Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024
Journal Homepage: https://djvs.uodiyala.edu.iq
https://doi.org/10.71375/djvs.2024.02404

Evaluation of Anti-bacterial Activity of Gold Nanoparticles *in-vitro*

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Abstract:

Gold nanoparticles were used in recent years in different applications for medicinal purposes. The dominant traits of GNPs include a great surface area, optoelectronic properties, limited toxicity, and excellent biocompatibility. The aim of this study was to investigate the antibacterial activity of gold nanoparticles. In this study bacterial isolates from humans and animals were applied to investigate the anti-bacterial effect of gold nanoparticles by using well diffusion method with different doses of gold nanoparticles solution. The result was shown that gold nanoparticles solution has anti-bacterial effect through the inhibition zone of bacterial species growth of *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. and *Serratia* spp. isolated from humans and E.*coli*, *Staphylococcus* spp. isolated from animals treated with gold nanoparticles at doses of 10 µg /mL and 20 µg /mL. The conclusion: Gold nanoparticles have anti-bacterial activity for different bacterial species that may be used in the future in the pharmacological applications and treatment of diseases.

Keywords: Gold nanoparticles, Antibacterial activity, Gram-negative, Gram-positive

Introduction

Nanotechnology refers to the scientific and engineering processes that encompass the synthesis, characterization, design, and utilization of devices and materials with at least one-dimensional functional structure on the nanometer scale, which is equivalent to one billionth of a meter (1). Materials that range in size from one nanometer to one hundred nanometers are referred to as nanoparticles (NPs). In Greek, the word "nanos" means "dwarf," and this is where the term "nano" gets its meaning (2). In the last few years, nanoparticles have garnered significant interest due to their remarkable characteristics, such as excellent resistance to oxidation, antimicrobial activity, and high thermal conductivity (3, 4). Additionally, materials in nanosizes demonstrated properties such as electrical, magnetic, mechanical, optical, and chemical quite different compared to their bulk form (5).

Three categories are often used to categorize nanoparticles (NPs) according to their composition: carbon-based, inorganic, and organic (6). Among the different kinds of nanomaterials, metal nanoparticles, particularly gold nanoparticles (GNPs), are attracting significant attention from diverse fields of science. Due to their distinctive features, they are extensively used in both non-medical and medical applications as ideal materials (7).

GNPs can be synthesized in different sizes and shapes based on the method by which they are synthesized. Including Au nanorods, nanocages and nanoshells (8). These differences can change their properties. Usually, these particles are produced using two main approaches, known as bottom-up and top-down. Furthermore, they may also be classified into chemical, physical and biological methods (9).

The dominant traits of GNPs include a great surface area, optoelectronic properties, limited toxicity, and excellent biocompatibility. Due to these properties, they are considered a remarkable instrument in the field of biotechnology (10).

The shape and size of GNPs have a significant impact on their features, hence influencing their compatibility, mobility and stability (11). The chemical and physical characteristics, such as fluorescence, chemical reactivity and electrical conductivity, of materials at the nanoscale often exhibit notable distinctions compared to their larger counterparts. An pattern for the note is the yellow color of gold when in bulk form, which turns red when its size is between 1 and 100 nm as well as blue or purple for sizes larger than 100 nm. Moreover, when comparing colloidal gold to bulk gold, its considered highly reactive. Hence expanding it is range of applications. providing antioxidant, catalytic, and optoelectronic properties, in addition to prospective for external modifications (12). In this study was evaluated the antibacterial potential for gold nanoparticles.

Method

Gold nanoparticles

In this study, rod gold nanoparticles coated with sodium citrate were used, their dimensions were 38 nanometers in length and 10 nanometers in width. This information is based on manufacturing Sigma, batch number MKCS7674. With the liquid form, brown color, PH 7, and SPR 785 nm properties.

Antibacterial activity of Gold nanoparticles

The antibacterial effectivity of gold nanoparticles was evaluated using the disc diffusion method against a variety of G+ and G- bacteria for different sources from human and animal.

The animal source microorganisms utilized in this study included *Escherichia coli*, *Staphylococcus* spp. and human source microorganisms, such as *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., and *Serratia* spp. were utilized and isolated in the microbiology lab, at College of Vet. Medicine, University of Diyala, Iraq. The procedure was carried out in the following manner: The isolated microorganisms cultured in a nutrient broth and then incubated for 24 hours to promote growth at a temperature of 37 ºC. From the nutrient broth tube suspension a volume of 0.1 ml of each bacterial mixture was spread uniformly over the nutrient agar surface, and then it was incubated for a period of 24 hours at 37 ºC. A particular bacterial cluster growing on the nutrient agar was transferred keen on a test tube containing 5 mL of normal saline. This created a bacterial

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E-ISSN:2958-6178
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suspension with a moderate level of turbidity, similar to that of a standard turbidity solution. The resulting concentration was approximately $1.5x10^8$ CFU/mL. A portion of the bacterial suspension was carefully transferred via a sterile cotton swab. It was then evenly spread across a previously prepared Mueller-Hinton agar medium. Then it was left undisturbed for 10 minutes. Three wells with a diameter of five millimeters were created on the surface of the Mueller Hinton agar medium (13). The agar discs were removed, and these wells were then filled with 50µl of GNPs suspensions of varying concentrations by using a micropipette, the first well filled with 10 µg/ml, the second well filled with 20 µg/ml and the third well filled with zero concentration (distal water). Subsequently, they were incubated overnight 18-24 hours at 37 ºC. Afterward the cultivation period, diameters for inhibited zones were measured in millimeters (mm) by using a ruler (14).

Analysis of statistic

Collected data was statistical analyzed by SPSS software. The results were by applying ANOVA and T tests and expressed as Mean ± SD with significance at P value ≤ 0.05.

Result

The result showed that the antibacterial activity for gold nanoparticles against both G+ and G- bacteria isolates from humans and animals exhibited an inhibition zone against the microorganisms used in this study, as shown at the Table 1 and 2 and Figure 1 and 2.

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Table (1): Presented the inhibition zone of bacterial species *E.coli*, *Staphylococcus*, *Streptococcus* and *Serratia* isolated from humans treated with gold nanoparticles (GNP) at doses of 10 µg /mL and 20 µg /mL

Human sources Bacterial Sp.	**Inhibition zone (mm)**	
	GNP 10 µg/ml	GNP 20 µg/ml
E.coli	15.33 ± 0.58	20.67 ± 1.15 #
Staphylococcus	14.67 ± 0.58	16.67 ± 0.58 #
Streptococcus	12.67 ± 0.58	21.33 ± 0.58 #
Serratia	13.33 ± 2.89	20 ± 3.46 #

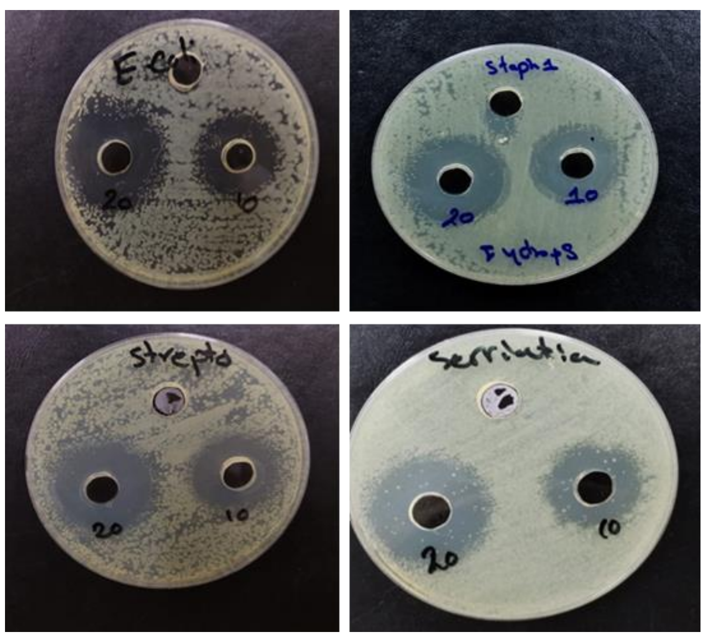

Figure (1) Presented the inhibition zone of bacterial species *E.coli*, *Staphylococcus*, *Streptococcus and Serratia* isolated from humans treated with gold nanoparticles (GNP) at doses of 10 µg /mL and 20 µg /mL

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Table (2) Presented inhibited zone for bacteria species *E. coli* and *Staphylococcus*, isolated from animal treated with gold nanoparticles (GNP) at dose of 10 µg /mL and 20 µg /mL

Animal sources Bacterial Sp.	Inhibition zone	
	GNP 10 µg/ml	GNP 20 µg/ml
E.coli	15.33 ± 0.58	15.67 ± 1.15
Staphylococcus	16.33 ± 0.58	16.67 ± 0.58

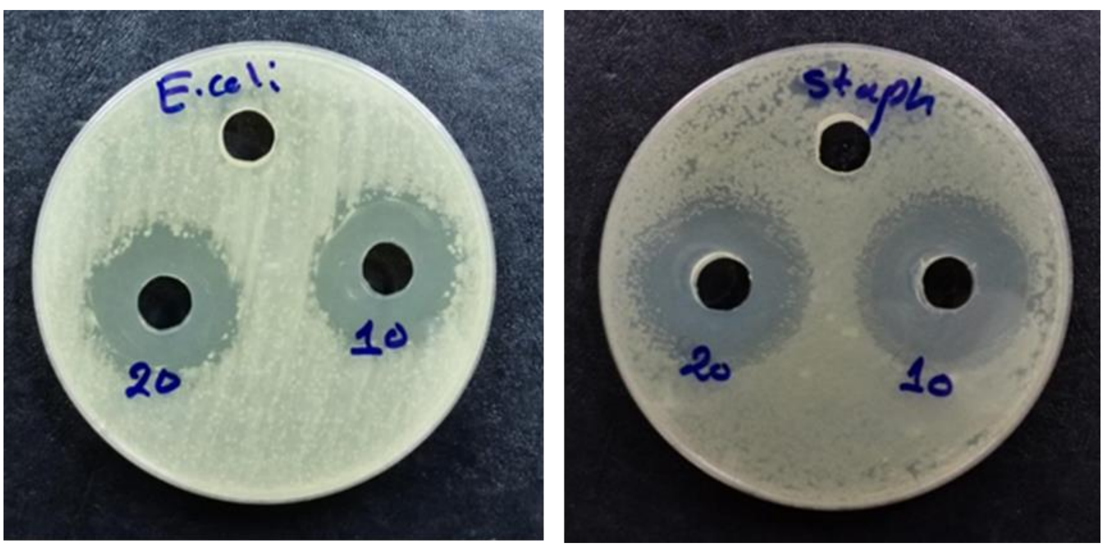

Figure (2) Presented the inhibition zone of bacterial species *E.coli* and *Staphylococcus* isolated from animals treated with gold nanoparticles (GNP) at dose of 10 µg /mL and 20 µg /mL

Discussion

Antibiotic resistance today is becoming the biggest threat to public health, the issue is especially related to the limitation of new effective antibiotic drug discovery (15, 16). The current work is for evaluation anti-bacterial potential for GNPs versus G+ and G- bacteria at different sources (animal and human). The antibacterial activity of GNPs in this study exhibited positive results against gram-negative and gram-positive bacteria including microorganisms such as *E.coli*, *Staphylococcus*, *Streptococcus* and *Serratia*. The inhibition zone at concentration 20 µg/ml was significantly greater than the inhibition zone at concentration 10 µg/ml. Shamaila, and Zafar (17) declared that the action of GNPs efficacy is size- and -dose dependent. The green synthesis of GNPs by Rahman and Khan (18) against *E.coli* showed an inhibition zone of 18.5 mm. Abdel-Raouf and Al-Enazi (19) synthesized GNPs whose sizes ranged from 3.85–77.13 nm. They found their impact on the growth of methicillin resistant *staphylococcus aureus* (MRSA) made an inhibition zone of 16 mm, Abd Noor and Ali (20) used GNPs at a concentration of 100 ppm to combat *streptococcus mitis* in dental plaque, making an inhibition zone of 19 mm, and Rajasekar and Karthika (21) synthesized GNPs by fish gut microbes to evaluate their antibacterial activity against *Serratia marcescens*, they found it made an inhibition zone of 13 mm.

The small sizes and large surface area of GNPs has an important role in the toxicity. The accumulation of the GNPs on the surface increasing at size was decreased, which in turn increases the toxicity against the bacteria by their effect on plasma membrane permeability, which ultimately results in the death of the bacterial cell (22). The mechanism in which nanoparticles interact with bacterial cells is that the metal oxides have a positive charge while the bacterial cells have a negative charge, resultant electromagnetic gravitation between the nanoparticle surfaces and bacteria. The nanoparticles releasing ions cooperating with the thiol (-SH) group of transport proteins that protrude for bacterial cell membrane, which leads to decreased cell membrane permeability and eventually leads to the death of the bacteria (23).

The antibacterial property of GNPs attained two methods that suppressed metabolic route by altering the membrane potential and depressing adenosine triphosphate (ATP) synthase activity. Secondly, dismantled the biological process of the ribosome by rejecting its subunit for tRNA binding. Additionally, it was found that they caused less damage to mammalian cells (24). The photothermal effect is an additional property, GNPs have an excellent ability to converted light to heat caused killing bacteria through changing infrared light into localized heat (25). Rod gold nanoparticles displayed a high rate of conversion of light to heat (26).

Conclusion

In the current study, rod-shape gold nanoparticles, 38 nanometers in length and 10 nanometers in width and sodium citrate-coated, exhibited a high antibacterial activity against several pathogenic bacterial positive and negative gram from animal and human sources. The result of this study demonstrated that GNPs can be the next therapy against these pathogenic bacteria.

References

1. Mohammed N, Nawar SH, Etawy MS, Nassar GE, Hassabo AG. Nanotechnology and its applications in industry and product design. Journal of Textiles, Coloration and Polymer Science. 2024;21(2):273-84.
2. Findik F. Nanomaterials and their applications. Periodicals of Engineering and Natural Sciences. 2021;9(3):62-75.

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3. Hasan S. A review on nanoparticles: their synthesis and types. Res J Recent Sci. 2015;2277:2502.
4. Harish V, Tewari D, Gaur M, Yadav AB, Swaroop S, Bechelany M, et al. Review on nanoparticles and nanostructured materials: Bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications. Nanomaterials. 2022;12(3):457.
5. Fan S, Feng X, Han Y, Fan Z, Lu Y. Nanomechanics of low-dimensional materials for functional applications. Nanoscale Horizons. 2019;4(4):781-8.
6. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. Journal of Nanobiotechnology. 2022;20(1):262.
7. Hammami I, Alabdallah NM. Gold nanoparticles: Synthesis properties and applications. Journal of king Saud university-science. 2021;33(7):101560.
8. Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology. 2009;84(2):151-7.
9. Elahi N, Kamali M, Baghersad MH. Recent biomedical applications of gold nanoparticles: A review. Talanta. 2018;184:537-56.
10. Yeh Y-C, Creran B, Rotello VM. Gold nanoparticles: preparation, properties, and applications in bionanotechnology. Nanoscale. 2012;4(6):1871-80.
11. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA. Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. The journal of physical chemistry B. 2006;110(14):7238-48.
12. Sztandera K, Gorzkiewicz M, Klajnert-Maculewicz B. Gold nanoparticles in cancer treatment. Molecular pharmaceutics. 2018;16(1):1-23.
13. mohammed Aj. Isolation Of Bacterial Pathogens From Ear, Nose And Mouth Of Domestic Rabbits With Antimicrobial Activity Of Pomegranate Peel, Melia Azedarach And, Lantana Cammra. Diyala Journal for Veterinary sciences-E-ISSN:2958-6178. 2023;1(3):1-8.
14. raheem RBA, Abood WN, Al-Obaidy ENJ. Antibacterial Activity of Senegalia greggii Seed Ethanolic Extracts using Agar Well Diffusion Assay. Diyala Journal for Veterinary Sciences-Print ISSN: 2410-8863. 2022;1(5):p: 1-7.
15. Abd El-Hamid MI, Y. El-Naenaeey E-s, M kandeel T, Hegazy WA, Mosbah RA, Nassar MS, et al. Promising antibiofilm agents: Recent breakthrough against biofilm producing methicillin-resistant Staphylococcus aureus. Antibiotics. 2020;9(10):667.
16. Zaboon SM, Al-Hayanni HS. DETECTION OF VIRULENCE FACTOR GENES AND ANTIBIOTIC RESISTANCE OF ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC) ISOLATED FROM CHILDREN WITH DIARRHEA. Biochemical & Cellular Archives. 2021;21(1).
17. Shamaila S, Zafar N, Riaz S, Sharif R, Nazir J, Naseem S. Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen. Nanomaterials. 2016;6(4):71.
18. Rahman TU, Khan H, Liaqat W, Zeb MA. Phytochemical screening, green synthesis

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P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

of gold nanoparticles, and antibacterial activity using seeds extract of Ricinus communis L. Microsc Res Tech. 2022;85(1):202-8.

19. Abdel-Raouf N, Al-Enazi NM, Ibraheem IB. Green biosynthesis of gold nanoparticles using Galaxaura elongata and characterization of their antibacterial activity. Arabian Journal of Chemistry. 2017;10:S3029-S39.

20. Abd Noor HJ, Ali B. Potential effect of gold nanoparticles against Streptococcus mitis (primary periodontal colonizer). Journal of Research in Medical and Dental Science. 2019;7(6):07-15.

21. Rajasekar T, Karthika K, Muralitharan G, Maryshamya A, Sabarika S, Anbarasu S, et al. Green synthesis of gold nanoparticles using extracellular metabolites of fish gut microbes and their antimicrobial properties. Brazilian journal of microbiology. 2020;51:957-67.

22. Seoudi R, Said DA. Studies on the effect of the capping materials on the spherical gold nanoparticles catalytic activity. World Journal of Nano Science and Engineering. 2011;1(02):51.

23. Chen X, Schluesener HJ. Nanosilver: a nanoproduct in medical application. Toxicology letters. 2008;176(1):1-12.

24. Parasuraman P, Anju V, Lal SS, Sharan A, Busi S, Kaviyarasu K, et al. Synthesis and antimicrobial photodynamic effect of methylene blue conjugated carbon nanotubes on E. coli and S. aureus. Photochemical & Photobiological Sciences. 2019;18:563-76.

25. Gharatape A, Davaran S, Salehi R, Hamishehkar H. Engineered gold nanoparticles for photothermal cancer therapy and bacteria killing. RSC advances. 2016;6(112):111482-516.

26. Von Maltzahn G, Park J-H, Agrawal A, Bandaru NK, Das SK, Sailor MJ, et al. Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. Cancer research. 2009;69(9):3892-900.