Acalypha Indica mediated MgO NP’s: A Novel Approach in Greener Route with its Antibacterial Activity against Pathogens

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ABSTRACT
The interest in miniaturization of particles revealed the hidden applications of metal oxides. The potential applications of the particles may vary when the size of the particle is reduced. One of the alternative routes to the conventional approach is the use of plant extract for the synthesis of metal oxides NP’s. In the framework of this study, the eco-friendly MgO nanoparticles were synthesized using Acalypha Indica leaf extract, functioning as reducing and capping agent by co-precipitation method. The predecessor taken here was Magnesium Nitrate. The biologically synthesized MgO NP’s were characterized by various techniques like X ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) with Energy Dispersive X-ray spectroscopy (EDX) profile and its antibacterial activity is evaluated against causative organisms. XRD studies confirmed the face centered cubic crystalline structure of MgO NP’s and the average crystalline size of MgO NP’s calculated using Scherer’s formula was found to be 13 nm. FTIR spectrum shows a significant Mg-O vibrational band. Purity, surface morphology and chemical composition of elements were confirmed by SEM with EDX. The SEM result shows the fine spherical morphology with the grain size range between 43nm to 62nm. Antimicrobial assay of MgO NP’s was examined against gram positive and negative bacteria. Appreciated activity was observed on the Staphylococcus aureus bacterial species. In general, the renewed attempt of this facile approach gave the optimum results of multifunctional MgO NP’s.

Keywords: MgO NP’s, greener route, XRD, antibacterial activity.

1. INTRODUCTION
Nanoparticles acquired a great interest due to their small size, in Greek ‘Nano’ means ‘a dwarf’. A revolution in tech world with diverse spectrum of application and its amenability caught the heed of biomedical research. As the impact of this, new medicines and technologies for human health are equipped with atoms and molecules in a controlled fashion. The only bridge that connects the solid matter in the visible scale and the material seen in a regular optical microscope is ‘Nano’. Changing the size of the particle, a researcher can literally tweak a material property of their own interest for e.g., changing fluorescence colour; the fluorescence colour of a particle can be used to identify various materials.

As particle size when reducing to nanoscale range, their surface area to volume ratio increases, so they can intake large amount of drugs and spreads them easily. When the size decreases, the particles physical, mechanical, chemical, electrical, magnetic, and optical properties also change. These qualities add up its advantage in Nano medicine. Nanotechnology is truly becoming a reality in reshaping the medical devices. Further explorations showed that the innate structure of wings of dragonflies and cicadas prevent bacterial growth, surface of their wings are like nanopillars which trap the microorganisms.[2] Stepping up to that, the innovative ideas are also implemented in nanotech by changing the material shapes in different forms like wires, tubes etc.,. Generally, Nanoparticles are classified based on their size, morphology and properties as Carbon-Based Nanoparticles, Metallic Nanoparticles, Polymeric Nanoparticles, Ceramic Nanoparticles and Lipid-Based Nanoparticles.[3]

Metal and metal oxide NP’s have inimitable properties and are different than their indigenous bulk materials. The metal
elements are able to form multifarious oxide compounds. These oxides can take up a cyclopean amount of structural geometries that can exhibit metallic, semiconductor or insulator character. There are numerous methods available for the synthesis of metal oxide NP’s. Some of the most commonly used methods are Laser Ablation, Ultrasonication, Spray Pyrolysis, Vaporization, Sol-Gel, Hydrothermal, co-precipitation etc., some inorganic metal oxides like ZnO, MgO, CaO, can be used as antibacterial agents. Since, they are stable under severe processing. J. Sawai made a Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay.[1-6] Among them MgO nanoparticle have strong antibacterial activity against G+ and G- bacteria. MgO has vast array of properties like high melting point, High temperature stability, refractory property etc., so it has diverse spectrum of industrial applications. According to United States Food and Drug Administration (21CFR184.1431) MgO is considered to be the safest metal for human use. It’s also notable that these metal oxides have antimicrobial activity without photo- activation activity. MgO nanoparticles in combine with other antimicrobials like nisin have effective antibacterial activity, it showed a growth inhibition of food borne pathogens and ultimately leads to cell death and thus enhance food safety. Apart from antimicrobial activity MgO NP’s are used as catalysts, in photonic and electronic devices, as they have high melting point and are highly functional. Researches also show that MgO can also be used as an alternative to the chemical pesticides. MgO thin films are acted as a shield in dielectrics, as secondary electron emission layer.[1-6]

Physical and Chemical means of synthesis cause catastrophe to our environment so there is need for an alternative route called greener route which will somehow regulate the toxicity of chemical method. There are various proposed materials available in greener route, some are plants, algae, bacteria, fungi etc... Priyanka Singh et al., had Biological Synthesis of Nanoparticles from Plants and Microorganisms.

Using plants in nanotechnology opened one of the new routes for researchers and their researches. During the past decade, it’s believed that the natural source that has an antimicrobial agent which provides remedies for new diseases is plants, in the large categories of plants; medicinal plants are often considered for solving the human health problems. These medicinal plants serve for various medicinal systems. As plants have secondary metabolites that can act as active ingredient in the development of drugs that incite effective antimicrobial agents to control the bacterial activities.

Metal and metal oxide nanoparticles are synthesized using plant extract, they intake less use of chemical agents. Various plants and other microbial organisms are used for the synthesis of gold and silver nanoparticles and some other NP’s. MgO NP’s was synthesized from various plant extracts. K. Jhansi et al., had Biosynthesized MgO nanoparticles using mushroom extract: effect on peanut (Arachis hypogaea L.) seed germination. S.L. Smitha et al., had Green synthesized gold nanoparticles using Cinnamomum Zeylanicum leaf broth, Panel Daizy Philip had undergone Green synthesis of gold and silver nanoparticles using Hibiscus Rosa Sinensis.

As biodegradable and with fewer side effects, MgO NP’s are used to mediate the freezing procedure in Nanocryosurgery for cancer treatment and also used as a therapy for leukaemia. Though the evolution of the technologies welcomed numerous treatments for manifold diseases yet there is lack of better addressing of certain diseases like autoimmune diseases, chronic pain & microbial infections.[6] There’s an impulse for the development of new medicines which will overcome the sufficiency caused by former one. Here is the study, which broken new ground to further investigate the antibacterial activity and efficacy of MgO NP’s against microorganisms using ACALYPHA INDICA plant extract by a simple and cost effective co precipitation method.[7]  

2. Experimental Procedure

All the chemicals used in this study were of analytical grade and utilized without any additional purifications. Magnesium Nitrate Hexahydrate [Mg (NO3)2.6H2O] and sodium hydroxide (NaOH) were the staple material for the synthesis of MgO nanoparticles. Fresh leaves of A. Indica (kuppaimeni) were collected from the local areas of Erode, Tamil Nadu. The fresh leaves were washed thoroughly with tap water for 2-3 times followed by washing in distilled water and used for further studies.

The leaves are shade dried for 3-4 days in a dust free ambience. The dried leaves were crushed into small chunks. Those fine pieces were boiled with 150ml of distilled water at 80° C for 3 hrs. The solution is then allowed to cool for few minutes and then filtered using whatmann filter paper. The obtained dark brown coloured extract was stored for further usage. Fig. 1 (a and b) represents the diagrammatic and graphical representation of extraction of A. Indica.

Preparation Of MgO NP’s:

0.2M of Magnesium Nitrate Hexahydrate [Mg (NO3)2.6H2O] solution was prepared in 50 ml of distilled water. The solution was then stirred unremittingly using magnetic stirrer until the opaque solution turned to be homogeneous. To the above solution, 50 ml of A. Indica leaf extract was added. The fusion is stirred vigorously for 2hrs followed by heating at 80°C. 25 ml of NaOH solution was added drop wise in the midst of stirring. The mixture was incubated, and the limpid solution followed by a precipitate was centrifuged and the precipitate was collected separately in a dish. The precipitate was then...
washed twice with ethanol to remove the impurities and it was kept in hot air oven at 100°C for 24hrs. The half dried brown coloured Mg (OH)₂ substrate were calcinated in muffle furnace at 450°C for 2hrs for dehydration process. The calcinated powder was pulverized well by mortar and pestle. The final white colored product obtained was MgO NP’s and they are hoarded in air tight container for further studies. Fig. 2 (a and b) represents the diagrammatic and graphical representation of biologically synthesized MgO NP’s.

**Characterization Techniques**

The size and crystalline structure of MgO NP’s were characterized by X RAY diffraction (LABX XRD 6000) using the Bruker Diffractometer with CuKα radiation having the wavelength of 1.5406Å operating with the voltage = 40.0 (kV) and current= 30.0 (mA). The sample was scanned in the range 2θ=0° to 90°. MODEL PHILIPS XL-30 Scanning Electron Microscope (SEM) with an attached

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**Fig. 1A and B:** (A) Diagrammatic Representation; (B) Graphical Representation of an indica leaf extract of an indica leaf extract

**Fig. 2 (a) diagrammatic representation of biologically synthesized MgO NP’s**
energy dispersive X-ray (EDX) analyser was used for the observation of sample's morphology and its chemical composition.

The sample was tested for antimicrobial activity by well diffusion method. Liquid Mueller Hinton agar media and the Petri plates were sterilized by autoclaving at 121°C for about 30 minutes at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 18 hrs culture of Gram positive microorganisms such as Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 3160), Gram negative microorganisms such as E. coli (MTCC 1698) and Klebsiella pneumoniae (MTCC10309) obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with loading of 50 µl and 100 µl of each sample to the distinct well with sterile distilled water as negative control and gentamycin (30mcg/disc) as positive control. The sample loaded plates were then incubated at 37°C for 24 hours to observe the zone of inhibition.

3. Results and Discussion

3.1 Visual Observation

The synthesis of nanoparticles is primarily observed by the colour change. Fig.2 (c) shows the changes in colour from dark brown to white in the synthesis of MgO NP's from aqueous solution of leaves of Acalypha Indica. The change in colour of the sample might be due to the parameters like extract concentrations, precursor concentration, temperature, and reaction time. The observed colour change demonstrates the degradation of phytochemicals in the mixture as reducing and stabilizing agents for the synthesis of MgO nanoparticles.

3.2 XRD Analysis

The powdered MgO NP’s was analyzed by X-ray diffractometer to investigate the structural parameters. The phase and the crystal structure of the biologically synthesized MgO NP’s using A. Indica plant extract has been investigated for better understanding of the position of atoms in lattice structure.

The result from the XRD analysis was a diffractogram showing the intensity as a function of diffraction angle. X-ray diffraction pattern was used to distinguish between amorphous and crystalline nature of material. The peaks show that the synthesized sample was polycrystalline in nature and the conformation of result was verified by comparing the 2θ values with the standard XRD data (JCPDS Card No.89-7746).\[8]\] XRD plot revealed the cubic phase of biologically synthesized MgO NP’s with three clear and distinct major diffraction peaks at 2θ=34.478, 42.6896, 62.0638 corresponding to the lattice planes (1 1 1), (200) and (220) respectively (Fig. 3). Moreover, the close investigation of XRD plot revealed that there were no additional secondary phases were present conform the
pure crystalline nature of biologically synthesized MgO NP’s. The crystalline size of MgO NP’s was obtained by Debye-Scherrer’s formula.\[9\]

\[
D = \frac{K \lambda}{\beta \cos \theta}
\] (1)

Where $K$ is the Dimensional constant depending on the particular geometry of the scattering object ($k=0.98$), $\lambda$ is Wavelength of the X-Ray radiation for CuKα ($\lambda=1.5406*10^{-10} \text{m}$), $\beta$ is the full/line width at half-maximum height and $\theta$ is the Bragg’s angle of the line profile correlating to half of the extract position of principle axis. The average crystalline size ($D$) of face centered cubic Crystal of MgO NP’s calculated was found to be 13 nm and the value was nearer to 13 nm had reported in the formation of MgO nanoparticles using Trigonella foenum-graecum leaf extract\[10\] and Rajesh Kumae et al., synthesis of MgO Nanoparticles by Co-Precipitation Method.\[11\]

The interplanar spacing is calculated from the Bragg’s equation,

\[
2d_{hkl} \sin \theta = m \lambda
\] (2)

Where, $d_{hkl}$ is the interplanar spacing ($m=1$), (hkl) lattice plane index, 2 is the lattice constant and h, k and l are the integers. Table 1 gives the crystallite size and the spacing between the consecutive planes along with the dislocation density and micro strain.

The lattice constants were calculated from the equation of cubic system using the method of least squares

\[
1/d^2 = (h^2 + k^2 + l^2)/a^2
\]

Where,

\[
a^2 = (h^2 + k^2 + l^2)/d^2
\] (3)

Substituting (2) in (3), we get

\[
a = \frac{n \lambda}{\sqrt{h^2 + k^2 + l^2}}
\]

Dislocation density is

\[
\delta = \frac{1}{D^2} \text{ Lines/m}^2
\] (4)

Micro strain arises due to the lattice misfit and then calculated by,

\[
\varepsilon = \frac{\beta \cos \theta}{4}
\] (5)

The calculated XRD parameters of biologically synthesized MgO NP’s were depicted in the Table.1. The calculated $a'$ value of the biologically synthesized MgO nanoparticles was well consistent with the standard values (JCPDS file: 89-7746).\[9\]

### 3.3 Fourier Transform Infrared Spectroscopy [FTIR] Analysis

Fig. 4. shows the FTIR spectrum for biologically synthesized MgO NP’s. The various biologically active functional groups hidden in both plant and the precursor revealed out by the characteristic bands of FTIR. The interaction of plant compounds with MgNO₃ has shifted the peak to different ranges that show the surface morphology of MgO NP’s. The FTIR spectrum bear witness for the presence of alkaloids, phenolic groups, polysaccharides, flavones, amino acids, terpenoids, flavonoids and steroids. The spectrum was carried out in the range of 400-4000 cm⁻¹. The absorption at ~3700 cm⁻¹ indicates O-H stretching mode of hydroxyl group (surface adsorbed). The peak at 1436 cm⁻¹ is attributed to NH₃ and the peak at 1060 cm⁻¹ corresponds to C-O stretch i.e. they might be an Alcohol, carboxylic acid, ester, and ether due to the absorption of CO₂. The peak at 2879 cm⁻¹ is assigned to aromatic symmetric CH₃ stretching band. In addition, a weak band corresponding to the adsorption of gas-phase CO₂ is visible at around 2358 cm⁻¹. The peak at 428 and 881 cm⁻¹

<table>
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<th>2θ (Deg)</th>
<th>FWHM (Deg)</th>
<th>Lattice planes Hkl</th>
<th>d-spacing (Å)</th>
<th>Crystallite Size (nm)</th>
<th>Micro Strain(S) X10⁻³ m</th>
<th>Dislocation Density (δ) 10¹⁵ lines/m²</th>
<th>Lattice Parameter (Å)</th>
<th>Unit cell Volume x10⁻³⁰ m³</th>
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<td>12.970</td>
<td>2.791</td>
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<td>34.4748</td>
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<td>2.5994</td>
<td>13.119</td>
<td>2.759</td>
<td>5.810</td>
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</table>
confirmed the formation of MgO molecules. The appearance of two peaks confirmed the presence of constituent elements (Mg and O) and the purity of the biologically synthesized MgO NP’s was checked and it’s well matched with the ‘Facile and Green Synthesis of MgO Nanostructures using Ionic liquids and Banana Stem Plant Extract Fig.6 shows the EDX spectrum of biologically synthesized MgO NP’s and the weight percentage of the elements were depicted in Table (Inset in Fig,5). Fig.7. shows the graphical representation of Atomic & weight percentage of biologically synthesized MgO NP’s.

3.6 Energy Dispersive X-Ray Analysis

Energy dispersive X-ray spectroscopy or energy dispersive X-ray analysis was used for the elemental scrutiny. The appearance of two peaks confirmed the presence of constituent elements (Mg and O) and the purity of the biologically synthesized MgO NP’s was checked and it’s well matched with the ‘Facile and Green Synthesis of MgO Nanostructures using Ionic liquids and Banana Stem Plant Extract Fig.6 shows the EDX spectrum of biologically synthesized MgO NP’s and the weight percentage of the elements were depicted in Table (Inset in Fig,5). Fig.7. shows the graphical representation of Atomic & weight percentage of biologically synthesized MgO NP’s.

3.7 Antimicrobial Activity

The nanomaterials are loaded into an inoculated agar plate and the inhibition was studied based on the inhibition zone. An inhibition zone appears around the disc be the nanomaterial’s bactericidal. The measure of the inhibition
activity against both gram positive and negative pathogens like Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia. The inhibitory effect of the synthesized MgO NP's against the bacteria increased with the increase of its concentration from 50 µl to 100 µl. The antibacterial activities of the MgO NP's evaluated against several pathogenic bacteria (gram positive and gram negative) at concentration range (50 µl & 100 µl) are presented in Table 3. The inhibitory effect on bacterial growth, biofilm formation and swimming motility has been found to be highly associated with bacterial survival and virulence. The obtained result shows the adverse effect on formation of biofilms and its growth. MgO nanoparticles caused obvious change in the morphology of pathogens, by damaging its cell membrane. MgO NP's might have been attached to the surface of the cell membrane of microorganisms, causing disturbance to its functions like permeability and respiration. Fig.8. The antibacterial effect was maximum in Staphylococcus aureus, marginal effect was seen in Escherichia coli, reduced outcome was observed in Klebsiella pneumonia. Fig. 9. shows the antibacterial effect of biologically synthesized MgO NP's in different bacteria's. Table.3 shows the rate of inhibition zone at two different concentration levels of sample.

**Table 3: Rate of inhibition zone at two Different concentration levels of sample**

<table>
<thead>
<tr>
<th>microorganisms</th>
<th>zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>50 µl</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
</tr>
</tbody>
</table>

zone is a measure of the antibacterial activity of the tested material. Based on the ability of the nanomaterials, it would control a broad spectrum or narrow spectrum of bacteria.

The result of this study indicated that the synthesized MgO NP's at two different concentrations had strong antibacterial
Synthesized sample rupture the cell wall of bacteria and interacts with the DNA, Protein, Ribosomes and Release Ros

4. Conclusion

This review has summarized the recent research work in the field of phytosynthesis of MgO NP’s. Owing to the rich biodiversity of plants, their potential for the synthesis of nanoparticles is yet to be fully explored. The above synthesis was found to be rapid, efficient, and environmentally benign. The green MgO NP’s were characterized by various techniques. The XRD result confirmed that face centered MgO NP’s has the crystalline size about 13 nm. The dislocation density has decreased with the increase in the crystal lattice size. Similarly, the micro strain has increased with the decrease in the crystal lattice size. The FTIR spectra confirmed the formation of cubic MgO NP’s phase by the characteristic peak appeared at 428 cm⁻¹ and 881 cm⁻¹. In this work, we present that the fabricated MgO NP’s from aqueous leaf extract of A. Indica plant have shown inhibition against the tested bacteria. We assured that our synthesis will bring a new protocol in the forthcoming studies of MgO NP’s.

References