Biopreparation for antimicrobial material from mixture of nano silver and olive leaves extract

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Abstract: Silver nanoparticles were synthesized using different aqueous concentrations of silver nitrate (0.5,1, 2, 3 ) with olive leaf extract as reductive factor. The reaction showed a strong change in color from yellow to dark brown. Antimicrobial activity against pathogenic bacteria like Staph aureus, E. coli was studied. Spectral properties were studied by using UV- visible spectrophotometer, all concentrations shown peaks at (435)nm .The nanosilver particles tested by Dynamic light scattering (DLS) so the size particles were about (80-126) nm , zeta potential values were (-17 - -23)m V . Biological silver nanoparticles gained interest in recent past owing to its simple preparation, low cost , ecofriendly and their products more stability .

Key words: Olive leaf, Silver nanoparticles, Biosynthesis

Introduction

Ecofriendly process in different disciplines have recently increased and become more demanding due to the increasing environmental problems in the world. Silver nanoparticles are estimated at 500 tons produced during a year [1]. Silver nanoparticles play a major role in the field of biosensor and drugs because of their great inhibitory and antibacterial ability, as well as antifungal, anti-inflammatory, many medical industries and many environmental applications. Many techniques are available for silver nanoparticle synthesis such as ion-sputtering, ion reduction, sol gel, etc. These methods are based on hazardous chemicals and require high energy which is difficult and involves high purification of impurities and will result in a lot of chemical pollution during the synthesis process. So attention has been turned to the technique of obtaining nanoparticles from natural materials being simple, inexpensive and environmentally friendly and the products are more stable.
Numerous plant extracts have been used for this purpose, such as Ezdracht or Neem trees, *Azadirachta indica*, marigold flower, *Ziziphora tenuior*, Olive, *Abutilon indicum*, *Solanum*, *Fig*, *Malava parviflora*, *Phoenix dactylifera*, Pine, Persimmone, Ginkgo, *Magnolia*, Platanus, etc.[2,3,4,5,6,1]. Solving many medical problems by finding nanomaterials that have an active role in biomaterials, due to the increase in new nanoparticles as minerals, metal oxides, polymers, and ceramics were used in the treatment of cancer and pathogens such as Bacteria etc. This is due to the unique characteristics enjoyed by nanomaterials as the ratio of surface to volume is very large, which alters the visual characteristics and structure [7]. Olive leaves contain biomolecules including phenols, alkaloids, proteins, amino acids, alcohol groups, polysaccharides and others. Bio-phenolic molecules such as caffeine and theophylline are responsible for the formation and stability of silver nanoparticles that act as a reducing agent to form silver particles and thus silver nanoparticles [8]. It was recorded that nanoparticles of silver and nanoparticles of silver oxide in their low concentrations are non-toxic to humans and very effective against viruses, bacteria and fungi. Excessive use of antibiotics has led to an increase in the emergence of antibiotic resistance and the emergence of resistance genes. The need for nanoparticles with inhibitory activity of microorganisms has emerged [9] and is therefore used in applications such as disinfection and ointments with external use to prevent infection in burns and open wounds [10]. Used in some medical instruments, textiles, food industry, agriculture, water treatment and also cosmetics.

**Materials and Methods**

**Preparation of Olive leaves extract**

Olive leaves were collected from Olive trees in Al-Jadria. For extract preparation, 20 g fresh leaves were cleaned with fresh water to remove dust and other particles, and then leaves were washed 2-3 times with de-ionized water and let them dried, chopped into small pieces and finally boiled for 30 min at 70 °C in 200 ml deionized water. After boiling, the aqueous extract was separated by filtration with Whatman No.1. Olive leaves extract was stored at room temperature to be used for biosynthesis of silver nanoparticles from silver nitrate.

**Biosynthesis of silver nanoparticles**

Different concentrations of silver nitrate solution were prepared in flasks of 50 ml for each concentration (0.5, 1, 2, and 3) mM, and 50 ml of ionic water were prepared as a control test. All flasks were then wrapped with aluminum foil to provide dark...
conditions to complete the reaction. After 60 minutes of reaction, a color change was observed compared to the control test, the color changed from orange yellow to dark brown. The solutions were taken after filtering through 0.2 micron microfiltration units to make the required measurements.

**Determination of antibacterial activity**

The study included testing of previously prepared solutions containing different concentrations of silver nitrate and olive leaf extract in addition to the control test in inhibiting the growth of Gram positive pathogenic bacteria such as *Staphylococcus aureus* and type of pathogenic bacteria Gram negative like *Escherichia coli* in vitro, this is depending on the McFarland method. The samples were loaded onto 5 mm cellulose disks and bacterial isolates were grown where 0.1 ml of the bacterial and equal suspension was transferred to (1.5 * 10^8) cells / ml, which represents the dilution of the bacteria when measured with a spectrophotometer at 600 nm wavelengths. Inject to the dish containing Muller-Hinton agar media and spread on its surface using the glass spreader. Leave the dish for 15-20 minutes at room temperature until the bacterial culture is diffusion. The tablets loaded with samples were placed and the plates were incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured around the disks for each type of bacteria [11].

**Diagnosis of nanoparticles using DLS**

DLS is an abbreviation for Dynamic Light Scattering. Dynamic light scattering is a device that measured particles size and Zeta potential. The device used for this purpose is Zeta plus American-made, model Brook haven (Zeta potential analyzer) .This measurement is a method to describe the surface charge or the probability of zeta of nanoparticles in solutions. This information is required and necessary to stabilize the appropriate system and prevent agglomeration and aggregation. In another way is the difference in potential voltage between the dispersion of the medium and the second layer of contact liquid [1].

**Diagnosis of nanoparticles using UV-Visible spectrophotometer**

The prepared nanoparticles were diagnosed using UV-Visible spectrophotometer sp-3000 nano( OPTIMA). The suspension was taken after the reaction was completed and measured with spectral range at (200-700) nm. Ionic water was used as a blank solution [13].

**Results and discussion**
Visual observation

A clear color change was observed to indicate the formation of silver nanoparticles compared to the control solution, where they shifted from yellow to dark brown and the intensity of the color increases with increasing concentration Fig. (2) [12].

![Image of color change](image)

Fig. (1): Chromatic change of silver nitrate solution and plant extract compared with plant extract alone

Determination of antimicrobial activity

Pathogenic bacteria show resistance to antibiotics and the emergence of resistant strains significantly therefore, the attention turned to the chemical antibacterial. Silver salts as a microbiological inhibitor were used in the past, in ancient time, but there are some limitations to the use of these salts, so the use of silver in its nanostructures may remove these determinants and this is due to the increase of the surface area of the element in its nanoparticles. In addition, cell membranes have pores of nanoscale so they are easy to penetrate and disrupt the permeability process, thus affecting DNA replication and gene expression [12, 13]. The results of the test showed the effect of silver nanoparticles clearly and more on the pathogenic bacteria of the Gram positive *Staph. aureus* than *E. coli* bacteria, this is consistent with [13] Fig. (2). Inhibition diameters were measured around nanomaterial discs while the extract alone showed no inhibitory activity Table (1.)

Table (1) shows the rate of bio-inhibition diameters against pathogenic bacteria

| Concentration of silver (mM) nanoparticles | *Staph. aureus* | *E. coli* |
|------------------------------------------|---------------|----------|
| 0                                        | 0             | 0        |
| 0.5                                      | 14            | 13       |
| 1                                        | 15            | 11       |
| 2                                        | 17            | 11       |
Fig. (2) shows the antimicrobial activity of silver nanoparticles compared to the extract alone.

### Diagnosing nanoparticles using DLS

Table (2) above and Figures (3-6) illustrate the sizes and distribution of silver nanoparticles, which are determined by the DLS (dynamic light scattering). It is noted that the nanoparticle sizes of the different concentrations used ranged from (80.60 - 126.54) nm. Increase the volume of nanoparticles by increasing the concentration relatively. As shown in Table (2) and Figures (7-10)) Zeta voltage of silver nanoparticles were at the values of Zeta voltage range from (-17.78 to -23.66) mV for different concentrations. This indicates that the surface of the nanoparticles is charged negatively and distributed in the media. The results indicate that concentration 0.5 and concentration 1 were preferable and concentration 0.5

| Concentration of silver nanoparticles(mM) | Effective diameter(nm) | Zeta potential(mV) |
|------------------------------------------|------------------------|--------------------|
| 0.5                                      | 94.59                  | -20.95             |
| 1                                        | 80.60                  | -17.78             |
| 2                                        | 102.42                 | -23.66             |
| 3                                        | 126.54                 | -21.10             |
outperforms due to the near zeta potential value within the range indicating the stability of the particle.

Fig. (3) The size of silver nanoparticles in the suspension at a concentration of 0.5mM

Fig. (4) The size of silver nanoparticles in the suspension at a concentration of 1mM
Fig. (5) The size of silver nanoparticles in the suspension at a concentration of 2mM

Fig. (6) The size of silver nanoparticles in the suspension at a concentration of 3mM

Fig. (7) Zeta potential of silver nanoparticles in the suspension at a concentration of 0.5mM
Fig. (8) Zeta potential of silver nanoparticles in the suspension at a concentration of 1mM

Fig. (9) Zeta potential of silver nanoparticles in the suspension at a concentration of 2Mm

Fig. (10) Zeta potential of silver nanoparticles in the suspension at a concentration of 3Mm
Diagnosis in the visible-ultraviolet spectrophotometer

Verification of the reduction of silver ions to silver nanoparticles was also done by taking the suspension after the reaction was completed and then measured in the UV-visible spectrophotometer light to measure the absorption spectrum at range 200-700 nm. The results showed that the absorption peak appeared at 435 nm wavelength, this is consistent with [12].

Conclusions

It is clear from the present study that the substances in the olive leaf extract have the ability to reduce the silver ion and convert it to nanosilver particles. The size of bio-prepared nanoparticles can be easily examined by using different concentrations of silver nitrate with Olive leaf extract.

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