Antioxidant and Antimicrobial Activities of *Nerium Oleander* Plant

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Abstract

The green synthesis of Ag nanoparticles was prepared biologically in the presence of *Nerium oleander* extract and examined by UV-Vis spectrophotometer, Infrared (IR) spectroscopy and scanning electron microscopic (SEM) to characterize Ag nanoparticles. The effect of alcoholic extract alone, Ag nanoparticles determined on some pathogenic microorganisms. The alcoholic extract showed no considerable influence on the growth of tested strains, while the synthesised Ag nanoparticles show different effect on the growth of the microbial species. The highest inhibition zone was shown on *E.coli* reaching (31mm) followed by *P. aeroginosa* (30 mm), *S. aurous* with inhibition zone reached to (23mm), *K. pneumonia* (22mm), and *C.albicans* (20mm). The effect of antibiotic (Gentamycin) has been studied alone on microbial growth with inhibition zone (17 mm) on *C. albicans* followed by *E.coli* with (13mm), and finally in *S.aurous* inhibition zone reached (15mm). The antimicrobial effect of (alcoholic extract- Gentamycin) was tested after mixing togathor which showed variotion with the highest effect on *C. albicans* in (19 mm), followed by *S. aurous, and E.coli* in (13 mm, 15 mm) respectively, *K. pneumonia*, *P. aeroginosa* showed no inhibiting effect by alcoholic extract- Gentamycin complex. The ability of crude alcoholic extract to remove free radicals was determined by estimating antioxidant activity. The activity of the plant extract on the DNA molecules *in vitro* showed low absorbance values related to the increase in the concentration of ethanolic extract.

Keyword: Ag nanoparticles, antimicrobial activity, antioxidant activity, green synthesis, *Nerium oleander*.

Introduction

Nerium oleander is an evergreen plant accomplishment up to four meters in height, belongs to family – Apocynaceae, the plant cultivated globally as an attractive plant infrequently tree dispersed in tropical Asia, Mediterranean area [1,2]. And in Southern Europe and Southwest Asia. There have been many reports and papers focesed on the pharmacological and antimicrobial effect of the *N. oleander* as medicinal plants [3]. Husain and Gorse, 2004 investigated the antimicrobial activity of leaves and roots of *N.oleander* extract gainst *Bacillus subtilis, Staphlococcus aurous, Bacillus pumilus, E.coli* and *Aspergillus niger*. The Ag nanoparticles has been synthesized using chemical or physical ways[5]. The current study demonstrates a ecofriendly, less cost approach to synthesis silver nanoparticles from *N.oleander* extract. In this study alcoholic extracts of *N.oleander* utilized as reducing and stabilizing agents. The antimicrobial and antioxidant activity *N. oleander* befor mixing with Ag ion and after mixing has been studied.

Materials and Methods

Preparation of *N. oleander* leaves extract

The leaves of plant were cleaned before dring at room temparture. Dried leaves were ground into powder form, (50) g of dried powder were then subjected to extraction with 80% ethanol by using a soxlatet apparatus. The crude extract obtained was concentrated using rotary evaporator under reduced pressure. Then; the concentrated extract was stored, in a refrigerator at 4°C until use.

The microbial strains and the growth conditions

The test microorganisms included the following gram positive bacteria: *S. aurous*. Gram negative bacteria: *E.coli, P. aueroginosa* & *K. pneumonia*, *Candida albicans* as well. These microorganism were diagnosed in microbiology lab. AL-Alwaya hospital. Baghdad-Iraq.

The test bacterial strains were inoculated into nutrient broth and were incubated at 37°C on a shaker. The inoculum size was maintained as per 0.5 Mc Farland standards ($1x10^8$ cfu/ml).

The activated inoculums were used for antimicrobial assay.

Green synthesis of silver nanoparticles

Leaf extract (10ml) was added to 90 ml of 1m M silver nitrate (AgNO3) solution, kept at room temperature. The synthesized silver nanoparticles were observed by changing the color to brownish [6].

Characterization of green silver nanoparticles

The reduction of Ag^+ ions was monitored by measuring the UV-Vis spectrophotometer with Shimadzu UV-1700 instrument at the range of wavelength 200-800 nm [7]. The shape and size of green synthesized silver nanoparticles was carried out using scanning electron microscopic (SEM) [8].

Determination the antimicrobial activity

The antimicrobial activity of alcoholic extracts, green sunthesized silver nanoparticles, Gentamysin and (alcoholic extracts-Gentamysin) complex were determined on pathogenic strains by agar well diffusion method. The nutrient Agar No.2 medium (Hi-Medium) was inoculated with 200 µl of the inoculums ($1x10^8$ cfu/ml). After solidified media, a well was prepared in the plates with cup - borer (6 mm). The well was filled with 100 µl of the extract, and kept in incubater at 37°C for 24 hr. Results were recorded by measuring the diameter of the inhibition zone. All samples were tested in triplicate.

Antioxidant activity test

Free radical scavenging activity was calculated by using two stages:

Preparation Standard DNA Solution

DNA sample was melted in a solution composed of (0.0015) M of sodium chloride and (0.00015) M of sodium citrate, and at final concentration (5) µg/ml of DNA in pH=7. To investige the effectiveness of plant extract on the DNA molecule *in-vitro* DNA solution recorded above which measured at A260/A280 absorbency equivalent 1.87. This indicates high purity and free of protein and then treated with five concentrations (0.0, 0.1, 0.2, 0.3, and 0.4) µg / ml mixed with (v/v) of DNA, kept in incubater at 37°C for 10 minutes, then the effectiveness of extract on the DNA was determined by measuring the absorbance at 260 nm wave length. The coefficient retail (QB) for nitrogen base pairs by using the following equation [9].

$$QB = \frac{X\,(Con) - X\,min}{X\,max - X\,min}$$

Where QB=Breaking index, X max = high absorbency, X min = less absorbency, and X (Con) = concentrations.

Results and Discussion

In our study, the synthesis of Ag nanoparticles was done by green synthesis method by *N. oleander* plant. Change the color of solution after 24 hours indicate the formation of Ag nanoparticles, also the results of the UV-visible spectroscopy were indicated the synthesis of nanoparticles of apparent broadening peak at 450 nm.

UV-visible spectra is one of the most widely used techniques for structural characterization of silver nanoparticles. Absorption measurement was carried out using UV-visible spectrophotometer at a resolution of 1 nm shown peak at 400 nm this analysis absorbance peak was found at 410 nm which was specific for Ag nanoparticles as shown in Fig.(1).

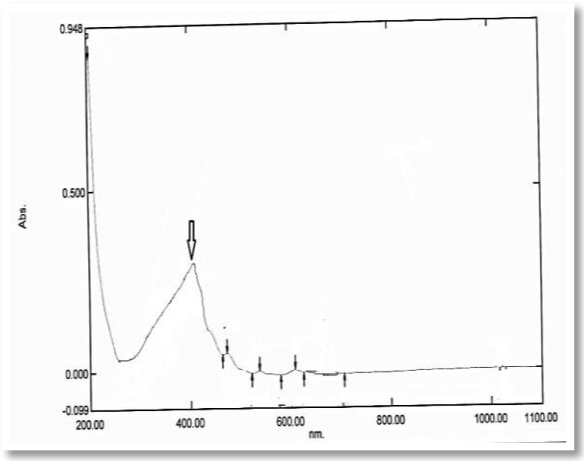

Fig.(1): UV-vis absorption spectra of silver nanoparticles suspension synthesize by N. oleander.

The size and shape of green synthesized Ag nanoparticles by *N.oleander* conformed by scan electron spectroscopy which indicate the formation of uniform nano sized molecules in Fig.(2).

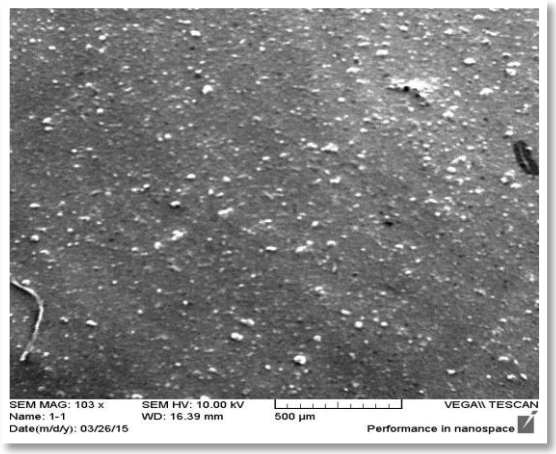

Fig.(2): SEM, Analysis Ag nanoparticles synthesis by of the N. oleander.

Infrared (IR) spectroscopy it is used to measure the functional groups in molecules. Fig.(3) showing absorption peaks at 3377.47 cm^{-1} represented to O-H group and the peak at 2922.25 cm-1 represented to C-H aromatic group, at 2850.88 cm-1 represented to C-H aliphatic group also peaks at 1735.99 represented to C=0 aliphatic ester group also at 1627.97 represented to C=N Lactame group, and at 1516.10 represented to C=C.

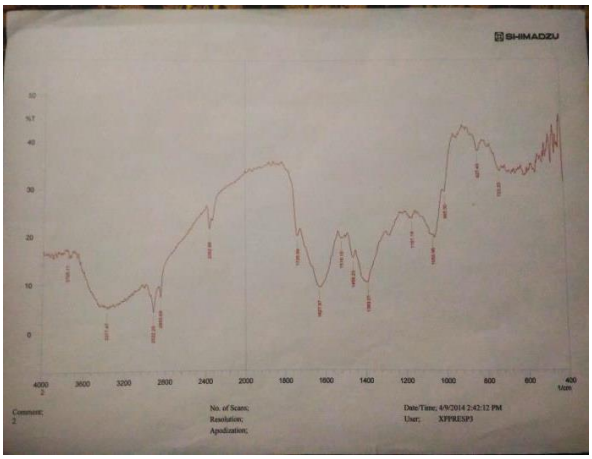

Fig.(3): Spectrum peak pick report.

The antimicrobial activity of *N. oleander* extract was determined by well diffusion method. The results in Table (1) show the effect of extract on growth of *S. aurous* with inhibition zone reached to 22mm, *E.coli* (20mm),*C.albicans* (19mm) finally, *K.pnuomonaie* by inhibition zone reached to 18mm. While the efficiency of green synthesized Ag nanoparticles by *N. oleander* showed the highest effect on *E.coli* with (31mm) and *P. auerogenosa* by inhibition zone reached to (30mm), (23mm) to *S. aurous, K. pnuomonaie* (22mm) and *C. albicans* (20mm).

Table (1)
Antimicrobial activities of the leaf extract of N.oleander expressed as dimeter of Inhibition zone (mm).

Micro - organisms	Inhibition zone in mm	
	Ethanol extract	Ag nanoparticles
S. aurous	22	23
P.auerogenosa	-	30
E. coli	20	31
K. pneumonia	18	22
C.albicans	19	20

The effect of gentamycin and (extract-Gentamycin) complex on tested strains presented in Table (2), the highest effects on the growth of yeast *C.albicans* by inhibiton zone reached to (19mm), then on the growth of *S.aurous* by inhibition zone reached to (15mm), finally in *E.coli* by inhibition zone (13mm), there is no effects on the growth of both *K. pneumonia*, and *P.aeroginosa* bacteria.

Table (2)
Antimicrobial effects of ethanolic extract of N.oleander with Gentamycin, expressed by dimeter of inhibion zone(mm).

Micro - organisms	Inhibition zone in mm	
	N.saturated	Saturated (G+E)
S. aurous	15	15
P. auerogenosa	-	-
E. coli	13	13
K. pneumonia	-	-
C.albicans	17	19

N. saturated = (Gentamycin antibiotic only), Saturated (G+E)=(Gentamycin antibiotic with extract), (-) no effects.

According to the results, the ethanolic extract of *N. oleander* have good effect on gram-positive bacteria and gram-negative bacteria, gram-positive bacteria is more susceptible to ethanolic extracts than gram-negative bacteria this may attribute to their cell wall structure. Several mechanisms have been studied the inhibitory effect of silver nanoparticles on bacteria, the effecincy of silver

nanoparticles towards sulfur and phosphorus is a key element of the antimicrobial effect Ag ions and Ag-based compounds have strong antimicrobial effects depend to its ability to bind with functional groups of proteins, resulting in protein denaturation[10]. The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag + treatment [11,12]. The present study concentrate on the use of plant synthesis of silver nanoparticles with potent antibacterial effect. In phytotherapy, there are potentially important compensation linked with the synergistic connections that might be of diverse antibiotics, or plant extracts or the synergy may be of antibiotic and plant extract. The compensation are (1) obtaining an sufficient healing achieve with moderately small doses, when compared with a artificial medication (2) increase in constancy or bioavailability of the free agents and (3) augmented effectiveness (4) drop of undesirable effects [8,10].

Plant antimicrobials have been established to be synergistic enhancers in that though they may not have some antimicrobial activity only, but when they are taken alongside with typical drugs they improve the outcome of that drug [9]. The synergistic effects obtained could lead to new choices for the treatment of infectious diseases [13].

The results in Table (3) exhibit the effect of different concs of ethanol extract of *N.oleander* with DNA molecule which observed evolution in absorbance with the increase in the concentration of extract, and that explain the effecincy of plant extract on DNase enzyme. Effect of ethanolic extract + DNA H_2O_2 represented in Table (3) shows the ability of plant to eliminate the free radicals from the liberated hydrogen peroxide composite and to increase the concentration of plant extract.

Table (3)
Effect of different on centrations of the plant extract on the pure DNA molecule and the effect of this extract in the removal of free radicals.

Concs. µg/ml	DNA + H_2O_2	Ethanol extract + DNA+ $H_20_2M(1*10^{-5})$
0.0	0.2006	0.275
0.1	0.2167	0.265
1	0.224	0.257
10	0.232	0.239
100	0.242	0.224

Conclusion

N.oleander ethanolic leafe extract showed a significant antimicrobial effect on most pathogenic strains in this study and its use may open new horizons as antioxidant in food industry.

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الخلاصة

تمت دراسة تاثير المستخلص الكحولي ودقائق الفضة النانوية المتكونة بواسطة اوراق نبات الدفلة وقد استخدم المجهري الإلكتروني الماسح (SEM) لتوصيف دقائق الفضة النانوية كما تمت دراسة تاثيرهما على نمو الاحياء المجهرية قيد الدراسة لم يظهر المستخلص الكحولي تاثيرا كبيرا في نمو الاحياء المجهرية المستخدمة في التجربة, في حين تباين تاثير المستخلص النانوي حيث سجلت اعلى منطقة تثبيط للمستخلص النانوي على نمو البكتريا المعوية *E.coli* بقطر (٣١) ملم تليها بكتريا *P. aeroginosa* بقطر (٣٠) ملم تليها بكتريا المكورات العنقودية بمنطقة تثبيط بلغت (٢٣) ملم ثم في بكتريا *K.pneumonia* بقطر (٢٢) ملم واخيرا في الخميرة *C. albicans* (٢٠) ملم. درس تاثير المضاد الحيوي (Gentamycin) على نمو الاحياء المجهرية قيد الدراسة وقد اعطى اعلى منطقة تثبيط في الخميرة *C. albicans* (١٧) ملم تلاها في ذلك البكتريا المعوية *E.coli* بمنطقة تثبيط بلغت (١٣) ملم واخيرا المكورات العنقودية بمنطقة تثبيط بلغت (١٥) ملم, وفيما يخص معقد المستخلص الكحولي والمضاد الحيوي Gentamycin فقد تباين تاثيره على الاحياء المجهرية فقد لوحظ بان هنالك تاثير في نمو الخميرة *C. albican*s بمنطقة تثبيط بلغت (١٩) ملم و *S. aurous, E.coli* بمنطقة تثبيط بلغت (١٥،١٣) ملم على التوالي ولكن لم يظهر اي تاثير يذكر على نمو كل من البكتريا *K. pneumonia , P. aeroginosa*. كما تم التحري عن قابلية هذا المستخلص الكحولي الخام لازاحة الجذور الحرة المتحررة من التحلل الضوئي من خلال انخفاض قيم الامتصاصية, وزيادة تركيز المستخلص المستخدم في هذه الدراسة باستخدام محلول الدنا القياسي.