Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue, 28-37

Enhancement of Antibiotic Activity Against Multidrug Resistant *Streptococcus mutans* Causing Dental Caries by Green Synthesized Silver Nanoparticles Using Cinnamon bark

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Received: May 4, 2024 / Accepted: June 2, 2024 / Published: March 2, 2025

Abstract: Dental caries is mostly caused by *Streptocuccus mutans*, and the pathogencity of this species is mainly due to the biofilm formation. Silver nanoparticles were produced using the cinnamon bark extract. It was noted that the potential for biosynthesizing silver nanoparticles occurred through color change, as the color of the mixture consisting of the extract of the cinnamon bark and silver nitrate changed from colorless to yellow within one hour, then after eight hours of incubation, to dark brown. The synthesized silver nanoparticles were characterized by the formation of a 431 nm wavelength absorption band, indicating the production of silver nanoparticles. The sizes of the silver nanoparticles were then determined through a scan electron microscope, where their average sizes ranged between 29.59-53.89 nm, while FTIR determined the functional groups for them and the proteins that can bind to silver nanoparticles have a ranged between 593.4 - 2978.4 cm^{-1}. Six (24%) isolates belonged to *Streptococcus. mutans* were obtained from the dental plaques of patients with tooth decay. *S. mutans* showed complete resistance to ampicillin, cefepime, and amoxicillin while demonstrated sensitivity to erythromycin, tetracycline, and to ciprofloxacin. Antibacterial activity of silver nanoparticle revealed that when the concentrations increase, the inhibition zone of multidrug resistant *S. mutant* isolates maximized until it reached a maximum inhibition of 200 µg/ml. when silver nanoparticle mixed with more resistant antibiotics, the antibacterial properties were strengthened by the presence of silver nanoparticles. of the majority of resistance antibiotics, such as ampicillin, gentamicin, cefepime, and amoxicillin against *S. mutant.*

Keywords: Cinnamon extract, silver nanoparticles, *S. mutans*, multidrug resistance.

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Introduction

Dental caries is a prevalent and expensive illness globally, and while it is not usually fatal, it poses a significant challenge for healthcare providers (1). A biofilm, or slime layer made of salivary polymers, food particles, and millions of bacterial cells, covers the surface of teeth (2). If left unchecked, the dental surfaces are covered with this biofilm can quickly thicken to hundreds of cells (3). Numerous bacterial species can colonize and flourish in the good adhesion site provided by the developed biofilm, often known as plaque (4).

It has been suggested that dental caries is mostly caused by *S. mutans* in humans (2). One of the most important aspects of the bacterium's pathogenicity is its ability to form dental plaque, a biofilm on tooth surfaces (5). The mouth, heart, joints, skin, muscle, and

central nervous system are all susceptible to pyogenic and other infections caused by *S. mutans* and other oral streptococci (4). The main habitats of *S. mutans* include the mouth, throat, and intestine The main habitats of *S. mutans* include the mouth, throat, and intestine (5,6). Adherence to enamel surfaces, the production of acidic metabolites, the capacity to store glycogen, and the synthesis of extracellular polysaccharides are some of the factors that lead to dental caries (6,7). *S. mutans* and *S. sobrinus* are important players in the development of dental caries because of their ability to adhere to other plaque bacteria as well as the enamel salivary pellicle *S. mutans* and *S. sobrinus* are important players in the development of dental caries because of their ability to adhere to other plaque bacteria as well as the enamel salivary pellicle (8). Because of their exceptional qualities, silver nanoparticles have been growing globally, especially in the industries of textiles, technology, food, and health (9,10). Of these, it is anticipated that the health industry will use silver nanoparticles extensively. It has been demonstrated that silver nanoparticles possess antiviral and antibacterial properties against a variety of microorganisms (11). In general, a range of methods, including chemical, biological, and physical ones, can be used to create silver nanoparticles (10). It is regrettable that environmental risks could arise from chemical or physical synthesis (9). As a result, new studies on the biological synthesis of silver nanoparticles with natural ingredients have been released. This type of synthesis is known as "green synthesis." Ag source, Often silver nitrate as a stabilizing and reducing agent are the three basic ingredients needed to synthesize silver nanoparticles Utilizing natural resources like bacteria, fungi, algae, plants, or yeast is essential for biological synthesis. Due of the readily available reducing and stabilizing chemicals, this synthesis process has several benefits, including low cost (9,12). Thus, the goal of this work was to create silver nanoparticles in an environmentally friendly way, characterize them using an extract of cinnamon bark, and assess how effective they were at preventing the growth of multidrug resistance *S. mutans* and increased antibiotic activity.

Materials and methods

Bark extract preparation

To make cinnamon plant extract, 2.5 grams of powdered cinnamon bark with 100 milliliters of distilled water were mixed then boiled it for two to seven minutes and left to cool. The process of filtering and filtration was carried out using filter paper and stored at 4°C (13).

Silver nitrate solution preparation

A solution of silver nitrate was made by dissolving 0.0421 in one hundred milliliters of deionized distilled water. and keep it in an opaque bottle to keep the silver from oxidizing until use (14).

Creation of silver nanoparticles with extract from cinnamon bark

To create silver nanoparticles, one milliliter of cinnamon bark extract and fifty milliliters of silver nitrate solution were combined and shaken at a rate of 200 revolutions per minute at room temperature for a duration of one to ten hours. The solution at the beginning appeared with pale yellow and then turned to dark brown (15).

Silver nanoparticles characterization

The silver nanoparticles that were generated were characterized according to (16) with:

1- UV-Visible Spectrophotometer

It was employed to ascertain the absorbance of silver nanoparticles, where 2 ml of the prepared silver nanoparticle solution was transferred after filtration to UV-Visible Spectrophotometer with a wavelength of 200-800 nm for determining the absorption band.

Fourier Transmission Infrared Spectroscopy (FTIR)

To ascertain the functional groups in silver nanoparticles, it was employed where 45°C was used to dry 125 milliliters of the produced silver nanoparticle solution and the resulting powder was placed in a special tablet-shaped mold inside the device.

Scan electron microscopy (SEM)

It was applied to ascertain the silver nanoparticles' size, where 45°C was used to dry 50 milliliters of the produced silver nanoparticle solution and the resulting powder was placed in the device's tube, covered with a layer of gold, and the model was examined.

Collection of dental caries samples

Twenty-five samples of dental plaques from patients who had tooth decay were gathered, and the samples were then transferred to brain heart infusion broth

Isolation and identification of *Streptococcus mutans*

Every sample was cultivated using the mitis salivarius selective medium. *S. mutans* was grown and isolated on mitis salivarius bacitracin agar (MSBA), which was prepared in accordance with (17) and incubated anaerobically for 48 hours at 37°C. Mannitol and triphenytetrazolium chloride (TTC) are applied to the dish's surface after incubation, and when the medium turns a dark pink color, the bacteria is likely *S. mutans*. To fully identify the samples, vitek2 system and biochemical tests were conducted. such as oxidase, catalase, blood hemolysis, carbohydrate fermentation, and growth at 4% NaCl (18).

Antibiotic susceptibility test for *Streptococcus mutans*

After preparing and pouring mitis salivarius bacitracin agar (MSBA) into petri dishes, *Streptococcus mutans* isolates were streaked onto the dish using a sterile swab. Then, the experimented antibiotic saturated in filter paper discs and added on agar surface and incubated for 24 hours at 37 °C (19). A meter ruler was used to measure the inhibitory zones' diameter (20).

Analyzing the effectiveness of silver nanoparticles in suppressing the growth of multi drug resistant *Streptococcus mutans*

Using agar well diffusion methods, the antibacterial properties of Silver NPs were investigated against *Streptococcus mutans* isolates that were resistant to many drugs. Four fresh culture colonies were isolated and cultured for eighteen hours at 37°C in five milliliters of mitis salivarius bacitracin broth (MSBB). Sterilized broth was used to calibrate the turbidity generated by growing culture in order to reach an optical density that was equivalent to 1.5 X 10^8 cells/ml, as 0.5 McFarland criteria. The suspensions were dipped into a sterile cotton swab. A mitis salivarius bacitracin agar plate was streaked all over using dipping cotton swabs. Next, porous pores with a diameter of 7 mm were made using a sterile cork core, and four concentrations of silver nanoparticles were added: 200, 100, 50, and 25 µg/mL. Next, After 24 hours of incubation at 37°C, the diameter of the inhibition zone was measured in petri plates (21).

Effect of combined silver nanoparticles with some antibiotics in preventing of multidrug resistance *Streptococcus mutans*

Antibacterial action of the silver nanoparticles with some resist antibiotics against (MDR) *Streptococcus mutans* isolates by disc diffusion method was determined and the optimum concentration of silver nanoparticles added with each resist antibiotics discs that included ampicillin, cefepime, amoxicillin and gentamicin, then the size of the inhibition zone for *Streptococcus mutans* isolates were measured using a meter ruler (22).

Results and discussion

Creation of silver nanoparticles with extract from cinnamon bark

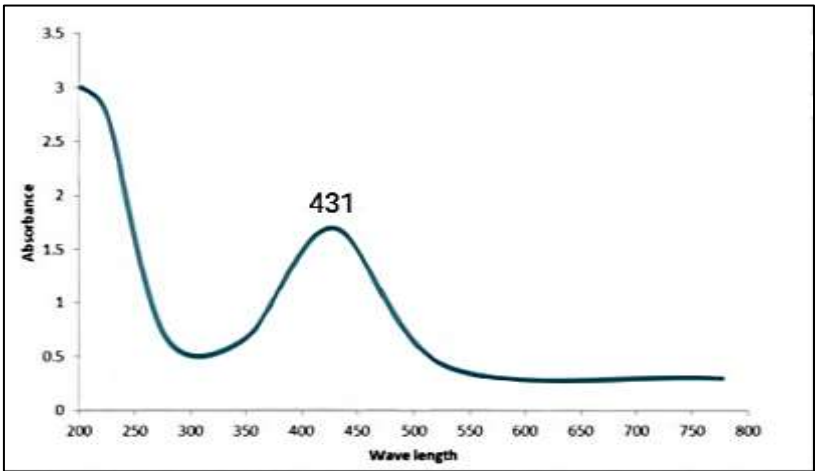
Silver nanoparticles were made using cinnamon bark extract *C. zeylanicum*, which has been described using:

Color change

Using *C. zeylanicum* cinnamon bark extract to produce silver nanoparticles, the reaction mixture's color altered following the incubation period in the shaking incubator from colorless to yellow within one hour and after 8 hours to dark brown, which indicated the manufacture of silver nanoparticles through changing the color of the medium, as the color change appeared. Silver nanoparticles were surface plasmon excited as a result through the active molecules of the substances present in cinnamon extract, where the silver nanoparticles are reduced and stabilized in large part by the active molecules that are present on the surface (23).

UV-Visible Spectrophotometer

UV-Visible Spectrophotometer was used, through which the absorption bands of silver nanoparticles were determined at a wavelength between 200 and 800 nm, this is a representation of the silver nanoparticle absorption peak. At 431 nm in wavelength, the absorption band is depicted in figure (1). which is an indicator of the appearance of silver nanoparticles.



Figure(1): UV-Visible Spectrophotometer for silver nanoparticles.

Fourier Transmission Infrared Spectroscopy (FTIR)

Identifying the relationship between silver nanoparticles and protein functional groups, as shown in figure (2), where seven absorption peaks appeared located in a region between 593.4 - 2978.4 cm^{-1}, where the

C-H bond and C-H_2 are indicated by the bands at 2978.3 and 1458.6 cm-1, respectively, while the bands at 1720.12 indicate a carboxyl group C=O, while the bands at 1371.56 cm indicate nitrate.

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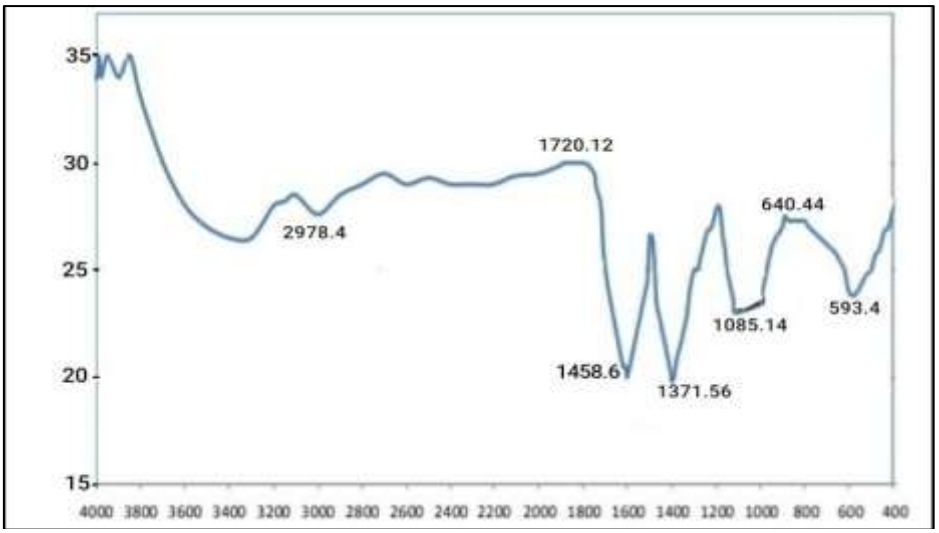
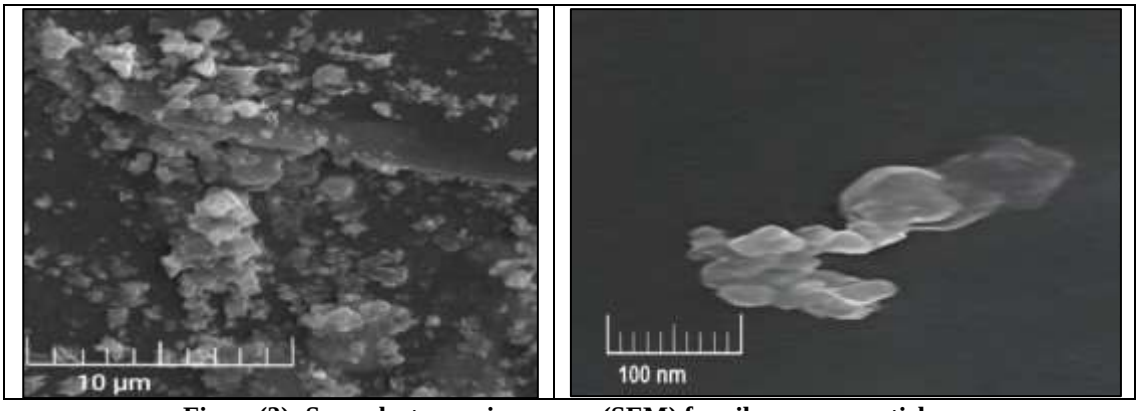


Figure(2): Fourier Transmission Infrared Spectroscopy (FTIR) for silver nanoparticles.

The silver particles' amine and carboxyl groups function as reducing agents and effectively stabilize the silver nanoparticles and preventing their aggregation in the medium through the binding of these groups to the surface of the silver nanoparticles and their formation of protein coat (24).

Scan electron microscopy (SEM)

According to the results in figure (3), the size of the nanoparticles ranged between 29.59-53.89 nm.



Figure(3): Scan electron microscopy (SEM) for silver nanoparticles.

Isolation and diagnosis of *Streptococcus mutans*

For the *S. mutans* isolation, the dental plaques of 25 patients with tooth decay were gathered. The samples were cultivated on mitis salivary agar (MSA), a selective medium that encourages *Streptococcus* growth. They were also cultivated on the extremely selective medium known as mitis salivary bacitracin agar (MSBA), which is made up of sucrose-supplemented MSA and an antibiotic called bacitracin that inhibits most bacteria with the

exception of *S. mutans* and *S. sobriuns*. Thus, based on cultural and biochemical testing, (24%) belonged to *S. mutant* and (76%) belonged to *S. sobrinus* respectively were obtained as in figure (4). Whereas *S. sobrinus* has smooth colony morphology, *S. mutans* has rough colony shape on plates containing mitis salivarius agar, a selective medium for *mutans streptococci* (8).

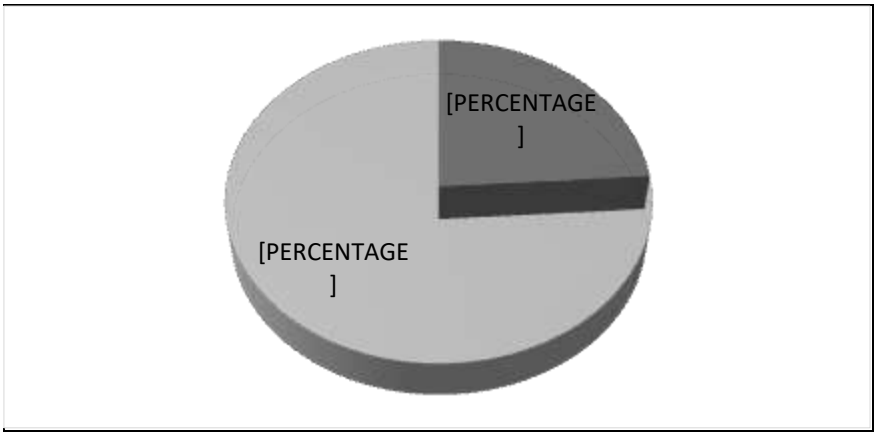


Figure (4): Isolation of from *Streptococci* from dental plaques of patients with tooth decay. (24%) belonged to *S. mutant* and (76%) belonged to *S. sobrinus.*

Due to their immature immune systems, lack of protection from environmental toxins, and frequent consumption of sugar-rich foods, children are among the age groups most susceptible to tooth decay (25). Due to the high concentration of proteins and glucose in saliva, factors influence the development of caries and the quantity of S. mutans present, the rate of S. mutant bacterial infection among diabetic people rises (26).

***Streptococcus mutans* antibiotic susceptibility test**

Because many antibiotics are used indiscriminately and widely to treat bacterial illnesses, the results of the susceptibility test demonstrated a distinct difference in the pattern of sensitivity and resistance to the antibiotics. 100% resistance to ampicillin, cefepime, and amoxicillin was demonstrated by *Streptococcus mutans*.. Additionally, 50% of the isolates demonstrated sensitivity to erythromycin, 67% to tetracycline, 50% to ciprofloxacin, and 17% to gentamycin (Table 1). There were high antibiotic resistance by *Streptococcus mutans* toward optochin and bacitracin and moderate resistance toward amoxicillin and ampicillin (27).

Table (1): Antibiotic susceptibility pattern for *Streptococcus mutans.*

Antibiotic	No. (%) Antibiotic susceptibility	
	Resistance	Sensitive
Amoxicillin	6 (100%)	-
Cefepime	6 (100%)	-
Ampicillin	6 (100%)	-
Erythromycin	3(50%)	3 (50%)
Tetracycline	2 (34%)	4 (67%)
Ciprofloxacin	3(50%)	3(50%)
Gentamycin	5 (83%)	1 (17%)

Analyzing the effectiveness of silver nanoparticles in suppressing the growth of *Streptococcus mutans* resistant to several drugs

The findings showed that, the highest inhibition zone of (MDR*)* *Streptococcus mutans* isolates recorded at 200 µg/ml silver nanoparticle, as shown in and figure (5 and 6). The two isolates, *S. mutans2* and *S. mutans6*, exhibited the highest rate of diameter inhibition, with diameters reaching 25 and 23.7 mm at 200 µg/ml of silver nanoparticles, respectively. This indicates that higher concentrations of silver nanoparticles are more effective than lower concentrations.

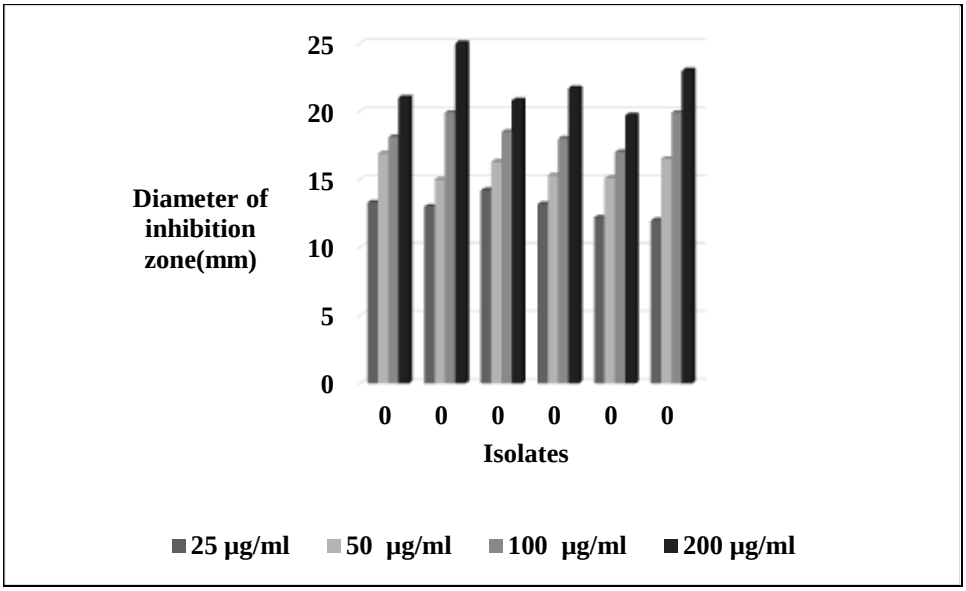


Figure (5): Diameter of inhibition zone of *Streptococcus mutans* isolates by silver nanoparticles.

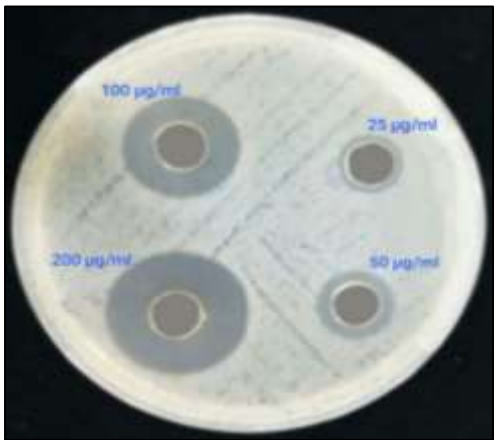


Figure (6): Effect of different concentrations of silver nanoparticles on *Streptococcus mutans* isolate.

A concentration gradient known as the PMF is created when certain carrier proteins and enzymes present in the bacterial cell membrane are bound by silver ions. This protein-enzyme chain transports electrons and protons from the cytoplasm into the periplasmic region. (28), When bacteria undergo aerobic respiration, the electron transport system across their cell

membrane serves as their main source of ATP production and the process is termed chemiosmosis, The ATP synthase complex, which produces ATP through a redox reaction between ADP and inorganic phosphate, is the only location where protons can leak back into the cytoplasm and the electrons arrive at the last location of the electron acceptor, which dissolves (29).

Effect of combined silver nanoparticles with some antibiotics in preventing of multidrug resistance *Streptococcus mutans*

The findings demonstrated that the bulk of drugs that are resistant to bacteria have stronger antibacterial properties when silver nanoparticles are present such as erythromycin, tetracycline, ciprofloxacin and gentamycin. In addition, a susceptibility shift detected of bacteria from resist to susceptible in case of ampicillin, cefepime, and amoxicillin after combination with silver nanoparticles as shown in table(2). The maximum amount of silver nanoparticles that exhibit antibacterial properties was 200µg/ml, which inhibited the growth of multi-resistance *Streptococcus mutans*, as shown in and figure (7).

Table (2): Values of the inhibition zones of the antibiotics on *Streptococcus mutans* before and after combination with synthesized silver nanoparticles.

Antibiotic	Inhibition zone diameter (mm.) (before combination with silver nanoparticles)	Inhibition zone diameter(mm.) (after combination with silver nanoparticles)
Amoxicillin	no	19-32
Cefepime	no	18-22
Ampicillin	no	17-22
Erythromycin	7-9	19-23
Tetracycline	7-10	18-25
Ciprofloxacin	7-9	18-32
Gentamycin	9-12	22-23

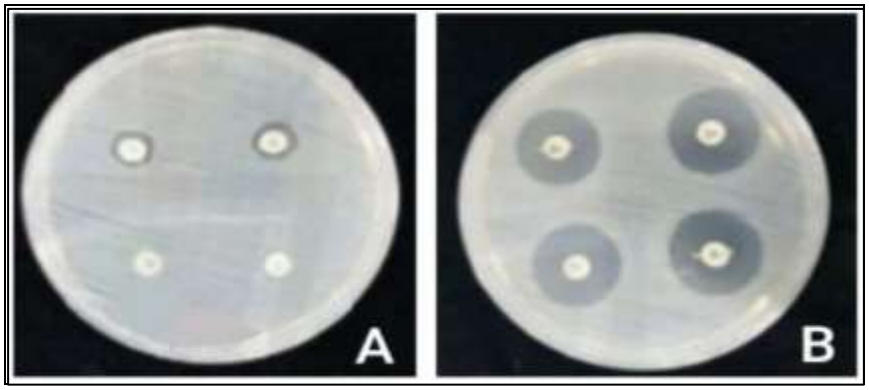


Figure (7): A) effect of antibiotics alone on *Streptococcus mutans* isolate, B) Effect a mixture of some drugs with silver nanoparticles on *Streptococcus mutans* isolate.

Thiol groups are bound by silver. Ag^{+} has antibacterial properties, one of which is its ability to bind efficiently to molecules on the surface of bacteria that contain sulfur and tear the cell wall, killing the bacterium (30). In various species, the effect silver nanoparticles-antibiotics activity can vary. These differences in the synergy between silver nanoparticles and antibiotics can

be attributed to a variety of factors that affect the capacity for interaction between silver nanoparticles and antibiotics (31). Since antibiotic compounds included groups like sulfur and starch (alpha acetal group) that may react readily with silver nanoparticles, the binding interaction between the two was most likely what generated the synergism (32).

Conclusions

It was concluded that silver nanoparticles were successfully created with the aid of cinnamon bark extract. And their enhancement of antibiotic effect on isolated bacteria was clearly noticed. Many further studies are recommended, especially those concerning with identification and purification the certain antibacterial compounds within the plant extract.

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