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# Bio-Green Synthesis Silver Nanoparticles Mediated by Cloves Seed Extract (Syzygium Aromaticum) and Antibacterial Activity on MDR *Staphylococcus Aureus*

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**Abstract:**

A systemic methodology was introduced in this study to investigate the enhanced antibacterial and anti-biofilm effects of broad-spectrum antibiotics with and without AgNPs. To that end, we synthesized AgNPs using cloves seed extract in an environmentally friendly manner (Syzygium aromaticum). The synthesized AgNPs were then characterized using a variety of analytical techniques. The AgNPs particles were uniform in size, with an average dimension of 32.8 nm. Furthermore, the antibacterial potency of the selected antibiotics has improved. AgNPs were discovered to be present in science. Tetracycline is a drug that is used to cure Gram-positive bacteria such as *Staphylococcus aureus*. Fortunately, the combination of sublethal concentrations proved successful. Antibiotic use in conjunction with AgNPs has resulted in a significant increase in antibiotic resistance.

**Keywords**: Extract, MDR, Cloves and Nanoparticles.

## Introduction

*Staphylococcus aureus* It is a very significant pathogen in the healthcare system that has not been removed from the community hospital or the environment. Since the 1880s when S. aureus was found, it has been considered a very important pathogenic gram-positive bacterium, causing numerous infections such as skin lesions, postoperative wounds, localized abscesses, osteomyelitis, endocarditis, arthritis, pneumonia, and urinary tract [1]. This bacteria are the leading source of infections associated with indwelling surgical instruments, such as catheters and artificial heart valves, due to the capacity of *S. aureus* to make biofilms on such products, resulting in chronic infections [2]. It is currently known to be the second most common etiological source of lower respiratory tract and bloodstream infections. Penicillin-resistant S. aureus emerged in hospitals just two years after the use of penicillin in the medical field however, after a few years, the presence of these resistant strains of S. aureus in the general community [3].

The fact is that antibiotic resistance will remain a very important problem in future medicine. The

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only solution for this problem is to found alternative drugs which pathogens have not earlier distinguished and then have not developed any resistance [4]. Among metal nanoparticles, silver nanoparticles (AgNPs) have drawn attention to the scientific area. The Antibacterial properties of silver are known since 1000 B.C. From that time silver containers were used to preserve water. Then silver nitrate and silver sulfadiazine have been used for the superficial and deep dermal burns treatment and for the elimination of warts [5]. It has been found that AgNPs are non-noxious to humans and active against bacteria at low-concentrations not having any side impacts on humans [6]. Many studies proposed that AgNPs link to the cell membrane surfaces dispersion permeability and respiration behavior of the cell [7, 8]. Silver nanoparticles have powerful antimicrobial activity toward many pathogenic bacteria –like *S. aureus, S. epidermis, L. mesenteroides, B. subtilis, E.coli, K. mobilis*, and *K. pneumonia* [7, 9]. The synthesis of silver nanoparticles as an antimicrobial agent has become more important in the face of the growing challenge posed by antibiotic-resistant microbes. The processing of silver nanoparticles using the bio-green method has been identified as having biomedical uses to monitor pathogenic microorganisms as cost-effective compared to widely used physical and chemical methods [10]. There are several experiments made to the synthesis silver nanoparticles using medicinal plants like *Helianthus annus, Sorghum bicolour, Saccharum officinarum, Zea mays, Aloe vera Capsicum annuum, Medicago sativa* and *Geranium* sp. [11, 12]. *Syzygium aromaticum* (clove) (pic. 1) is usually cultivated in Indonesia, Tanzania, Madagascar, Brazil. The clove oil of this plant has been described as having beneficial analgesic, antiseptic, and anesthetic effects [13] and is widely used in dental medicine. Many studies have been done to find the components of *S. aromaticum* [14]. The buds of this plant contain 15–20% essential oil, such as eugenol, eugenyl acetate, and β-caryophyllene. Additional essential oil components of clove oil are vanillin, methyl salicylate, tannins, crategolic acid, and triterpenoids. The minor ingredients such as methyl amyl ketone, methyl salicylate, etc., are responsible for the characteristic pleasant odor of cloves [15].

**Pic. 1: Syzygium aromaticum seeds (clove)**

The aim of the current study is to evalute the antibacterial activity of silver nanoparticles synthesized by green method using extraction of *Syzygium aromaticum* seeds (clove).

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## 2. Materials and methods

### 2.1. Isolation and characterization of bacterial isolates

A total of 154 samples obtained from various sites (urine and wounds) from patients in Al-Qasim Hospital in Babylon, Iraq. Samples were inoculated on nutrient agar and blood agar and then incubated at 37°C overnight below aerobic-circumstances. Many of these isolates were established for microscopic morphological, cultural and biochemical studies. The Vitek2 device has been used to confirm the characterization of the isolates.

2.1.preparation of silver nanoparticles using extract of clove seed

Dry clove seeds were purchased from the local Al-Hilla market in Iraq and AgNO3 was used as a silver precursor in this analysis. Natural dry clove seeds have been used as a bio-reducing agent. In the preparation stage of the extracttwenty-five grams of dried cloves seeds were added to 100 ml distilled water in 250 mL Erlenmeyer flask. The preparation of aqueous cloves seeds extract was done by using a magnetic heating stirrer at 80 °C for 10 min. The extract was then filtered through Whatman filter paper no.1 and stored at 4 °C for further work, Then,Bio-green synthesis of silver nanoparticle by the bio-green method was carried out by adding 25 ml of cloves seeds extract to 10 ml of 0.1 M silver nitrate solution and incubated it at room temperature for 2 h. The overall reaction process was done in a dark place to avoid unnecessary photochemical reactions. Change the color of this solution to brownish-yellow was observed by the naked eye.

### 2.3. Measurement of Antimicrobial Activity of AgNPs: Evaluation was done by two methods

2.3.1. **agar well diffusion method**: the method was used to assess the antimicrobial activity of AgNPs using Muller Hinton plate inoculated with tested bacteria at inoculum 1.5 x 108 CFU/ml . A cork borer was used to make wells in the center of plate, these wells were filled with 100 µl of filtered AgNPs and incubated at 37°C for 24 hrs. at dark conditions. After that, the diameter of the inhibition zone was measured.

**2.3.2. Microtiter plate dilution**: this method used to evaluate the antibacterial activity of synthesized AgNPs more specific than above. serial dilution of AgNPs add to 0.1 ml of McFarland turbidity *S. aureus* (4 isolates) to each one of 12 wells with control (bacterial suspension without AgNPs). After incubation for 24 hours. At 37 ° C, crystal violet (1%) was added to the inoculated wells to read the bacterial cell viability of each well by reading it with an ELIZA reader at wave length 680 nm.

### 2.4. Characterization of silver nanoparticles product:

2.4.1. size analyzer: The synthesis of AgNPs was characterized using UV Spectrophotometer and a size analyzer. The characterization was carried out at the veterinary college of Al-Qasim green university.

2.4.2. Measuring the Surface Plasmon Resonance (SPR) by UV–visible spectroscopy: The SPR of

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silver nanoparticles was measured using UV–visible spectroscopy at a wavelength ranging from 300-500 nm according to (14).

**3. Results and discussion:**

After the culturing and diagnosis of the samples, the results revealed in the table (1).

Table (1):- The numbers and percentage of *S. aureus* isolated from clinical specimens

| Source of samples | No. of samples | No.(%) of *S. aureus* isolates |
|---|---|---|
| Urine | 100 | 42(42.00%) |
| Wounds swab | 54 | 13(24.00%) |

*S. aureus* is a type of Gram-positive bacteria that can cause different healthcare-associated infections such as skin lesions and urinary tract infections. The current study showed the occurrence of tested bacteria in urine is 42 % while another study [16] showed a lower incidence of the same bacteria in the urine. Other study [17] found the distribution of this bacteria in wounds is (15.7%).

**Antibiotics sensitivity test:**

**Table 2: Antibiotic Susceptibility of *S. aureus* Isolated from different source**

| Antibiotic | Sensitive No. (%) | Intermediate No. (%) | Resistance No. (%) |
|---|---|---|---|
| Ampicillin(10µg) | _ | _ | 55 (100%) |
| Cefoxitin(30µg) | 10(18.1%) | _ | 45(81.8%) |
| Imipenem(10µg ) | 22(40.0%) | - | 33(60.0%) |
| Clindamycin( 2 µg ) | 20(36.36 %) | - | 35(63.63%) |
| Cefotaxime( µg) | 25(45.45%) | _ | 30(54.54%) |
| Oxacillin(1 µg) | 7(12.7%) | - | 48(%87.27) |
| Tetracyclin(30 µg) | 27(49.09%) | 1(1.8%) | 27(49.09%) |

**Preparation and characterization of green synthesized AgNPs:**

1. **Optical observation:**

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nanoparticles. The brown color of the mixture of aqueous clove seed extract with AgNo3 solution changed to dark brown which is the initial indicator of the formation of AgNPs. Clove seed extract in the present study is considered a reducing agent for the reduction of AgNo3 to AgNPs. Due to the dark brown coloration of AgNPs produced correlates with strong light absorption as well as excitation of Plasmon resonance (SPR).

## 2. Uv-spectrophotometer:

To confirm the AgNPs formation and stability, we used UV-spectroscopy. The UV-spectrophotometer result revealed the peak of absorbance of surface Plasmon resonan (SPR) of synthesized AgNPs at 400 nm (fig. 1). The Narrow peak shape pointed to narrow range of product nanoparticles size less than100 nm [18]. The result has closely similarity with . The range of absorbance peak at 400 nm mean the vibration of free electron from synthesized AgNPs with wave light which really indicate AgNPs formation [19].

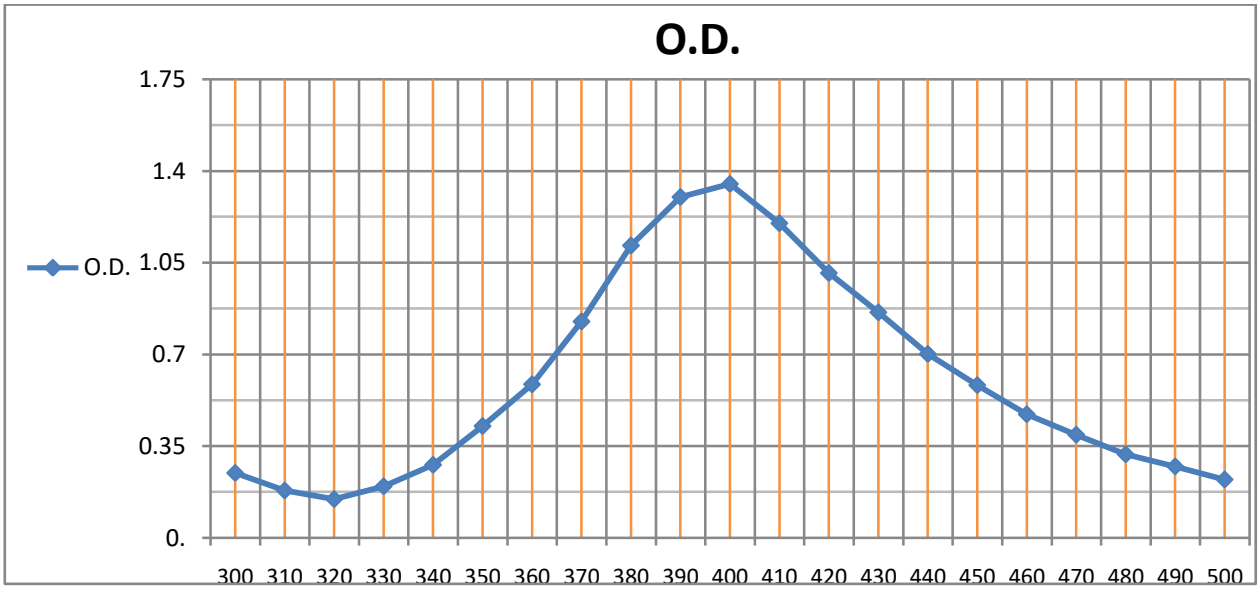


Figure 1: Optical density peak of green synthesized AgNPs using UV spectrophotometer at 400 nm.

## 3. Size analyzer:

The size of green synthesized AgNPs was determined by size analyzer advice were depend on dynamic light scattering. The distribution of size analysis revealed the effective diameter of synthesized AgNPs was 32.6 nm (fig. 2)

The size of the synthesized AgNPs affects its antibacterial activity, and the small size of the particles is more effective as an antibacterial than large one [20]. Many previous studies reported that antibacterial activity based on particles size of AgNPs [21, 22].

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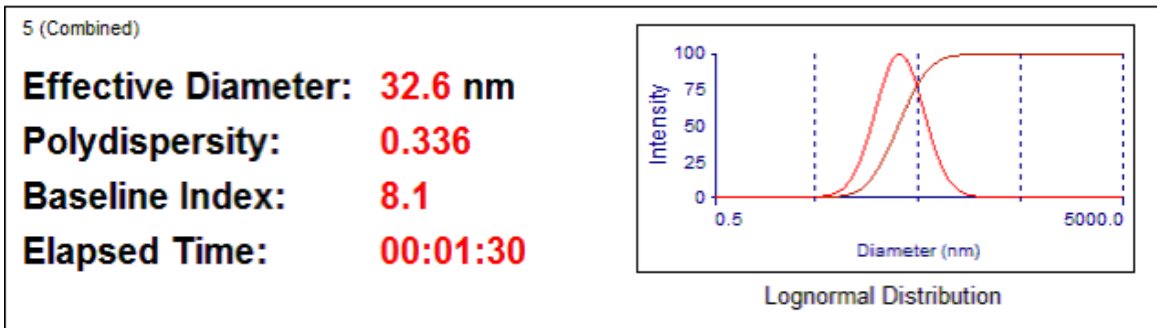


Figure 2: Size distribution of green synthesized AgNPs determined by dynamic light scattering g. Note that, the particle size distribution analysis revealed the average particle size was approximately 32.6 nm.

**Antibacterial activity of green synthesized silver nanoparticles:**

**Agar well diffusion:**

The antibacterial activity of silver nanoparticles (AgNPs) against *S. aureus* isolates was examined by agar well diffusion with different concentrations of synthesized silver nanoparticles diluted with distilled water (table 2)(fig. 3). MIC and sublethal concentrations of AgNPs and antibiotics were determined. MIC and sublethal concentrations (Table ) for each bacteria was calculated against different type of Antibiotics and AgNPs alone. The findings have shown. The disparity in effective doses

Figure 3: Antibacterial effect of different concentrations of green synthesized silver nanoparticles (1-5 with stock solution) against four isolates of MDR *S. aureus* by agar well diffusion.

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Table (2):-The inhibition zone (mm) of green silver nanoparticles (AgNPs) against MDR *S. aureus*.

| Dilution | Nanoparticle (ml): Distal water(ml) | Inhibition zone (mm) |
|---|---|---|
| Stock solution | - | 25 |
| 1 | 8:2 | 22 |
| 2 | 6:4 | 20 |
| 3 | 4:6 | 20 |
| 4 | 2:8 | 18 |
| 5 | 5:5 | 17 |

From table above, the stock solution of green AgNPs shows the highest zone of inhibition against tested bacteria (25 mm) followed by less zone of inhibition with lower concentrations of AgNPs, antibacterial activity decreases dramatically with AgNPs concentration decreases.

**Microtiter plate dilution method:**

Different concentrations of green biosynthesized AgNPs in 12 wells (fig. 4) mixed with one volume of MDR *S. aureus* to 4 isolates showing the gradient of the anti-bacterial effect from the highest concentration to the lowest by reading the wavelength in the ELISA reader device compared to the control wells that represent bacterial isolates without addition green synthesized AgNPs solution (fig. 5).

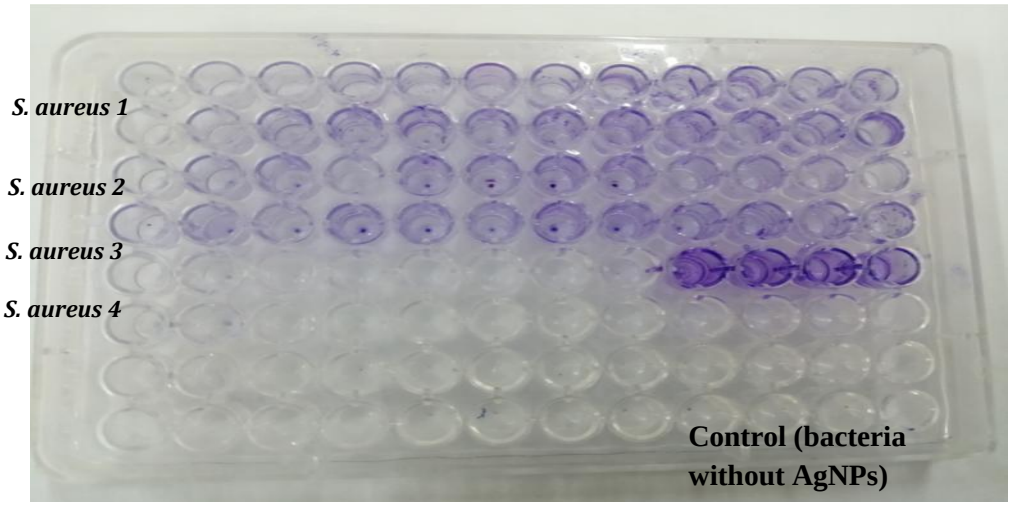


Figure 4: Antibacterial activity of green synthesized AgNPs in different concentrations against

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MDR *S. aureus* (4 isolates) in a microtiter plate

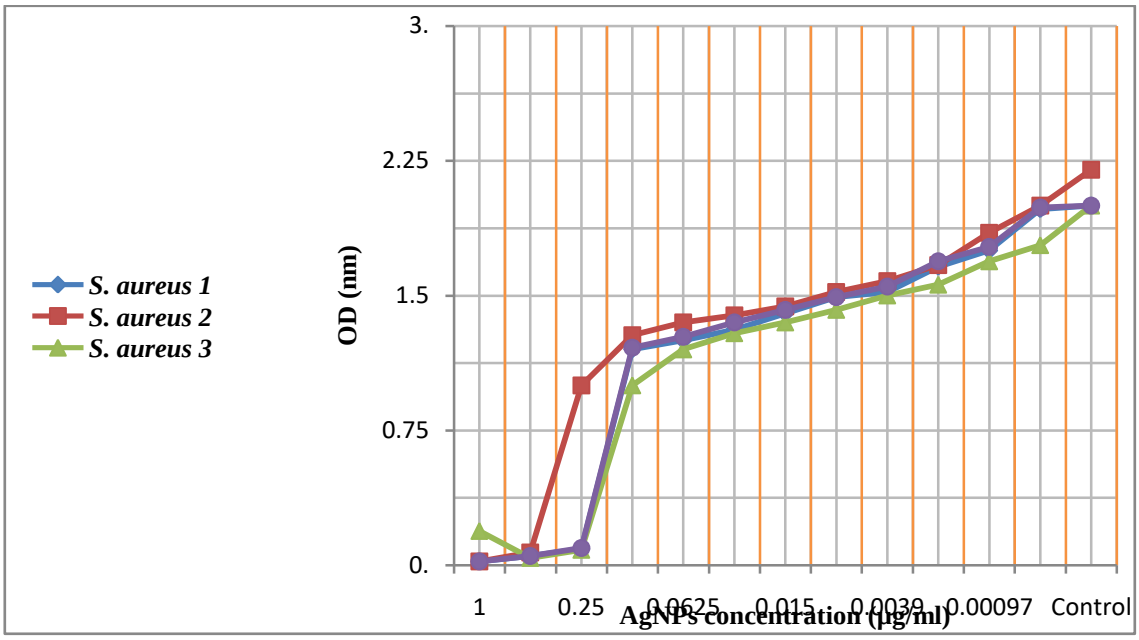


Figure 5: optical density of bacterial growth (*S. aureus*) affect by different concentrations of green synthesized AgNPs.

In vitro killing experiments have been undertaken to investigate the feasibility of using AgNPs as an antibiotic adjuvant, and the impact of both AgNPs and antibiotics has been enhanced by sublethal concentrations. In order to do so analyzed, the bacterial assay were treated with ampicillin and vancomycin sublethal concentrations. This is the added to these sublethal amounts of AgNPs Antibiotics treatments resulted in substantially improved antimicrobial activity which mentioned in recent research that AgNPs have been used to suppress biofilm activity[23].In the current research, the dose-dependent capacity of AgNPs to inhibit the activity of biofilms produced by *s aurus*. The antibacterial activity of AgNPs still unknown, Many researchers proposed that AgNPs disturb the permeability and functions of cell respiration by interacting with the bacterial cell membrane (Kvitek et al., 2008) and may penetrate the bacteria cell (Morones et al., 2005). Other researchers suppose that Ag+ ions may interact with the thiol groups in bacteria proteins, affecting on DNA replication (Marini et al., 2007).

Silver nanoparticles have also an exhaustive tendency to react with phosphorus and sulfur groups. Therefore, the proteins of cell membrane having sulfur and compounds containing phosphorus such as deoxyribonucleic acid (DNA) are possible to be the favored sites for silver nanoparticles (McDonnell and Russell, 1999).

The antibacterial effect of AgNPs on gram positive bacteria and especially *S. aureus* was targeted the cell wall peptidoglycan which destructed it [24]. Moreover, AgNPs may damage or fusion the glycan strand with each other which due to bacterial death via pores formation and that observed as inhibition zone on culture media [25, 26]. Other researchers have found that clove-synthesized

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AgNPs have the ability to break the cell wall and membrane, penetrate the cytoplasmic membrane, and inhibit normal synthesis of DNA and protein in S. aureus [25, 27]

**Conclusions**:

In this work, a systematic approach was developed to elucidate the improved antibacterial and anti-biofilm effects of broad-spectrum antibiotics with or without AgNPs. To this end, we have synthesized AgNPs using an environmentally sustainable approach using cloves seed extract (**Syzygium aromaticum**). Synthesized AgNPs were then characterized using a number of analytical techniques.The synthesized particles of the AgNPs were uniform in size Average dimension of 32.8 nm. In addition, the antibacterial efficacy of the chosen antibiotics has increased AgNPs were found to be present in research. Tetracycline is used to treat Gram-positive bacteria Staphylococcus aureus . Fortunately, the mixture of sublethal concentrations was found to be efficient. The use of antibiotics in conjunction with AgNPs has resulted in a substantial increase in antibiotic resistance.

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