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# A comparative study between commercially manufactured silver nanoparticles and alcoholic leaf extracts of Ziziphus spina-christi (Sedr) against human pathogenic bacteria

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***Abstract*---**Through this study, we show the biological activities of commercially manufactured silver nanoparticles from US Research nano. (USA) and compared it with alcoholic leaf extract Ziziphus spina-christi (Sedr) against Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Proteus mirabilis*, *Pseudomonas aerginosa*) The efficacy of ten antibiotics against these studied bacteria such as Ampicillin, Erythromycin, Azithromycin, Gentamicin, Vancomycin, Tetracycline, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Chloramphenicol and Clindamycin was also evaluated. The results showed that the manufactured silver particles had inhibitory activity against *S. aureus*, *E. faecalis, P. mirabilis* and *Ps. aerginosa* is higher than the inhibitory activity of alcoholic leaf extract of Ziziphus spina-christi (Sedr).

***Keywords*---**silver nanoparticles , alcoholic leaf extract Ziziphus spina-christi , Sedr

## Introduction

Nano is a word derived from the Greek ( Nanos ) which means dwarf, which represents things the size of one hundred millionths of a meter ($10^{-9}$). Nanoparticles have received a lot of attention due to their use in various fields, including Nanomedicine  This is due to the special properties possessed by these nanoparticles, These advantages led to the provision of important techniques for the production of mineral particles in their very small size. These materials provide solutions to environmental and technological challenges in various fields.

These characteristics depend significantly on the total particle size, shape and distribution (Plaza *et al.*, 2014) Bio-nanotechnology is formed as a complementary factor between biotechnology and nanotechnology to develop biological and environmentally friendly production for the synthesis and numbers of materials and nanoparticles. ( Sahayaraj and Rajesh, 2011). Silver nanoparticles are metal particles that are within the nanoscale size range of 1-100 nm and have physical properties different from those of the original metal ions and enter into wide applications

( Ferdous and Nemmar,2020 ). With the increasing number of infections caused by multidrug-resistant bacteria, there is a need for effective antibacterial alternatives so Silver was a better alternative because it is a disinfectant and targets a wide range of positive and negative bacteria ( Sangiliyandi *et al* ., 2014 ), Sedr trees belong to the Nabaqi family (Rhamnaceae ) Which includes 58 genera, 600 species between trees, shrubs and climbers, its scientific name *Ziziphus – spina –christiwilld* Sidr is a fast-growing spiny tree, medium to large, ranging in height from (10-3) meters. The leaves are oval in shape and range from 4 to 2 cm in length. (Habib,2020 ) Sedr leaves contain active substances such as soaps, tannins and phenols (Ahmed *et al* ..,2019 ) It has been reported that *Ziziphus – spina – Christi* has antimicrobial activity against pathogens that are usually quite resistant to modern drugs (Hafiz and Mubaraki,2016 )

**Materials and methods**

**Dissolving AgNPs nanoparticles powder:**

Weigh 1gm of AgNPs powder and dissolve it in 5ml of DMSO to obtain At a concentration of 200 mg/ml as a standard concentration put the solution in a small beaker in a water bath on a Magnetic stirrer hot plate At a temperature of 40 degrees Celsius at speed 80 for 30 minutes Then move it on Ultrasonic For an hour, it was then placed in a sterile tube until used And before use mixes by Vortex Mixer.

**Preparation of alcoholic leaf extract of Ziziphus spina-christi (Sedr):**

The alcoholic extract of Sedr leaves was prepared with a weight of (30gm) from the leaves powder and (300ml) of ethanol alcohol was added to it at a concentration of 99%, Any ratio (1:10) weight/volume (Boskabady *et al* ., 2006 ) The extract was left for 24 hours at room temperature ( Rios *et al* ., 1988 ) Then the extract was filtered by several layers of gauze Then by centrifugation, then concentrate the extract by evaporating alcohol Using a rotary evaporator At a temperature not exceeding 40°C Where a thick extract is obtained Then finish drying it using a convection oven at a degree temperature not exceeding 40°C, Then save the final extract until use ( Schneider and Ermel,1986 )

**Inhibitory activity test:**

The diffusion method was used On the agar by Drilling on the media of Muller Hinton With a cork drill to make holes of equal dimensions to prevent the overlap of the damping diameters and a diameter of 6 mm to contain the solutions By

0.5ml per hole after spreading 0.1ml of the bacteria suspension on the medium to test the sensitivity of bacteria to Sedr leaf alcohol extract and silver nanoparticle solution at concentrations ( 25, 50, 75,100 mg/ml ) Then the plates were left in the refrigerator for one hour to spread the plant solutions, and then the plates were incubated at 37 °C for 24 hours and the results were read by measuring the diameter of the inhibition zone. (Sexena *et al.*, 1999 )

**Bacterial samples:**

**Table No. (1)**
**Bacterial isolates**

| **Species Bacteria** | **Gram Stian** | **Disease focus** |
|---|---|---|
| *Staphylococcus aureus* | + | pharynx |
| *Enterococcus faecalis* | + | kidney stones |
| *Proteus mirabilis* | - | reproductive tract |
| *Pseudomonas aeruginosa* | - | wounds |

**Test for sensitivity to bacteria:**

**Table No. (2)**
**shows the sensitivity and resistance of bacteria to antibiotics**

| **Bacterial Isolates Antibiotics** | ***S. aureus*** | ***E. faecalis*** | ***P. mirabilis*** | ***P. aeruginosa*** |
|---|---|---|---|---|
| Ampicillin | **S** | **R** | **R** | **R** |
| Erythromycin | **S** | **S** | **S** | **R** |
| Gentamicin | **S** | **S** | **S** | **S** |
| Azithromycin | **S** | **S** | **S** | **S** |
| Vancomycin | **S** | **S** | **S** | **R** |
| Tetracyline | **S** | **I** | **S** | **R** |
| Trimethoprim/ Sulfamethoxazole | **S** | **I** | **S** | **R** |
| Clindamycin | **I** | **S** | **S** | **R** |
| Ciprofloxacin | **I** | **S** | **I** | **S** |
| Chloramphenicol | **S** | **S** | **S** | **R** |

**S: Sensitive , R : Resistance , I : Intermediate Sensitive**

**Results and Discussion**

Table No. (3) showed that there were significant differences between the inhibitory effect of all concentrations of commercial silver nanoparticles and alcoholic leaf extracts of Ziziphus spina-christi (Sedr) Whereas silver nanoparticles recorded the highest inhibitory activity against positive bacteria *Staphylococcus aureus* With a diameter of 20.0±0.0mm at a concentration of 100 mg/ml And the least inhibition

is 11.3±0.0 mm in diameter at a concentration of 50 mg/ml It agrees with the results of the researcher ( Saleh *et al* ., 2020 ) who recorded 15.25 mm inhibitory diameters against bacteria *S. aureus* At the same concentration of 100 mg/ml and with a diameter of inhibition of 13.25mm at a concentration of 50 mg/ml. The ethanolic extract of Sedr leaves showed a high inhibitory effect on *S. aureus* bacteria at a concentration of 100 mg/ml with a diameter of 18.0 ± 0.0 and the effect was stopped at a concentration of 50 mg/ml with a diameter of 14.0 ± 0.0mm

As for *Enterococcus faecalis*, the highest effect of silver nanoparticles was with the inhibition diameter of 20.0±0.0 mm at the concentration of 100 mg/ml and the lowest effect with the inhibition diameter of 11.3±1.1 mm at the concentration of 25 mg/ml. And it is consistent with the results of the researcher (Nayyar *et al.*, 2021), which showed that silver nanoparticles affect the bacteria *Enterococcus faecalis* with an inhibition diameter of 14.22 mm. The molecular mechanism behind the antibacterial activity of AgNPs is believed to be silver Ag+ ions, where interaction occurs between them and the cell envelope, DNA, enzymes and proteins (particularly those containing a sulfhydryl group) inside the cell as well as the production of reactive oxygen species ROS. From this, bacteria can The development of resistance against AgNPs through various mechanisms such as the active influx of ions from the cell (Wu *et al.*, 2021), The results showed that the alcoholic extract of Sidr leaves has a high inhibitory effect against *E. faecalis*, with a diameter of 20.3 ± 0.5mm at a concentration of 100 mg/ml.

Table (3) shows the highest inhibitory activity of silver AgNPs nanoparticles against *Proteus mirabilis* bacteria with a diameter of 22.3±0.5mm at a concentration of 100 mg/ml, and researchers (Addul Hussein and Alsharifi, 2021) obtained effective results for AgNPs particles on Gram-negative bacteria *P. mirabilis* with a diameter of 35 mm, and the ethanolic Sedr leaf extract showed an inhibitory activity on *Proteus mirabilis* bacteria, as the highest inhibition was at the concentration of 100 mg/ml with a diameter of 18.0 ± 0.5mm and the lowest inhibition at the concentration of 50 mg/ml with a diameter of 16.0 ± 0.0mm

The bacteria *Pseudomonas aeruginosa* was affected by AgNPs particles with an inhibitory diameter of 17.3±1.1 mm at the highest concentration of 100 mg/ml and considered *P. aeruginosa* is the primary pathogen of chronic wounds and studies have shown that silver is highly effective against the biofilms of *P. aeruginosa* ( Bjarnsholt *et al.*, 2007), The researcher (Saleh et al., 2019) obtained results in agreement with the results of the current study, where the effectiveness of AgNPs on *Pseudomonas aeruginosa* bacteria with inhibition diameter was 17.75 mm at a concentration of 100mg/ml *Ps. aeruginosa* was affected by ethanolic Sedr leaf extract with a diameter of 11.3±1.1mm at a concentration of 75 mg/ml

**Table No. (3)**
**Effectiveness of silver nanoparticles and Sidr leaf extract**

| Bacterial species | Con | AgNPs | Sedr leaf extract |
|---|---|---|---|
| *S. aureus* | **100** | 20.0∓0.0a | 18.0∓0.0a |
| | **75** | 0.0∓0.0c | 15.0∓0.0b |
| | **50** | 11.3∓0.5b | 14.3∓0.5c |
| | **25** | 0.0∓0.0c | 0.0∓0.0d |
| *E. faecalis* | **100** | 20.0∓0.0a | 20.3∓0.5a |
| | **75** | 19.3∓1.1a | 18.6∓1.1b |
| | **50** | 16.0∓0.0b | 17.0∓0.0c |
| | **25** | 11.3∓1.1c | 15.3∓0.5d |
| *P. mirabilis* | **100** | 22.3∓0.5a | 18.6∓0.5a |
| | **75** | 19.0∓1.0c | 18.0∓1.0ab |
| | **50** | 20.0∓0.0b | 16.0∓0.0b |
| | **25** | 14.6∓0.5d | 0.0∓0.0c |
| *P. aeruginosa* | **100** | 17.3∓1.1a | 10.0∓0.0b |
| | **75** | 17.3∓1.5a | 11.3∓0.0a |
| | **50** | 16.0∓0.0b | 0.0∓0.0c |
| | **25** | 17.0∓0.0a | 0.0∓0.0c |

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