Plant Archives Volume 20 No. 1, 2020 pp. 579-584 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETECTION THE ANTIMICROBIAL ACTIVITY OF AGNPS SYNTHESIZED BY *QUERCUS INFECTORIA* PLANT

Reem M. A. Al Majeed[1], Nehia N. Hussein[1] and Khalida F.AL-Azawi[2]

[1] Biotechnology Division, Department of Applied science, University of Technology, Baghdad, Iraq.
[2]Applied Chemistry Division, Department of Applied science,University of Technology, Baghdad, Iraq.

Abstract

Antimicrobial activity of plant extract of *Quercus infectoria* gall plant and silver nanoparticles (AgNPs) synthesized by *Quercus infectoria* galls with hot plat stirrer at 30 minutes. Silver nanoparticles were characterized using UV-visible, X-Ray, and SEM. Antimicrobial activity was determined by well diffusion methods against some pathogenic microbes (*Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Candida albicans*). Biosynthesized silver nanoparticles were observed that the greatest effect on the growth of all pathogenic microbes compared with plant extracts. Also the synergistic effect of plant extract and silver nanoparticles with antibiotics was elevated.

Key word : Antibiotics test; Green synthesis; Medicinal plant; Synergistic effect.

Introduction

Q. infectoria generally known as gall oak. It is a small tree growing to 4 to 6 feet tall, crooked, by smooth and bright leaves (Dhiman, 2006) (Fig. 1). Phytochemical analyses of *Q.infectoria* galls extract revealed the presence of phenolic, tannins, flavonoids, alkaloids and saponins, these are strong reducing agents due to their abundant OH-groups that increase their antibacterial and antioxidant activity (Zaki, *et al*., 2015). Creation of nanoparticles has increased great significance during the last few years due to their unique properties and application (Garlapati, *et al*., 2010). Today, synthesis of nanoparticles is carried out mainly on biological systems such as bacteria, fungi, yeast, algae and plant extracts (Philip, *et al*., 2011). This study amid to biosynthesis of silver nanoparticles AgNPs by *Q.infectoria* galls alcoholic extract, Determination of antimicrobial activity for AgNPs, and determination of antibiotic, and determination of the Synergistic effect for plant, and AgNPs with antibiotics.

Materials and Methods

Preparation of extract

Q. infectoria galls were purchased from the nearby market Baghdad/Iraq in May 2018. Plant material were dried and pulverized with motor and pestle or electric mill. 100 g of gall powder were extracted with 70% ethanol by soxholet for 7 hr., and filtered through Whatman filter paper, and after evaporated by a rotary evaporator under vacuum to obtain the extract, the extract was stored at 4°C until use. (Ghassan[a] *et al*., 2013).

Fig. 1: *Q.infectoria* plant.

Synthesis of AgNPs

***Author for correspondence :** E-mail: nehiahussein@yahoo.com

1 mM of Aqueous solution of $AgNO_3$ was prepared by adding it to 90 ml of (D.W.) at room temp, and mixed with ten ml of extracts at 70°C temperature while stirring magnetically at 1000 rpm for 3min (Ghassan[b] *et al.*, 2013).

Characterization of silver nanoparticles

UV-Vis spectral analysis was done by using spectrophotometer (PG-T80+ UV/Vis spectrophotometer, England) from 350-700 nm at a resolution of 1 nm. The crystallite space size was determined from the width of the XRD peaks, supposing that they are free from non-uniform strains, by the Scherrer recipe (Ghassan[b] *et al.*, 2013).

$$D = 0.94 \lambda / \beta \cos \theta$$

The surface picture and the size of the created silver nanoparticle was done by SEM analysis in the Labs of Applied science of university of technology/ Iraq.

Isolates preparation

Different pathogenic microorganisms *E. coli*, *P. aeruginosa*, (G-ve), *S. aureus* (G+ve) and *C. albicans* (Yeast) were taken from isolated specimens. The isolates were cultured overnight at 37 ^{0}C on nutrient broth. The microbial suspensions were homogenized and adjusted to 0.5 McFarland standards tube (5×10^5 CFU/mL).

Evaluation the antimicrobial activity

This analysis was done by used agar well diffusion assay against different pathogenic microbes *E. coli*, *P. aeruginosa*, (G-ve), *S. aureus* (G+ve) and (yeast) *C. albicans*. NB was inoculated with isolates for 3 hr at 37 °C. The turbidity was compared with 0.5 McFarland standards. 100 µL of the culture was spread on the MHA and left to dry for 10 min. Wells with 6 mm diameter were poured with 50 µL from Plant extract and AgNPs solution, the diameter of inhibition zone was calculated and recorded as mean ± SE of the triplicate experiment after incubation (Kumar and Mamidyala, 2011).

Antibiotic susceptibility test

Detected the microbial isolates sensitivity or resistance to antibiotics were compared with the standard tables (Table 3). Tests for antimicrobial activity were performed with standard antibiotic discs such as Ampicillin, Azithromycin, Erythromycin, Ciprofloxacin, Carbencillin, Tobramycin, Doxycycline, and Trimethoprim. The zone of inhibition was measured after overnight incubation at 37°C.

Calculation of increase in fold area

This assay was evaluated by calculated the mean of the zone of inhibition made by an antibiotic only and mixture of the antibiotic with AgNPs (Fayaz *et al.*, 2010).

Determination of minimum inhibitory concentration

MIC for isolates' under study by serial dilution method was determined according to (Mazzola *et al.*, 2009). Sterilized brain heart infusion broth (0.8 mL) (Difco USA) was distributed in test tubes, inoculated with 0.1 mL of each isolates culture and compared with 0.5 McFarland standards tube. Then 0.1 mL of the different plant extract concs. (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) were added into all tubes, except the control tube, Normal saline (0.1 mL) was added instead of plant extract. The result was recorded depending on the turbidity after incubation period.

Statistical analysis

The results are reported as mean ± SD of three independent replicates. Statistical analysis of data was done by computer using SPSS version 11.5 software. Level of significant was measured by the Analysis of Variance (ANOVA) test. The level of significance was shown using the least significant difference (LSD) test.

Results and Discussion

Biosynthesis of silver nanoparticles

The fresh suspension of *Q. infectoria* was yellowish in color. When adding of $AgNO_3$ with heated with 30 min, alteration to deep greenish color was observed for the indication to formation of AgNPs (Lu, *et al.*, 2014). (Fig. 2). 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, a strong peak was observed at 450 nm, and the surface Plasmon resonance (SPR) confirmed successful formation of AgNps (Fig. 3). The X-ray diffraction test was performed to approve the crystalline structure of biosynthesized silver nanoparticles. XRD spectrum of alcoholic extract reduced silver nanoparticle was exhibited the strongest three peaks at 2θ (66.40^0, 30.64^0, 24.73^0) (Φιγ. 4) the average sizes of AgNPs were observed after applied Scherrer equation was found to be 6.0 nm (Fig. 4). The created Silver nanoparticles were spherically in shape, and size between 26 and 80 nm (Fig. 5).

Determination of the antimicrobial Activity

The well diffusion method was used to determine the inhibition zones of the different concentrations from *Q.infectoria* alcoholic extract as shown in table 1, Fig. 6. The three G+ve, G-ve bacterial strains and one yeast were used. The result of the inhibitory effect of the extract on the growth of microbes showed a considerable diversity. The results show the highest effect for the alcoholic extract against yeast *C.albicans* by zone of

Fig. 2: Show the biosynthesis of AgNPs by *Q.infectoria* galls. Left tube: alcoholic extract. Right tube: AgNPs.

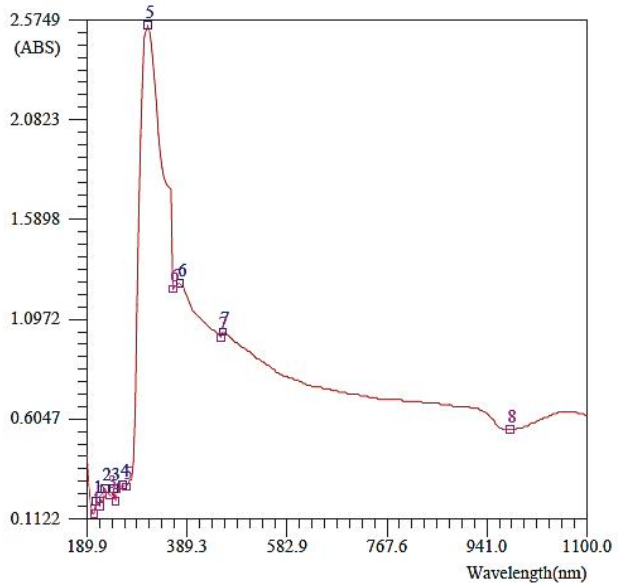

Fig. 3: UV vis spectra of alcoholics extract only (left pics.) And UV- vis spectra of AgNps (right pics) show strong peak at 450 nm.

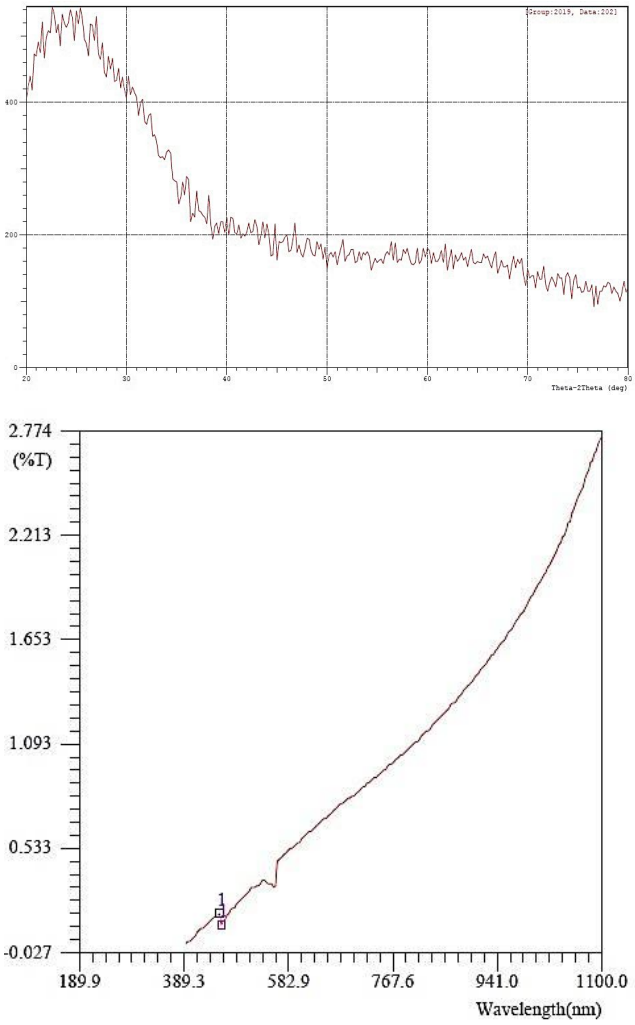

Fig. 4: XRD patterns recorded from drop-coated films on glass substrate of silver nanoparticles synthesized by treating *Q.infectoria* extract with $AgNO_3$ aqueous solutions.

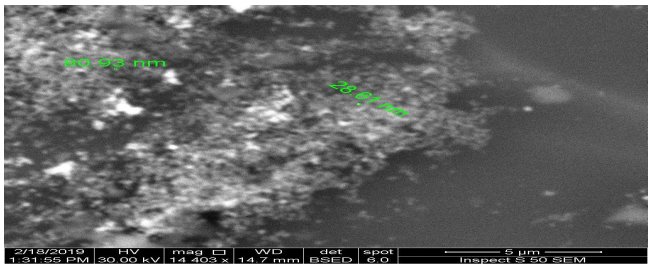

Fig. 5: Show the spherical shape of nanoparticles by SEM.

inhibition reached to (26.6mm), and (25.33mm) on the growth of *S.aureus*, and (24.33mm) of inhibition zone for both of *E.coli* and *P.aeroginosa* , results in this study was agreed with previous work by (Ayub, *et al.,* 2015). Ethanolic crude extracts of plant inhibited G+ve bacteria relatively better than the G-ve bacteria. The antimicrobial properties shown are mainly due to the presence of tannin which is the major constituent present in this plant (Ikram & Nowshad, 2009).

The application of silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against all human pathogens. However, the antimicrobial effect was dose-dependent, and was more pronounced against G+ve bacteria than G-ve microbes. Additionally, the silver nanoparticles showed good inhibition activity towards *C. albicans* this agreed with (Ghassan *et al.,* 2013)**,** Also the results indicate that the biosynthesized AgNPs more effect on the growth of pathogenic microbes than the plant extract only. This is consistent with what exists by (Hungund, *et al.,* 2015) (Table 2, Fig. 7).

Tests for antimicrobial activity were performed with standard antibiotic discs such as Ampicillin, Azithromycin, Erythromycin, Ciprofloxacin, Carbencillin, Tobramycin, Doxycycline, and Trimethoprim. (Table 3). *P.aerogimosa* show high resistance against Erythromycin, Doxycycline, Ampicillin, Trimethoprim, Carbencilin with ratio (97%,

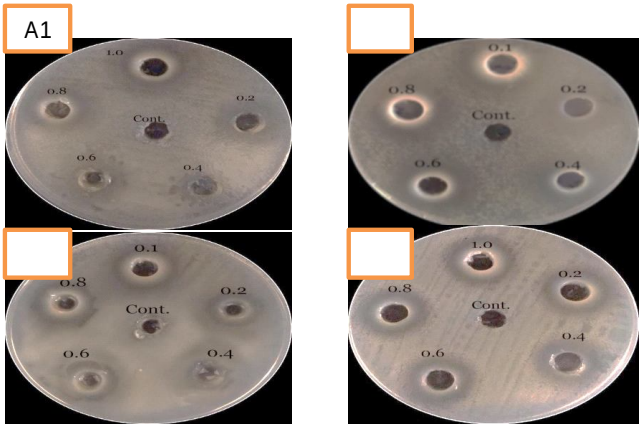

Fig. 6: The antimicrobial activity of the ethanolic extract of *Q.infectoria* plant on some pathogenic microbes. (A1, B1, C1,and D1) *C.albicans* , *S.aureus* ,*P.aeruginosa* ,and *E.coli* respectively.

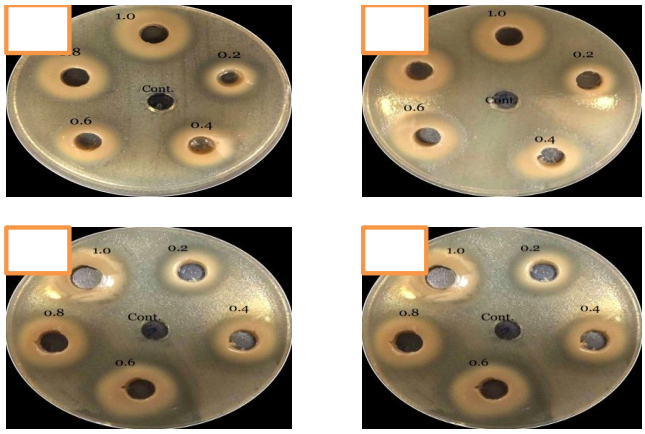

Fig. 7: The antimicrobial activity of biosynthesized AgNPs on some pathogenic microbes. (A2, B2, C2, and D2) *C.albicans* , *S.aureus* ,*P.aeruginosa* ,and *E.coli* respectively.

Table 1: Effect of *Q.infectoria* plant extract against some pathogenic microbes.

M.O name	inhibition of zone of µg/ml				
	0.2	0.4	0.6	0.8	stock
S.aureus	16.3±1.15	19.3±1.15	20.6±0.57	24.3±0.57	25.3±0.50
E.coli	16.6±0.50	19.3±0.57	20.6±1.15	22.6±0.50	24.3±0.50
P.aeruginosa	16.3±1.15	18.6±0.50	20.3±0.57	22.6±0.57	24.3±1.15
C.albicans	17.0±1.15	20.6±1.15	23.6± 1.15	25.3±0.57	26.6±1.15

Table 2: Effect of biosynthesized AgNPs against some pathogenic D microbes.

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

Table 3: Effects of Antibiotics against some pathogenic microbes.

M.O. / Antibiotics	Inhibition zone of Antibiotics (mm)			
	E.coli	*S.aureus*	*P.aeruginosa*	*C.albicans*
Ciprofloxacin	35	34	30	32
Carbencillin	15	13	6	14
Erythromycin	6	6	6	6
Ampicillin	6	6	6	6
Azithromycin	18	13	10	15
Tobramycin	30	25	21	20
Doxycycline	6	6	6	6
Trimethoprim	45	15	6	6

98% and 100%). Also its show sensitivity against Ciprofoxacin by inhibition zone reached to (30mm) and Tobramycin by inhibition zone reached to (21mm), and zone of inhibition reached to (10 mm) against Azithromycin. *E.coli* and *S.aureus* shows high resistance against Erythromycin, Doxycycline, and Ampicillin with ratio 100%. The highest effect in Trimethoprim by inhibition zone reached to (45mm), follow in Ciprofoxacin by inhibition zone reached to (35mm), Tobramycin (30mm) and, Azithromycinm by zone of inhibition (18 mm), and finally in Carbencillin by zone of inhibition reached to (15 mm). *S.aureus* show sensitivity against Ciprofoxacin by zone of inhibition reached to (34 mm), Tobramycin by zone of inhibition reached to (25mm) and (15 mm) for Trimethoprim, finally (13 mm) for Azithromycin and Carbencillin. *C.albicans* show high resistance against Erythromycin, Doxycycline, Ampicillin, Trimethoprim, with ratio) 97%, 98% and 100%). Also it show sensitive against other antibiotics. The highest effect of Ciprofloxacin by inhibition zone reached to (32mm)., and for Tobramycin by inhibition zone reached to (20 mm), finally in against Azithromycin by zone of inhibition reached to (15mm), finally for Carbencillin by inhibition zone reached to (14 mm).

Evaluation of the synergistic effect

According to the results in Tables 4 and 5, a synergistic effect was observed for the mixture of antibiotic with the plant extract, and the mixture of antibiotic with the silver nanoparticles, by increasing the diameter of the inhibition zone of some antibodies to which the bacteria have shown resistance against it, The effects of(PY, AM, E, TMP, and DO) antibiotics was increased in the presence of AgNPs against all tested pathogens. The highest effect for the combination of Antibiotics with

Table 4: Synergistic effect for different antibiotics with and without extracellularly alcoholic extract and biosynthesized AgNPs against *P.aeruginosa* , and *C.albicans* microbes.

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

Ab- Antibiotic, A-alcoholic extract, F.I-Fold Increase F= ((b-a)/a)*100.

Table 5: Synergistic effect for different antibiotics with and without extracellularly alcoholic extract and biosynthesized AgNPs against *S.aureus and E.coli* bacteria.

M.O		*P.aeruginosa*				*C.albicans*				
Ab		Ab+A	F.I	Ab+AgNPs	F.I	Ab	Ab+A	F.I	Ab+AgNPs	F.I
AM 10mg	6	6	0.00	10	66.66	6	6	0.00	12	100
E 15mg	6	10	150	15	66.66	6	14	133.33	10	66.66
DO 30mg	6	9	50.00	13	116.66	6	11	83.33	10	66.66

Ab- Antibiotic, A-alcoholic extract, F.I-Fold Increase F= ((b-a)/a)*100

Note: In the absence of bacterial growth inhibition zone, the disc diameter (6 mm) were used to calculate the fold increase.

silver nanoparticles compared to effects of the combination of Antibiotics with plant extract. The biosynthesized AgNPs inhibited all pathogenic microorganisms. The DNA loses its replication ability and cellular proteins become inactivated upon silver ion treatment (Kumar *et al.*, 2008). Furthermore, higher concentrations of Ag^+ ions have been shown to interact with cytoplasmic components and nucleic acids (Kim, 2007). The antibacterial activities of Erythromycin, Erythromycin, Doxycycline, Ampicillin, Trimethoprim, and Carbencilin antibiotics increased in the presence of AgNPs against all pathogens.

Determination of minimum inhibitory concentration

The MIC of the plant extract detection for *all* isolates was carried out with the following concentrations (0.2, 0.4, 0.6, 0.8 and 0.1 mg/mL). After the incubation period, the result showed that the MIC of the plant extract was 0.8 mg/ml against all microorganisms tested, while the MIC of the Silver nanoparticles synthesized was 0.6 mg/ml against all microorganisms test.

Conclusion

Galls of *Q. infectoria* have been in use since times immemorial to treat wide range of indications, because it contains a lot of active compounds, especially tannin. Also the combination of antibiotic with plant extract and silver nanoparticles would avoid development of resistance by microorganisms and improve the antimicrobial activity of the antibiotic and minimize the dosage of antibiotics against multi drug resistance pathogens.

Acknowledgment

Authors would like to extend their sincere appreciation to the Department of Applied Science Laboratories, University of Technology, Baghdad, Iraq for its experimental assistance. No funding support by our institution. The authors are responsible for their funding support.

Conflict of interest statement

We declare that we have no conflict of interest.

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