RESEARCH ARTICLE

Detection of combination effect of silver nanoparticles prepared with *Pimpinella anisum* and some antibiotics on some pathogenic bacteria

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Received: 08th July, 19; Revised: 12th August, 19, Accepted: 08th September, 19; Available Online: 12th September, 2019

ABSTRACT

This article aims to detect the effect of combining silver nanoparticles prepared with the extract of the *Pimpinella anisum* seeds and was obtained from the local markets in the city of Kirkuk. Iraq, with some antibiotics on antibiotic resistance bacteria (*E.coli* and *Staphylococcus aureus*), were obtained from Kirkuk General Hospital and re-diagnosed based on reliable diagnostic sources. This study demonstrates that the *P. anisum* seeds extract reduced the silver ion through changing the solution color to reddish-brown. The examination of silver nanoparticles synthesized by *P. anisum* the extract of seeds was carried out using X-ray and Scanning electron microscopy (SEM) and X-ray showed spherical shape particles with 44 nm in size with three diffractions which are indexed as (111), (200) and (220) and the SEM images showed clear particles. The synergistic effects of biosynthesis AgNPs at different concentrations with different standard antibiotic discs (amoxicillin, chloramphenicol, erythromycin, ciprofloxacin) against multi-antibiotic resistance bacteria were also investigated. The result showed the synergistic action of AgNPs and antibiotics leading to enhance antibacterial activity.

Keyword: Pathogenic bacteria, *Pimpinella anisum*, Silver nanoparticals.

International Journal of Drug Delivery Technology (2019); DOI: 10.25258/ijddt.v9i3.24

How to cite this article: Abbood1, H.A.R. and Zain alabden, S.S. (2019). Detection of combination effect of silver nanoparticles prepared with *Pimpinella anisum* and some antibiotics on some pathogenic bacteria. International Journal of Drug Delivery Technology, 39 (3): 460-464.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Nanotechnology is the science and application that deals deal with the materials smaller than 100 nanometers. This hot topic has developed rapidly in different fields.[1]

The science of nanotechnology has changed the way to words the diagnosis, treatment, and prevention of various diseases in all aspects of human life. Silver nanoparticles are one of the common nanomaterials among several metallic nanoparticles that are involved in biomedical applications.[2]

Applying nanotechnology represents an important step in developing bioactivities during the synthesis of this particle by biological, chemical, and physical methods.[3]

It is necessary to use low cost and environmentally friendly methods for obtaining these particles.[4,5] Various studies deal with the use of nanotechnology for obtaining the alternative to antibiotics using biological methods.[6-8]

Pimpinella anisum L. is an annual herb with white flowers and a small seed whose color is green to yellow. The plant is self-fertile and prefers light, sandy medium loamy and well-drained soil. It possesses sweet and spicy taste, and the odor is aromatic and agreeable.[9]

Pimpinella anisum seeds showed the antimicrobial activity in the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[10]

Misuse of antibiotics represents a common problem worldwide; besides their negative side effects, it leads to developing resistant bacteria against powerful antibiotics.[11] Therefore, the current study aims to detect alternatives to antibiotics and studying the combination effect silver nanoparticles and some antibiotics on *E.coli* and *Staphylococcus aureus*

MATERIALS AND METHODS

Microorganisms and materials

The researchers obtained the tested resistance bacteria (*Staphylococcus aureus* and *E.coli*) from Kirkuk general hospital and re-diagnosed depending on the reliable diagnosis sources.[12-14]

The *Pimpinella anisum* seeds were obtained from local markets in Kirkuk city, Iraq.

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While antibiotic discs were purchased from Sigma Aldrich, Bangalore.

Preparation of the seed extract

The researchers collected fresh, well seeds of *P. anisum* seeds that were collected from local markets in Kirkuk-Iraq. Double distilled water was utilized to wash these seeds repeatedly ten times for removing any surface contamination. After washing, those seeds were placed at room temperature for 2 days to be dried. Then, they were grounded into a fine powder.[15]

The powdered samples were prepared to be extracted. After that, a 250 mL conical bottle was used to dissolve 10g of the sample in 100 mL of distilled water. Then, the sample was heated at 50°C for 1hour.[16] Next, the filtration of the mixture was done the use of Whatman filter paper No. 1. Employed as a standard solution, the storage temperature of extracts was at 4°C.[15]

Synthesis of silver nanoparticles

1mM Silver nitrate ($AgNO_3$) was prepared by dissolving 17mg of $AgNO_3$ in 100mL of double-distilled water; it was stored in a brown bottle at 4^0C away from light.[17] One mL of concentrated extract of plants was added to 9 mL of 1mM ($AgNO_3$). The presence of brown color demonstrated the AgNPs formation from the plant extract.[18] Purification of particles was conducted using centrifugation at 10,000 rpm for 15 minutes for removing extra silver ions. The process of centrifugation was reiterated thrice 8 in order for all silver colloids to be removed using double-distilled water.[15]

Characterization of silver nanoparticles

Formation of nanoparticles was examined by X-RD (Shimatzu) in Physics Department, Collage of Science Baghdad University and SEM (TESCAN.VEGA) in (Nanotechnology and Advanced Materials Research Center, University Of Technology, Iraq) to detect the size and shape of the particles.[8,19]

Antibiotic sensitivity

A sterile cotton swab was used to uniformly spread the bacterial inoculum (approximately 1-2 X 108 CFU/mL) on a sterile Petri dish containing Mueller Hinton agar. The media surface, with the help of sterile forceps. The plates were incubated for 24 hours at 37^0C.[20]

Detection of Antibacterial Activity of silver nanoparticles:

The evaluation of antibacterial activities of silver nanoparticles prepared with *P. anisum* extract was conducted through the use of a well diffusion method on Mueller-Hinton agar. The zones of inhibition were described in millimeter (mm). Briefly, plates of MHA agar were inoculated with bacterial strain under aseptic conditions. A 50 µL nanoparticles were used to fill the wells (with 6mm diameter). Then, these wells were incubated at 37°C for twenty-four hours.[21]

Combination of silver nanoparticles with antibiotics:

The antibiotics discs were immersed in 20 µL absolute silver nanoparticles prepared with *P. anisum* extract, and other discs were immersed in 20 µL of distilled water as a control. All discs were fully dried before the application, then the discs were placed on the Muller Hinton ager surface. The plates were incubated at 37 C ^{0} for twenty-four hours. Then, the zone of inhibition was measured in millimeter.[22]

RESULTS AND DISCUSSION

The silver nanoparticles synthesis represents the process of reduction whereby the silver ion is reduced to silver nanoparticles.[6] The findings demonstrated that the synthesis of the silver nanoparticles through the use of the extract of *P. anisum* seeds, which reduced the silver ion. This result is consistent with.[15]

In this study, silver nanoparticles production was firstly detected by changing the color to reddish brown[23-24] (Figure 1)

The synthesis of silver nanoparticles through the use of the extract of *P. anisum* seeds was examined using X-ray diffraction and SEM (see Figures 2 and 3). The full width at half maximum data was employed in line with Scherrer equation for determining the mean size of the particle. This equation was: $d = 0.^9\ \lambda/\beta.\ \mathrm{Cos}(\theta)$; Where d refers to a crystallite size of AgNPs particles, λ represents the wavelength of the X-ray radiation source, β denotes angular. The XRD spectrum and SEM showed the synthesis of silver nanoparticles with three diffractions, which were indexed as 111, 200 and 220 of nano-

Figure l: changing the color of the solution to reddish-brown

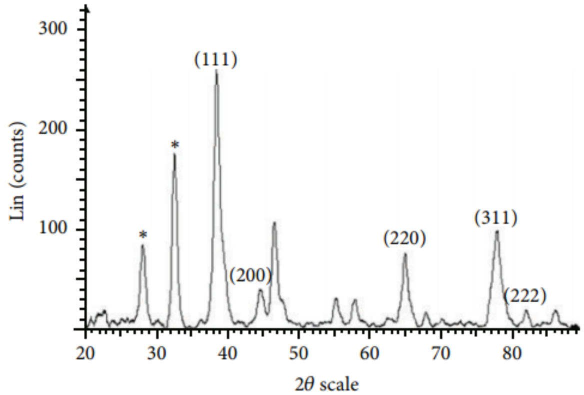

Figure 2: XRD pattern of the silver nanoparticles

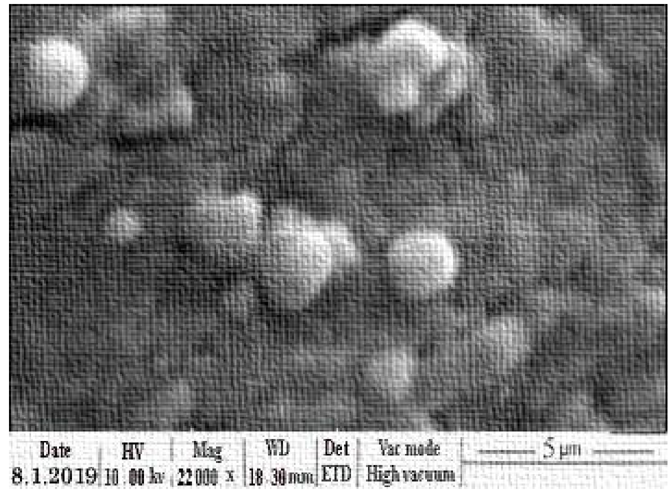

Figure 3: SEM images of bio-synthesized AgNPs by using *Pimpinella anisum* (L.) seed extract

silver. This result disagrees with [7,25]. The size of particles was 44 nm with a spherical shape.

The results of antibiotics sensitivity and the combined effects of the particles and the antibiotics illustrated in Figure 4 and Figure 5.

The results showed that there is an increase in the diameter of inhibition zone around the antibiotic discs from that found with the use of silver nanoparticles alone which used in this study, while the effect of combination between antibiotics and silver nanoparticles showed an increase in the diameter of zone of inhibition than that of antibiotics alone or the silver nanoparticles alone against *E.coli* and *Staph.aruse*, and these results are inconsistent with Barapatre, A., et al., Pirtarighat, S., et al., Ildiz, N., et al., [26-28]. So the combinations could represent therapeutic alternatives for the treatment of resistant bacteria.[29] In this research, the combination of silver nanoparticles prepared with plant extract with antibiotics showed a good effect on the (*E.coli* and *Staph.aureus*) and these results are inconsistent with.[24,25,28]

Finally, Different types of antibiotics were used in this study with different action against bacteria: amoxicillin, chloramphenicol, erythromycin, and Ciprofloxacin act by inhibiting bacterial cell wall synthesis, protein synthesis, and bacterial DNA replication respectively.[30]

CONCLUSION

In this paper, we evaluated the impact of combining silver nanoparticles prepared with the extract of *P. anisum* and some antibiotics on *E. coli* and *Staphylococcus aureus*. This study

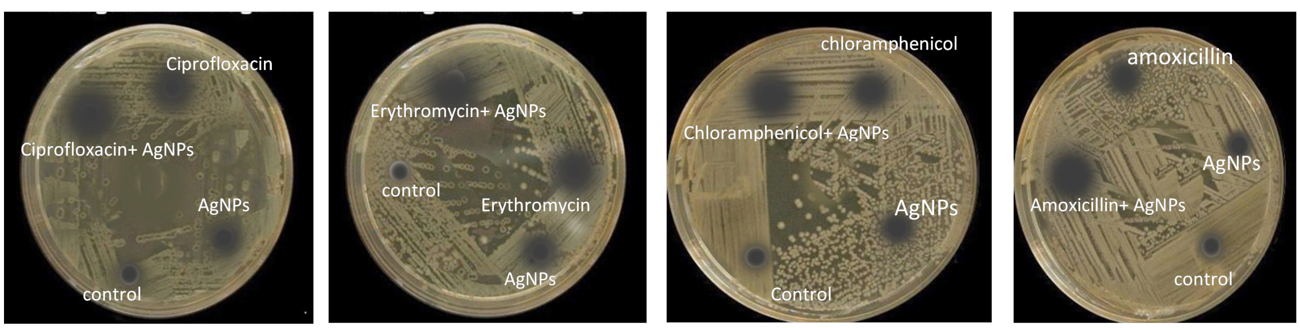

Figure 4: Antibacterial activity of AgNPs of *Pimpinella anisum* alone and it's combined action with four different antibiotics (amoxicillin, chloramphenicol, erythromycin and Ciprofloxacin) against the growth of *E.coli* . The inhibition zone measured in (mm)

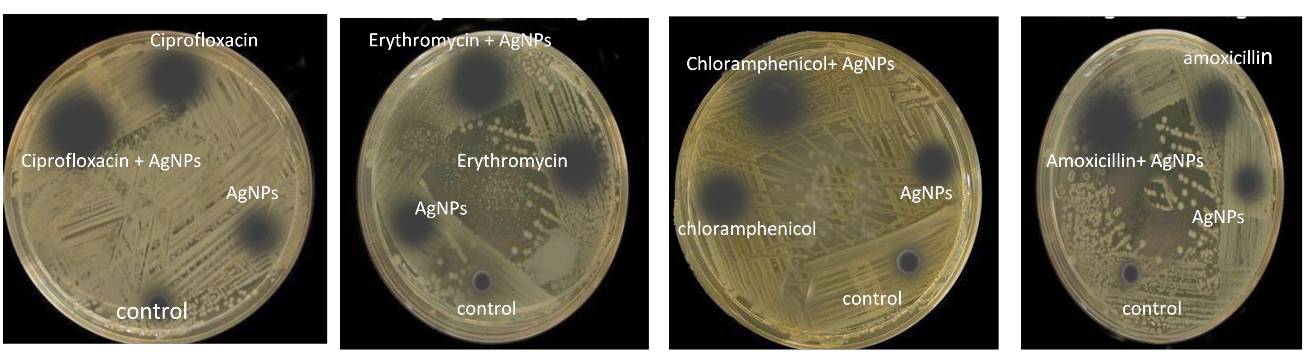

Figure 5: Antibacterial activity of AgNPs of *Pimpinella anisum* alone and it's combined action with four different antibiotics (amoxicillin, chloramphenicol, erythromycin and ciprofloxacin) against the growth of *Staph.aureus*. The inhibition zone measured in (mm)

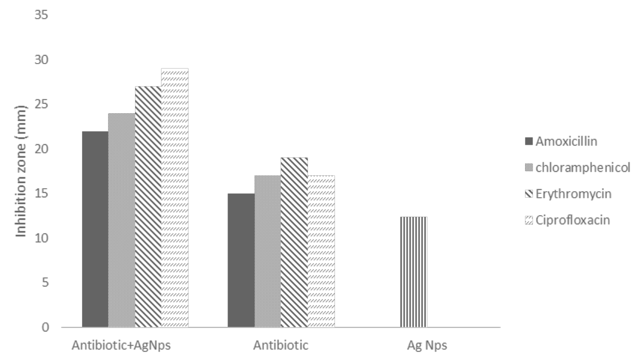

Figure 6: Sensitivity of *E.coli* to antibiotics and silver nanoparticles

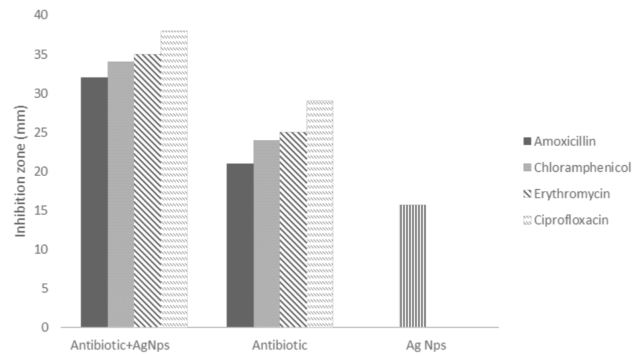

Figure 7: Sensitivity of *Staph.aureus* to antibiotics and silver nanoparticles

demonstrates that the *P. anisum* seeds extract reduced the silver ion through changing the solution color to reddish-brown.

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