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Green Synthesis of Silver Nanoparticles Using *Dianthus Caryophyllus* Extract: A Novel Antibacterial Approach Against Oral *Streptococcus* Species

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Abstract: The present study details the synthesis of silver nanoparticles (Ag NPs) from *Dianthus caryoophyllus* plants extract and study its effect on the growth of *Streptococcus* isolated from the oral cavity. The synthesized silver nanoparticles (Ag NPs) were analyzed by UV visible spectroscopy, diffraction of X-rays (XRD) and Infrared Spectroscopy with Fourier Transform (FTIR). The ability to fight bacteria was also studied by diffusion technique using Agar wells against wild and mutant isolates of *different* species of *Streptococcus* including *S. mutans*, *S. salivarius* and *S. sobrinus*. The results showed the most potent antibacterial effect of *D. caryophyllus* extract was 30 mm in diameter at a concentration of 100 mg/ml against non-mutated *S. salivarius* and mutated isolates of *S. salivarius* and *S. sobrinus*, while the lowest value is 9 mm in diameter at a concentration of 25 mg/ml against non- mutated *S. mutans*. also findings from the study indicated that silver nanoparticles exhibit antimicrobial properties and the greatest number of the inhibitory zone estimated 35 mm in diameter at a 100 mg/ml concentration against non-mutated isolate of *S. salivarius* and 15 mm in diameter at concentration of 25 mg/ml against mutated isolate of *S. mutans* was showed the lowest value of antibacterial effects. The origin of nanoparticles can be useful in industry alternatives to antibiotics as an economic and safe source, but more studies are needs to ensure that they do not effect on human cells.

Keywords: *Dianthus caryophyllus* extract, Silver nanoparticles, Antibacterial activity, *Streptococcus* spps.

INTRODUCTION

"Nano" which refers to "dwarf," is derived from a Greek word for things that are one billionth in size. Due to their numerous uses, such as Nano- medicine, and their features in various domains which based on their overall size, content, shape, and distribution, to differentiate them, nanomaterial possess drawn a lot of attention. Nanoparticles have been created using a variety of chemical, physical and biological methods [1]. Nanoparticles have different applications in medicine such as in the treatment of tuberculosis by chemotherapy, the treatment of kidney diseases, , drug delivery for skin diseases, the treating of Alzheimer's disease, drug targeting to infectious diseases, nanoparticles containing different anticancer agents and nanoparticles used in vaccination against COVID-19 [2].

The use of fungi, bacteria, algae, plants, or extracts from them in biological processes to create nanoparticles has been investigated as an environmentally acceptable substitute for chemical and physical approaches [3, 4]. One of the known types of metallic nanoparticles is noble metal nanoparticles, which include gold, silver, and platinum [5]. Due to their chemical stability and other unique characteristics, silver nanoparticles (Ag NPs) have drawn more attention and have been the subject of substantial research. They are widely used as catalytic agents or agents that kill bacteria. They are utilized in antiseptic sprays, topical moisturizers and wound dressings, in addition to their anti-inflammatory properties and biomedicine. They are effective in the detection and treatment of cancer [6-8]. Ag NPs are used in medicine for various activities such as drug-delivery systems, anticancer, antiparasite, antiviral, antibiofilm and antimicrobial [9].

Plants can produce nanoparticles far more effectively than other biological processes. The produced silver nanoparticles have shown action against numerous clinical strains of bacteria, and various types of nanoparticles have been utilized in various ways to combat microbial resistance [10]. Plant extracts are more advantageous than microorganisms because they are less expensive, require less particular setup for culture and isolation procedures, and are simple to scale up for large-scale nanoparticle synthesis [11, 12]. One advantage of using plants and their extracts to create metal nanoparticles is that they are readily available, safe to utilize, and contain a range of metabolites that could assist with the reduction process [8]. Metal nanoparticles are reduced and stabilized by plant extracts [13]. Combining biomolecules including vitamins, terpens, saponins, alkaloids, proteins, amino acids, enzymes, polysaccharides, and tannins and phenols to reduce and stabilize the product [11].

However, the kind and concentration of phytochemicals in the plant part, temperature during synthesis and the duration of the reaction all influence its size, form, and antibacterial properties of nanoparticles synthesized by plants [12]. Numerous plants, including Arabica coffee, Acalypha indica, Sphaeranthus indicus, Matricaria chamomile, and Salvia officinalis [12]. Alfalfa sprouts [13], starch [14] and Aloe vera plant extract [15] has been used to synthesize mineral nanoparticles using plant extracts.

D. caryophyllus was chosen for this research study. Dianthus belongs to the family Caryophyllaceae. It's widespread in the Mediterranean countries of Spain, Turkey, Italy, Croatia, Greece and Albania [16]. According to reports, Dianthus plant has a large number of secondary metabolites such as phenolic

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acids and flavonoids [17]. According to phytochemical assays, Triterpenes, alkaloids, coumaruns, and cyanogenic glycoside were found in D. caryophyllus, [18].

This study reveals an ecofriendly, more economical as well as effective method of producing silver nanoparticles using D. caryoophyllus plant extract and study the effect it is on the growth of Streptococcus isolated from the oral cavity.

MATERIALS AND METHODS

Bacterial isolates: All samples were collected from gingiva from healthy people after drying around teeth with cotton to prevent contamination from normal flora and the samples were cultures on blood agar medium by loop under anaerobic conditions. Biochemical tests were performed on bacterial isolates. The assays made the isolates' identification and characterization easier. The biochemical assays employed in this research were as follows and confirmed the identification of bacteria by the Vitek 2 compact system [19].

Plant collection and grinding: The D. caryophyllus cloves were obtained from a local market in March 2023, then the plant sample was identified in the herbarium of the Department of Biology-College of Science- University of Baghdad. After that the plant sample was ground using an electric blender to prepare fine powder, the prepared powder was put in an airtight container and stored under dry conditions for further use.

Preparing of plant extract: Aqueous extract of D. caryophyllus cloves was done according to [20] with some modification, 250 ml of boiled distilled water with 50 g of clove powder was suspended in it, and the beaker was placed in an electrical shaker for 24 hr., then muslin was used for filtration then used of filter paper, the supernatant placed in Petri dishes for drying by oven at 40 ℃. After drying the extract was collected and three concentrations were prepared (25, 50 and 100) mg/ml using distilled water and kept cold at 4 ℃ to be used later in silver nanoparticles (Ag NPs) production [20].

Preparation of silver nanoparticles (Ag NPs): About 10 ml of plant extract in each concentration alone (25, 50 and 100) mg/ml and 5 ml of a (10mM AgNO3) solution were combined to create the silver nanoparticles, while control was applied without silver nitrate. These solutions were kept in the dark for 24 hours at 30°C. The color of the solution converted to brown (dark color) after 24 h. as a result of the production of Ag NPs that were recovered for further characterization after being centrifuged twice for 5 min. at 10,000 rpm [21, 22].

Ag NPs' characteristic feature: UV–visible spectroscopy is used characterize the produced Ag NPs [23]. At room temperature, 1 mL of Ag NPs was added to 4 mL of deionized water using an absorbance of 300–800 nm [24, 25]. Then the crystalline structure of Ag NPs was investigated using an X-ray spectrometer (XRD-6000, Shimadzu, Japan) [26, 27]. Also, FTIR assay was done using the technique described in [28], the FTIR 8400S, Shimadzu, Japan, was utilized for investigating significant alterations in the surface structure and bonding of Ag NPs.

Making mutant isolates of bacteria: The antibiotic Ciprofloxacin was used in making of the spontaneous mutants of the three bacterial isolates. The necessary quantity of medium (nutrient agar) was dissolved in D.W. to make the antibiotic nutrients medium, sterilize it, and then leave it to cool until it reached to 40°C. A certain amount of the antibiotic solution was introduced to the medium and gently mixed ever after. In order to ensure sterility, after adding the antibiotic medium to the plates they were incubated for 24hr. [29]. This medium was used to isolate the mutant bacteria.

Detection of the antibacterial effect of D. caryophyllus extract: The Blood agar plates for Streptococcus isolates were prepared. In brain-heart broths, the bacteria were cultivated, properly labeled, and incubated overnight at 37°C. The inoculum was applied to each bacterium's agar plate in an aseptic condition. A sterilized cork-borer was used to create cups in each Petri plate. Each cup was filled with the plant extract in different concentrations. In one of the cups, the identical volume of solvent was pipetted and used as a control. After that, these plates were stored at 37°C for 24 hours in an incubator. The plates were checked after 24 h. to determine the zone of inhibition, which was measured in millimeters [30].

Detection of the antibacterial effect of Ag NPs against mutated and wild bacterial isolates: The antibacterial activity of the crude solution of silver nanoparticles derived from the five wild and mutant Streptococcus isolates was evaluated using the well diffusion method [30]. A number of Streptococcus species, such as S. mutans, S. salivarius, and S. sobrinus, were employed to evaluate the antibacterial efficacy of the silver nanoparticles solution. Ag NPs (100 µl) was applied to each well. The diameters of the inhibitory zones were measured after the plates were incubated for 24 hours.

Statistical Analysis: The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors in study parameters. This study used the least significant difference-LSD to significantly compare between means (ANOVA/ Two ways) [31].

Results and Discussion

Identifying the isolated bacteria: Streptococci were grown on Mites Salivarius Agar plates and their shape and appearance were employed for identification. It was observed as a violet or light blue in color and the diameter was about 1-2 µm. S. salivarius appeared as ovoid or spherical in shape with a convex, flat, or raised surface. In contrast, S. sobrinus appeared flat and spherical with smooth surface, blue colonies on MSA Agar, as shown in figure (1). This study detected three species of Streptococci (2 isolates S. mutans, 4 isolates S. sobrinus, and 5 isolates S. salivarius) isolated from gingival infections. They are Gram- positive, ovoid, or spherical in shape, arranged in short or medium- length, non-spore forming chains. After that, recognition of the isolates was confirmed by the Vitek 2 System.

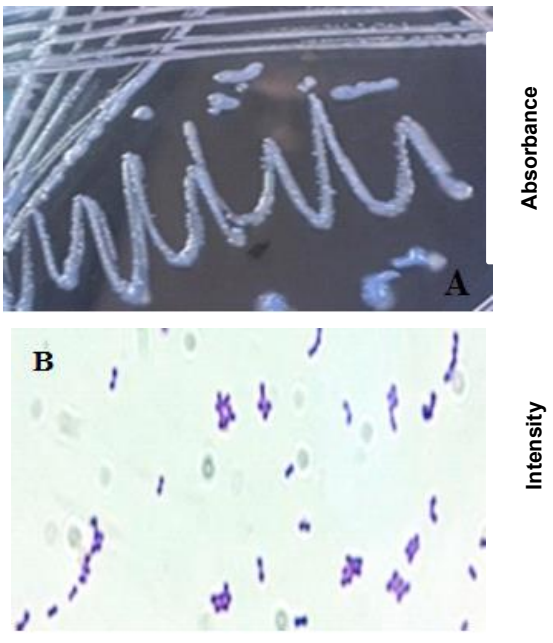

Figure (1): A: *S. spps.* on Mites Salivarius Agar, B: Gram stain of *S. spps.*

Characterization of Ag NPs by alteration of Color: Figure (2) illustrates how the color of the combination solution changes from light dark to deep dark, showing the formation of Ag NPs.

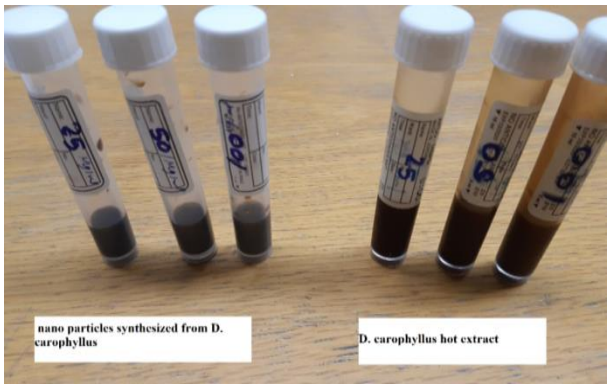

Figure (2): Change in color of plant extract after formation of Ag NPs.

UV using spectrophotometer: Figure (3) shows the reported absorbance of Ag NPs at 350 nm. A higher absorbance peak indicates a drop in the amount of Ag NPs.

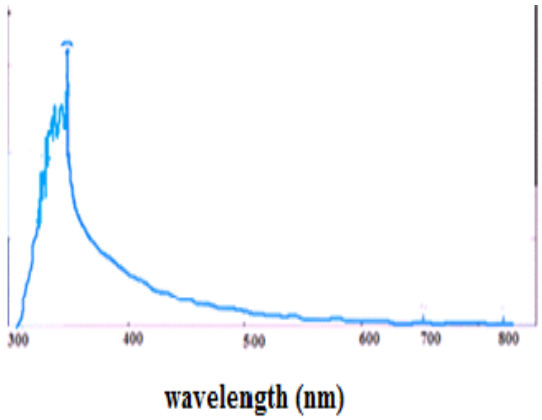

Figure (3): UV-Vis spectrophotometer applied to Ag NPs production.

XRD technique: An x-ray spectrometer is used to perform diffraction of x-rays on a solution containing Ag NPs, as shown in figure (4) to demonstrate the crystalline nature of the Ag NPs. The refracting spectra were obtained at angles ranging from 20° to 80° from the pattern. Reflections were seen at four significant angles: 38.52°, 45.39°, 64.79°, and 78.18.

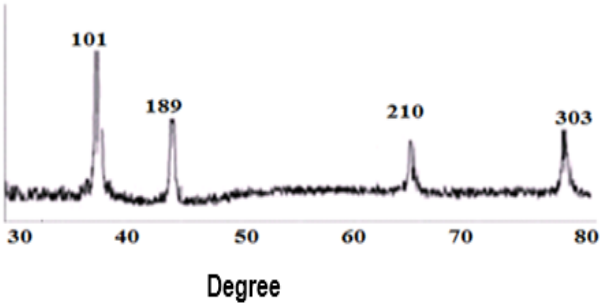

Figure (4): XRD assay of Ag NPs from *D. caryophyllus*.

FTIR examination: Findings indicated that solutions responsible for Ag NPs reduction and Ag ion bio- reduction were analyzed using an FTIR spectrum. Transmission bands found at the following wavelengths—3427, 2920, 2856, 1747, 1634, 1450, 1379, 1279, 1048, and 494 cm−1 [33] were indicative of the presence of a capping agent containing Ag NPs, as figure (5) shows.

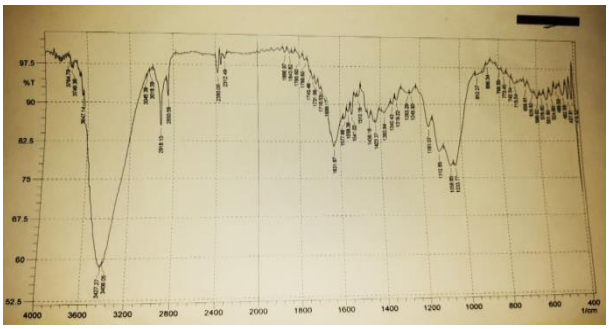

Figure (5): FTIR results of Ag NPs from *D. caryophyllus*

Antibacterial activity of D. caryophyllus aqueous extract against mutated and non-mutated Streptococcus spps: The extract of D. caryophyllus has a good activity against Streptococcus spps.The data showed an inhibitory zone of D.carophyllus flower extract at the concentration of 100 mg/mlwas 30 mm and at a concentration of 50, 25 mg/ml were 27 and 25 mm, respectively against S. salivarius. In contrast the inhibitory activity at concentration of 100 mg/ml was 16 mm and in 50 and 5 mg/ml were 14 and 9mm, respectively, against S. mutans. At the same time, least effective against S. sorbinus were 15, 13, and 11mm in diameter of inhibition zone at concentrations used in this study of D. caryophyllus. The result is shown in table (1) and figures (6 and 7).

Table (1): Antibacterial activity of D. caryophyllus aqueous extract against Streptococcus spps.

Bacterial isolates		Conc. 100µg/ml	Conc. 50µg/ml	Conc. 25µg/ml	Control	L.S.D (P-value)
Non -mutated	*S. salivarius*	30.00 ±1.84	27.05 ±1.47	25.19 ±1.24	0.00 ±00	6.961 ** (0.0001)
	S. mutans	16.07 ±0.57	14.15 ±0.74	9.00 ±0.67	0.00 ±.00	5.226 ** (0.0001)
	S. sorbinus	15.26 ±0.42	13.00 ±0.56	11.14 ±0.54	0.00 ±00	4.968 ** (0.0001)
Mutated	*S. salivarius*	30.09 ±2.17	25.22 ±1.08	20.07 ±1.13	0.00 ±.00	6.308 ** (0.0001)
	S. mutans	21.15 ±0.85	23.29 ±1.14	20.17 ±0.82	0.00 ±00	5.982 ** (0.0001)
	S. sorbinus	30.04 ±1.63	20.17 ±0.78	18.08 ±0.69	0.00 ±.00	6.170 ** (0.0001)
L.S.D. (P-value)		6.029 ** (0.0001)	5.833 ** (0.0001)	6.752 ** (0.0001)	0.00 NS	---

** (P≤0.01).

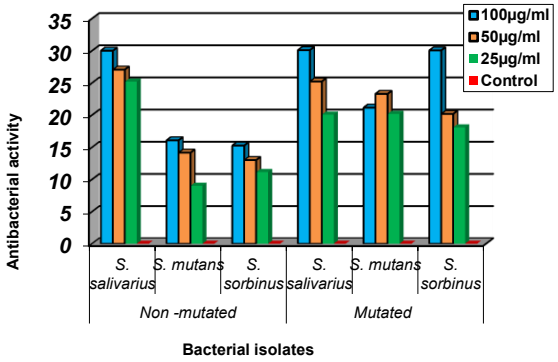

Figure (6): Antimicrobial activity of *D. caryophyllus aqueous extract against Streptococcus spps.*

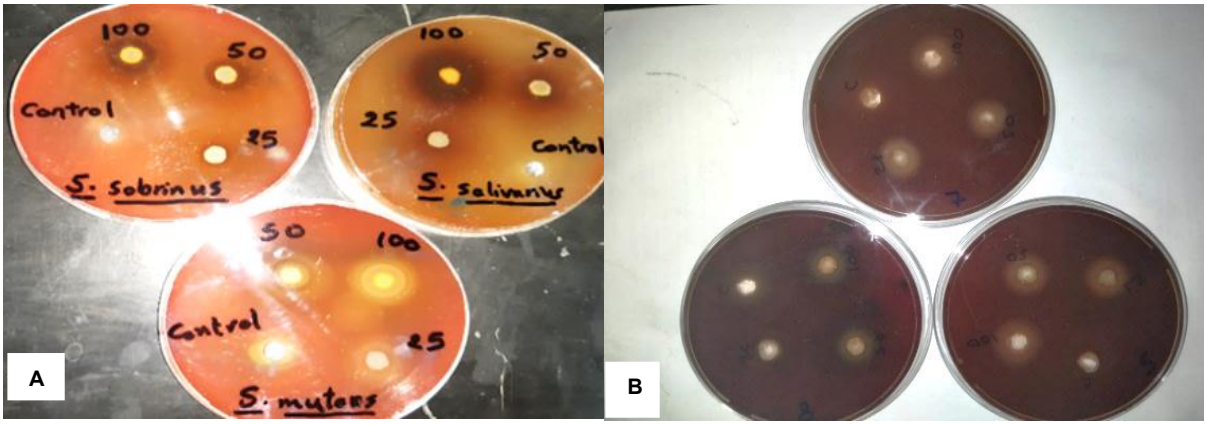

Figure (7): A. Aqueous extract of *D. caryophyllus* against non-mutated *Streptococcus* spps., B. extract of *D. caryophyllus* against mutated *Streptococcus* spps.

Antibacterial action of Ag NPs from D. caryophyllus against mutated and non-mutated Streptococcus spps: This study showed good activity of nanoparticles from D. caryophyllus against Streptococcus spps. isolated from gingiva, as demonstrated in table (2) and figures (8 and 9).

Table (2): Antibacterial activity of Ag NPs from *D. caryophyllus* against *Streptococcus* spps. isolated from gingiva.

Bacterial isolates		Conc. 100µg/ml	Conc. 50µg/ml	Conc. 25µg/ml	Control	L.S.D. (P-value)
Non-mutated	*S. salivarius*	35.21 ±2.36	30.35 ±1.14	29.16 ±1.26	0.00 ±00	7.028 ** (0.0001)
	S. mutans	30.08 ±1.72	25.19 ±0.93	23.02 ±1.07	0.00 ±.00	6.447 ** (0.0001)
	S. sorbinus	25.21 ±1.19	23.00 ±0.88	20.11 ±0.79	0.00 ±.00	5.793 ** (0.0001)
Mutated	*S. salivarius*	30.08 ±0.91	30.17 ±1.05	25.07 ±1.14	0.00 ±00	6.026 ** (0.0001)
	S. mutans	20.00 ±0.87	18.24 ±0.75	15.00 ±0.66	0.00 ±00	5.163 ** (0.0001)
	S. sorbinus	25.19 ±1.02	20.03 ±0.79	18.12 ±0.69	0.00 ±.00	5.508 ** (0.0001)
L.S.D. (P-value)		5.809 ** (0.0001)	5.874 ** (0.0001)	6.054 ** (0.0001)	0.00 NS	---

** (P≤0.01).

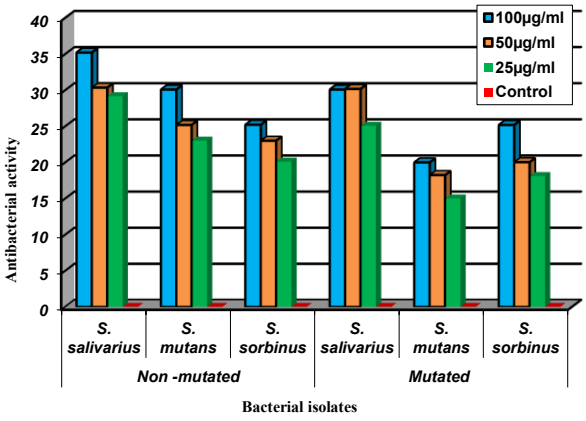

Figure (7): Antibacterial activity of Ag NPs from *D. caryophyllus* against *Streptococcus* spps. isolated from gingiva.

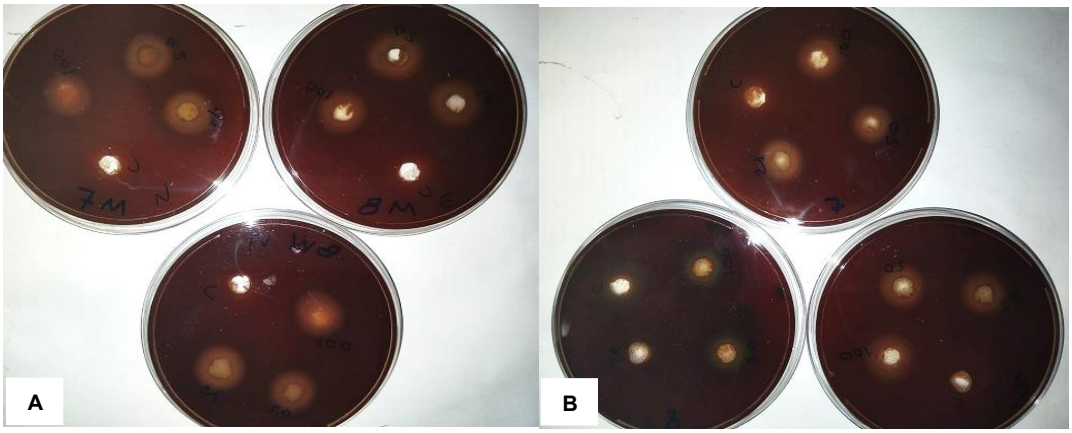

Figure (9): A. Ag NPs of *D. caryophyllus* against non-mutated *Streptococcus* spps., B. Ag NPs of *D. caryophyllus* against mutated *Streptococcus* spps.

Ag NPs have been shown in previous research to have the ability to successfully fight both Gram- negative and Gram-positive bacteria, including those that are extremely resistant to antibiotics. The mechanism of Ag NPs is demonstrated to penetrate the bacterial cell wall, alter the permeability of the cell membrane, and ultimately result in the death of the cells G.M. [34]. The research results were encouraging, and it was shown that cloves have outstanding antibacterial activity in addition to low amounts of zinc oxide nanoparticles working against these bacteria.

Streptococci were grown on Mites Salivarius agar plates, and their shape and appearance were employed to identify them. This result agrees with [35]. About 69% of normal healthy individuals found were carriers for Streptococcus spp. This result agrees with another study that reported that about 20-70 % of adult individuals carry Streptococcus ssp. in the gingival cavity, which found almost all gingiva pathogens have been identified and also found at a high rate in children [36, 37]. Microscopically, the surface of silver nanoparticles form, plasmon palpitations show signs of irritation, as seen by the change in color of the aqueous solution [38, 39]. In the Ag NPs solution, the plasmon band surface absorbance typically ranges from 300 to 450 nm [40, 41]. In comparison to the standard, the generated XRD spectrum matched the planes of 101, 189, 210, and 303. The results obtained agreed with those found in [36]. Extracts rich in phytochemicals and pure compounds have shown inhibitory effects against plaque, adhesion, and biofilm formation of Streptococci [42].

Streptococci can cause a wide range of diseases, from non-life-threatening illnesses like dental caries, and pharyngitis to potentially fatal illnesses like necrosis of the fascia and meningitis [43]. S. mutans is recognized as the oral Streptococci's primary cause of dental caries; as stated by [41]. 5%–10% of public health budgets in advanced nations are allocated to periodontal disease, related dental care, and dental caries [43]. Dental caries is among the most widely prevalent disorders in the world [44]. When administered to pathogenic bacteria such as P. aeruginosa and E. coli, plant extract in ethanol solvent (400 mg/ml) significantly outperformed the antibiotic ($P \leq 0.05$), while having no effect on S. aureus and K. pneumoniae. At last, plant extract from D. caryophyllus L. flower buds are thought to be an effective exporter for managing harmful germs in hospitals [45]. D. caryophyllus L. flower buds contain phytochemical substances that are thought to be an excellent source for controlling hospital pathogenic bacteria [46]. Also used in toothpaste as an antibacterial agent against Streptococcus spps. [47]. When the separate extracts were combined with ineffective antibiotics at lower dosages, this inhibition was seen [48]. The antibacterial effects of various ZnONPs and clove extract concentrations on S. mutans isolates from dental caries cases were investigated in this study. A total of 31 isolates of S. mutans were collected, representing 20.52% of the 151 isolates in total. The selection of this particular nanomaterial was based on its strong affinity for human cells and its lack of toxicity when compared to other nanoparticles.

Excellent results were obtained from both the well diffusion method and the MIC experiment. In the well diffusion method, the effectiveness increased as the concentration of ZnONPs and clove extract increased. It was found in the ZnONP that the minimum inhibitory concentration is equivalent to 0. 312 mg/ml for S. mutans, and in the MIC experiment, the alcoholic and aqueous extracts of clove were found to have MICs in the third and fourth tubes, indicating that their respective MICs for S. mutans are 25 mg/ml and 12.5 mg/ml [49].

This study also agrees with another local study, which reported that the Ag NPs synthesized from Streptococcus spps. have antibacterial activity against Gram-negative and Gram-positive bacteria [50, 51]. However, the Ag NPs had a good antibacterial effect not only against Streptococcus spps. but also, against K. pneumoniae and their biofilm formation [52, 53]. In callus extract, adding AgNO3 Nanoparticles (0.5 mg/ml) resulted in a substantial rise of Apigenin. However, adding AgNO3 nanoparticles (2 mg/ml) caused a considerable increase in Quercetin and Luetolin production, according to the results of [54]. Ag NPs increase the activity of Apigenin in callus extract, which has a synergistic effect. These findings suggest that clove oil may have an influence on the physical, chemical and antibacterial properties of toothpaste made with hydroxyapatite, Nano silver, and clove oil. Nanoparticles had not only antimicrobial activity against bacteria but also anti-cancer activity [55].

The extract of D. caryophyllus and AgNPS of this plant extract can be used as antibacterial drugs against Streptococcus spp. in gingiva and stop the destruction of our teeth by these types of bacteria, but further studies are also needed to improve its safety.

CONCLUSION

The D. caryophyllus had a good antibacterial agent against Streptococcus spps., that caused dental caries, therefore, can be used as herbal medicine for the treatment of these detrimental bacteria. Because of this low side effects for healthy. AgNPs synthesized from the hot extract of D. caryophyllus were also used, and they had good antimicrobial activity against Streptococcus spps. (mutated and non-mutated). Nanoparticles of Ag are especially now used in treatment of teeth infected with Streptococcus spps. (mutated and non-mutated), which cause

dental caries. Finally, this type of research needs more studies in vivo to study AgNPs effect on the tissue of the mouth.

Disclosure Statements

- **Ethics approval and consent to participate:** The protocol for the present study was approved by the Ethics Committee at the Department of Biology (University of Baghdad) and the Iraqi Ministry of Health (Reference: CSEV/0921/0099). Written informed consent was obtained from all the patients. The work was carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki).
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- **Author's contribution:** The authors confirm contribution to the paper as follows: study conception and design: Al-Haidari, A M Dh, Sweedan, E Gh; theoretical calculations and modeling: Sweedan, E Gh; data analysis and validation: Hasan, J M, Sweedan, E Gh; draft manuscript preparation: Sweedan, E. Gh, Al-Haidari A M Dh, Hasan, J M. All authors reviewed the results and approved the final version of the manuscript.
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