Green Synthesis of Magnesium Oxide Nanoparticles with Antioxidant Potential Using the Leaf Extract of *Piper nigrum*

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Green fabrication is an environmentally friendly and innovative method, and an attractive research field for the production of magnesium oxide nanoparticles (MgO NPs) in clinical and environmental applications. In this study, a viable green synthetic approach has been carried out to produce MgO NPs by using leaf aqueous extract of *Piper nigrum* as a capping agent. The formation and physicochemical properties of the MgO NPs were confirmed through standard characterization methods. All the analytical data revealed the formation of pure, rod-shaped, and crystalline MgO NPs with an average size of 20 nm. The low concentration of 50 μg/mL MgO NPs shows 29.99% free radical scavenging, whereas the highest concentration of 500 μg/mL MgO NPs exhibits 91.99% free radical scavenging compared with ascorbic acid. The IC₅₀ of the MgO NPs was found to be 89.5 μg/mL. This study shows that the synthesized MgO NPs exhibit good anti-oxidant activity that can be used for biomedical applications.

**INTRODUCTION**

The advancements made in the field of biotechnology and nanotechnology pave the way for research in the field of nanoparticle synthesis. Nanoparticles (NPs) are extensively used for the treatments of cancer, diabetes, allergies, infections, and inflammation. They are used in various applications in the fields of medicine,¹ cosmetics,² renewable energy,³ environmental remediation,⁴ and biomedical devices.⁵ Magnesium oxide (MgO) is known as a widely used compound that has unique optical, electronic, thermal, mechanical, and chemical properties.⁶ It is an important and functional metal oxide that has been widely used in various fields, such as catalysis, refractory materials, paints, and superconductors.⁷ Several methods have been used for the synthesis of nano-MgO, including sol–gel, chemical gas phase deposition, laser vaporization, hydrothermal synthesis, and combustion aerosol synthesis.⁸ Biological methods for the synthesis of MgO NPs of plant materials have not been widely exploited.⁹ Green synthesis of NPs is an ecofriendly approach, because the biological components act as reducing and capping agents, and there is no need for high energy, toxic chemicals, high pressures, etc. Biological synthesis can be used for the large-scale production of NPs. *Piper nigrum* Linn., or black pepper, is a perennial woody climbing liana belonging to the family Piperaceae. It is native to India, Indonesia, Malaysia, South America, and West Indies, and is also widely cultivated in tropical regions. It is considered the ‘King of Spices’.¹⁰ The presence of a pungent alkaloid piperine contributes a spicy taste to the seeds, leaves, and other parts of *P. nigrum*, which contain small amounts of safrole, pinene, sabinene, limonene, caryophyllene, and linalool compounds. Fruits of *P. nigrum* show different activities, such
as hepatoprotective, antipyretic, CNS depressant, analgesic, bioavailability enhancer, anti-oxidant, and anti-inflammatory.\textsuperscript{11}

The distinguishing of NPs is decided by the source of plant extracts. Plant extracts act as both reducing and stabilizing agents. Mallikaarujuna et al.\textsuperscript{12} reported the investigation of phytochemical fabrication and characterization of silver nanoparticles by using an extract of pepper leaves. A study was performed by Thangapandi et al.\textsuperscript{13} on the antibacterial and photocatalytic activity of \textit{P. nigrum} seed-extract-mediated zinc oxide nano-rods. They concluded that \textit{P. nigrum} seed-extract-mediated zinc oxide NPs show better antibacterial activity and photocatalytic activity. Biosynthesis of metal and semiconductor nanomaterials using the green chemistry approach has vital applications in the production of novel medicines and other products. Nontoxic-mediated green routes produce unique MgO nanomaterials. Up to now, no study has been reported on the synthesis of MgO NPs using the leaf extract of \textit{P. nigrum}. In the present study, MgO NPs were synthesized using \textit{P. nigrum} leaves and characterized. In addition, the anti-oxidant potential of \textit{P. nigrum}-leaf-mediated MgO NPs was assessed.

**MATERIALS AND REAGENTS**

**Collection of \textit{P. nigrum} and the Preparation of Extract**

\textit{P. nigrum} leaves were collected from a pepper garden in the Thrissur district (10°31’49.2420”N, 76°12’53.0244”E) Kerala, India. The leaves were washed with running tap water and cut into small pieces. The extract of \textit{P. nigrum} was prepared using 100 ml distilled water which was boiled at 100 °C for 30 min. The obtained extract was filtered using Whatman No.1 filter paper and stored at 4 °C for further use.\textsuperscript{14}

**Synthesis of MgO NPs**

An amount of 10 g of magnesium nitrate was dissolved in 100 ml distilled water and allowed to boil at 60 °C for 20 min. Then, 50 ml of \textit{P. nigrum} extract was added to 100 ml of the magnesium nitrate solution and boiled at 60 °C for 30 min. Next, the solution was incubated for 24 h, resulting in a dark greenish precipitate. Finally, the obtained precipitate was annealed at 400–500 °C for 3 h. After the annealing process, a fine powder was obtained and stored at 4 °C for further studies.\textsuperscript{15}

**Characterization of MgO NPs**

The optical property of MgO NPs was observed using UV-vis, at a wavelength range of 200–800 nm. The functional groups and chemical residues on the surface of MgO NPs were detected using Fourier-transform infrared FTIR analysis (Perkin Elmer Spectrum 1000). The crystalline nature of the prepared MgO NPs was identified using an x-ray diffractometer (XRD). The size of MgO NPs was calculated on the basis of Scherrer’s formula.\textsuperscript{16} The microstructure, particle distribution, elemental composition, and purity of the MgO NPs were determined by a scanning electron microscope (SEM; JEOL JSM 7660F) equipped with an energy-dispersive x-ray analyzer (EDX) unit. The stability of the synthesized MgO NPs was analyzed by using a zeta potential analyzer (Malvern Zetasizer nano-ZS90). Thermogravimetric analysis (TGA) was performed with a TA Instrument (TGA-SDT 2960). For this purpose, the samples were heated in flowing air (100 mL/min) from 35 to 900 °C at a heating rate of 10 °C/min.\textsuperscript{17}

**Anti-oxidant Activity**

The anti-oxidant potential of the MgO NPs was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Different concentrations of MgO NPs, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 500 µg/ml, were prepared. Water (0.1 ml) and methanol (0.7 ml) were used as references. The measurements were made by measuring the absorbance of 0.1 ml of deionized water and 0.7 ml of DPPH solution. The ability to reduce free DPPH radicals was calculated based on the formula: \( Aa = (A_o - A_i/A_o) \times 100\% \), where \( Aa \) is the anti-oxidant activity [%], \( A_i \) the average absorbance of the tested solution, and \( A_o \) the average absorbance of the DPPH solution.

**RESULTS AND DISCUSSION**

**Characterization and Physicochemical Properties of MgO NPs**

The UV-spectra of the MgO NPs are presented in Fig. 1, showing the peak at 364–571 nm. The color change depicts the existence of the MgO NPs. The surface plasmon resonance bands of colloidal Mg were observed in the range of 364–571 nm. Prasanth et al.\textsuperscript{18} have reported the formation of MgO NPs by green synthesis, which was confirmed by UV–vis spectrophotometry. They obtained a peak at

![Fig. 1. Analysis of the optical properties for \textit{P. nigrum}-mediated MgO NPs.](image-url)
301 nm in the UV–vis spectrum. The FTIR spectrum of MgO NPs is depicted in Fig. 2. Peaks at 3366 cm\(^{-1}\), 2839 cm\(^{-1}\), 2672 cm\(^{-1}\), 1759 cm\(^{-1}\), 1548 cm\(^{-1}\), 783 cm\(^{-1}\), and 470 cm\(^{-1}\) can be seen in the \(P. nigrum\)-mediated MgO NPs. The peaks of 783 cm\(^{-1}\) and 470 cm\(^{-1}\) refer to the group of Mg-O. A peak at 3366 cm\(^{-1}\) corresponds to O–H/N–H vibrations. The peaks at 2839 and 2672 cm\(^{-1}\) indicate the presence of C-H asymmetric and symmetric stretching modes. The bands at 1759 and 1548 cm\(^{-1}\) correspond to O–H stretching vibrations.

Nguyen et al. produced MgO NPs from different extracts (flower, bark, leaf) of \(Tecoma stans\) (L.) by biogenic synthesis, and proved the formation of MgO NPs by FTIR analysis through the existence of Mg-O functional groups at 514–658 cm\(^{-1}\).

Figure 3 shows the XRD pattern of green-synthesized MgO NPs further confirming the formation of MgO NPs. The intense peaks represent the (111), (200), (311), and (420) planes. Bragg’s reflection angles are based on the crystal of Mg NPs. These phases were indexed to the crystalline nature, which was compared to the data from JCPDS card #00–004–0829. \(D = \frac{\lambda}{2\beta\cos \theta}\), where \(D\) is the average particles size, \(\lambda\) is the wavelength (1.5418 Å), \(\theta\) is the Bragg’s angle, and \(\beta\) is the full width half-maximum of the corresponding peak. Scherrer’s formula was used to estimate the particle sizes, which were found to be in the range of 15–30 nm. The average size of the MgO NPs was calculated and found to range from 15 nm to 30 nm. Srivastava et al. determined the green synthesis of MgO nanoflowers and used XRD to study the crystalline nature of bio-inspired MgO NPs. SEM images of MgO NPs have determined that the particles are rod-shaped (Fig. 4). The SEM image shows well-dispersed nano-rods of MgO-NPs without any aggregation. Hassan et al. produced spherical-shaped \(Rhizopus oryzae\)-mediated MgO NPs, and studied their morphology with help of SEM. Their elemental composition was investigated with the help of EDX spectra. The EDX graph showed that the phytomediated MgO NPs were extremely pure, and holding Mg and O ions, which refer to the formation of MgO through an aqueous extract of \(P. nigrum\). Fouda et al. synthesized biogenic MgO NPs via \(Cystoseira crinita\) (brown algae), which revealed the weight percentages of Mg and O by EDX analysis.

The zeta potential is an important analysis method for determining the stability of MgO NP suspensions. The zeta potential analysis revealed that the MgO NPs hold a high negative potential value of –75.1 mV (Fig. 5), which indicates the physical stability of the MgO NPs. Ammulu et al. used phyto-assisted synthesis of MgO NPs using \(Pterocarpus marsupium\) roxb heartwood extract, which has a zeta potential of –2.9 mV. The
thermal stability of MgO NPs was analyzed up to 900 °C (Fig. 6a and b). An initial weight loss of 22.3% was observed up to 300 °C due to dehydration. Overall, 59% of weight loss occurred up to 900 °C. The removal of phytochemicals or decomposition biomolecules formed NPs at 300–900 °C. Figure 6a represents the exothermic peaks at 300 and 400 °C, and Table I describes the comparison between *P. nigrum*-mediated MgO NPs and *Trigonella foenumgraecum*-mediated MgO NPs.\textsuperscript{26}

**Anti-oxidant Activity**

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay was used to determine the radical scavenging activity of *P. nigrum*-synthesized MgO NPs (Fig. 7). The low concentration of 50 μg/mL MgO NPs shows 29.99% free radical scavenging, whereas at the highest concentration of 500 μg/mL the MgO NPs exhibit 91.99% free radical scavenging compared with ascorbic acid. The IC\textsubscript{50} of MgO NPs was found to be 89.5 μg/mL. Younis et al.\textsuperscript{27} produced MgO NPs using *Rosa floribunda charisma* extract, and evaluated its anti-oxidant activities by the DPPH method. They concluded that green-synthesized MgO NPs have good anti-oxidant activity. The DPPH assay result suggests that biologically synthesized MgO NPs can act as active anti-oxidant agents in the field of biomedicine.

**CONCLUSION**

An aqueous extract of *P. nigrum* was employed as a capping/stabilizing and reducing agent for the biosynthesis of MgO NPs. The MgO NPs were characterized by FTIR, SEM, EDX, TGA, and zeta potential analyses. Crystalline and rod MgO NPs with an average size of 26 nm were prepared by the biological method. *P. nigrum*-assisted MgO NPs

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**Table I. Comparison between *Piper nigrum*-mediated MgO nanoparticles and *Trigonella foenumgraecum*-mediated MgO nanoparticles.**

| Properties          | *Piper nigrum*-mediated MgO nanoparticles | *Trigonella foenumgraecum*-mediated MgO nanoparticles\textsuperscript{26} |
|---------------------|------------------------------------------|---------------------------------------------------------------|
| Size                | 15–30 nm                                  | 36–69 nm                                                     |
| Shape               | Rod                                      | Spherical                                                   |
| Nature              | Crystalline                               | Crystalline                                                 |
| Functional groups   | O–H/N–H vibrations, C-H asymmetric and symmetric stretching modes | C = O Stretching, C = C stretching, and –CH stretching     |
hold good anti-oxidant properties, proved by the DPPH assay. The IC$_{50}$ of MgO NPs was found to be 89.5 µg/mL. Green-synthesized MgO NPs may be used for medical applications, such as the production of nano-drugs.

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**AUTHORS CONTRIBUTIONS**

PR: Supervision, Funding acquisition and Project administration. NV: Investigation, Methodology, Data Curation and Writing- Original draft preparation. JD: Data Curation and Writing.

**CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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