Biosynthesis and antibacterial activity of MgO-NPs produced from Camellia-sinensis leaves extract

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Keywords: Magnesium-Oxide, Green-Synthesis, Camellia-sinensis, antibacterial-activity

Abstract
Magnesium oxides nanoparticles (MgO-NPs) were synthesized by a novel technique based on the leaf extract of Camellia sinensis (Green tea). The synthesized nanoparticles were evaluated for antibacterial activity (against both gram-positive and gram-negative pathogens) and therefore can be a suitable therapeutic alternative to the usage of antibiotics. The antibacterial activity of synthesized MgO-NPs is tested against clinical isolates of gram-negative (Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae) and gram-positive (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes) pathogenic bacteria. Agar well diffusion assay data indicate that MgO-NPs exhibit antibacterial activity at all concentrations tested against both gram-negative and gram-positive pathogenic bacteria, producing zone of inhibition (ZOI) in the range of 9.6 ± 1.1 to 21.0 ± 1.5 mm diameters. The maximum response is observed at 25 μg ml⁻¹ concentration of MgO-NPs, producing a zone of inhibition ranging from 15 ± 1.2 mm (E.coli) mm to 21.0 ± 1.5 mm (S. marcescens).

1. Introduction

The rapid emergence and dissemination of antimicrobial-resistant (AMR) bacteria are posing serious challenges to global public health. This may increase mortality risk, treatment duration, health care budget, and low life expectancy. In the United States, each year, more than two million people are infected by multidrug-resistant (MDR) bacterial pathogens, resulting in more than 23,000 deaths [1]. In Europe, it costs about 1.5 billion Euros per year to overcome drug-resistant bacterial infections [2]. The increasing prevalence of infections caused by drug-resistant bacteria, thus limiting the options for effective antibiotic therapy. In exploring various options to address this problem, nanomaterials, due to their greater durability, lower toxicity, higher stability, and selectivity, have emerged as promising candidates.

Nanomaterials can be synthesized by many methods [3–6]. Nanoparticle (especially nanoparticles) synthesized via natural processes involving plants etc (as precursors or reducing agents) are highly biocompatible [7]. The green techniques thus developed for the synthesis of nanoparticles have got importance in various branches of nanotechnology [8]. Several green synthetic method using biological materials such as microorganisms, marine organisms, micro-fluids, and plant extracts as a reducing or capping agent, have been well explored for nano particles synthesis (Azizí S., 2013). Green methods of NP synthesis are simple, efficient, ecofriendly, and cost effective as compared to other methods of nanoparticle synthesis and the products are safe for medical applications (Gopalkrishnin 2012). During recent times several groups have achieved success in the synthesis of nanoparticles using extracts from unicellular organisms (like bacteria and fungi) as well as from...
different plants [9]. Plant extracts based methods are relatively easy to handle, readily available, low cost, as compared to other biological methods (Khan M., 2013). Plant extracts possess various sorts of biologically active molecules serving as important bioreductants for synthesis of nanoparticles. Biologically active molecules present in plant extracts, have high affinity for the surface of nano-structures, and act as functionalizing ligand increasing the efficiency and suitability of nanoparticles for biomedical application (LU AH., 2007 … Shabestarian H., 2017).

Metal oxides have been reported for their antibacterial activities [10–16]. Oxide-based nanomaterials are famous for their exceptional physical and chemical properties [17]. Amongst the metal oxides MgO have been utilized extensively in the literature [18, 19]. Magnesium oxide nanoparticles (MgO-NPs) are one of the well-known zero-dimension (0D) oxide-based nanomaterial with excellent properties in comparison with its bulk counterpart. These included high chemical stability, electrical permittivity, photocatalytic efficiency, lower toxicity, and excellent biocompatibility. As a result, it has a wide range of potential applications in catalysis, remediation of toxic waste, antibiotics, paints, mechanical, electrical, digital, antibacterial activities, semiconductors, superconductive materials and catalytic devices [20]. Besides, it can be utilized in therapeutic uses like band-aids, coated capsules, biological labeling, and blood collecting vessels, etc [21].

Recent investigations have demonstrated that MgO-NPs exhibit strong antimicrobial activity against pathogenic bacteria [22], yeasts [21], and viruses [23], making it a suitable therapeutic alternative to the usage of antibiotics.

So far, numerous chemical procedures have been reported for the synthesis of MgO-NPs. However, the chemicals or precursors used in these techniques are not only highly toxic and harmful to living species but may also lead to serious environmental problems [23]. To overcome these problems, some alternate techniques are needed to be sought to provide low toxic waste [24].

Green synthesis techniques are somehow found to be cost-effective, eco-friendly, and less likely to be exposed to dangerous chemicals or their products [25]. The synthesized NPs by these techniques are highly, biocompatible as they are being prepared by biological means (which cover functional biomolecules to reduce metal ions dynamically) [7].

During recent times several groups have achieved success in the synthesis of MgO-NPs nanoparticles using extracts from various plants such as Rosmarinus officinalis L/ Rosmary) [26], Manihot esculanta (Crantz) [27], Aloe vera (A. vera) [28], and Neem (16,., Moorthy SK 2015), etc [29]. However, despite these valuable efforts, there are still a large number of medicinal plants needed to be explored for the synthesis of MgO-NPs. Similarly, in green synthesis, one has to be careful in the choice of plants and other chemicals as precursors. All the plants and chosen chemicals may not be non-toxic or biocompatible. Besides, some plants might only be available in particular season or territory and the procedure involved in their extraction may not only difficult but also lengthy or time-consuming.

Keeping in mind all the pros and cons of these points in our investigation, we use leaf extract of ‘Camellia sinensis’ as a nontoxic green precursor. Green tea (Camellia sinensis), is an evergreen shrub or plant, belongs to the family Theaceae. Its leaves and leaf buds are used to make tea (one of the most ancient and famous beverages consumed globally with ample health benefits). Green tea possesses different classes of chemical compounds, including polyphenols, polysaccharides, alkaloids, proteins, minerals, amino acids, and vitamins [30]. It has been shown that green tea possesses anti-cancer [31], anti-oxidant [32], anti-hypercholesterolemic [33], anti-obesogenic [34], and antimicrobial properties [35].

This work reports a novel, simple and cost-effective technique for the green synthesis of Mg-NPs. The synthesis technique is based on the green tea extract. As a result it avoids the use of toxic chemicals. This made the final product more biocompatible and safe. In addition the as-synthesized Mg-NPs are more effective in antibacterial activities against gram positive and negative bacteria as compared to the NPs synthesized by other chemical techniques. To the best of our knowledge, this will be the first study to demonstrate the biosynthesis and antibacterial activity of MgO-NPs produced from Camellia sinensis leaves extract.

2. Experimental details

2.1. Preparations of camellia sinensis leaf extract

Camellia sinensis leaves were dried and grinded to powder with the help of a domestic blender. Five grams dried leaves powder of Camellia sinensis was mixed with 200 ml of distilled water and boiled at 100 °C for an hour to obtain an extract solution. The obtained solution is filtered through Whatman’s No.1 filter paper (As shown in figure 1). For the synthesis of MgO-NPs, the freshly prepared green tea leaf extract was used.

2.2. Synthesis of MgO-NPs

Wet chemical method was employed for MgO-NPs synthesis [36–39]. 0.1 M of Magnesium nitrate hexahydrate, Mg(NO₃)₂·6H₂O solution was prepared in a beaker. Consequently, 10 ml of green tea extract was added in a
dropwise manner to the as-prepared solution and magnetically stirred for 2-hours at 80 °C. Sodium hydroxide (NaOH) was used to adjust the pH in the range between 10–12. The solution was centrifuged (10,000 rpm) for 10 min and MgO-NPs were collected as a precipitate. The MgO-NPs precipitate is then three times washed with ethanol to remove the excess of Mg(NO₃)₂ and plant material. MgO-NPs were dried for 8 h at 40 °C to. The dried material was then crushed with mortar and pestle. The powdered specimen thus obtained is calcinated at 450 °C for 30 min in the muffle furnace to obtain MgO-NPs The entire process and the products obtained are shown in figure 2.

2.3. Screening of antibacterial activity of MgO-NPs.

The antibacterial potential of green synthesized MgO-NPs was tested by agar well diffusion method [40] against clinical isolates of both gram-positive and gram-negative bacteria, obtained from Combined Military Hospital (CMH) Muzaffarabad. Total of seven bacterial species i.e. E. coli (UTI), P. aeruginosa (UTI), S. mercescens (UTI), K. pneumonia (Wound) as Gram-negative and S. aureus (UTI), S. epidermidis (wound), S. pyogenes (throat swab) as Gram-positive bacteria are used in this study. All bacterial strains are identified by various biochemical tests [41]. Pure culture of all bacterial strains is kept at 4 °C in agar slants in freeze-dried condition until later use.

All bacterial strains are subcultured overnight at 37 °C from their pure cultures on Mueller–Hinton Broth. The turbidity of bacterial culture was adjusted to freshly prepared 0.5 McFarland turbidity standard [42] equivalents to (1.5 × 10⁸ CFU ml⁻¹) bacteria. Each bacterial strain is swabbed uniformly onto separate Muller–Hinton agar plates with the help of sterile cotton swabs under aseptic conditions. Wells are made with a sterile polystyrene tip (4 mm). Different concentrations of MgO-NPs (5, 10, 15, 20, 25 μg ml⁻¹) are prepared separately in dimethyl sulfoxide (DMSO) and used. Fifty microliters (50 μl) of each concentration are then added to each well. All the plates were incubated overnight, at 37 °C, and the zone of inhibition (ZOI) around each well is measured in millimeters by using a caliper. Clindamycin phosphate at a concentration of 20 μg ml⁻¹ is used as a standard reference antibiotic. Each experiment is performed in triplicate (N = 3) and the mean value is calculated.

3. Results and discussion

The chemical compounds of tea mainly contain polyphenols, polysaccharides, amino acids, and vitamins [43] and play a key role in preventing the risk of cancer, hypercholesterolemia, atherosclerosis, Parkinson’s disease, Alzheimer’s disease, and other aging-related disorders [44]. Green tea polyphenolic catechins can inhibit the growth of a wide range of oral pathogens and may be useful in the treatment of common oral infections, such as
dental caries, periodontal disease, and oral malodor [45, 46]. C. sinensis can also be used as a reducing and capping agent in nanoparticle synthesis [47].

Using green tea leaves extract, the Magnesium nitrate ions are reduced to Magnesium oxide (MgO-NPs). MgO-NPs formation is observed from the solution’s color change from green to yellowish-brown, as can be seen in figure 2. The overall chemical reaction thus occurred can be written in the form of the chemical reactions as below:

\[
\text{Mg(NO}_3\text{)}_2 + 2\text{NaOH} \rightarrow \text{Mg(OH)}_2 + 2\text{NaNO}_3
\]

\[(1)\]

\[
\text{Mg(OH)}_2 \xrightarrow{\Delta} \text{MgO} + \text{H}_2\text{O}
\]

\[(2)\]

3.1. Morphology and compositions

The morphology and shape of the synthesized MgO-NPs are observed via field emission scanning electron microscopy (FESEM) in different magnification. Figure 3 shows Low magnification FESEM micrograph of the synthesized nanoparticles of MgO. The particle-like morphologies can be seen in the micrograph. Almost all of the particles are well-dispersed but closely related or linked with each other. Figure 4 shows the high magnification FESEM micrograph of the synthesized nanoparticles of MgO. High magnification confirmed the particles’ structures in the nano dimension. The synthesized particles vary in size from few nanometers up to 80 nm. The smaller size particles are so closely packed that it seems embedded on the surface. Except for MgO-NPs, no other structures or morphologies can be seen in the synthesized sample.
Energy dispersive x-ray spectroscopy (EDX) is used to study the elemental contents of the synthesized sample. Figure 5 shows the EDX spectrum of the synthesized MgO nanoparticles. The spectrum shows different peaks tagged with Mg and O correspond to Magnesium and Oxygen contents of the synthesized sample [28].

Nanomaterials are found to have majority of the atoms at the surface as compare to volume. In other words, they have a ‘large surface to volume ratio’. Therefore, x-ray photoelectron spectroscopy (XPS) has been used to analyze the elemental contents at the surface of the as-synthesized MgONPs. The obtained XPS survey is displayed in figure 6. The survey has several peaks at ~50 eV (labeled as Mg 2p), 90 eV (labeled as Mg 2s), 531 eV (labeled as O 1s), and at ~1305 eV (labeled as Mg 1s) correspond to MgO [48, 49]. A shoulder peak at 285 eV (labeled as C 1s) correspond to a minute quantity of carbon, which might have included due to sample exposure in the air before characterization [50]. The peaks detected in the range of 300–380 eV (labeled as Mg KLL), and at 980 eV (labeled as O KVV) are Auger signals corresponds to oxidation of polycrystalline Mg [50].
3.2. Antibacterial activity of MgO-NPs

The antibacterial effects of green synthesized MgO-NPs are evaluated against seven bacterial strains, of which three were gram-positive (S. aureus, S. epidermidis, S. pyogenes) and four were gram-negative (E. coli, P. aeruginosa, S. marcescens, K. pneumonia). The bacterial cultures were treated with various doses of MgO-NPs (5, 10, 15, 20, 25 μg ml$^{-1}$) dissolved in DMSO. The zones of inhibition of MgO-NPs against all bacteria are shown in figure 7(a) and the inhibition values are listed in table 1. The results demonstrate that, in the presence of MgO-NPs, the growth is significantly inhibited against all the tested species, and the values of ZOI increase with an increase in the concentration of MgO-NPs. The maximum response against all the tested bacteria is observed at 25 μg ml$^{-1}$ concentration of MgO-NPs. We observed that among members of the gram-negative group, at 25 μg ml$^{-1}$ concentration MgO-NPs, the least response is displayed by E. coli (15 ± 1.2 mm), whereas, the highest response is observed for S. marcescens (21 ± 1.5 mm). Similarly among gram-positive bacteria, at the same concentration, the lowest ZOI is displayed by S. aureus (18.8 ± 1.0 mm) whereas; S. pyogenes showed the

![Figure 5. EDX analysis of the as-synthesized MgO nanoparticles showing the elemental contents of the synthesized sample.](image1)

| Element | Weight % | Atomic % |
|---------|----------|----------|
| O, K    | 48.64    | 59.00    |
| Mg, K   | 51.36    | 41.00    |
| Totals  | 100.00   |          |

![Figure 6. XPS survey of the as-synthesized MgO nanoparticles showing peaks for the elemental contents in the synthesized sample.](image2)
highest sensitivity (21 ± 1.2 mm) at the same concentration of MgO-NPs. The results demonstrate MgO-NPs exhibited antibacterial activity against the tested microorganisms in the following manner, S. marcescens followed by S. pyogenes, P. aeruginosa, S. epidermidis, S. aureus, K. pneumonia, and then E. coli. MgO-NPs inhibited the growth of both gram negative and gram positive bacteria on all the tested concentration, the effect is more pronounced with an increase in concentration as sown in figure 7(b). In vitro antibacterial action of
MgO-NPs has been reported by several research groups [18, 19, 21]. The exact mechanism of antibacterial action of MgO-NPs is not known, however various mechanisms, such as formations of reactive oxygen species (ROS), nanoparticles interaction with bacterial cell and alkaline effects have been proposed to explain the antibacterial effect of MgO-NPs.

Accumulating number of studies suggest that the bactericidal effect of MgO generally has been ascribed to Reactive oxygen species (ROS) generation, thereby inducing membrane damages and leakage of internal cellular contents. It has been found that the bacterial cells after treatment with MgO nanoparticles revealed the development of deep craters on their membrane surface, indicating damages to membrane structure. The cell size gets shorter and more compact, suggesting leakage of the cellular contents in response to treatment [21, 51]. The reverse transcription-q PCR analysis also revealed the up-regulation of genes involved in oxidative stress response [51].

Another possible mechanisms pertaining to antibacterial effect of MgO-NPs could be due to electrostatic interaction between bacterial surface and MgO-NPs, subsequently inducing damage. It has been shown that MgO-NPs particles due to their positive charge strongly interaction with negatively charged bacterial surface (Stoimenov et al, 2002). Further support to this hypothesis has been provided by the findings of Makhluf et al (2005) that high bactericidal activity of nano-MgO is due to the interaction of MgO-NPs and bacterial cell (Makhluf et al 2005).

The alkaline effect of MgO-NPs has also been reported by several research groups as an important factor contributing to the antibacterial action of MgO nanoparticles (Sawai et al, 2001; Yamamoto et al, 2000). Adsorption of water moisture on the MgO nanoparticle surfaces form a thin water layer around the particles resulting in a much higher pH than its equilibrium value in solution. The high pH in this thin surface water layer could damage the bacterial membrane upon contact, resulting in cells death (Sawai et al 1997).

### Table 1. ZOI produced by different concentrations of MgO-NPs.

| Organisms       | Clindamycin phosphate (20 μg ml⁻¹) | ZOI (mm) of MgO-NPs at different concentration |
|-----------------|------------------------------------|-----------------------------------------------|
|                 | 5 μg ml⁻¹ | 10 μg ml⁻¹ | 15 μg ml⁻¹ | 20 μg ml⁻¹ | 25 μg ml⁻¹ |
| S. aureus       | 30.5 ± 1.8 | 10.6 ± 0.5 | 12.6 ± 1.0 | 15.8 ± 1.1 | 18.2 ± 1.2 | 18.8 ± 1.0 |
| E. coli         | 29.8 ± 1.0 | 10 ± 1.3   | 11.8 ± 1.4 | 12.6 ± 1.3 | 15.3 ± 1.0 | 15 ± 1.2   |
| S. pyogenes     | 32 ± 1.6  | 12 ± 1.1   | 14 ± 1.0   | 17 ± 1.6   | 18 ± 1.4   | 21 ± 1.2   |
| P. aeruginosa   | 30 ± 1.1  | 10.6 ± 1.0 | 12.6 ± 0.5 | 15.6 ± 0.5 | 17.6 ± 1.0 | 19 ± 1.1   |
| K. pneumonia    | 27.3 ± 1.3| 9.6 ± 1.1  | 12 ± 1.0   | 13 ± 1.0   | 14 ± 1.3   | 18 ± 1.1   |
| S. epidermidis  | 28 ± 0.5  | 10 ± 1.1   | 12 ± 0.5   | 15 ± 0.5   | 16.3 ± 1.2 | 19 ± 1.0   |
| S. marcescens   | 33.6 ± 0.7| 11.6 ± 1.3 | 14 ± 1.6   | 18 ± 1.0   | 19.3 ± 1.2 | 21 ± 1.5   |

4. Conclusions

Green synthesized MgO nanoparticles from leaf extract of Camellia sinensis are found to be cost-effective, eco-friendly, and less likely to be exposed to dangerous chemicals or their products. MgO-NPs synthesis employing leaf extract of Camellia sinensis (Green tea), thereby eliminating the need for chemical and physical methodologies. The plant material used in this study is cheap and easily available throughout the world and is free from seasonal limitations. The as-synthesized MgO nanoparticles are found to have greater durability, excellent biocompatibility, higher stability, and selectivity, have proven to be a promising candidate in different antibacterial activities. In addition to this extracellular synthesis of nanoparticles could be easily optimized for downstream processing in large-scale operations. The as-synthesized MgO nanoparticles have shown excellent antibacterial effects against various pathogenic microorganisms of both gram-positive and gram-negative group. Thus, it can be an effective antibiotic therapy in the increasing prevalence of infections caused by drug-resistant bacteria.

Acknowledgments

This work was supported by the Research Universiti Grant, Universiti Kebangsaan Malaysia, Geran Universiti Penyelidikan (GUP), code: 2018-134.
Author contributions

Conceptualization, Abdulhameed Khan and M R I Faruque; Data curation, Dana Shabbier and Mayeen Uddin Khandaker; Formal analysis, Abdulhameed Khan, Dana Shabbier and Israf Ud Din; Funding acquisition, M R I Faruque; Investigation, Pervaiz Ahmad and Mayeen Uddin Khandaker; Methodology, Pervaiz Ahmad; Resources, Pervaiz Ahmad and MRI Faruque; Supervision, Abdulhameed Khan; Visualization, Dana Shabbier; Writing—original draft, Abdulhameed Khan; Writing—review & editing, Pervaiz Ahmad, Mayeen Uddin Khandaker, MRI Faruque and Israf Ud Din.

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