Biotechnology Journal International
18(1): 1-7, 2017; Article no.BJI.29534
Previously known as British Biotechnology Journal
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
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Antibacterial Potential of Magnesium Oxide Nanoparticles Synthesized by *Aspergillus niger*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2017/29534
Editor(s):
(1) Chan Yean Yean, Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Malaysia.
(2) Sukesh Voruganti, Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, USA.
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(5) P. Rameshthangam, Alagappa University, Karaikudi, Tamilnadu, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/18677

Original Research Article

Received 16th September 2016
Accepted 14th April 2017
Published 18th April 2017

ABSTRACT

A total of 280 urinary tract infection samples were collected in this investigation. Out of them 212(75.7%) samples showed a positive response.to bacterial isolates. Morphological, cultural and biochemical testes were confirmed using VITEK 2 System. Its revealed that 54 (30.2%) of the bacterial isolates were gram positive and 158 (69.8%) gram negative. The bacterial isolates were distributed as *Escherichia coli* 96 (45.2%), followed by *Klebsiella pneumonia* 48 (22.6%), *Staphylococcus aureus* 43(20.3%), *Pseudomonas aeruginosa* 14 (6.6%), and *Staphylococcus epidermidis* 11 (5.2%). The synthesis of magnesium oxide nanoparticles (MgO NPs) was performed using *Aspergillus niger* Method. Agar Wells Diffusion Method was applied for the evaluation of antibacterial activity against gram positive *S. aureus*, and gram negative *P. aeruginosa* bacteria isolated from Urine tracts infection (UTI). The results showed that the biosynthesized MgO NPs appeared to be an extracellular with a size range of 43-91 nm as confirmed by Scanning Electron Microscopy (SEM) and UV-Visible spectroscopy for the

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absorbance band at 256.5 nm. Besides, MgO NPs were found to be an effective antibacterial agent against Gram positive *S. aureus*, and the zone inhibition diameter at 27 mm. Howover, the zone inhibition diameter against gram negative *P. aeruginosa* bacteria was at 24 mm, compared with inhibition effects of ciprofloxacin antibiotics at 24 and 20 mm, respectively.

Keywords: Nanoparticles; biosynthesis of MgO NPs; Aspergillus niger; antimicrobial activity.

1. INTRODUCTION

Nanotechnology is a field of study that have been greatly developed during the last three decades which have made great advancement in many aspects of human life and in the scientific techniques of biomedical science and biotechnology [1]. It is offers a way to develop potential antibacterial agents to overcome the multi-drug resistance of bacteria [2]. A nanoparticle is a body having a size of about 100 nm or less [3]. Many research workers have attempted to correlate the biological activity of inorganic antibacterial agents with the size of the constituent particles [4,5]. In particular, an inorganic oxide nanomaterial's like CaO, ZnO and MgO have s have been reported to be a potential effective alternatives in addressing some of these challenges [6]. MgO NPs have the advantage of non-toxicity, high thermal stability, biocompatible, low cost, and have considerable potential as an antibacterial agent. Magnesium plays several vital roles in human biology [7,8]. The mechanism of metal oxide nanoparticle action on bacteria is complicated and not fully understood. It has been reported that the antibacterial activity of MgO nanoparticles is attributed to the production of reactive oxygen species (ROS) which induce lipid peroxidation in bacteria [9]. Several studies have shown that smaller particles have greater antibacterial activity due to higher reactive surface area [10]. Biological methods for nanoparticle synthesis exploiting micro-organisms, enzymes, and plants or plant extracts have been suggested as a possible eco-friendly alternative to chemical and physical methods [11,12].

The aims of this investigation were to identify bacterial isolates from UTI samples, the biosynthesis of MgO NPs through a biological method and the description and characterisation of the bacterial isolates through performing the SEM and UV vis spectrophotometer analysis. Besides, a well diffusion method was used for the assessment of the ability of MgO NPs as an Antibacterial *E. coli* and *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Samples Collections and Bacterial Identification

Urine samples were collected from patients admitted to the Central laboratory in Sulaimani Teaching Hospital, Sulaimani, Iraq. All samples were processed and cultivated at 37℃ for 24 hrs on different media including MacConkey agar, Blood agar, Mannitol salt agar, and the Mueller Hinton agar (Oxoid, UK). They were treatments to isolation and identification of pathogenic bacterial isolates according to the standard methods which recommended by [13,14]. Confirmed by VITEK 2 System (Version 5.01 BioMerieux).

2.2 Biosynthesis of MgO Nanoparticles

The MgO NPs were prepared through exploiting a method of [15] with some modification. The synthesis of MgO NPs was carried out with a Niger ATCC 16404 method where the active culture of the isolate was inoculated into Sabouraud dextrose broth (Sigma-Aldrich, Germany) and the flasks were incubated at 28℃ ±2, 150 rpm for 3 days. Following incubation, the fungal filtrate was obtained by passing through Whatman No.1filter paper. The collected supernatant was added to deionize water treated with 1% of mM MgCL2.Futhermore,the collected supernatant was incubated with shaker incubator at 150 rpm for 96 hrs at 28℃. Conical flasks with either fungal filtrate or MgCL2 served as positive and negative control respectively.

2.3 UV-vis Spectra Analysis

The MgO NPs characterisation was performed through UV VIS spectrophotometer using a Systronics UV double-beam spectrophotometer method (India). The scanning range for the samples was 200-600 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference [16].

2.4 Scanning Electron Microscope (SEM)

A scanning electron microscope (Cam Scan--3200 LV SEM machine, USA) was used to record the micrograph images, particle size and morphology of synthesized MgO NPs. A thin layer of gold was coated in an auto fine coater to make the samples conductive. After that the material was subjected to analysis by SEM machine [17].

2.5 Antibacterial Effects of MgO Nanoparticles

The antibacterial effects of MgO NPs were investigated by exposing *S. aureus, and P. aeruginosa* using agar well diffusion method in [18]. Approximately, 20 ml of Mueller Hinton agar media was poured in sterilized petri dishes. The samples were determined and tested inoculums adjusted to 1×10^5 cells/ml, matching with 0.5 McFarland. Inoculums (100 µl) were applied on the surface of the agar plates and spread using swabbed onto the plates. Agar wells of 5 mm diameter were prepared with the sterilized cork borer. Three wells were bored, one well containing the extract alone (control negative) and the others loaded with 1.0 mg/ml from synthesized MgO NPs. Ciprofloxacin antibiotics used as positive control against bacterial isolates. Then the plates were incubated at 37℃ for 24 hrs, where an inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale [19].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacterial Isolates

It is evident from Table 1 that the most frequently isolated bacteria from urine source were *Escherichia coli* 96 (45.2%) followed by, *K. pneumoniae* 48 (22.6%), *S. aureus* 43(20.3%), *P. aeruginosa* 14 (6.6%), and *S. epidermidis* 11 (5.2%). This result was found to be in agreement with the findings of Inbaneson et al. (2011). The isolates were cultured on Blood, MacConkey and Mannitol Salt agar plates and incubated at 37℃ for 24 hours. They were identified according to colony characteristics and microscopic examination of stained smear that demonstrate microbial shape, structure, agreement, gram stain reaction, and biochemical tests like indol (I), methyl red (M.R), Vogas proskauer (V.P), citrate utilization, oxidase (C), motility test, catalase, coagulase, and urease production; according to [20] as in Table 2. Then the identification was confirmed by using VITEK 2 compact system as recommended by Biomerieux. Our results were compared with the resource reported by [20,13].

Table 1. Accounts of isolated bacteria from UTI samples

Bacterial strain	No. of bacteria isolates	%
E. coli	96	45.2
K. pneumoniae	48	22.6
S. aureus	43	20.3
S. epidermidis	11	5.2
P. aeruginosa	14	6.6
Total	212	100

3.2 Biosynthesis of MgO Nanoparticles

The results in Fig. 1 illustrate the process implemented for the extracellular synthesis of MgO NPs by cultivation of *Aspergillus niger* ATCC 16404 mycelia biomass (Fig. 1A), with an optimal media enrichment by MgCL2 (Fig. 1B). The amount of MgO NPs was 56 mg/100 ml which were collected after centrifuge and dried on 60℃. In the biosynthesis of metal oxide nanoparticle by a fungus, the enzymes in metabolic pathway caused a reduction of metal to its metallic solid nanoparticles through the catalytic effects [21,15].

3.3 Characteristic Features of MgO NPs

The features of MgO NPs were performed using UV-VIS spectroscopy as shown in Fig. 2. The absorption band was shown to be at the level of 256.5 nm. Our findings were in an agreement with the results reported by [22,23].

The size range of MgO NPs biosynthesized with *A. niger* used Scanning electron microscope analysis was 47.35 to 98.46 nm (Fig. 3). Our finding was in agreement with the result obtained by [24]. The biosynthesis of nanoparticles induced the microbial cells to produce the biological agents that secrete a large amount of enzymes, which are capable of hydrolyzing metals, and produced the metals ions [25].

Table 2. Biochemical tests used for identification of bacteria

Isolated bacteria	Biochemical tests						
	Catalase	Coagulase	Oxidase	Indole	M.R	V.P	Citrate
E. coli	N	N	+	+	+	-	-
K. pneumonia	N	N	+	-	-	+	+
P. aeruginosa	N	N	+	-	-	-	+
S. aureus	+	+	-	N	N	N	N
S. epidermidis	+	-	-	N	N	N	N

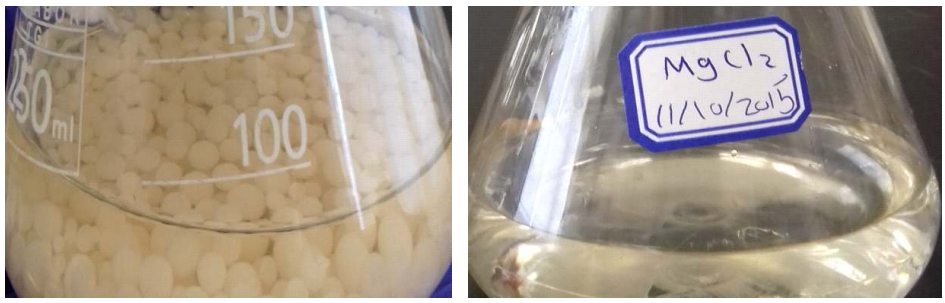

Fig. 1. Biosynthesis of MgO NPs (A) *A. niger* extract (B) $MgCL_2$ with *A. niger* extract

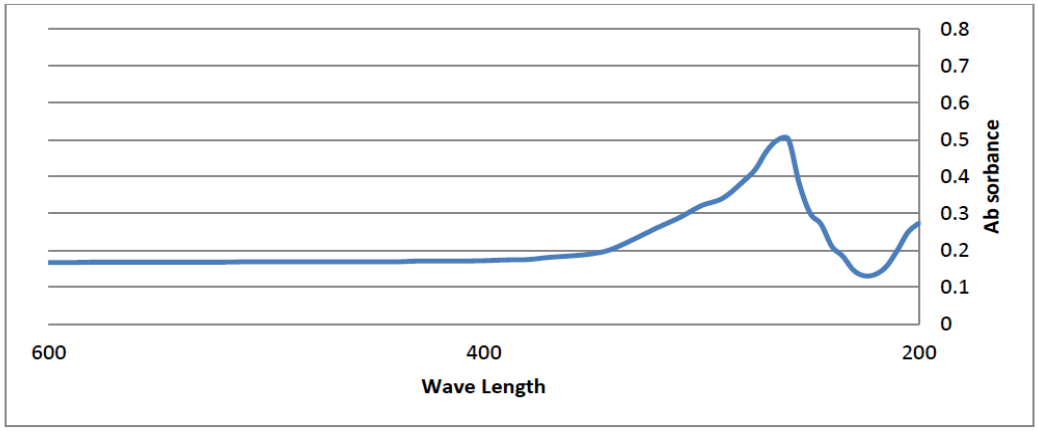

Fig. 2. UV–visible spectra of MgO NPs synthesized by *A. niger*

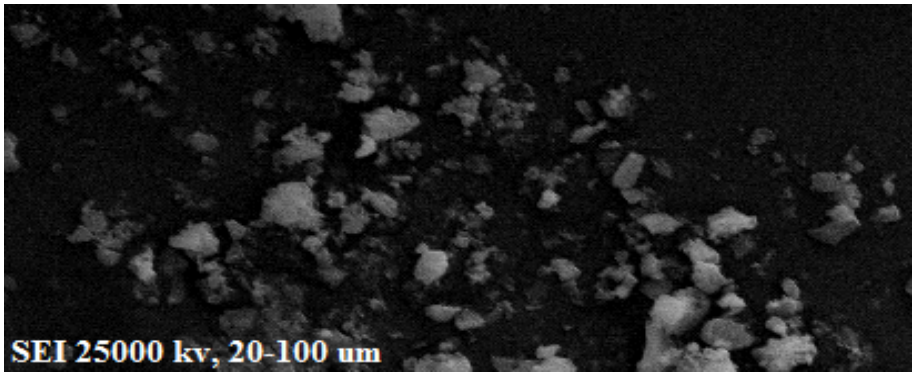

Fig. 3. The SEM image of biosynthesized MgO nanoparticles synthesized by *A. niger*

Fig. 4. Antibacterial activity of MgO Nps. 1.0 mg/ml on used well diffusion test (A) *S. aureus* (B) *P. aeruginosa*

Table 3. Antibacterial activity of MgO NPs synthesized by *A. niger*

Microorganism species	Zone of inhibition (mm)		Cell-free filtrate
	MgO NPs 1.0 mg/ml	CIP 0.5 mg/ml	
S. aureus	27	22	4
P. aeruginosa	24	20	4

3.4 Antibacterial Activities of MgO NPs

Antibacterial activity of 1.0 mg from MgO NPs was tested against gram positive *S. aureus*, and gram negative *P. aeruginosa* bacteria (Fig. 4a and b). The results showed that highly inhibition activity of MgO NPs against these microbial pathogens and the zone inhibition diameter (ZID) against *S. aureus* was at 27 mm compared with the ZID from ciprofloxacin at 0.5 mg/ml at 22 mm. while the inhibition effects against *P. aeruginosa* was at 24 mm compared with the used of ciprofloxacin which appear at 20 mm (Table 3).

A slightly higher antibacterial activity against *S. aureus* than *P. aeruginosa*. Were observed (Fig. 4). A similar finding was reported by (22) whom found that the amount of MgO NPs ability for bacterial inhibition was strongly dependent on particle size [26].

The antibacterial activity of MgO NPs on cell membrane integrity and permeability have resulted in stress induction on the bacterial cells, subsequently resulting in the inhibition of cell growth and eventually in cell death [4,24].

4. CONCLUSION

Its concluded from this study, that the biosynthesized MgO NPs using *A. niger* appeared as a spherical morphology with a particle size ranged between 40–95 nm An antibacterial activity was performed against *S. aureus* and *P. aeruginosa* confirmed that ZID assay found to be highly inhibition effects and there effects were more in gram positive compared with gram negative bacteria.

ACKNOWLEDGEMENTS

A deep gratitude is presented to the staff at the Biology Department Laboratory, Faculty of Science, Sulaimani University for their cooperation in making available all the needed facilities to perform our work regarding cell lines tests successfully.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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