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Short Communication

Green Synthesis of Silver Nanoparticles by Using *Salvadora persica* Leaf Extract and Evaluation of their Antifungal Activities

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ABSTRACT
In present study, silver nanoparticles were produced from interaction the ethanolic extract of *Salvadora persica* leaf with silver nitrate solution. UV-vis spectroscopy and PSA device confirmed size and formation of silver nanoparticles. The results showed the average size of silver nanoparticles synthesized is 6.2 nm and the minimum size of silver nanoparticles is 4.7 nm. Furthermore, the antifungal activity of silver nanoparticles and ethanolic extract of *Salvadora persica* leaf against *Aspergillus niger* and Penicillium *digitatum* were investigated by using disk diffusion method. The results showed that silver nanoparticles have significant antifungal activities in comparison to other treatmeants. So these silver nanoparticles have unique antifungal activity and can be used of them in pharmaceutical Industries.
Key words: *Salvadora persica*, Silver Nanoparticles, Antifungal Activities, Silver Nitrate.

INTRODUCTION

The silver nanoparticles shows special physicochemical properties, therefore they use many applications such as catalysis, health, electronic and optical.[1] Silver nanoparticles also act as a potential antifungal agent. The exact mechanism of antifungal effect of silver nanoparticles is still not known. The studies indicated that the silver nanoparticles interact with the organisms and controlled the growth and destroy it.[2] Also another mechanism is silver nanoparticles may serve as a vehicle to deliver Ag+ more effectively to the bacteria cytoplasm and membrane, whose proton motive force would decrease the local pH (as low as pH 3.0) and enhance Ag+ release.[3] Generally, the method for the silver nanoparticles preparation involves the reduction of silver ions in the solution or in high temperature in gaseous .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 by using environment-friendly solvents and nontoxic reagents is of great interest. Huang *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 present in the extract.[4] *Salvadora persica* L. is a large shrub with opposite branches, sometimes growing as dense thickets on sand hummock, belonging to family Salvadoraceae, commonly known as 'Pilu', 'Jal' and 'Tooth brush tree' and is widely distributed in Iran, India, Africa, Saudi Arabia and Pakistan. Leaves and flowers also used for toothache, gumproblems, skin diseases, kidney stones, constipation and anthelmentic. Also the plant has been incorporated into commercially available toothpaste.[5] *Salvadora persica* L. extract showed presence of carbohydrates, alkaloid (salvadorine), steroids, terpenoids, saponins, flavonoids and glycosides. The leaf extracts of *Salvadora persica* L. was showed significant free radical scavenging activity. Potent antioxidant activity of *Salvadora persica* L. increased its width as useful traditional medicine.[2] The based on reported silver nanoparticles synthesized using aqueous extracts of bark and root of *Salvadora persica*. Also they evaluated the antibacterial activity of silver nanoparticles synthesized. The results showed that silver nanoparticles synthesized have favorable antibacterial activities.[1-6] In present study we used the ethanolic extract of *Salvadora persica* leaf and silver nitrate solution for synthesis silver nanoparticles and also the antifungal activities of silver nanoparticles and ethanolic extract of *Salvadora persica* leaf against *Aspergillus niger* and Penicillium *digitatum* by disk diffusion method were investigated.

MATERIALS AND METHOD

Salvadora persica leaf was prepared and dried in spring from Bandar Abas, Iran. The formation of silver nanoparticles in size of 400 to 500 nm was prepared using UV-Vis spectroscopy device in the model of DR 5000-HACH and also we used PSA device in the model of HORIBA Light Scattering Particle Size Analyzer LB-550 to specify size of silver nanoparticles. The *Aspergillus niger* and Penicillium *digitatum* was prepared from Shiraz branch, Islamic Azad University. in this study, the materials including the ethanol, Dimethyl sulfoxide (DMSO), potato dextrose agar (PDA) and potato dextrose broth (PDB) medium of Merck Company, silver nitrate of Sigma-Aldrich Company and Co-trimoxazol of Rouz Daru Company were used.

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The extraction of *Salvadora persica* leaf

Dried *Salvadora persica* leaves (10 gr) was mixed with 100 ml of ethanol (96%) and was extracted by reflux method. Then after the ethanolic extract was formed, filtered and used without any further purification.[7]

Synthesis of Silver Nanoparticles

In order to synthesis the silver nanoparticles, ethanolic extract of *Salvadora persica* leaf was mixed with silver nitrate solution (0.1 M) in different ratios. After that, these mixtures were heated at 70 ^{0}C until reduced by half. Then the silver nanoparticles were separated. Finally for characterizing formation and size of silver nanoparticles used instrumental methods such as the PSA and UV-visible spectroscopy analysis.[7]

Antifungal Activity of Silver Nanoparticels

Silver nanoparticles that were synthesized in the previous steps dried and powdered. After that silver nanoparticles synthesized (and other treatment) was mixed with DMSO solvent in 512, 256, 128, 64 µg/ml concentrations. Then, the disks contains four concentration of treatments against *Aspergillus niger* and Penicillium *digitatum* on PDA medium that was placed inside the incubator for 48 to 72 h at 29^{0}C. finally, the inhibition zone was measured in millimeter. The size of the disks was 5mm.[7]

RESULTS

In present study, silver nanoparticles were produced from interaction the ethanolic extract of *Salvadora persica* leaf with silver nitrate solution(0.1 M). In this regard, changing solution color from light green (Figure 1A) to brown (Figure 1B) was a good sign in the progress of this experiment.

UV-Vis Analysis

UV–visible spectrum was showed only 1 to 1 ratio of ethanol extract of *Salvadora persica* leaf (10 ml) and silver nitrate solution(10ml), the maximum absorbance peak was 430 nm that it's the sign of silver nanoparticles synthesized (Figure1).

PSA Analysis

The result of PSA analysis was showed the average size of silver nanoparticles is 6.2 nm and the minimum size of silver nanoparticles is 4.7 nm (Figure 2).

Antifungal Activity Analysis

The antifungal activity of silver nanoparticels , ethanolic extract of *Salvadora persica* leaf, co-trimoxazol and silver nitrate against *Aspergillus niger* and penicillium *digitatum* were investigated. The results showed inhibition zone of silver nanoparticles synthesized in concentrations of 512, 256, 128 and 64 µg/ml against Penicillium *digitatum* were 17.00 ± 1.00 mm, 16.50 ± 0.50 mm, 15.00 ± 0.00 mm and 13.00 ± 0.00 mm and against *Aspergillus niger* was 21.00 ± 0. mm, 20.00 ± 0.00 mm, 16.00 ± 1.00 mm and 14.00 ± 1.00 mm, respectively. Also the results showed ethanolic extract of *Salvadora persica* leaf has antifungal activity against fungi. The inhibition zone of *Salvadora persica* leaf extract against Penicillium *digitatum* in the concentrations of 512, 256, 128 and 64 µg/ml were 14.00 ± 1.00 mm, 14.00 ± 1.00 mm, 13.00 ± 0.00 mm and 11.00 ± 0.00 mm, respectively and against *Aspergillus niger* were 14.50 ± 2.50 mm, 12.50 ± 2.50 mm, 10.16 ± 4.53 mm and 9.00 ± 1.00 mm, respectively. Co-trimoxazol and silver nitrate treatments were used as a control (Table 1) (Figure 3).

DISCUSSION

In this study, ethanolic extract of *Salvadora persica* leaf was used as a reducing agent to reduce Ag+ to Ag^0. The size of silver nanoparticles

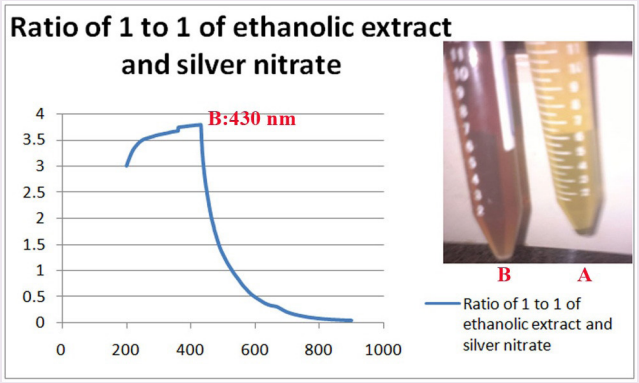

Figure 1: Schematic of UV-Vis spectra after reaction (B) with the maximum absorbance peak at 430 nm.

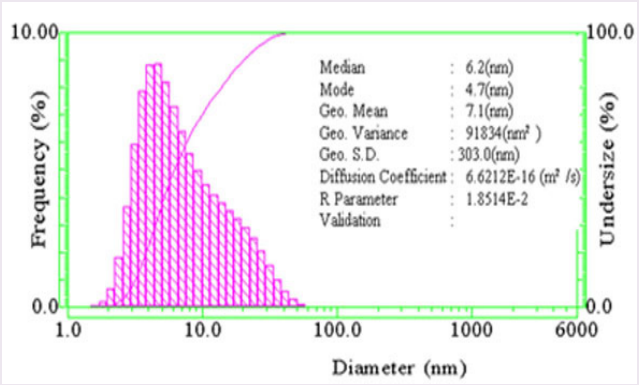

Figure 2: PSA analysis of silver nanoparticles showed obtained nanoparticles with average size of 6.2 nm.

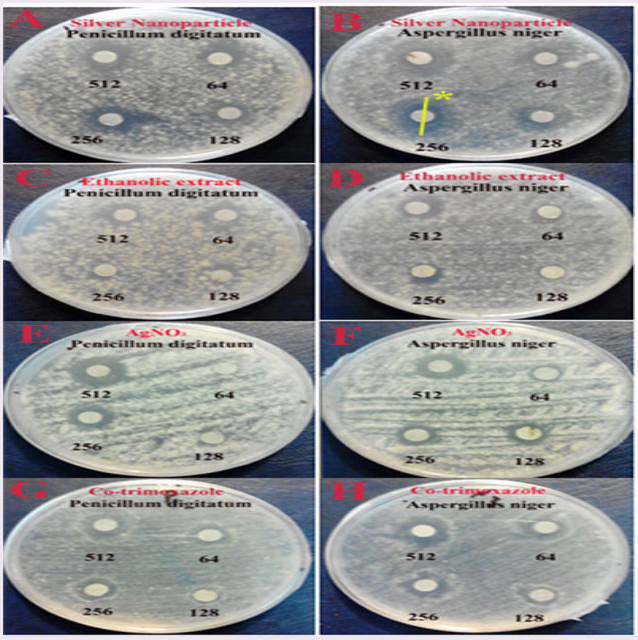

Figure 3: Antifungal activity of treatments in 512,256, 128 and 64 µg/ml concentrations against *Aspergillus nager* and Penicillium *digitatum* (*Inhibition zone in millimeter).

Table 1: The inhibition zone of treatments against *Penicillium digitatum* and *Aspergillus niger* in (mm±SD). Co-trimoxazol and silver nitrate treatments were used as a control. DMSO did not show any antifungal activity.

	Penicillium digitatum			
	512 µg/ml	256 µg/ml	128 µg/ml	64 µg/ml
Silver nanoparticle synthesized	17.00±1.00	16.50±0.50	15.00±0.00	13.00±0.00
ethanolic extract of *Salvadora persica* leaf	14.00±1.00	14.00±1.00	13.00±0.00	11.00±0.00
Silver nitrate	15.0 ± 0.00	13.67 ± 0.57	*5.00 ± 0.00	5.00 ± 0.00
Co-trimoxazol	14.0 ± 2.0	12.33 ± 2.08	11.33 ± 2.30	12.00 ± 0.00
	Aspergillus niger			
Silver nanoparticle synthesized	21.00±0.00	20.00±0.00	16.00±1.00	14.00±1.00
ethanolic extract of *Salvadora persica* leaf	14.5 0±2.50	12.50±2.50	10.16±4.53	9.00±1.00
Silver nitrate	13.00 ± 0.00	11.00 ±0.00	11.00 ± 0.00	9.00 ± 0.00
Co-trimoxazol	14.00 ± 1.73	14.33 ± 1.15	12.33 ± 2.51	15.00 ± 0.00

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

synthesized using ethanolic extract of *Salvadora persica* leaf (6.2 nm) compared to silver nanoparticles synthesized using methanolic extract of *Zygophyllum Qatarense* Hadidi leaf (47 nm) are significant because ethanolic extract of *Salvadora persica* leaf contains carbohydrates, alkaloid, steroids, terpenoids, saponins, flavonoids and glycosides. The concentration of these compounds are very important to reducing Ag+ to Ag^0 and also the size of silver nanoparticles.[2-7] The result showed silver nanoparticels have a good activity against fungi even better than other treatment. The average results of treatments was showed the highest antifungal activity of treatments respectively related to silver nanoparticle (16.56 mm), co-trimaxozol (13.16 mm), ethanolic extract of *Salvadora persica* leaf (12.27 mm) and silver nitrate (10.33 mm). The based on our results and the reported of Barzegar and *et al* silver nanoparticles synthesized using green methods have a good potential against fungi even better than co-trimaxozol. The power of antifungal activity of silver nanoparticles synthesized using methanolic extract of *Zygophyllum Qatarense* Hadidi leaf (18.2 mm) are better than silver nanoparticles synthesized using ethanolic extract of *Salvadora persica* leaf (16.56 mm). We believe these variations related to size, shape and structure of silver nanoparticles.[7]

CONCLUSION

The results showed ethanolic extract of *Salvadora persica* leaf could be used as a reducing agent to reduce Ag+ to Ag^0 and synthesis silver nanoparticles. In this method silver nanoparticles was synthesized without needs to chemical compounds. The size of silver nanoparticle that produce using ethanolic extract of *Salvadora persica* leaf are very significant (6.2 nm). These silver nanoparticles have unique antifungal activity and can be used in pharmaceutical Industries.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

ABBREVIATION USED

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