NTU Journal of Pure Sceinces (2024) 3 (1) : 36-41
DOI: https://doi.org/10.56286/ntujps.v3i1.769

IRAQI Academic Scientific Journals

ISSN: 2789-1089 EISSN: 2789-1097
NTU Journal of Pure Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JPS/index

Treatment and Prevention of Oral Biofilms by Clove _Gold Nanoparticles (Comparative Study)

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Article Informations

Received: 09-11-2023
Accepted: 13-01-2024
Published online: 06-04-2024

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Key Words:
Enterococcus faecalis, Streptococcus mutans, lactobacillus spp., gold nanoparticles, clove buds.

A B S T R A C T

The aim of the study **is** to **assess** the antibiofilm activity of biosynthesizing gold nanoparticles derived from clove buds extracts against certain oral bacteria to compare between preventive and treatment protocols. The antibiofilm activity was tested using the Microtiter plate method against oral bacteria (*Enterococcus faecalis, Streptococcus mutans and lactobacillus spp.)*. This study has showed a significant antibiofilm effect on oral bacteria.

Introduction

Nanotechnology is the physical, chemical, and biological manipulation of materials at the atomic level to achieve the nano size range of 1 to 100 nm. Recently, it's regarded as one of the most important science and technologies. With the exponential advancements in biomedical applications using nanoparticles for imaging, diagnostics, drug delivery, and therapies, the production of nanoparticles is a broad area of research in the field of nano technology, however, nanotechnology is expanding and has implications for energy, electronics, food, and medicine [1] and [2].

Plants nanoparticles (NPs) production is better than other biological processes because cell culture is not disrupted. Plant NPs synthesis is a one-step process without mutation issues, unlike microbe NPs synthesis. Plants extract components (bud extracts, flower extracts, fruit extracts, and seed extracts) synthesise NPs using reducing agents, secondary metabolites, enzymes and proteins [3].

Clove (Syzygium aromaticum) is one of the healthiest spices, has been used for centuries as a food preservative and for a number of medical purposes. This plant is one of the best sources for medical, cosmetic, food and agricultural uses, since it contains large amounts of the phenolic compounds' eugenol, eugenol acetate and gallic acid. The antioxidant and antibacterial properties of clove exceed those of many other fruits, vegetables, and spices.

Dental caries is a common disease of the mouth that is mostly caused by Streptococcus mutans (S. mutans), a facultative anaerobic bacterium. The ability to make biofilm and make it easier for germs to stick to tooth enamel and each other is a key factor in the development of dental caries, which can lead to a serious disease, i.e. infective endocarditis. Preventing the infection is still one of the hardest things about being a dentist. The toxic factor comes from the ability of bacterium to make itself more resistant to different antibiotics and avoid being destroyed by defense cells. The main way that S. mutans sticks and clings to tooth enamel is by making glucan substance, which is an important part of the polysaccharide matrix. This matrix, which is made from sucrose, makes adhesion and bonding preferable. The glucosyltransferase (GTF) enzyme in S. mutans facilitate the breakdown of sugar into fructose and glucose. After that, simple sugars are added to the growing glucan polymer, which helps make the exopolysaccharide matrix [4].

The complex system, i.e., the oral cavity is the habitat of more than 300 different bacterial species, including streptococci, which accounts for roughly (47-85%) of the biofilm found in the first four hours following a dental cleaning. Under typical settings, when biofilm oxygen levels decrease, the proportions of anaerobic bacterial growth increase, resulting in the emergence of many species from the genera. A variety of oral disorders are caused by Streptococcus, Lactococcus, Lactobacillus, Enterococcus, Corynebacterium, Staphylococcus, Bacteroides and Veillonella species [5].

Enterococcus faecalis

It is a gram positive anaerobic bacteria. It is frequently found in the human oral cavity, gastrointestinal tract and vaginal tract, evolved to such nutrient-rich, low-oxygen requirements and a complex ecology. E. faecalis is a common sign of endodontics. It is also very resistant to antimicrobial treatments and can live in difficult environments with a small amount of food and a high alkaline pH of 11.5 for a long time [6].

Chronic periodontitis has been linked to Enterococcus species, especially Enterococcus faecalis, while inflammatory root canal treatment has been linked to chronic apical periodontitis. E. faecalis can grow as a biofilm on the walls of root canals and as a single infection in the canals without help from other bacteria. Another study found that the bacteria in the mouth are the source of the microorganisms that found in the root canal space [7].

Oral Streptococci that live in the mouth and can be acquired soon after birth. They are an important part of the oral microbiome as they live in the mouth. They are active in fermenting carbohydrates and producing acids as a byproduct. Acidic species in the mouth, such as, Streptococcus mutans, play an important role in the growth of dental caries. Oral Streptococci is also important because it can make hydrogen peroxide, which can stop S. mutans from growing. In the human oral cavity, there are 25 species of the genus Oral Streptococci, which accounts for around 20% of all oral bacteria. Although S. mutans are naturally present in human oral microbes and the microbial species are associated with carious lesions, commensal Streptococci have been described as the most significant bacteria associated with the development of dental caries. These bacteria have the ability to transform into opportunistic pathogens that cause disease and harm the host [8].

Lactobacillus spp.

This bacterium is often found at active carious sites in both adults and children, especially in areas with advanced caries. Lactobacilli are germs that can be found in the mouth soon after birth. Lactobacilli in babies' mouths are thought to have come from their parents' mouths, especially their mothers', because they are so close to them. They can also come from foods that babies eat, like milk and other fermented foods. There is no evidence to distinguish between different types of Lactobacillus spp. or identify where they came from. In the mouth, Lactobacilli are usually found in saliva inside the cheeks, the hard palate and the back of the tongue. Most people

think they only stay in the mouth for a short time and are only found in very small amounts on the teeth of healthy babies and young children. [9].

Chlorhexidine

Chlorhexidine was produced as an antiseptic agent by Imperial Chemical Industries (Manchester, UK) in the 1950s. Chlorhexidine is a common antiseptic ingredient used in medicine and personal hygiene products like toothpaste and mouthwash [10].

Two chloroguanide chains joined by a hexamethylene chain form chlorhexidine. It is a strong base and di-cation at physiological pH. Water-insoluble chlorhexidine must be mixed with gluconic or acetic acid to generate digluconate or diacetate salts. Chlorhexidine solutions are colourless, odourless and harsh taste. Topically applied chlorhexidine N-chlorinated derivative binds covalently to skin and mucosa proteins, providing long-lasting antibacterial effects with low systemic absorption [11].

Aims of study

The aim of the study is to assess the antibiofilm activity of biosynthesizing gold nanoparticles derived from clove buds extracts against certain oral bacteria, to compare between preventive and treatment protocols.

Materials and methods:

Bacterial Strains:

Pathogenic oral bacterial: Enterococcus faecalis, Streptococcus mutans and lactobacillus spp. were obtained from the University of Mosul /College of Dentistry's Microbiology Laboratory (where they had been previously isolated by a team researcher and identified both morphologically and genetically).

Chemicals:

The chemicals used in the current study included: glycerol, glacial acetic acid and saline, crystal violet powder, methanol, chlorhexidine 0.2%, sucrose 2% and brain heart infusion broth. McFarland tube no.1 (0.5) which equal 1.0x108 CFU/ml.

Preparation of clove buds aqueous extract:

Clove buds were bought from shops and kept at a temperature of 25˚C, away from the sun and heat. Aqueous solution was prepared [12].

Preparation of clove- gold nanoparticles:

An aqueous solution of (gold salt) Hydrogen tetrachloroaurate (III) trihydrate (HAuCl4.3H2O) 0.001M was prepared [3]. The prepared nanoparticles were characterized by UV- Vis Spectroscopy, Fourier-Transform Infrared Spectrum (FTIR), Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Analysis (EDX), Zeta Potential Analysis, DLS measurements and XRD Analysis, which were carried out at the laboratory of Ministry of Sciences and Technology-Baghdad/Iraq and Alkhora company lab. Baghdad/Iraq.

Microtiter plate method

A pure single colony of each strains was used for inoculation in tubes containing 3 ml of brain heart infusion-broth supplemented with 2% sucrose. Bacterial cultures were then incubated at 37˚C for 48 hrs. Determination of antibiofilm activity was conducted by using two microtiter plate assay. The culture of each strains (Enterococcus faecalis, Streptococcus mutans and lactobacillus spp.) was adjusted to 0.5 McFarland using sterile brain heart infusion-broth (1.5 × 108). For each bacterium, the experiment was set by two major groups, treatment group: clove-gold nanoparticles (C-GNps) and clove buds extract were added into the wells after the addition of bacteria and prevention group: C-GNp and clove buds extract before the addition of bacteria in the wells. Two concentrations of C-GNps and clove buds extract were used. Two wells in each plate as control negative for biofilm formation level of untreated bacterial strains and two well in each plate as control positive represented the biofilm inhibition by 0.2% chlorohexidine. To check sterility of medium, 200 µl of brain heart infusion broth containing 2% sucrose were added to two wells in each plate. The experiment was conducted by adding aliquots of 100 µl of previously prepared bacterial culture distributed the addition of bacterial suspension was before the addition of clove-gold nanoparticles and clove buds extract in part of treatment then Aliquots of 100 µl of gold nanoparticles and cloves extract, while this was done before bacteria in part of prevention [13]. The microtiter wells where designed and arranged as in table 1.

Table 1. Bacteria examined in the current study (E. faecalis, S. mutans, lactobacillus spp.)

Treatment		**Prevention**	
G.N- 1	Clove-1	G.N- 1	Clove- 1
G.N-1	Clove-1	G.N- 1	Clove- 1
G.N- 1	Clove-1	G.N- 1	Clove- 1
G.N- 0.5	Clove-0.5	G.N- 0.5	Clove- 0.5
G.N- 0.5	Clove-0.5	G.N- 0.5	Clove- 0.5
G.N- 0.5	Clove-0.5	G.N- 0.5	Clove- 0.5
Bacteria + CHX 0.2		Bacteria	Broth
Bacteria + CHX 0.2		Bacteria	Broth

*GN-Gold nanoparticles, *Clove – Clove buds extract, *CHX- Chlorohexidine

The plates were stored at 37˚C for 48 hrs. Following incubation, the liquid from each well in the plate was aseptically aspirated with a micropipette, and the plates were washed three times with sterilised normal saline (0.9%). 200 µl of methanol was poured to the washed wells, and the plates were allowed to sit at room temperature for 20 minutes before poring the fixative methanol and drying the plates. The plates were stained by pouring 200 µl of 0.1% crystal violet into each well and leaving them for 15 minutes before washing three times with distilled water and drying at room temperature. The adherent cells were resolubilized in each well with 200 µl of 33% glacial acetic acid, and the optical density was measured at 600 nm with a microplate reader. Each clinical isolate was tested three times, and the mean and standard deviation were computed for statistical purposes. [14].

Statistical analysis:

One Way Analysis of Variance and Duncan's Method was used to compare CHX, C-GNps, and clove buds extract three times. All the tests were done the same way. A p-value of less than 0.5 was thought to be important.

Results and Discussion:

The anti-biofilm effect of C-GNps and clove buds extract of both concentration (1:1 and 0.5) were done against the oral bacterial isolates (Enterococcus faecalis, Streptococcus mutans, and lactobacillus spp.) in therapeutic and preventive ways, using the microtiter plate compared with 0.2% chlorohexidine.

Results showed that both C-GNps and clove buds extract have the antibiofilm effect in different degrees as shown in figures 1 ,2 and 3.

The significant effect on Enterococcus faecalis and Streptococcus mutans in treatment group was given by CHX, C-GNps respectively, while the best effect on lactobacillus spp. was given by CHX, C-GNps and clove buds extract (1:1).

In case of prevention study group, a significant effect on biofilms from Streptococcus mutans and lactobacillus spp. was shown using CHX and all the extracts used, while the biofilms synthesized by Enterococcus faecalis was affected by CHX and C-GNps significantly.

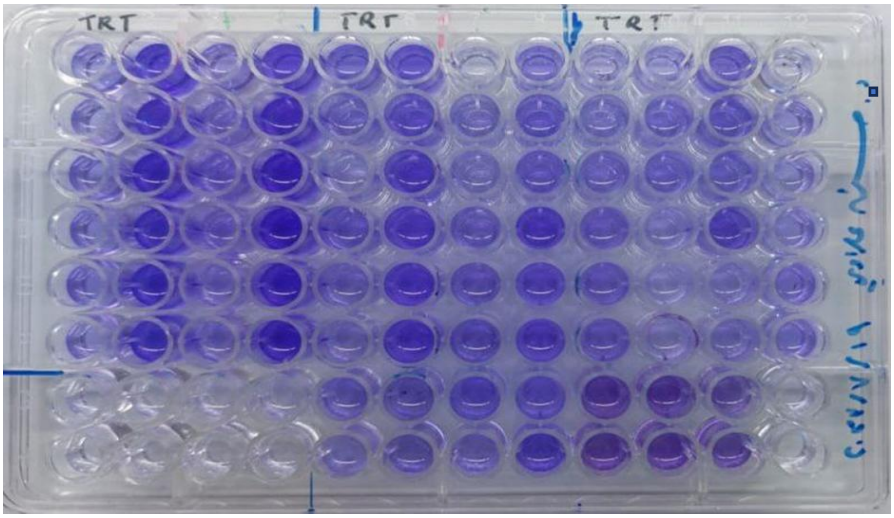

Figure 1: Microtiter plate of antibiofilm effect of CHX, clove extract and C-GNps.

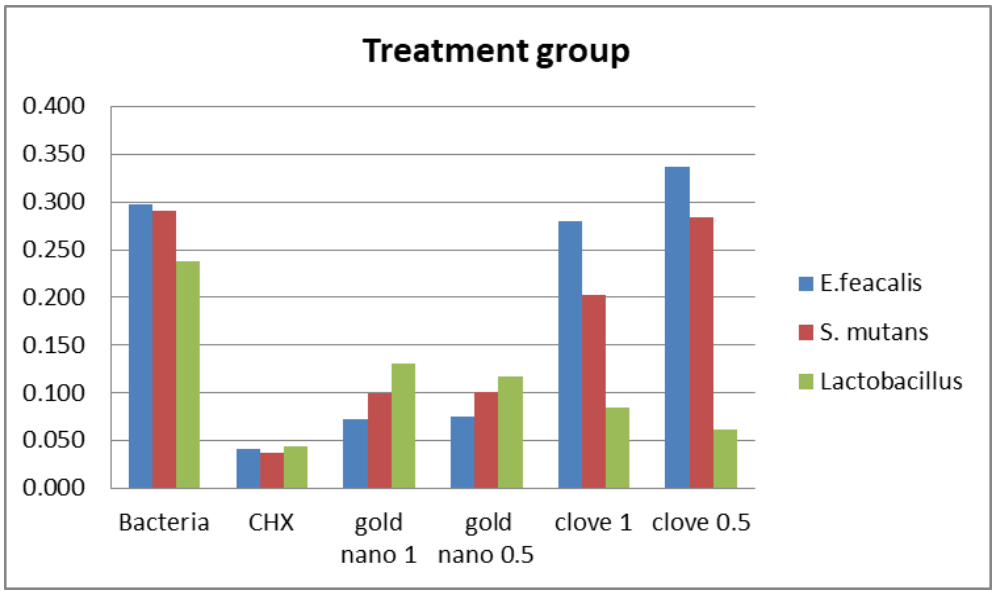

Figure 2: antibiofilm effect of CHX, clove extract and C-GNps. (treatment methods)

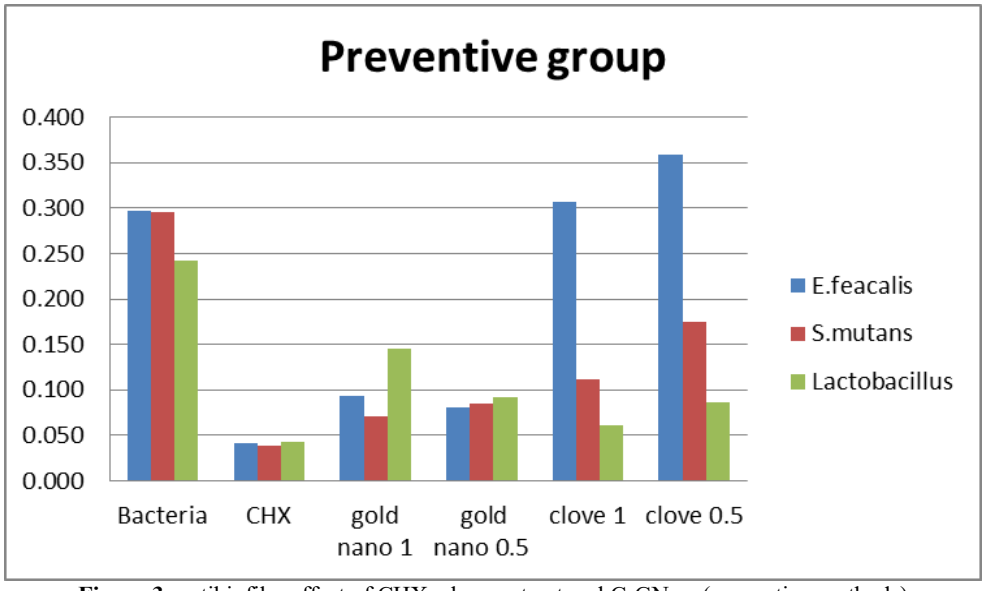

Figure 3: antibiofilm effect of CHX, clove extract and C-GNps. (prevention methods)

The literature has recorded many studies for antibiofilm activity of medicinal plant extract from various plant components, including the roots, flowers, buds, and leaves, were also discovered to have anti-biofilm properties [15]. The antibiofilm activity of gold nanoparticles from medicinal plants have been studied [16] and [17]. According to the previous studies it has been found that clove buds extract affected but they found the clove buds nanoparticles has better effect on biofilm of bacteria. Anti-biofilm efficacy of AuNPs was investigated on the glass surfaces. In this study, morphological changes were observed in the structures of biofilm due to the stress created by AuNPs. Data analysis of biofilm formation by fluorescence microscopy revealed that the two-tailed p value to be \0.05 indicating that biofilm reduction (%) at 0.002 mol/L of AuNPs was statistically significant when compared with control biofilm (without AuNPs). This indicates that AuNPs have efficient anti-biofilm activity against several pathogenic bacteria [18].

Conclusion

This study has shown gold nanoparticles biosynthesized by aqueous extract of clove buds with great stability, showed a good antibiofilm effect on Enterococcus faecalis, Streptococcus mutans, and lactobacillus spp. also aqueous extracts of clove buds have antibiofilm activity against cariogenic microorganisms.

Acknowledgment:

This investigation was carried out in various scientific labs in Mosul City, Iraq, including the Microbiology Laboratory of the College of Dentistry/Mosul University.

Ethical approval:

The scientific and ethical academic committees of the Department of Basic Science, College of Dentistry/University of Mosul approved the project under approval number UoM.Dent.23/9.

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