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Study of effect bio synthesized silver nanoparticles from *Proteus mirabilis* and on anti-oxidant activity

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***Abstract*---**Microbes that cause disease and have developed resistance to pharmacological treatment are becoming an increasingly serious threat to public health. Because of this, the research and development of novel bactericides is a pressing necessity. This work focuses on the production of silver nanoparticles, which were synthesized from *Proteus mirabilis* and then had their shape and size distribution evaluated by particle size distribution analysis. Investigation using an analyzer and a scanning electron microscope (SEM). Aqueous medium's ultraviolet-visible (UV-VIS) spectrum. The silver nanoparticles made from *Proteus mirabilis* showed their antioxidant ability in vitro by scavenging DPPH free radicals. The highest inhibition titer was found in the mixture of DPPH and silver nanoparticles at 512g/ml (70.001%), while the lowest was found at 32mg/ml (59.597 %).

***Keywords*---**Proteus mirabilis, anti-oxidant, silver nanoparticles.

Introduction

Anti-microbial resistance to many different classes of antibiotics has happened because antibiotics have been given to people in the wrong way. This will definitely have a big effect on the future effectiveness and use of antibiotics in both community and hospital care around the world. (Ventola, 2015).

Nanotechnology has brought a fresh perspective on the creation of new processes and goods. It can be used for multiple purposes, including promoting human health benefits and increasing agricultural and industrial output. As a result, nanoparticles have been utilized for several purposes, including drug delivery, the

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development of antimicrobials to combat bacterial resistance, and insect control (Sekhon,2014; De Oliveira ,2021).

The emergence of pathogens resistant to antibiotics poses a global hazard to public health. In some instances, bacteria exhibit resistance to various drugs. This has necessitated the creation of novel therapeutic agents. As nanotechnology emerges as a cutting-edge field in interdisciplinary science, the application of NP-based medicines to combat pathogenic bacteria has emerged as an essential option. Numerous NPs have been investigated for their antibacterial capabilities. (Zinjarde, 2012)

Today, the evolution of biotic systems, particularly microorganisms (MOs), is used as a novel method for the synthesis of Nano materials. (NMs) (Ibrahim, 2015; Saha *et al*, 2017).

Biological approaches to the synthesis of NPs are superior to chemical and physical procedures because they are environmentally friendly, sustainable, and cost-effective. Therefore, the utilization of prokaryotic organisms and their supernatant in the synthesis of NPs attracts the interest of Nano biotechnology experts (Sengani *et al.*, 2017).

Materials and Methods

Source of microorganism

Urine, burns, and wounds were some of the types of samples that were collected. the urine specimens collected mid-stream from bladder and kidney stones and urinary tract catheter patients, in sterile plastic bottle ,while the sample burns and wounds collected by sterile cotton swab, all samples transfer to laboratory and culture on to blood agar and macConkey agar medium and incubation at 37°C for 24 hours. Isolated, then repeatedly purified until pure isolates were obtained, and then subjected to macroscopic, microscopic, and specialized biochemical testing (MacFaddin ,2000).

Confirmation of *proteus mirabils* identification

Isolates were analyzed morphologically and biochemically, then compared to the identification table for confirmation of their identities. in addition to the confirmatory tests based on morphology and biochemistry

Bio Synthesis of silver nanoparticles

For the biosynthesis of silver nanoparticles, the bacteria *Proteus mirabilis* used cell-free supernatant and silver nitrate (AgNO3) as precursors. the concentration of AgNO3 in the cell-free supernatant of *Proteus mirabilis*, 1 mM was added. In order to prevent the oxidation of the AgNO3, this step had to be prepared in a dark environment. The pH of the mixture produced by the reaction was adjusted to (8.3). The resulting solutions were then put into an incubator with shaking at a rate of 200 rpm and 37 degrees Celsius for a period of 24 hours.

After incubation, a change in color was observed, and the reaction mixture was centrifuged at 6000 rpm for 20 minutes. The supernatant was then discarded and replaced with deionized distill water, and the centrifuge was repeated three times at the same speed and time to remove any remaining supernatant. The pellet that deposited at the bottom of the tube represented the collection of nanoparticles, and it was then dried in an oven at 40 °C for 18 to 24 hours The powder was collected carefully, and then it was placed in sample vials so that it could be analyzed further (maria *et al.*,2015).

Characterization of silver nanoparticles
UV-Visible spectroscopy

After periodically diluting the reaction mixture with deionized distill water and then measuring it with a UV-Vis spectrophotometer, a UV-Vis spectrometer was used to monitor the color change of the reaction mixtures. This was done by measuring the UV-visible spectrum of the reaction mixture (Caroling *et al.*,2013)

Scanning electron microscope (SEM) analysis

Scanning electron microscopy, also known as SEM, was utilized in order to determine the morphology of nanoparticles as well as their sizes. The unit of electron microscopy in the Science faculty building at the University of Kufa was responsible for analyzing the biologic AgNPs. The specimen was prepared by first grinding up some silver nanoparticles (AgNPs), then creating a colloidal suspension of the AgNPs, and finally adding a droplet of the suspension to the matrix that was being fixed. After that, the sample was dried and analyzed with a SEM. Imaging was performed with an accelerating voltage of 12.5-15 KV, a low vacuum mode, a spot size of 5, working distances of 5-10 mm, and various magnification powers (Caroling *et al.,* 2013).

Antioxidant activity of silver nanoparticles

The DPPH assay was used to evaluate the amount of free radical (anti-oxidant) scavenging activity exhibited by the AgNPs (Thaipong *et al* .,2006). As a means of providing a negative control, an ethanolic solution of DPPH that was brought to its full concentration of 100 µL was prepared. In a similar manner, water containing various concentrations of silver nanoparticles (32, 64, 128, 256, and 512 g /mL) was prepared. The reaction mixture was prepared by combining 100 µL of silver nanoparticle solution with 100 µL of DPPH solution. The silver nanoparticle solution had a range of concentrations. The reaction mixture was shaken for the entirety of the thirty minute incubation period that took place at room temperature, and the absorbance was measured at 517 nm using a 96-well plate. The percentage of scavenging activity can be calculated based on the change in absorbance in comparison to the control (which only contained DPPH). The TROLOX solution (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control throughout this experiment. The experiment was carried out twice for accuracy.

The amount of free radical inhibition provided by AgNPs was determined using the following formula (Lee *et al.,* 2015) the formula for calculating the percent of inhibition is =((OD control-OD sample))/ODcontrol x 100.

Result and Discussion

Isolation of *Proteus mirabilis* by chemical method

The Proteus isolates were first described as belonging to the genus Proteus by swarming on blood agar, and the bacteria on McConkey agar were pale and didn't lactose fermented and on chromogenic agar good growth light brown colony According to the biochemical properties, the species of *Proteus mirabilis* were identified in this study and used Bio synthesis AgNPs.

Biochemical Tests		
test	*P. mirabilis*	*P. vulgaris*
Catalase production	+	+
Oxidase production	-	-
Urease production	+	+
Gelatinase production	+	+
Motility Test	+	+
(Triple sugar iron agar) TSI	Acidic slant / alkaline bottom, H2S production	Acidic slant / alkaline bottom, H2S production
Indole production	-	+
Methyl red test	+	+

Bio Synthesis of silver nanoparticles

The reduction caused the color of the bacterial supernatant to change from light yellow to dark brown, and the color got darker as the incubation time went on. This shows that SNPS can be made through the bio-reduction of Ag ions(Kalu *et al* .,2012)

The findings agreed with those of Karthick *et al* (2013). The color change of the supernatant solution from pale yellow to dark brown observed by naked eye after the addition of AgNO3 indicated the formation of Ag Nano-particles in the reaction mixture.

A B

Fig (1) Showed (A) Bacteria Culture Supernatant (BCS) without $AgNO_3$
(B) Culture Supernatant of bacteria Mixture with 1mM $AgNO_3$

UV- Visible spectrophotometer analysis

As a result of this, the greatest absorption peak of the silver nanoparticles in the colloidal solution was found to be centered at 420 nm during the UV spectroscopic analysis of the colloidal solution. The plasmon resonance band demonstrates a strong absorption, which suggests that the particles in solution do not aggregate to a significant degree. The brown hue that is absorbed is caused by the activation of surface plasmon vibrations in the particles, and surface plasmon absorption is highly dependent on the particle size, shape, dielectric medium, and chemical environment around the nanoparticles that are disseminated in water (Mariselvam *et al.,*2013; Vasyliev *et al* .,2020).

The result agree with Desai,*et al* (2012) ware said The increase in intensity of the SPR peak could be due to increasing number of nanoparticles formed as a result of reduction of silver ions present in the aqueous solution.The surface Plasmon band in the silver nanoparticles solution remains close to 410 nm throughout the reaction period, indicating that the particles are dispersed in the aqueous solution with no evidence for aggregation.

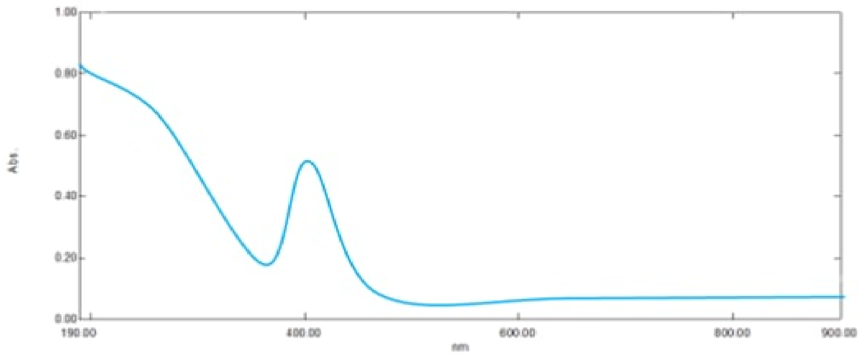

Figure (2):UV–visible absorption spectrum of AgNPs synthesized *Proteus mirabilis*

Scanning Electron Microscopy (SEM) analysis

The results showed well-dispersed nanoparticles and homogenous with diameter of (35-100nm) for silver nanoparticles with variable shapes most of them present in spherical form Figure (3).

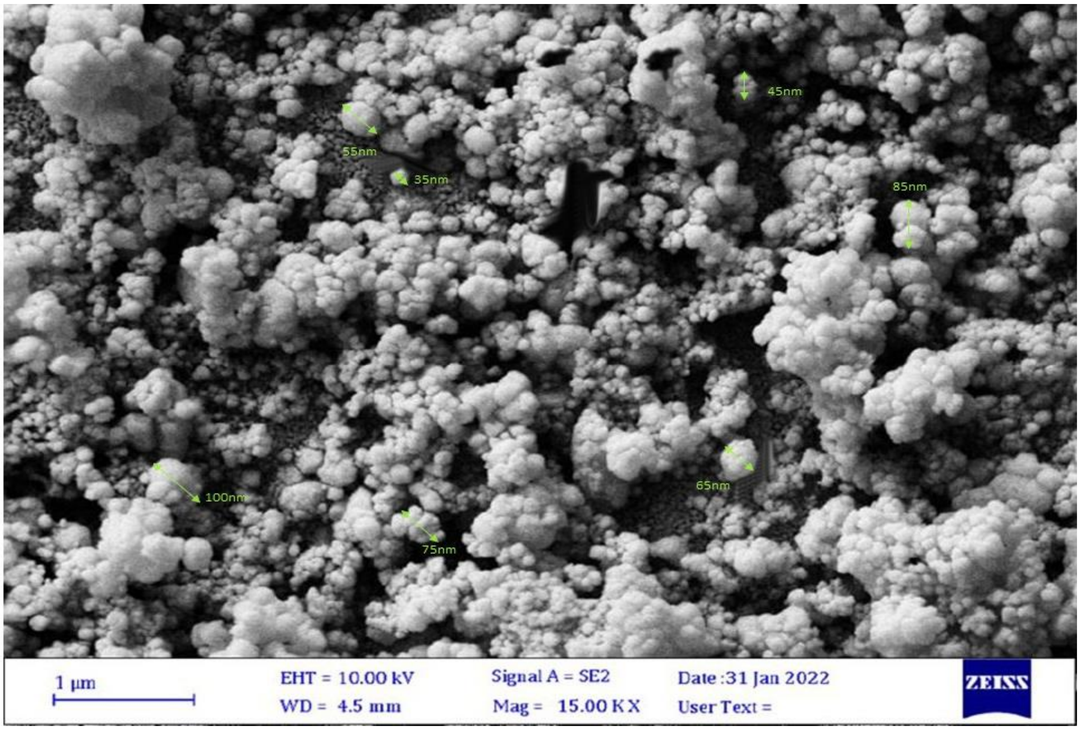

Figure (3) : SEM Micrograph of AgNPs Bio synthesis from *Proteus mirabilis*

The Proteus mirabilis were responsible for the synthesis of the AgNPs, which were spherical in shape and ranged in size from 35 to 100 nm. The amount of AgNO3 that was added to the supernatant had an effect on the characterization of the nanoparticles as well; the 1 mM concentration was the optimal amount for the biogenesis of AgNPs when compared to the other concentrations.

The size and shape of biogenic nanoparticles were analyzed with a scanning electron microscope. The results of the experiments showed well-dispersed nanoparticles with a diameter of (35-100nm) for silver nanoparticles biosynthesized from Proteus mirabilis. A previous study on the biosynthesize of silver nanoparticles from Lactobacillus sp. produced nanoparticles with a size of (30-100 nm) (Aldujaili *et al.*,2015 ; Abdulhassan .;2016)

Antioxidant activity of silver nanoparticles

The antioxidant capacity of the AgNPs was evaluated in vitro using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. This was accomplished by lowering the concentration of DPPH free radicals. After introducing the nanoparticles to a solution of DPPH (100 M), the absorbance (A) was measured at 517 nm after being kept in the dark for 30 minutes. The findings demonstrated that nanoparticles had the potential to scavenge DPPH free radicals, as determined by the following formula (Lee *et al.,* 2015; Kiran *et al.*,2020)

$$\%\ \text{of inhibition} = \frac{(OD\ control - OD\ sample)}{\text{ODcontrol}} x\ 100$$

Table (1)
The results of antioxidant % for AgNPs at deferent concentration

AgNPs co.	OD sample	OD control	Result antioxidant%
512 μg/ml	0.4354	1.4514	70.001
256 μg/ml	0.4854	1.4514	66.556
128 μg/ml	0.5264	1.4514	63.731
64 μg/ml	0.5642	1.4514	61.127
32 μg/ml	0.5864	1.4514	59.597

In vitro data showed that biosynthesized AgNPs had antioxidant properties. These findings are in line with what he had previously discovered. Positive DPPH tests performed by Khan *et al.* (2013) showed that AgNPs are free radical scavengers. The DPPH scavenging assay showed that the AgNPs were more active than the standard in this case (i.e., ascorbic acid) Researchers discovered that the nanoparticles' DPPH activity increased with increasing concentration. As the concentration of AgNPs rose, so did their reducing power. Antioxidants with good activity were found in silver nanoparticles, according to Gecer, E. N. (2021). However, further study into medical applications, such as cancer and in vivo, is required.

Conclusion

Conclude our findings, we can say that *Proteus mirabilis* is capable of creating silver nanoparticles and that those nanoparticles are highly stable in solution. The produced silver nanoparticles demonstrated significant antioxidant, through their ability to inhibit free radicals represented of (DPPH) which have a bad effect on the body The biogenesis of silver nanoparticles has shown that these particles have the potential to be candidates for use in medicinal applications where antibacterial activity is needed. Additional research in this area might one day result in advances in the medical practices used to treat illnesses caused by microorganisms.

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