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Study of biosynthesized silver nanoparticles from *pseudomonas aeruginosa* and antioxidant activity

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***Abstract*---**This research, it was briefly described how biosynthesis silver nanoparticles from Pseudomonas aeruginosa, the synthesized AgNPs were initially noticed through visual color change from yellow to reddish brown and the synthesis was confirmed by multiple tests like surface plasmonic resonance (SPR) UV-visible spectrophotometer, Morphology and size of AgNPs were determined by scanning electron microscope (SEM)to verify its properties this material is nano, and this material was also tested in DPPH assay To ensure that it kills free radicals when used in the medical field like used silver nanoparticles in the treatment.

***Keywords*---**silver nanoparticles, pseudomonas aeruginosa, SEM, DPPH assay.

Introduction

Nanotechnology is a science with various specializations that deals with different aspects of research and technology at the nano level (Kannan *et al.*,2010) is a new branch of nanotechnology that combines physical and chemical procedures with biological principles to be used to synthesize nano-sized particles with specific functions represents an economical alternative to chemical and physical methods of nanotechnology(Ahmed *et al.*,2003)

Nanoparticles have special and enhanced chemical and physical properties characteristics compared to its bulk materials, these properties of nanoparticles include their different shape, size and structure, as well as crystallization, in addition to its large surface area of small volume ratio homogeneity and other

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features will give us useful information about nano systems (Ramezani *et al.*,2008)

There are types of nanoparticles including (silver, gold, magnetic, Tio2) studies have shown that silver nanoparticles are one of the most effective materials because of its strong antimicrobial efficacy against bacteria viruses and other true microorganisms (Rai *et al* ,2009) undoubtedly, it is one of the most widely used nanomaterials among all so it is used in textile industries for water treatment, sunscreen lotions, etc (Sharma *et al* ,2009)

Nanotechnology is described as two different processes: bottom to top and top to bottom, in the process of top-down nano the scale structure is made of large scale structure for different technologies in the bottom-up process, nanoparticle structures consist of an atom by atom or molecule by molecule by self-assembly or self-organization (Kasthuri and Veera pandiran,2009 & Dubey *et al.*,2010)

There are different types of methods that are used, including physical and chemical methods in the synthesis of nanoparticles, but in order to use these methods requires different factors, including strong and weak chemical reducing agents and protective agents most of them are toxic and flammable and cannot be disposed of easily due to environmental factors issues, low production rate and high temperatures for synthesis Practical in addition to being capital intensive and inefficient in it materials and energy use(Mohanpuria *et al.*,2008&Rai *et al.*2008)

one of the biological methods used to synthesis nanoparticles biological factors namely green algae, fungi (Bhainsa and D'Souza ,2006) bacteria(Chaudhari *et al.*,2012)Actinomyces (Mohanpuria *et al.*,2008) and plant extracts (Singhal *et al.*, 2011). the biological method uses a wide range of resources for the synthesis of nanoparticles, biological agents secrete a large amount of enzymes capable of decomposing metals and thus producing them enzymatic reduction of metal ions (Rai *et al.*, 2009)

Material and Method

Biosynthesized silver –nanoparticles

We have used silver nitrate (AgNO3) as a precursor to nanoparticles for the biosynthesis of silver by *Pseudomonas aeruginosa* this bacteria isolated from different sources and confirmed by biochemical test Whereas, silver nitrate (1 mm/L) was added to the cell-free supernatant of *Pseudomonas aeruginosa,* which is used in flask, this step was made under dark conditions in order to avoid the oxidation of AgNO3, the pH of the reaction mixture was adjusted to 8 with Naoh (2M),the result was incubated in a 150 rpm shaking incubator at 37°C for 24 hours, the mixture was centrifuged at 6000 rpm for 25 minute at 4 C then supernatant replacing it with deionized distilled water and re-centrifuging for three times at the same speed and time to remove the supernatant, the granules were deposited at the bottom of the tube which is an array of nanoparticles and then dried in an oven at 40°C for 18- 24 hours. the dried powder was carefully collected and the samples stored in vials for further analysis (Chaudhari *et al.*,2012; Sarvamangala *et al.*,2013; Sumi *et al.*,2015)

Charecterization of Sliver –Nanoparticle
UV-Visible spectrophotometer analysis

After periodically diluting the reaction mixture with deionized distill water and subsequently measuring the UV-visible spectrum of the reaction mixture with a UV-Vis spectrophotometer, the color change of the reaction mixtures was monitored,silver nanoparticles were analyzed using UV-Vis spectrophotometer spectrophotometer (Shimadzu 1600) at the chemical department laboratory in al Qadisiyah University College (Caroling *et al.*,2013)

Scaning electron microscope (SEM)

We used scanning electron microscopy (SEM) to characterize the morphology and size of nanoparticles in the electron microscopy unit at the College of Science/University of Kufa, and then we prepared the sample by adding a small drop of nanoparticle bioparticle suspension onto a silicon wafer. left to dry and then analyzed by (SEM),(Caroling *et al.,* 2013)

DPPH assay

The radical cation DPPH method was transformed in order to evaluate the free radical removal effect of 100 pure Chemical compounds DPPH reagent was DPPH (8 mg) dissolved in MeOH (100 mL) to concentrate the solution80 µl/ml in order to determine the scavenging efficacy, 100 µl we did by mixing DPPH reagent with 100 µl of the sample at 96 A fine microplate where it was incubated at room temperature For 30 min after incubation, absorbance was measured we used a 514 nm ELISA reader and 100% methanol was used as a control

$$\text{Radical scavenging } \% = \frac{(A)\text{control} - (A)\text{sample}}{(A)\text{control}} * 100$$

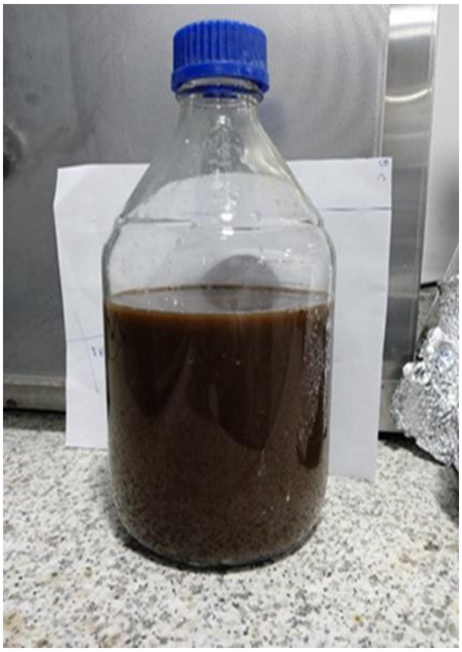
The IC50 DPPH values (the concentration of sample required for inhibition of 50% of DPPH radicals) were obtained through extrapolation from regression analysis. the antioxidant was evaluated based on this IC50 value(Lee *et al* .,2015)

Result and Discussion
Biosynthesized silver –nanoparticles

After choosing the efficient isolate from *pseudomonas aeruginosa* and appear the bacteria in MaconCky agar pale and have grape odor , beta heamolysis in blood agar and positive for oxidase , we use it to synthesis a large amount of silver nanomaterial by adding 1 mm of silver nitrate to each liter of BHI, where we incubate it in the shacking incubator for 24 hours, as well as equilibrating the mixture to PH (8) in order to obtain an alkaline medium after the incubation period, the color will change from yellow to brown shown in figure (1), then we wash this solution through the centrifuge three times, get rid of the supernatant and collect the precipitate, and then use the oven to dry similar to (Singh *et al*

.,2018) the organisms generally have mechanisms for detoxifying metals this includes isolating the metal in the cytoplasm by concentrating it in transforming it (e.g., by oxidation or reduction) into a harmless form in the organism (Brown& Moses.,2020) the use of biological methods to biosynthesize is a developing part and the scientists have been viewing with interest microorganisms as probable eco-friendly nano-factories(Iravani.,2011) the color that metallic nanoparticles show is a result of cohesion excitation of free electrons completely within the conduction band, resulting in Surface plasmon resonance (SPR) (Sarvamangala *et al.*,2013)

A



B

Figure (1) biosynthesis of silver nanoparticles

A-original color(yellow)for supernatant with out $AgNO_3$
B-brown color after adding $AgNO_3$

Charecterization of Sliver –Nanoparticle
UV-Visible spectrophotometer analysis

UV - Visible Spectrum is a proven method for nanoparticle analysis. 24 hours after the reaction was incubated the mixture, we will notice the color change which indicates the composition nanoparticles in the reaction mixture,biosynthesis of nanoparticles it can be confirmed by visual observation and measuring the surface plasmon resonance (SPR)using UV visible Spectroscopy , the absorption spectrum of nanoparticles formed in the reaction mixture has an absorption peak at(420 nm) shown in figure (2)his an evidence of the presence of surface plasmon resonance (SPR) of nanoparticles and a single SPR band indicates that nanoparticles have spherical shape that is nearby (Kumari *et al.*, 2017)

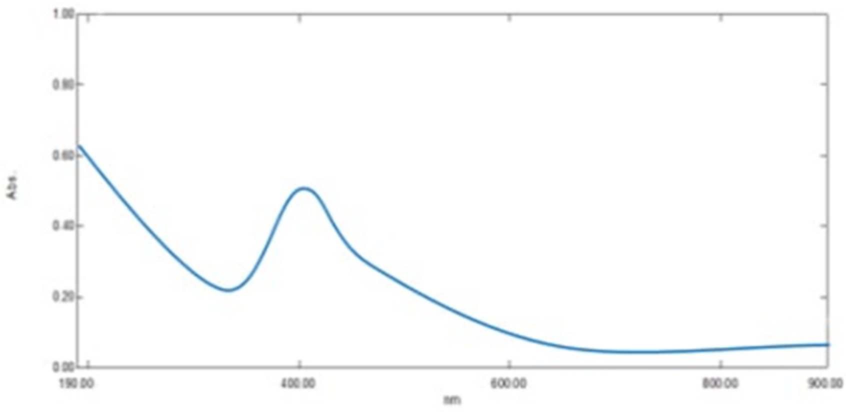


Figure (2) UV–visible absorption spectrum of AgNPs synthesized by *pseudomonas aeruginosa*

Scaning electron microscope

Scanning electron microscopy (SEM) was used to characterize the shape, size and distribution of nanoparticles, after biosynthesis of silver nanoparticles using cell-free supernatant of *pseudomonas aeruginosa*, and the results showed that nanoparticles are well dispersed and homogeneous with a diameter (22.33-73.80 nm)shown in figure (3) relative to silver nanoparticles with the variable shapes are mostly spherical, the average total sizes of the silver nanomaterial that appeared in the scanning electron microscope is (40.18) and its shape is spherical the concentration of AgNO3 which was added to the supernatant also have effect on characterization of formed nanoparticles, the best concentration in the biosynthesis of AgNPs was 1mM AgNO3 and our result agreed with (Geoprincy *et al* .,2013, Ramalingam *et al.*,2014)

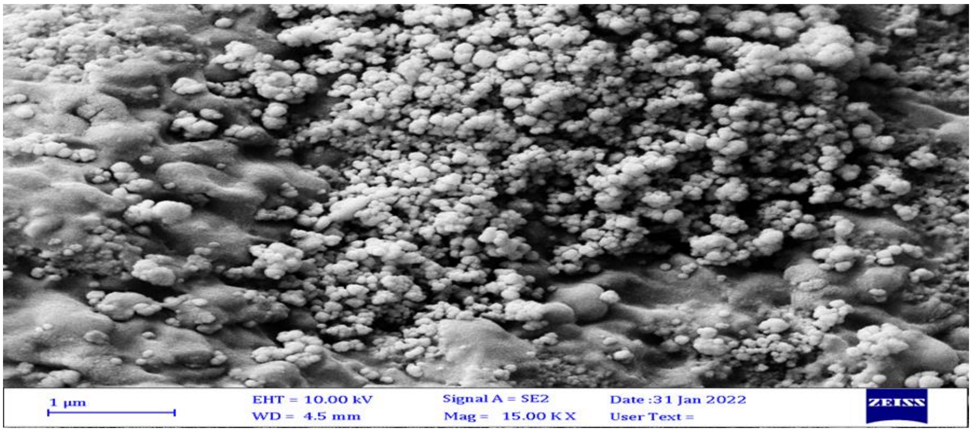


Figure (3) SEM of silver nanoparticles synthesized by *pseudomonas aeruginosa*

The shape of AgNPs was spherical and homogenous, size between (22.33-73.80nm)

DPPH assay

DPPH is the most stable and well-known free radical base concerning the reduction of hydrogen or electron acceptance from donors, the DPPH reducing ability of the AgNPs was evaluated by observing the color change, that indicated by observing the color change from the original color of DPPH (purple) into (yellow) color and the control does not show any color change, DPPH scavenging assay showed effective inhibitory activity of AgNPs when compared to the standard when addition of AgNPs in DPPH solution, the color change was occur as a result of DPPH scavenging due to donate a hydrogen atom to stabilize the DPPH molecule it is responsible for absorbing 517 nm that result shows(67.2% in 514 µg),(64.1% in 256 µg),(58.3% in 128 µg)and (45.1 % in 64 µg) figure (5) nearyby to (Manikandan *et al.*,2021) DPPH scavenging activity of nanoparticles increased with increasing their concentration that indicated by the elevated percentage of inhibition of DPPH which increased with increase concentration of AgNPs that exhibited more inhibition (67.2% in 514),(64.1% in 256) for AgNPs respectively due to more an electron donated and accepts by DPPH(Bhakya *et al.,*2015) the different mechanisms involvement in radical antioxidant reactions may explain the difference in Vehicle scavenging capabilities. antioxidant mechanisms not only does it remove free radicals, but also inhibits production from free radicals (Xing *et al.,*2015)

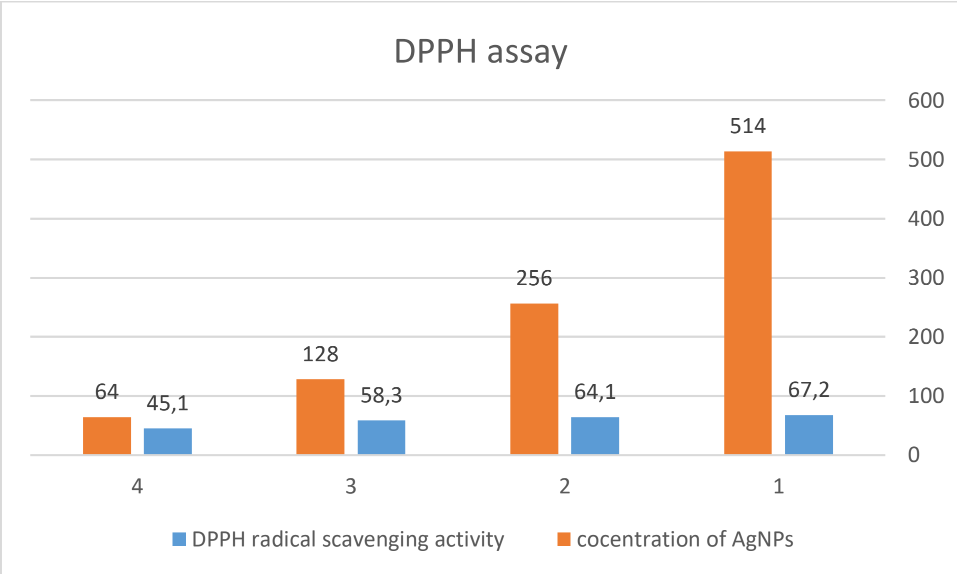


Figure (5) show effect silver nanoparticles to DPPH assay

Conclusion

AgNPs have emerged as a typical antimicrobial nanomaterials applied in industry, daily life and medicine. due to the strong activity of AgNPs and the release of Ag ions, and attracted biological properties and safety huge interest from scientists in the modern era, a simple, stable and environmentally friendly method of biosythesis silver nanoparticles were synthesized from *pseudomonas aeruginosa* and it was confirmed by UV-spectrophotometer, SEM studies, that nanoparticles are well dispersed and homogeneous with a diameter (22.33-73.80 nm) . biocombined AgNPs have been found to be so multifunctional with good antioxidant activities, this is amazing the biosynthesis method facilitates a better alternative to both other chemical and physical methods. hence this method it can be used in large-scale production and can be used in many medical and technological applications

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