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ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIS OF SILVER NANOPARTICLES BY PLANTS

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Abstract

Antibacterial activity of biosynthesized silver nanoparticles (AgNPs) was significant in therapeutic application of nanotechnology. These researchers studied an ecofriendly and rapid method for the first time to synthesize silver nanoparticles using *Quercus infectoria* galls alcoholic extract and their antibacterial properties. The extract was found to have the potential to form silver nanoparticles on hot plat stirrer within 30 minutes. The green synthesized silver nanoparticles were characterized using different techniques. The UV-visible spectrum of the solution containing AgNPs showed a peak at 450 nm corresponding to the plasmon absorbance of silver nanoparticles. X-Ray Diffraction patterns (XRD) showed that they could be indexed as face-centered-cubic structure of silver and Scanning electron microscope SEM micrograph indicates the uniform spherical particles at the size range of 40 to 60 nm. Antibacterial activity of alcoholic extract and AgNPs biosynthesized from *Q. infectoria* galls was determined by well diffusion and micro plate assay methods against *Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa* and *Escherichia coli*. Also evaluated for their synergistic effect with antibiotics against the multi drug resistant pathogenic microorganisms. The antibacterial efficacy of various antibiotics was found to be enhanced in the combination of silver nanoparticles.

***Key word*:** Synthesis of silver nanoparticles, Antibacterial, Green chemistry, *Quercus infectoria* galls.

Introduction

Synthesis of nanoparticles has gained great significance during the last few years due to their unique properties and application (Garlapati *et al*., 2010) Chemical methods are among the most important approaches in metallic nanoparticles synthesis. However, these methods use high cost and toxic reagents as reducing and stabilizing agents (Prabhu and Poulose, 2012).

Currently, there is a growing need to develop inexpensive and environmentally friendly nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol (Philip *et al.*, 2011). Today, synthesis of nanoparticles is carried out mainly on biological systems such as bacteria, fungi, yeast, algae and plant extracts. In microorganism-mediated methods, the synthesis reaction takes a long time (24-124 h.) and the process of sub-culturing cell cultures is time-consuming (Zaki *et al*., 2012) when in plant-mediated methods, reaction time is greatly short and cultivation of microbial cells step is eliminated, also no need to focus on cross contamination as like microbes.

Phytochemical analyses of *Q. infectoria* galls extract revealed the presence of phenolic, tannins, flavonoids, alkaloids and saponins. These phytochemical compounds in *Q. infectoria* galls are strong reducing agents due to their abundant OH-groups that enhance their antibacterial and antioxidant activity (Vermani, 2009).

Hence, this research work is mainly focused on simple process as a green technology using aqueous extract of *Q. infectoria* galls as a first time for the biosynthesis of silver nanoparticles without the usage of hazardous and toxic solvent. The process has several advantages with low cost, compatibility, stability and also has proved antimicrobial activity against pathogenic microorganism.

The aim of the study:

1. Biosynthesis of silver nanoparticles AgNPs by *Q. infectoria* galls alcoholic extract.
2. Determination of Antimicrobial Activity for AgNPs by *Q. infectoria* galls.
3. Determination of antibiotic and antibiotic-modifying activity.

Fig. 1: Show the biosynthesis of AgNPs by *Q. infectoria* galls. Left tube: alcoholic extract, Right tube: AgNPs.

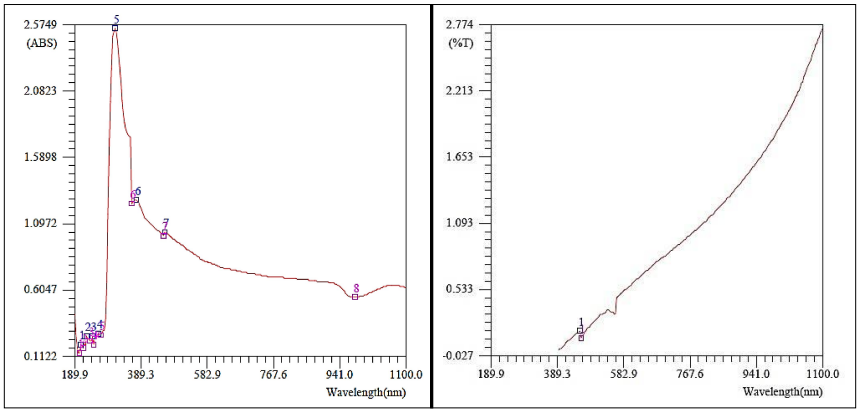

Fig. 2: UV-vis spectra of alcoholics extract only (left pics.) and UV-vis spectra of AgNps (right pics.) show strong peak at 450 nm.

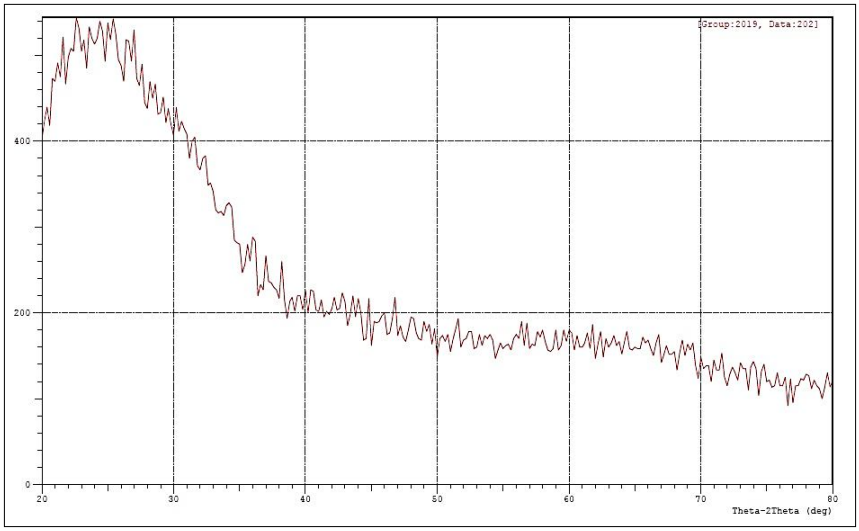

Fig. 3: X-ray diffraction pattern of Ag nanoparticles prepared with alcoholic extract, Peaks reveal to organic compound of extract.

Materials and Methods

Collection, Processing and Preparation of plant Extract

Quercus infectoria galls were purchased from the local herbal shop in Baghdad and identified based on their physical appearances which were globular in shape, 0.8 cm to 2.5 cm in diameter, green-yellow in color, odor is slight, strongly pungent taste and tuberculate surface (Ansari *et al*., 2012). The galls were washed with distilled water, left dry at room temperature before they were crushed and ground prior to the extraction. Extraction was done by taking 50g of dried plants powder added to it 300 ml ethanol using soxhlet distillation for 7 hour.

Synthesis of Silver Nanoparticles

Ten milliliter of *Q. infectoria* galls alcoholic extract was gradually added into 90ml of 1mM silver nitrate ($AgNO_3$) in a 250ml Erlenmeyer flask and put on hot plat stirrer under dark conditions. The reaction solution was checked for 30 minutes and monitoring the change in color of $AgNO_3$ solution from green color to brown.

Characterization of AgNPs

UV-Vis Spectra Analysis: Analysis of UV-Vis spectra was done by using a double beam spectrophotometer (Shimadzu uv-1650 pc spectrophotometer) (Tajdidzadeh *et al*., 2014).

The solution of silver nanoparticles was monitored by measuring the UV-Vis spectrum of the reaction solution in the 300-900 wavelength rang after diluting a small aliquot of the solution into deionized water. The solution was pipetted into a test tube and diluted four times by deionized water and analyzed at room temperature.

X-Ray Diffraction (XRD)

X-ray diffraction analysis was detected to examine the crystallographic structure of the purified AgNPs (Moharram *et al*., 2014). The XRD grids were coated with dried biosynthesized nanoparticles and the synthesized nanoparticles diffraction pattern was measured by X-ray diffractometer (Shimadzu XD-3A).

Scanning electron microscope (SEM)

(SEM) was used to obtain the surface image and the size of the microbially synthesized silver nanoparticle.

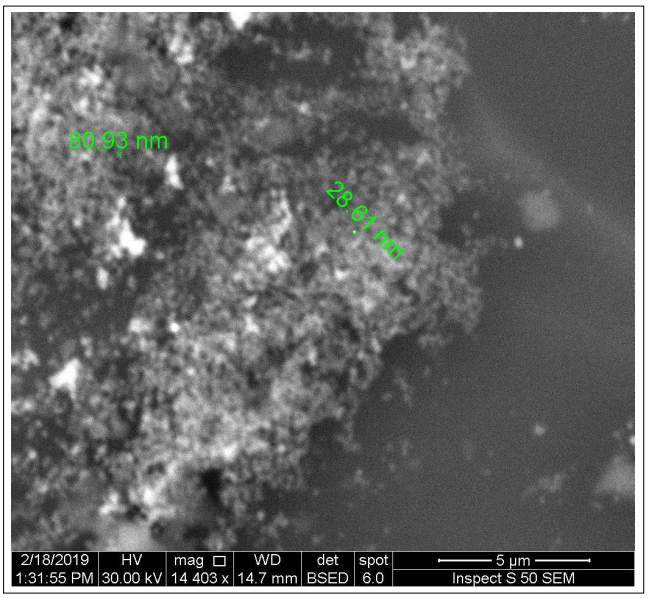

Fig. 4: Show the spherical shape of nanoparticles by SEM.

Determination of Antimicrobial Activity

The AgNPs and alcoholic extract prepared by *Q. infectoria* galls were used to evaluate antimicrobial activity against Gram positive bacteria, (*Staphylococcus aureus*, *Candida albicans*) and Gram negative bacteria, (*Echerichia coli, Pseudomonas aeruginosa*) on Mueller Hinton Agar plates by agar well diffusion method (Sharma *et al*., 2014). The Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) methods for all test bacterial strains were also determined.

Determination of antibiotic and antibiotic-modifying activity

Tests for antibacterial activity were performed with standard antibiotic discs such as Ampicillin, Azithromycin, Erythromycin, Ciprofloxacin, Carbencillin, Tobramycin, Doxycycline, Trimethoprim. The combined formulation of alcoholic extract prepared by *Q. infectoria* and AgNPs with antibiotic discs which did not show any effect on microorganism were used to find out the synergistic effect against the pathogens. The zone of inhibition was measured after overnight incubation at 37°C.

Results and Discussion

Biosynthesis of AgNPs

The formation of silver nanoparticles was monitored with color change and UV-Vis spectroscopy. The biosynthesis of AgNPs was prepared using 90ml of $AgNO_3$ solution and 10ml of *Q. infectoria* gall alcoholic extract. Change in color of solution from green color to deep brown was observed for the formation of silver nanoparticles in the solution after 30 mint under hot plat stirrer (Fig. 1), indicating the formation of AgNPs (Lu *et al*., 2014).

Characterization of AgNPs

Uv-Visible Spectroscopic Analysis: In this study, AgNPs were successfully synthesized by *Q.infectoria* galls. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by a UV-vis spectrophotometer (Ayub *et al*., 2015). In the UV-visible spectrum (Fig. 2), a strong peak was observed at 450nm and the Surface Plasmon Resonance (SPR) confirmed successful formation of AgNps.

X-Ray Diffraction (XRD)

The X-ray diffraction studies were performed to confirm the crystalline structure of synthesized silver nanoparticles. XRD spectrum of alcoholic extract reduced silver nanoparticle was showed the strongest three peaks at 2θ (66.40°, 30.64°, 24.73°) (Fig. 4) the average sizes of AgNPs were observed after applied Scherrer equation was found to be 6.0nm.

Table 1: Diameter of zone of inhibition by alcoholic extract against multi drug resistant pathogenic microorganisms.

1.0µg/ml	0.8µg/ml	0.6µg/ml	0.4µg/ml	0.2µg/ml	M.O
25.3±0.50	24.3±0.57	20.6±0.57	19.3±1.15	16.3±1.15	*S.aureus*
24.3±0.57	22.6±0.50	20.6±1.15	19.3±0.57	16.6±0.50	*E.coli*
24.3±1.15	22.6±0.57	20.3±0.57	18.6±0.50	16.3±1.15	*P. aeruginosa*
26.6±1.15	25.3±0.57	23.6±1.15	20.6±1.15	17±1.15	*C.albicans*

Table 2: Diameter of zone of inhibition by AgNPs synthesized from alcoholic extract against multi drug resistant pathogenic microorganisms.

1.0µg/ml	0.8µg/ml	0.6µg/ml	0.4µg/ml	0.2µg/ml	M.O
28.3±0.50	26.6±0.57	25.3±0.56	22.3±1.15	20.6±0.50	*S.aureus*
28.0±0.57	27.6±0.50	25.3±1.15	23.3±1.15	21.3±1.15	*E.coli*
26.6±0.57	25.3±1.15	23.6±0.57	21.3±1.15	20.3±1.15	*P. aeruginosa*
28.6±0.57	26.6±0.50	25.3±1.15	22.3±1.15	20.6±0.57	*C.albicans*

Scanning electron microscope (SEM)

The size and shape of the silver nanoparticles biosynthesized was studied by SEM (Fig. 4).

Antimicrobial Assay

The antibacterial effect of alcoholic extract and biosynthesized AgNPs was studied against multi drug resistant microorganism (gram positive and gram negative) displayed excellent antibacterial activity against all tested microorganism strains at the volume of 100 µL/ well. Alcoholic extract showed zone of inhibition range from 26.6-24.3 mm (24.3 mm for both *E. coli* and *P. aeruginosa*, 25.3 mm for *S.aureus*, 26.6 mm for *C. albicans*) (Table 1). These

Table 3: Synergistic effect of different antibiotics with and without extracellularly alcoholic extract and biosynthesized AgNPs against pathogens. X-alcoholic extract F.I-Fold Increase F= ((b-a)/a)×100.

M.O		*P. aeruginosa*				*C. albicans*					
AB		**AB+X**	**F.I.**	**AB+AgNPs**	**F.I.**	**AB**		**AB+X**	**F.I.**	**AB+AgNPs**	**F.I.**
PY 100mg	6	13	116.66	16	166.66	AM 10mg	6	116.66	13	83.33	11
AM 10mg	6	10	66.66	15	150.00	E 15mg	6	133.33	14	116.66	13
E 15mg	6	13	116.66	14	133.33	TMP 10mg	6	66.66	10	0	6
TMP 10mg	6	17	183.33	20	233.33	DO 30mg	6	100	12	50.00	9
DO 30mg	6	9	50.00	14	133.33						

M.O		*S. aureus*				*E. coli*				
AB		**AB+X**	**F.I.**	**AB+AgNPs**	**F.I.**	**AB**	**AB+X**	**F.I.**	**AB+AgNPs**	**F.I.**
AM 10mg	6	66.66	10	0.00	6	6	100	12	0.00	6
E 15mg	6	150	15	66.66	10	6	133.33	14	66.66	10
DO 30mg	6	116.66	13	66.66	10	6	83.33	11	50.00	9

results agreed with previous work carried out by (11). The antibacterial activity of AgNPs synthesized from *Q.infectoria* galls showed zone of inhibition range from 28.6-26.6 mm (26.6 mm for *P. aeruginosa*, 28.0 mm for *E. coli*, 28.3 mm for *S. aureus*, 28.6 mm for *C. albicans*) (Table 2). These results agreed with previous work carried out by (Hungund *et al*., 2015).

The MIC of alcoholic extract was found to be 80 µg/ml against pathogenic microorganism also MBC of alcoholic extract was found to be 100 µg/ml against pathogenic microorganism.

The MIC of AgNPs from *Q.infectoria* galls was found to be 60 µg/ml against pathogenic microorganism also MBC of AgNPs from *Q. infectoria* galls was found to be 80 µg/ml against pathogenic microorganism.

Determination of antibiotic and antibiotic-modifying activity

Tests for antibacterial activity were performed with standard antibiotic discs such as Ampicillin, Azithromycin, Erythromycin, Ciprofloxacin, Carbencillin, Tobramycin, Doxycycline, Trimethoprim.

P.aeruginosa show high resistance against Erythromycin, Doxycycline, Ampicillin, Trimethoprim, Carbencilin with ratio (100, 99, 97, 100.98)% while it show sensitivity against Ciprofoxacin and Tobramycin inhibition rate 30.20mm, 21.4mm and medium sensitivity against Azithromycin inhibition rate 10.1mm.

C. albicans show high resistance against Erythromycin, Doxycycline, Ampicillin, Trimethoprim, with ratio (100, 99, 97.98)% while it show sensitivity against Ciprofoxacin and Tobramycin inhibition rate 0.32mm, 20.6mm and medium sensitivity against Azithromycin and Carbencillin inhibition rate 15.0mm and 14.2mm.

E.coli and *S. aureus* shows high resistance against Erythromycin, Doxycycline, Ampicillin with ratio 100% while *E.coli* show sensitivity against Ciprofoxacin, Tobramycin and Trimethoprim inhibition rate 35.1mm, 30.2 and 24.5mm and medium sensitivity against Azithromycin and Carbencillin inhibition rate 18.3mm and 15.7mm.

S.aureus show sensitivity against Ciprofoxacin and Tobramycin inhibition rate 34.1mm and 25.3mm and medium sensitivity against Trimethoprim, Azithromycin and Carbencillin inhibition rate 15.4mm, 13.7mm and 13.2mm.

The antibacterial activities of Erythromycin, Doxycycline, Ampicillin, Trimethoprim, Carbencilin increased in the presence of alcoholic extract and AgNPs biosynthesized from *Q.infectoria* gall alcoholic extract against the test pathogens (Table 3). The synergistic effect of silver nanoparticles represents the highest percentage of increase in inhibition.

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