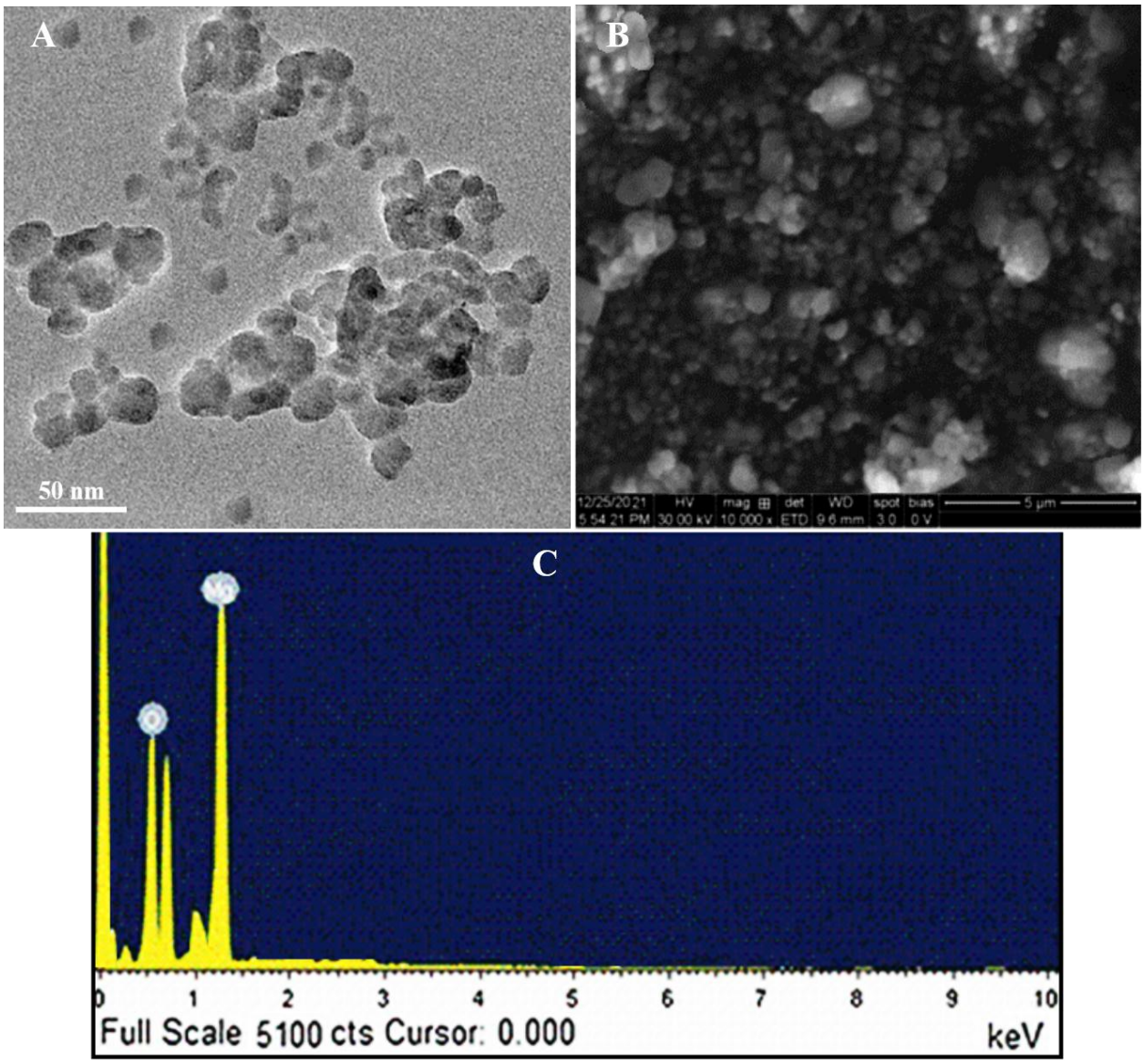

Figure 5: (A) HRTEM analysis, **(B)** SEM analysis, and **(C)** EDS analysis of Biosynthesized MgO NPs

3.5 Antibacterial Activity

Antibacterial activities of plant extract and greener MgO NPs were examined against *S. aureus* and *E. coli* bacteria. The biodirected MgO NPs exhibited higher inhibition efficiency than plant extract against the tested bacteria. The results depicted that MgO NPs significantly apprehend bacterial growth with inhibition zones of 17(± 0.4 mm) and 16(± 0.5 mm) against *S. aureus* and *E. coli* which are more effective than a plant extract of 11(± 0.2 mm) and 12(± 0.4 mm) respectively as given in Table 1. The higher anti-bacterial action of biodirected MgO NPs may be assigned to their nano size, spherical shape, and high dispersal. The nanoparticles of smaller size and sphere morphology offer greater surface regions and are consequently highly effective than their bulk particles. This high bacterial inhibition efficiency of the tested NPs can be assigned to producing ROS released by metal oxide ions (47,48). According to literature, control elimination of metal oxides from metal oxide nanoparticles generate superoxide ($\bullet O_2^-$) and hydroxide (•OH) radicals in harmful microbes (49). When the concentration of these ROS upsurges, then the scavenging ability of bacteria cells will lead to cell death.

Table 1: Bacterial Inhibition Efficiency of MgO NPs and Plant Extract against *S.aureus* and *E.coli*

Bacteria	Zones of Inhibition	
	MgO NPs	Plant Extract
S. aureus	17(±0.4 mm)	11(±0.2 mm)
E.coli	16(±0.5 mm)	12(±0.4 mm)

3.5.1. MIC Determination

MIC is a useful method for quantitatively evaluating the tested material's antibacterial activity. MIC is the least amount of greener MgO NPs essential to obstruct the growth of bacteria. A dilute suspension of MgO NPs was incubated with *S. aureus*, and *E. coli* strains at 37 ^{o}C for 24 hrs in a shaking incubator. As shown in Table 2, after incubation, it was discovered that S. aureus and E. coli showed MICs of 0.125 mg/mL.

Table 2: MIC of MgO NPs against *S. aureus and E. coli.*

Bacteria	MgO NPs(mg/mL)			
	1	0.5	0.25	0.125
S. aureus	-	-	-	+
E.coli	-	-	-	+

4. Conclusion

Among other conservative protocols to synthesize nano-scale materials, biogenic routes are a promising field that offers an innocuous, biodegradable, and valuable procedure to manufacture innovative materials. In this work, we present a biogenic production of magnesium oxide NPs using leaf extract of *C. erectus*. The phyto constituents of plant extract performed a prominent role in the formation and stabilization magnesium oxide NPs. The size and shape of MgO NPs were optimized at 70 ^{o}C. The greener MgO nanoparticles were inspected for bacterial inhibition activity against *S. aureus* and *E. coli*. Thus, MgO NPs have actively reserved the *S. aureus* and *E. coli* growth with zones of inhibition of 17(±0.4) mm and 16(±0.5) mm, respectively. This high efficiency of greener MgO NPs can be attributed to their nano size and a high degree of dispersion. The as-synthesized MgO NPs may necessitate further investigation and additional applications.

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Conflict of Interest There is no conflict of interest

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