J. Appl. Environ. Biol. Sci., 7(8)94-101, 2017

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Antibacterial Activity of Ag and MgO Nanoparticles Synthesized By *Trichoderma viride*

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Received: March 9, 2017
Accepted: May 23, 2017

ABSTRACT

The current study demonstrated the biological method for the synthesis of magnesium oxide nanoparticles (MgO NPs) and Silver nanoparticles (Ag NPs) using *Trichoderma viride*. Thereafter, the characterized objects were determined using Scanning Electron Microscopy (SEM), and UV-Visible spectroscopy. In addition, the antibacterial activity was evaluated against gram negative *E. coli* and gram positive *Staphylococcus aureus* bacteria by agar well diffusion method. The results showed that biosynthesized of MgO NPs and Ag NPs were found as extracellular and the absorbance band was apparent at 256.5 and 420 nm respectively using UV-Visible spectroscopy, and in the range size of 45.12 to 95.37 nm and 5 to 40 nm, respectively through characterization technique using Scanning Electron Microscopy (SEM). The results revealed that both MgO NPs and Ag NPs are effective antibacterial agents against gram negative *E. coli* and gram positive *S. aureus* bacteria. The inhibition zone diameter of MgO NPs was 30 and 49 mm, against *E. coli* and *S. aureus*, respectively. While Ag NPs showed inhibition effects at 30 and 51 mm, compared with inhibition effects of amoxicillin antibiotics against *E. coli and S. aureus* at 27 and 45 mm, respectively.

KEYWORDS: *Nanoparticles, MgO NPs, Ag NPs, Trichoderma viride, Antimicrobial activity.*

INTRODUCTION

Bio nanotechnology combines between nanotechnology and biotechnology to develop diverse biosynthetic and environmental eco-friendly technologies for the synthesis of different nanomaterial's [1]. Nanoparticles are basically categorized into organic and nonorganic nanoparticles. The nonorganic nanoparticles have vital importance because they can resist adverse processing conditions [2].

The exceptional properties of metal NPs are critical by their shape and size [3]. The basic Nano scale which is crucial in Nano science and nanotechnology is usually on the 0.2 to 100 nm. Moreover, the percentage of atoms at the surface of a material become more significant [4]. Regardless of composition, each material shows diverse characteristics during their size is decreased to less than 100 nm [5].

There is several processing to synthesis different types of nanoparticles by use of physical, chemical, biological, and other hybrid methods [6]. Nevertheless, physical and chemical methods are more common for nanoparticle synthesis, the use of toxic compounds limit their applications. The advantage of using safe eco-friendly methods for biogenetic production is represented by the simplicity of their procedures and flexibility [7-8].

Nanotechnology is widely applied in the areas of biology, medicine, optical, electrical, mechanical, optoelectronics etc. [9]. The most vastly produced nanomaterials are metal oxide nanomaterials; their existing applications involve catalysis, sensors, environmental remediation and personal care products [10].

A central inorganic material with a widespread band-gap is Magnesium [11]. It has many uses in medicine like the relief of heartburn, sore stomach, and for bone regeneration [12-13]. In recent times, MgO nanoparticles have displayed prospect for application in tumor treatment [14].

The antimicrobial activity of Nano silver has higher poisonous potential on all microorganisms comparing to other nanoparticles. Additionally, some microorganisms are more susceptible to silver than others and the choice of capping agent is important in the toxicity. The antimicrobial effect of Nano silver can be utilized to prevent microbial colonization of medical devices and to determine the fate of nanoparticles in the environment [15].

The mechanism of metal oxide nanoparticle action on bacteria is sophisticated and ambiguous. It has been found that the antibacterial effect of nanoparticles refers to the production of reactive oxygen species (ROS) which induce lipid peroxidation in bacteria [16]. Numerous studies have reported that smaller particles have larger antibacterial effect because of higher reactive surface area [17].

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This research aims to synthesize MgO and Ag NPs by biological method, and describe the characterized objects by SEM and UV vis spectrophotometer analysis. Moreover, the study aims to assay the antibacterial activity of NPs against *E. coli* and *S. aureus* using well diffusion method.

MATERIALS AND METHODS

Bacterial isolates: Bacterial isolates were procured from Department of Biological Sciences, College of Science, Yarmouk University, Jordan. They were comfortable through cultivated at 37 °C for 24 hrs on different media including MacConkey agar, Blood agar and Mannitol salt agar (Oxoid, UK). The bacterial isolates were identified according to the standard methods which recommended by [18-19].

Biosynthesis of nanoparticles: The MgO and Ag NPs were prepared using method of [20], with some modification. The synthesis of NPs was carried out with *Trichoderma viride* ATCC 16404, the active culture of the isolate was inoculated into Sabouraud dextrose broth (Sigma-Aldrich, Germany) and the flasks were incubated at 28 °C ±2, 150 rpm for 3 days. After incubation, the fungal filtrate was procured by passing through Whatman No.1 filter paper. The collected supernatant was added to deionized water treated with each of 1% of mM $MgCL_2$ and $AgNO_3$ further incubated with shaker incubator at 150 rpm for 96 hrs at 28 °C. Conical flasks with either fungal filtrate or $MgCL_2$ and $AgNO_3$ served as positive and negative control, respectively.

UV-VIS spectra analysis: The MgO and Ag nanoparticles were characterized by UV-VIS spectrophotometer (Systronics, South Korea). The scanning range for the samples was 200-600 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UV Winlab" software to record and analyze data. Base line correction of the spectrophotometer was performed using a blank reference [21].

Scanning electron microscope (SEM): A scanning electron microscope (Cam Scan-3200 LV SEM machine, USA) was used to record the micrograph images, characterize mean 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 subject to analysis by SEM machine which was operated at a vacuum [22-23].

Antibacterial effects of MgO and Ag nanoparticles: The antibacterial effects of MgO and Ag NPs were investigated by exposing *S. aureus*, and *E. coli* using agar well diffusion method [24]. Approximately 15 ml of Mueller Hinton agar media (Oxoid, UK) was poured in sterilized petri dishes. The samples were determined and tested inoculums were adjusted to 1×10^5 cells/ml, matching with 0.5 McFarland. Inoculums (100µl) were put on the surface of the agar plates and were disseminated via swabbing onto the plates. Agar wells of 4 mm diameter were prepared with the sterilized cork borer. Three wells were bored each in a certain Petri dish, one well contained the extract only, control positive and the other well loaded with the synthesized MgO and Ag NPs. The well added of MgO, Ag NPs at 100 µg/ml and 0.50 mg/ml Ciprofloxacin antibiotics used as positive control against bacterial isolates. Then the plates were incubated at 37˚C for 24 hrs, where upon inhibitory activity was noticed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm by the ruler scale [25-26].

Statistical Analysis: ANOVA analysis was used to analyze data, in specific, the general linear model of the Statically Analysis System was utilized [27]. Duncan's multiple range test was employed to evaluate significant treatment differences [28]. The 0.05 level of probability was considered to determine the level of significance.

RESULTS AND DISCUSSION

Nano-particles synthesis: The usage of the fungus *Trichoderma viride* for the extracellular biosynthesis of MgO from MgCl2 and AgNPs from $AgNO_3$ solution is reported. The synthesis of MgO NPs extracellular was carried out by cultivation of *Trichoderma viride* mycelia biomass on optimal media enrichment with $MgCL_2$. The quantity of MgO NPs with a concentration of 70 mg/100 ml was collected after centrifuge and drying on 60 °C.

The prepared MgO NPs was identified using UV-VIS spectroscopy. Figure 1. shows the UV–visible spectra of MgO NPs produced by *T.viride*. The absorption was appearing as a band at 256.5 nm. These results were in agreement with the UV-visible spectrum for MgO NPs at 260 nm in the results of [29]. The optical properties of the MgO NPs were investigated in depth using the UV–Vis absorption spectra in the wavelength range of 200–600 nm at room temperature.

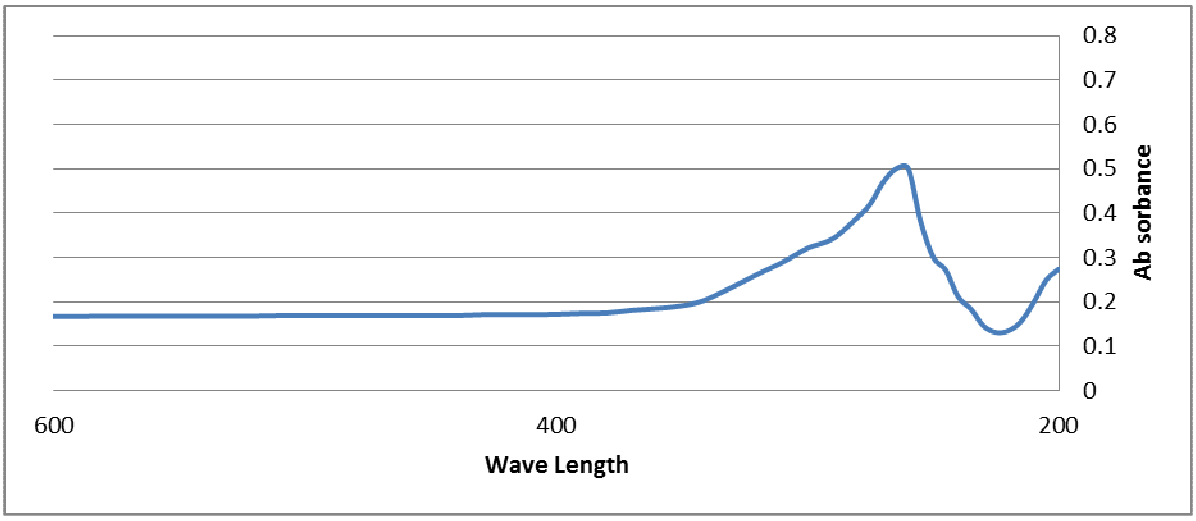


Figure 1. UV–visible spectra of MgO NPs produced by *T.viride*.

The purity and the size range of MgO NPs biosynthesized with *T. viride* were determined using scanning electron microscope analysis. The size appeared range from 45.12 to 95.37 nm. Figure 2 shows the SEM image of MgO NPs produced by *T.viride*.

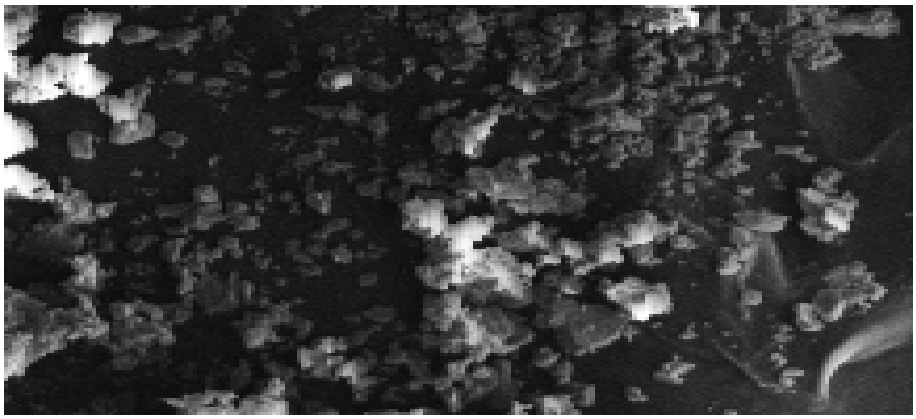


Figure 2. The SEM image of MgO NPs produced by *T.viride*.

The results showed that the aqueous silver (Ag (+)) ions, when exposed to a filtrate of *Trichoderma viride*, and reduced in solution, thus causing formation of very stable Ag NPs. The total amounts of nanoparticles produced from *T.viride* cultivations were at 162.3mg/100 ml. These Ag NPs were characterized using some techniques. The maximum absorbance of nanoparticles was at 420 nm on ultraviolet-visible spectra, and the nanoparticles size was at range between 5-40 nm, appeared using the SEM techniques.

These results were compatible with those obtained by [30]. The biosynthesis of nanoparticles was induced by the microbial cells to produce the biological agents that excrete many enzymes, which can hydrolyze metals, and produced the Nano metals ions [20, 31].

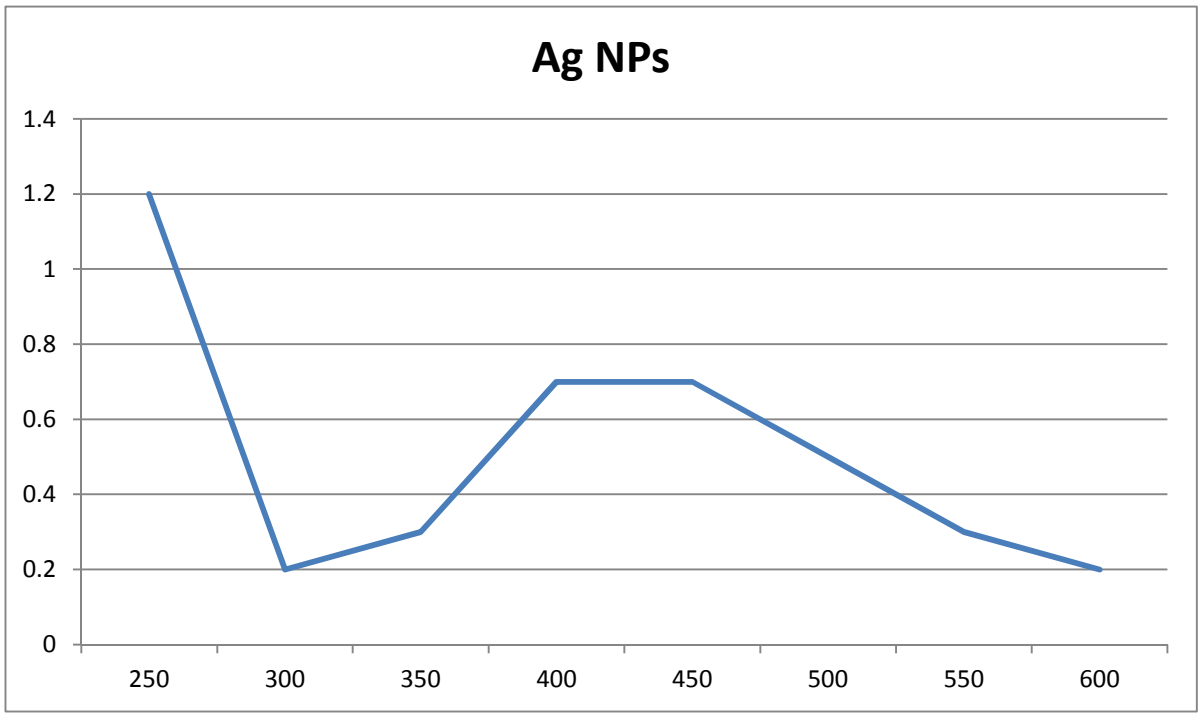


Figure 3. UV–visible spectra of Ag NPs produced by *T.viride*.

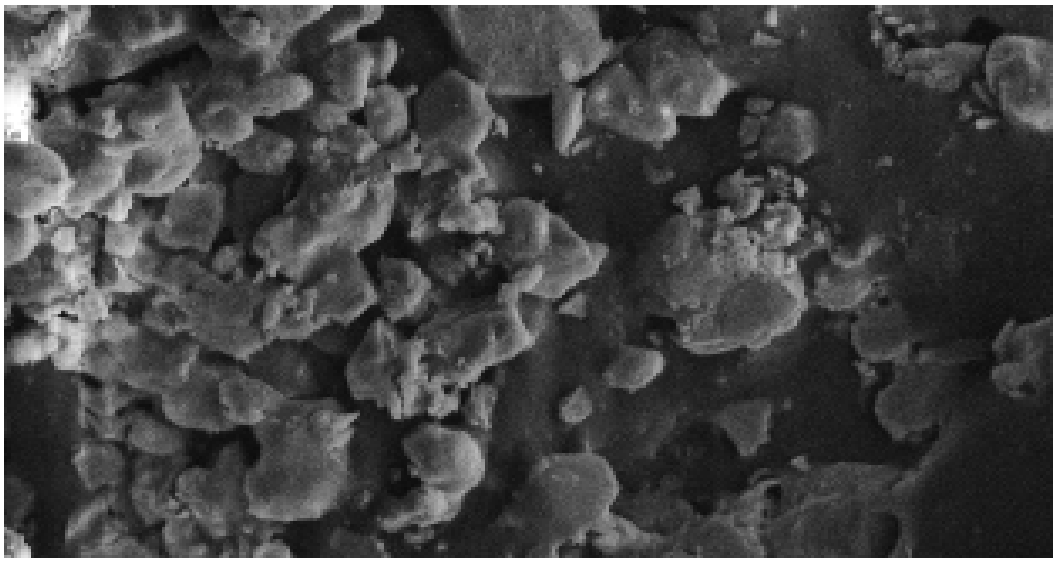


Figure 4. The SEM image of Ag NPs produced by *T.viride*.

Antibacterial activity of MgO NPs: The two bacterial isolates *E. coli* and *S. aureus* were inspected for their sensitivity to MgO NPs and antibiotics by well diffusion method (Table 1 and Figure 5). The diameters of inhibited zones, were measured and compared with the standard manual suggested by the national committee for clinical laboratory standards (NCCLs). The results showed that the bacterial isolates of *E.coli* and *S. aureus* were sensitive to MgO NPs and the inhibition zone diameter was at 30 and 49 mm, respectively compared with the antibiotic of amoxicillin effects against two strains which appeared at 27 and 45 mm, respectively.

Table 1. Inhibition Zone Diameter (mm) of MgO NPs Synthesized by *T.viridie* against *E.coli* and *S. aureus*.

Bacterial species	Zone Inhibition Diameter (mm)			
	MgO NPs	Amoxicillin	MgO NPs+Amoxillin	*T. viridie* Extract
E.coli	30^{b}±2.85	27^{c}±1.94	33^{a}±2.48	4^{d}±0.51
S. aureus	49^{b}±2.62	45^{c}±3.47	52^{a}±4.27	4^{d}±0.26

a-d: The values with dissimilar letters are significantly different at a critical level of 0.05.

The interaction of MgO NPs with Amoxicillin had significantly increased the inhibitions effects on *E. coli* and *S. aureus* and the inhibition zone diameter was 33 and 52 mm, respectively ($p<0.05$). [32] have reviewed the studies that investigated the antimicrobial activity of nanoparticles. They argue that nanoparticles are more and more used to target bacteria as a substitute to antibiotics. [33] found that MgF_2 NP-coated catheters were able to significantly decrease bacterial colonization of *E.coli* and *S. aureus*. The results were contrasting with the *T.viridie* extract without nanoparticles which had no significant antibacterial effects against any of the two bacterial isolates through the inhibition zone diameter which was 4 mm. These results illustrated the inhibition ability MgO NPs against bacterial isolates and it was evident that MgO NPs had higher effect against gram positive compared with the gram negative bacteria. [34] have been attributed the higher susceptibility of Gram-positive bacteria to differences in cell wall structure, cell physiology, metabolism or degree of contact.

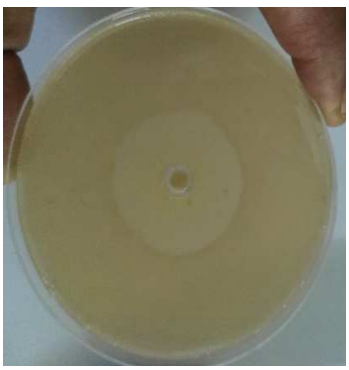
The antibacterial activity of Ag NPs towards *E. coli* and *S. aureus* bacteria was tested using the well diffusion agar method (Tables 2 and Figure 6). The inhibition zone diameter for the effect of AgNPs alone against *E. coli* and *S. aureus* bacteria was 30 and 51 mm, respectively compared with the amoxicillin effect which showed an inhibition zone diameter of 27 and 45 mm, respectively. The use of Ag NPs combination with amoxicillin had significantly increased the inhibition zone diameter which became 33 and 55 mm against *E. coli* and *S. aureus*, respectively. Alternatively, the *T.viridie* Extract which was not treated with Ag ions did not significantly affect *E. coli* and *S. aureus*, the inhibition zone diameter was 4 mm for each of them. [35] have discussed the effect of AgNPs as an alternative strategy against bacterial biofilms. [36] found that Ag-NPs can be used as an effective antibacterial agent against both *E. coli* and *S. aureus* bacteria. [37-38] found that the bactericidal effect of AgNPs depends on the size and shape of the particles. In specific, the surface area of a dose of AgNPs increases as the particle size decreases, allowing greater material interaction with the surrounding environment. In addition, [38] argued that triangular-shaped particles of silver display more bacterial killing activity than rods or spherical particles. [39] have documented other characteristics that affect the biological activity of nanoparticles like zeta potential and particle chemistry, with the former probably has a significant role in the ability of particles to penetrate into the cell.

Table 2. Inhibition Zone Diameter (mm) of Ag NPs synthesized by *T.viridie* against *E.coli* and *S. aureus*.

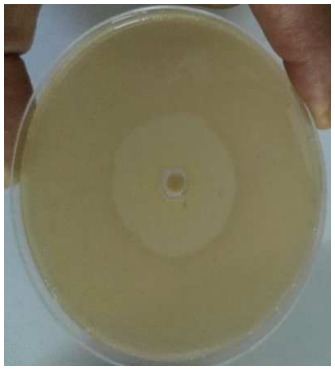
Bacterial species	Zone Inhibition Diameter (mm)			
	Ag NPs	Amoxillin	Ag NPs+Amoxillin	*T.viridie* Extract
E. coli	30b±1.18	27^{c}±1.94	33a±2.13	4^{d}±0.51
S. aureus	51b±3.74	45^{c}±3.47	55a±2.60	4^{d}±0.26

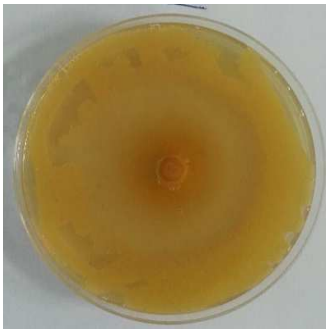
a-d: The values with dissimilar letters are significantly different at a critical level of 0.05.

In the present results, the inhibition zone size differed for diverse types of bacteria, the inhibition zone appeared higher in gram positive bacteria than gram negative bacteria. The interaction of metals nanoparticles with bacteria resulted from the electro static interaction between the bacteria surface walls and the metals nanoparticles resulting in the damage of the bacterial cells [40]. Moreover, the death of bacterial cells was caused by the production of active oxygen such as H_2O_2 because of the presence of metals nanoparticles and caused to adherence of the surface of the cell membrane which resulted in disturbance in its respiration and interaction with enzymes of the respiration chains of bacteria due to the cells death [41].


MgO NPs against *E. coli*


MgO NPs against *S. aureus*


MgO NPs + Amoxillin against *E. coli*


MgO NPs+Amoxillin against *S. aureus*

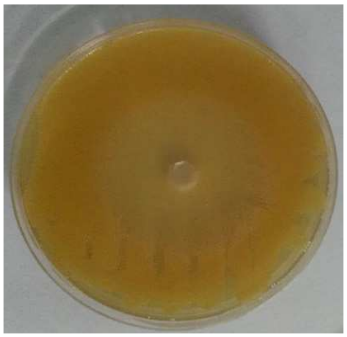
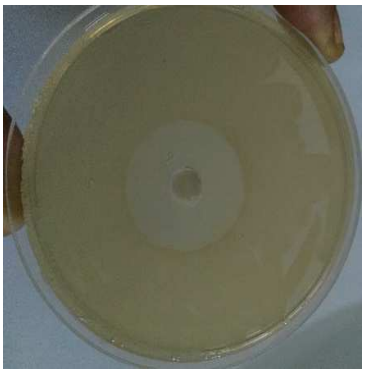
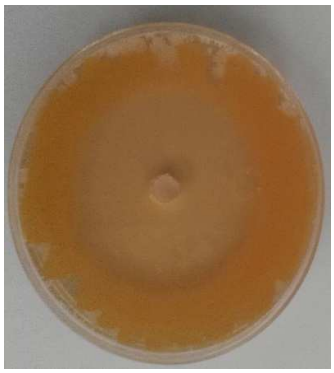
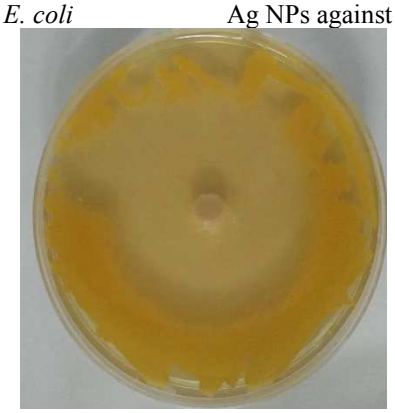

Amoxillin against *S. aureus*

Figure 5: Inhibition Zone of MgO NPs Synthesized by *T.viridie* against *S. aureus and E.coli*..


Ag NPs against *E. coli*


Ag NPs against *S. aureus*


Ag NPs + Amoxillin against *E. coli*


Ag NPs + Amoxillin against *S. aureus*


Amoxillin against *E. coli*

Figure 6: Inhibition Zone of Ag NPs Synthesized by *T.viridie* against *S. aureus and E.coli*.

CONCLUSION

In this study, MgO NPs and Ag NPs were synthesized biologically by *T. viride*. Thereafter, the antibacterial activity of both nanomaterials was estimated against gram negative *E. coli* and gram positive *S. aureus* bacteria by agar well diffusion method. The results revealed that both MgO NPs and Ag NPs are effective antibacterial agents against *E. coli* and *S. aureus* bacteria. In addition, a mixture of nanoparticles and amoxicillin was more effective than each one alone in inhibiting the bacterial activity of *E. coli* and *S. aureus*. Future research could be conducted on different nanomaterials and antibiotics.

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