



Green synthesis of silver nanoparticles using *Zygodium qatarense* Hadidi leaf extract and evaluation of their antifungal activities

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ABSTRACT

In this study, silver nanoparticles were biosynthesized from the methanolic extract of *Zygodium qatarense* Hadidi leaf extract. The methanolic extract of *Zygodium qatarense* Hadidi leaf was used as a stabilizer and reducing agent to reduce Ag⁺ to metallic silver. The produced silver nanoparticles have the average size of 47 nm as confirmed with UV-visible spectroscopy and scanning electron microscope. Furthermore, the antifungal activity of synthesized nanoparticles, methanolic extract of *Zygodium qatarense* Hadidi leaf, and silver nitrate were investigated against *Aspergillus niger* and *Penicillium digitatum* by disk diffusion and micro broth dilution methods. All of the treatments showed antifungal activity, but silver nanoparticles when compared with other treatments had a significant effect against the *Aspergillus niger* and *Penicillium digitatum*.

INTRODUCTION

Silver nanoparticles have been widely used for various purposes, such as medical devices, cleaning agents etc., due to its unique antimicrobial properties. Generally, the method for the silver nanoparticles preparation involves the reduction of silver ions in the solution. However, the reducing reagents, such as sodium borohydride, may increase the environmental toxicity or biological hazards. Hence, the development of a green synthesis of silver nanoparticles using environment-friendly solvents and nontoxic reagents is of great interest. Sun *et al.* described the silver nanoparticles synthesis using a leaf extract of *Cinnamomum camphora*, while the reduction was considered due to the phenolics, terpenoids, polysaccharides and flavonoids

and flavonoids (Sun *et al.*, 2014). *Zygodium qatarense* Hadidi is reported to contains alkaloids, sterols, and coumarins (Cybulska *et al.*, 2014). The plant possess anti-inflammatory properties and also used to treats intestinal pains (Mahasneh *et al.*, 2002). High antimicrobial activity was observed in butanol and ethanol extracts from *Zygodium qatarense* Hadidi against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* (Cybulska *et al.*, 2014). Also, fresh organ leaves powder and twigs from *Zygodium qatarense* Hadidi were used for wound healing and earache (Safa *et al.*, 2013). All the above properties makes this plant a suitable candidate for this study.

In this study, the methanolic extract of *Zygodium qatarense* Hadidi leaf and silver nitrate solution were used for synthesis of silver nanoparticles. Further, the antifungal activity of silver nanoparticles, methanolic extract of *Zygodium qatarense* Hadidi leaf, and silver nitrate were evaluated by standard disk diffusion, the minimum inhibitory concentration (MIC), and the minimum fungicidal concentration (MFC) methods.

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MATERIALS AND METHODS

Zygothellium qatarense Hadidi leaf was collected from Bandar Abbas, Iran. The plant material was thoroughly washed and dried in shade.

The formation of silver nanoparticles in size of 400 to 500 nm was determined using UV-Vis spectroscopy device (model DR 5000-HACH) and also the scanning electron microscope (SEM) device (KYKYDIGITAL-EM3200) is used to specify size and shape of silver nanoparticles. The *Aspergillus niger* and *Penicillium digitatum* was procured from Shiraz branch, Islamic Azad University. In this study methanol, DMSO, potato dextrose agar (PDA) and potato dextrose broth (PDB) medium were procured from Merck & Co, USA, whereas the silver nitrate was procured from Sigma-Aldrich, USA.

The extraction of *Zygothellium qatarense* Hadidi leaf

The 10 g of dried *Zygothellium qatarense* Hadidi leaves were mixed with 100 ml of methanol (95%) and refluxed for 30 min. The extract was filtered through whatman (no. 1) filter paper.

Synthesis of Silver Nanoparticles

The methanolic extract of *Zygothellium qatarense* Hadidi leaf and silver nitrate solution (0.1 M) were mixed together in different ratios. These mixtures were heated at 70 °C until reduced by half and then the synthesized nanoparticles were separated.

Antifungal activity

The synthesized nanoparticles, *Zygothellium qatarense* Hadidi leaf extracts, silver nitrate and Cotrimoxazol were dissolved in DMSO and the final concentration were made to 512, 256, 128, 64 µg/ml concentrations. The plates contain four concentration of treatments were incubated for 48 to 72 hours at 29 °C. Finally, the inhibition zone was measured in millimeter.

The minimum inhibition concentration (MIC)

The 95µl of potato dextrose broth (PDB) was placed into the cells of micro plate and 100µl of each sample was added to the first cell of each row. After mixing the contents of the first cells, 100 µl was removed and added to the next cell and so till ninth cells of every row. Finally, the 5 µl of fungal suspension was added to all cells of micro plate except tenth cells. The tenth cells of each rows was served as a control.

The minimum fungicidal concentration (MFC)

MFC was determined on cultured fungal cells in potato dextrose agar (PDA) medium and incubated for 48 to 72 hours at 29 °C. The lowest concentration of treatments that inhibited 99.9 % of fungal cell growth was considered as minimum fungicidal concentration (MFC) (Ahmadi *et al.*, 2015).

RESULTS AND DISCUSSION

In this experiment, methanol extract of *Zygothellium Qatarense Hadidi* leaf and silver nitrate solution were mixed with each other in different ratios. The changing solution color from dark green to brown was considered as progress of this reaction (Fig. 1).

UV-Vis Analysis

UV-visible spectrum showed only 1 to 1 ratio of methanol extract of *Zygothellium Qatarense Hadidi* leaf and silver nitrate solution, the maximum absorbance peak was 454 nm. (Fig.1). The methanolic extract of *Zygothellium Qatarense Hadidi* leaf was used as a stabilizer and reducing agent to reduce Ag⁺ to metallic silver because *Zygothellium Qatarense Hadidi* leaf contain some phytochemical compounds that can be reducing Ag⁺ to Ag⁰.

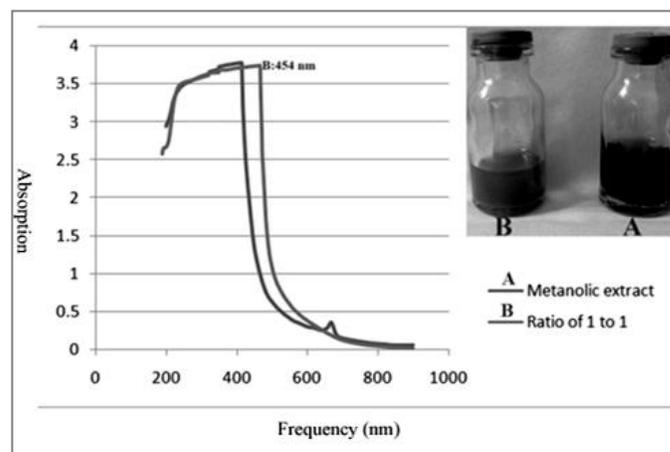


Fig 1: Schematic of UV-Vis spectra before (A) and after reaction (B).

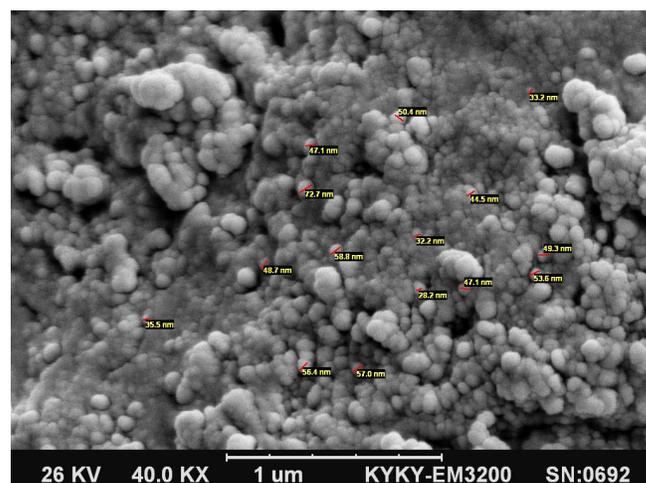


Fig. 2: Scanned image of silver nanoparticles synthesized by using the Scanning electron microscope (SEM).

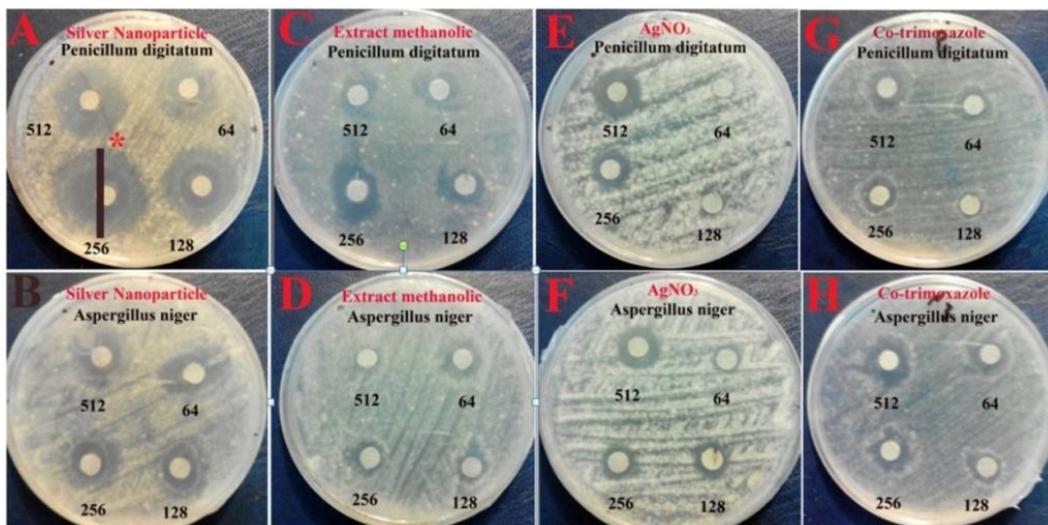


Fig. 3: Antifungal activity of treatments in 512,256, 128 and 64 µg/ml concentrations against *Aspergillusnige* and *Penicillium digitatum* (*Inhibition zone in millimeter).

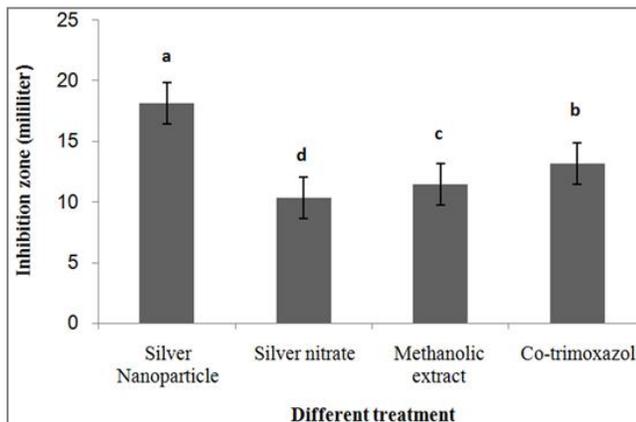


Fig. 4: Comparison of antifungal activity of different treatments against *Penicillium digitatum* and *Aspergillus niger*.

Table 1: The inhibition zone size of disks contain treatments against *Penicillium digitatum* and *Aspergillus niger* in millimeter and the results of MIC and MFC in µg/ml. Silver nitrate and Co-trimoxazol were as a control.

	Disk diffusion method (mm±SD)				MIC (µg/ml)	MFC (µg/ml)
	512 µg/ml	256 µg/ml	128 µg/ml	64 µg/ml		
Penicillium digitatum						
Silver nanoparticle	25.66 ± 1.15	22.00 ± 1.73	19.33 ± 2.30	17.00 ± 1.73	8	32
Methanolic extract of <i>Zygothryllum Qatarense</i> Hadidi	11.33 ± 1.52	13.16 ± 3.25	12.33 ± 1.52	12.00 ± 3.00	16	128
Silver nitrate	15.0 ± 0.00	13.67 ± 0.57	*5.00 ± 0.00	5.00 ± 0.00	16	64
Co-trimoxazol	14.0 ± 2.0	12.33 ± 2.08	11.33 ± 2.30	12.00 ± 0.00	32	64
Aspergillus niger						
Silver nanoparticle	16.67 ± 2.88	15.67 ± 2.08	13.67 ± 1.52	15.00 ± 1.00	8	32
Methanolic extract of <i>Zygothryllum Qatarense</i> Hadidi	9.33 ± 3.78	11.66 ± 0.57	7.33 ± 4.04	14.33 ± 0.57	16	128
Silver nitrate	13.00 ± 0.00	11.00 ± 0.00	11.00 ± 0.00	9.00 ± 0.00	16	64
Co-trimoxazol	14.00 ± 1.73	14.33 ± 1.15	12.33 ± 2.51	15.00 ± 0.00	16	128

*Inhibition zone in millimeter and the size of disks was 5 mm.

SEM Analysis

The shape and average size of silver nanoparticles have been found 47 nm by scanning electron microscope (SEM) (Fig. 2).

The antifungal activity by disk diffusion method

The results showed inhibition zone of silver nanoparticles in the concentrations of 512, 256, 128 and 64 µg/ml for *Penicillium digitatum* respectively was 25.66 ± 1.15 mm, 22.00 ± 1.73 mm, 19.33 ± 2.30 mm and 17.00 ± 1.73 mm. Whereas for *Aspergillus niger* the diameter of inhibition zone was 16.67 ± 2.88 mm, 15.67 ± 2.08 mm, 13.67 ± 1.52 mm and 15.00 ± 1.00 mm. The inhibition zone of methanol extract against *Penicillium digitatum* in the concentrations of 512, 256, 128 and 64 µg/ml was 11.33 ± 1.52 mm, 13.16 ± 3.25 mm, 12.33 ± 1.52 mm and 12.00 ± 3.00 mm respectively and against *Aspergillus niger* was 9.33 ± 3.78, 11.66 ± 0.57, 7.33 ± 4.04 and 14.33 ± 0.57 mm. Co-trimoxazol and Silver nitrate were as a control (Fig.4) (Table 1).

Determination of MIC and MFC

The results showed the minimum inhibitory concentration (MIC) of silver nanoparticles against *Penicillium digitatum* and *Aspergillus niger* was 8 µg/ml and for methanolic extract of *Zygophyllum qatarense* Hadidi leaf was 16 µg/ml. Also the minimum fungicidal concentration (MFC) of silver nanoparticles against *Penicillium digitatum* and *Aspergillus niger* was 32 µg/ml and for methanolic extract of *Zygophyllum qatarense* Hadidi was 128 µg/ml. Co-trimoxazol and silver nitrate were served as a control. Silver nanoparticles had more effects in compared with other treatments in the MIC and MFC methods (Table 1).

CONCLUSIONS

Our results were showed methanolic extract of *Zygophyllum qatarense* Hadidi leaf can be used for synthesis silver nanoparticles as an efficient green reagent without needing to chemical compounds. The synthesis method is very easy and cheap. The silver nanoparticles and methanolic extract of *Zygophyllum qatarense* Hadidi leaf has unique biological properties and can be used in Pharmaceutical industries.

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