Effect of gold nanoparticles and Plant thymus extract against *Streptococcus mutans* biofilm formation.

Mays Yasir Younis [1], Faysal Ghaze Hassen [2]

[1-2] Department of Pathological analyzes- College of Applied Sciences- Samarra University

Corresponding author email:Maysyasir27@gmail.com

[2]Faysal.alsamarrie@uosamarra.edu.iq

ABSTRACT

Dental caries is a biofilm-associated, sugar-dependent, and multifactorial infectious disease, leading to a dynamic demineralization-remineralization process in dental hard tissues. It is primarily mediated by acidogenic bacteria, with Streptococcus mutans recognized as a key etiological agent due to its strong adhesion capabilities and biofilm formation This study evaluates the antimicrobial and antibiofilm efficacy of gold nanoparticles (AuNPs) and plant extracts against *S. mutans*, employing a serial dilution assay at concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL. Furthermore, antibiotic susceptibility was assessed against a range of antimicrobial agents. Results demonstrated that AuNPs exhibited a statistically significant reduction in biofilm viability, with greater inhibition observed in root infections compared to dental caries-associated biofilms ($P > 0.05$).Antibiotic susceptibility testing revealed high resistance levels to Ampicillin (80%), Amoxicillin (92%), and Penicillin (72%) in dental caries isolates, while root-associated isolates exhibited even greater resistance to Amoxicillin (100%) and Penicillin (87%). Conversely, Metronidazole and Cefotaxime demonstrated complete inhibitory efficacy (100%) against both dental caries and root infections. These findings underscore the potential application of gold nanoparticles as an alternative antimicrobial strategy for mitigating biofilm-associated dental infections

Keywords: Dental caries, *Streptococcus mutans,* Biofilm inhibition, Gold nanoparticles, Antibiotic resistance.

INTRODUCTION

Dental caries is one of the most prevalent chronic infectious diseases, affecting individuals across all age groups. The World Health Organization (WHO) recognizes it as a major public health concern, emphasizing the need for preventive and therapeutic strategies.(1) Carious lesions result from microbial biofilm formation, driven by the fermentation of dietary carbohydrates, leading to the progressive dissolution of enamel and dentin .Among the microbial species implicated in caries pathogenesis(2), *Streptococcus mutans* plays a dominant role due to its acidogenicity, acidic, and extracellular polysaccharide production,

which facilitate adherence and biofilm stability(3). The virulence factors of *S. mutans* enable it to survive within cariogenic environments, further complicating treatment outcomes. Tradtional antimicrobial approaches, including antibiotic therapy(4), have shown limited efficacy due to the emergence of bacterial resistance mechanisms. As a result, there has been growing interest in nanotechnology-based interventions, particularly gold nanoparticles (AuNPs), which exhibit broad-spectrum antimicrobial properties through disruption of bacterial membranes and inhibition of biofilm formation(5). This study aims to Isolate and characterize S. mutans strains from dental caries and root infections .Evaluate the biofilm-inhibitory effects of gold nanoparticles (AuNPs) and plant extracts. Assess antibiotic susceptibility patterns of S. mutans isolates.

Materials and Methods

2.1 Collecting samples and isolating bacteria

A total of 150 clinical samples were collected from patients diagnosed with dental caries and root infections at the Specialized Dental Center in Tikrit and outpatient clinics between October 2023 and August 2024. Using sterile swabs, samples were collected and transported under aseptic conditions for microbiological analysis. Samples were cultured on:

- Mitis Salivarius Bacitracin Agar (MSBA) – Selective for S. mutans.
- Blood Agar – To assess hemolytic activity.
- Muller Hinton Agar – For antibiotic susceptibility testing.

Plates were incubated at 37°C for 24-48 hours, following which bacterial colonies were morphologically and biochemically characterized using VITEK-2 automated bacterial identification system.

2.2 Preparation of Culture Media

2.2.1Preparation Mitis salivarius bacitracin agar(MSB)

The MSBA medium was prepared by dissolving 90.7 g of dehydrated powder in 1000 mL of distilled water, supplemented with 1 mL selective supplement, sterilized by autoclaving at 121°C for 15 minutes, and poured into sterile plates(6)

2.2.2 Preparation blood agar

The medium was prepared by dissolving 40 g of dehydrated powder in 1000 mL of distilled water, sterilized, and cooled to 45-50°C before the addition of 5% human blood.(7).

2.2.3 PreparationMuller Hinton Agar

MHA was prepared by dissolving 38 g of dehydrated medium in 1000 mL of sterile distilled water, autoclaved at 121°C under 15 psi for 15 minutes, and poured into Petri plates for bacterial susceptibility testing**.**

2.2.4 Preparation of plant thymus extract

Prepared 3g of plant extract powder was dispersed in 300ml DW. below the mixture placed on the magnetic stirrer for 3hr at 100 ºC. The glass flask must be sealed with Aluminum Foil. After that, the precipitation was separated by filter papers (Whatmann No. 1 filter paper (pore size 25 µm)). Then the extract is kept in the refrigerator**.**

2.2.5 Preparation of Gold Nano particles by Laser

gold nanoparticles were prepared by the laser ablation method, which is considered one of the most important methods in the preparation of nanomaterial's as well as it's considered as an eco-friendly method Au nanoparticles were prepared Nd-Yag laser immersing a pellet of Au in 10ml of deionized water, and then laser radiation was focusing on the surface of the target with an energy of 900 mJ wavelength 1064nm1 Hz, with a number of pulses (1000)pulses(8).

2.3 Antibiotic test

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, adhering to Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotics were teste (9), The inhibition zones were measured after 24 hours of incubation at 37°C, and results were interpreted using CLSI standards (10).

2.4 Biofilm Formation and Inhibition Assay

The biofilm formation ability of S. mutans was quantified using the 96-well microtiter plate method, with gold nanoparticles (AuNPs) ,thyme plant extract and gold nanoparticales with thymus plant extract as inhibitory agents.

1. A bacterial suspension was prepared from brain-heart infusion (BHI) broth. The sample was diluted and compared with McFarland's standard solution, then incubated at 37°C for 24 hours.

2- added the gold nanoparticles (AuNPs) ,thyme plant extract and gold nanoparticales with thymus plant extract and use Different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) were introduced.

3- he titration dish was closed, then covered with Parafilm to prevent contamination. It was incubated at 37°C for 24 hours. After incubation, the contents of the wells were discarded, and the wells were washed three times with phosphate-buffered saline (PBS) to a pH of 7.2 to remove bacterial cells. They were then left to dry at room temperature.

4- Adherent live cells were fixed by adding 200 µL of absolute methanol to each hole and left for 15 minutes, after which the contents of the holes were discarded and left to dry.

5- 200 microliters of 1% crystal violet stain was added to each hole and left for 20 minutes. The stain was then removed and washed three times with phosphate-buffered saline (PBS) to remove any remaining stain. The stain was then left to dry at laboratory temperature.

6- Add 200 microliters of 96% ethanol to each well

7. Using the ELISA device the Optical density (OD) was measured at the wavelength at 595 nm using a microplate reader. (11)(12)

Results

3.1 Bacterial Isolation and Identification

Of the 150 clinical samples, 40 isolates (26%) were identified as *S. mutans* based on colony morphology, biochemical tests, and VITEK-2 analysis. Colonies on MSBA appeared as small blue spherical colonies, characteristic of *S. mutans*.

3.2 Cultivation Characters

The isolates showed growth on the MSBA medium, and they were cultured under aerobic and anaerobic conditions at an aerobic and at a temperature of 37°C for 24-48 hours. The colonies appeared as small blue dots or droplets.As Shown in Figure 1.

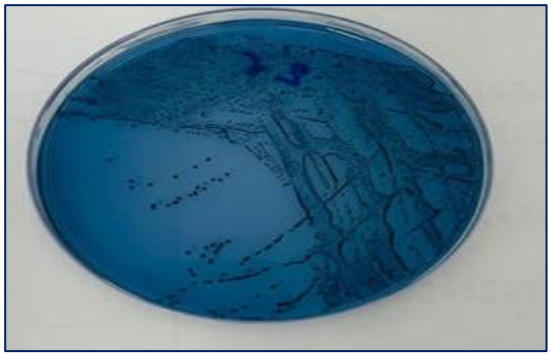


Figure (1) shape of the *S.mutan*

3.3 Biochemical tests

It included several tests to confirm the diagnosis of *Strep.mutans* as shown in Table (1).

Table (1) shows the biochemical tests for diagnosis *Streptococcus mtans*.

Biochemical test	Result
Catalysis test	Negative
Oxidase test	Negative
Coagulase test	Negative
Urease test	Negative
Hemolysis test	alpha-hemolysis
sugars (sucrose, mannitol, sorbitol, inulin)	positive
Indole	Negative
Simmon citrate	Negative
Melthy red	Positive
Voges-Proskauer	Positive

3.4 Diagnosis of *Streptococcus mutans* by VITEK-2

VITEK2 tests showed that the Probability of Streptococcus mutans is 96%, and this is an excellentpercentage that confirms that the bacteria isolated from caries are Streptococcus mutans, as noted in Figure (3).

Organism Quantity:
Selected Organism : Streptococcus mutans

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.60 hours	**Status:**	Final
Selected Organism	90% Probability	**Streptococcus mutans**		
	Bionumber:	120011177754522		
ID Analysis Messages				

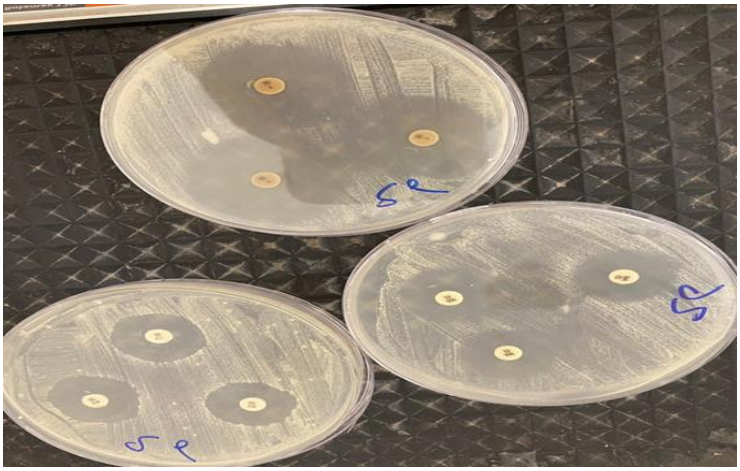
Figure 2. VITEK-2 test result.

3.5 Antibiotic Susceptibility Test

Susceptibility testing conducted on isolates of *S.mutans* using 7 different antibiotics, they were classified based on the NCLSI guideline (13) shows in table (2)and figure(3)

Table 2: the number and percentage of MDR-Streptococcus mutans

antibiotics	sample	frequency	Percent (%)
Amoxicilin	Cavity	23	92
	Root	15	100
Ampicilin	Cavity	20	80
	Root	9	60
Penicilin	Cavity	18	72
	Root	13	87
Vancomycin	Cavity	5	20
	Root	5	33
Tetracycline	Cavity	11	44
	Root	7	47
Metronidazole	Cavity	25	100
	Root	15	100
Cefotaxime	Cavity	25	100
	Root	12	80



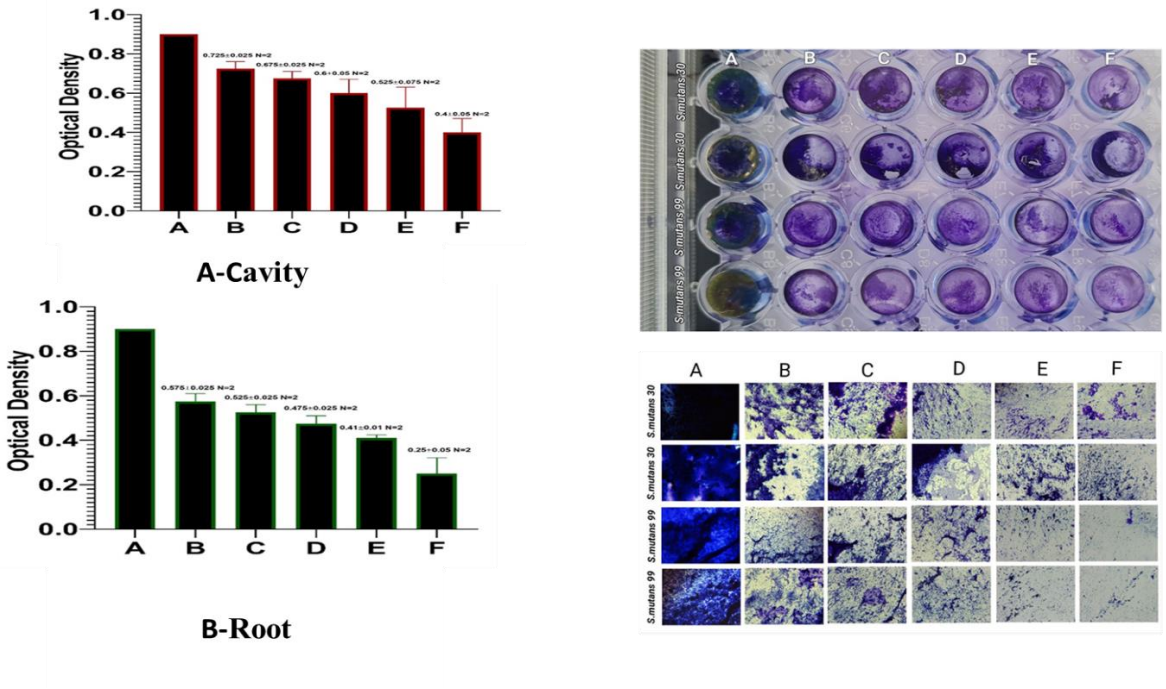
Figure(3) Antibiotic Susceptibility Test of *S.mutans*

3.6.1 Biofilm Inhibition Plant thymus extract

Plant thymus exhibited significant biofilm inhibition, with greater efficacy observed in root-associated biofilms compared to dental caries-related biofilms ($P > 0.05$). The mechanism involves cell wall penetration, disruption of biofilm architecture, and inhibition of quorum sensing pathways.As shown table(3) and on figure(4)

Table (3) effect *Strep.mutans* bacterial on biofilm production using thyme plant

NO	Sample	Materials	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.5mg/ml	Control	Average type
1	Cavity	Plant	0.35 ij	0.45 gh	0.55ef	0.65 cd	0.70 bc	0.90 a	0.600 b
2	Cavity repetition	Plant	0.45 gh	0.60 ed	0.65 cd	0.70 bc	0.75 b	0.90 a	0.675 a
3	Root	Plant	0.30 j	0.40 hi	0.50 fg	0.55 ef	0.60de	0.90 a	0.542 c
4	Root repetition	Plant	0.20 k	0.42 h	0.45 gh	0.50 fg	0.55ef	0.90 a	0.503 c
	Average concentration		0.325 e	0.468 d	0.538 c	0.600 b	0.650 b	0.900 a	



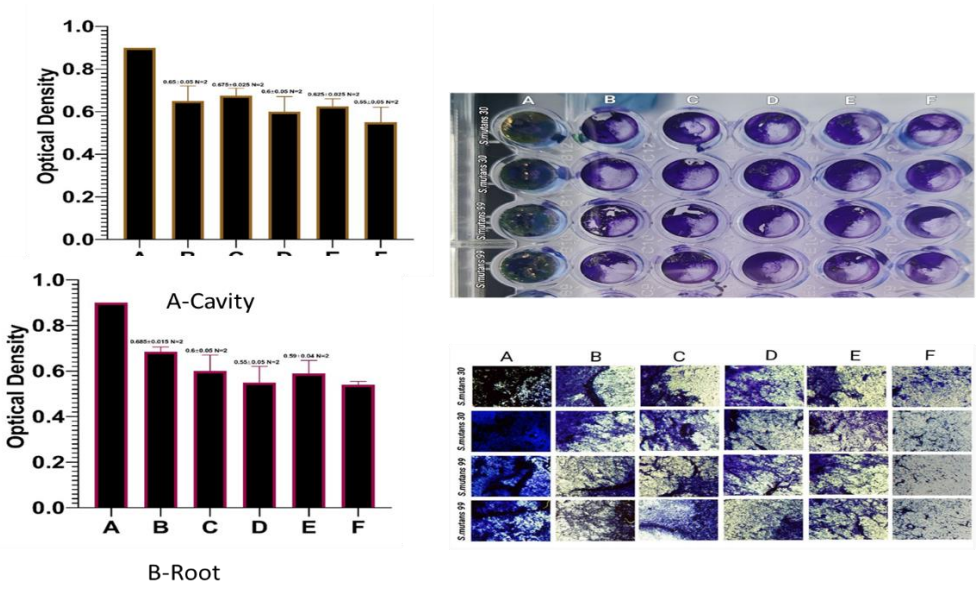
Figrue(4) Reduces biofilm formation in (Plant extract) *in S.mutans* (A)cavity (B)root

3.6.2 Biofilm Inhibition by Gold Nanoparticles

Gold nanoparticles exhibited significant biofilm inhibition, with greater efficacy observed in root-associated biofilms compared to dental caries-related biofilms ($P > 0.05$). The mechanism involves cell wall penetration, disruption of biofilm architecture, and inhibition of quorum sensing pathways and the effect gold on the *S,mutans* greater than plant thymus s .As shown table(4) and on figure(5).

Table (4) effect *Strep .mutans* bacterial on biofilm production using gold nanoparticles

NO	Sample	Materials	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.5mg/ml	Control	Average type
1	Cavity	Gold	0.50 fg	0.60 de	0.65 cd	0.70 bc	0.75 b	0.90 a	0.683 a
2	Cavity repetition	Gold	0.45 gh	0.50 fg	0.55 ef	0.60 de	0.65 cd	0.90 a	0.608 b
3	Root	Gold	0.20 k	0.35 ij	0.45 gh	0.55 ef	0.60 de	0.90 a	0.508 c
4	Root repetition	Gold	0.15 k	0.30 j	0.40 hi	0.50 fg	0.55 ef	0.90 a	0.467 d
	Average concentration		0.325 E	0.438 d	0.513 c	0.588 b	0.638 b	0.900 a	



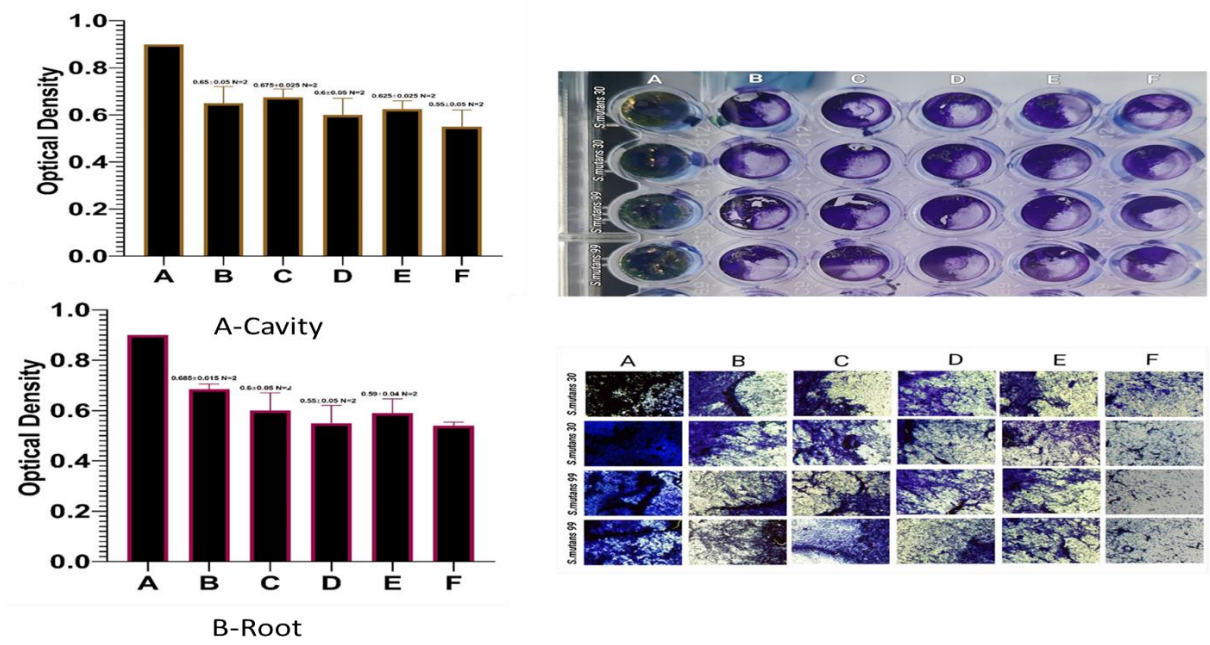
Figrue(5) Reduces biofilm formation in (gold nanopartical) in *S.mutans* (A)cavity (B)root

3.6.3 Biofilm Inhibition by Gold Nanoparticles with plant thymus

Gold nanoparticles exhibited significant biofilm inhibition, with greater efficacy observed in root-associated biofilms compared to dental caries-related biofilms ($P > 0.05$). The mechanism involves cell wall penetration, disruption of biofilm architecture, and inhibition of quorum sensing pathways and the effect gold nanoparticals with thymus plant on the *S,mutans* greater than plant thymus , gold nanoparticals. As shown table(5) and on figure(6)

Table (5) effect*Strep.mutans* bacterial on biofilm production using gold nanoparticles with thymus plant

NO	Sample	Material	100mg/ml	50mg /ml	25mg /ml	12.5mg/ml	6.5mg/ml	Control	Average type
1	Cavity	Plant+Gold	0.50 e	0.60 cd	0.55 de	0.65 bc	0.70 b	0.90 a	0.650 a
2	Cavity repetition	Plant+Gold	0.60 cd	0.65 bc	0.65 bc	0.70 b	0.60 cd	0.90 a	0.683 a
3	Root	Plant+Gold	0.55 de	0.63 c	0.50 e	0.55 de	0.70 b	0.90 a	0.638 a
4	Root repetition	Plant+Gold	0.53 e	0.55 de	0.60 cd	0.65 bc	0.67 bc	0.90 a	0.650 a
	Average concentration		0.545 d	0.608 c	0.575 cd	0.638 bc	0.668 b	0.900 a	



Figrue(6) Reduces biofilm formation in (gold nanopartical with thymus plant) in *S.mutans* (A)cavity (B)root

Discussion

As the chornic disease in the wide world, dental caries is a serious healthcare issue. Dental caries affect all age . For this reason, it is thought that dental caries is a microbiological caused by food, drink , smoking and it requires a cariogenic biofilm and frequent consumption of carbohydrates that ferment, such as sucrose ,mannitol ,glucose(14). The bacteria of causes dental caries is *streptococcus mutans* is Gram-positive facultative anaerobic bacteria(15).

In the study , specimens collected from the oral cavity isolated from dental caries and root caries were plated on Mitis salivarius bacitracin agar(MSBA) Special for insulation of *streptococcus mutans* The findings of this study were consistent with the observations made by (16) in samara. n this study when study to gram staining, these colonies shown as Gram positive cocci, exhibiting arranged in pairs or short chains and a spherical shape ,catalase negative , oxidase negative , urease negative , and triple sugar iron positive .consistent with

the observations made by Zadeh(17). while the Identification and delection according to the VITEK2 system results, and only forty isolates (26.7%) were identified as *Streptococcus mutans*. The findings nearal presented in this study are consistent with researcher(18) in Baghdad city .

This study concluded that a total of 40 *S.mtuans* clinical isolates of dental caries had a high resistance to Amoxicillin(92%) the study are consistent with researcher (19) the rate(100%).The antibiotic Penicillin is rate (72%) the study are consistent with researcher with (20) the rate (66.15) and disagree with(21) the rate (96%) , The antibiotic Ampicilin is rate(80%) consistent with researcher(22) , The antibioticVancomycin is rate(20%) , The antibiotic Tetracycline is rate (44%) , The antibiotic Metronidazole is rate (100%), , The antibiotic Cefotaxiame is rate(100%) . they study isolates of root caries had a high resistance to Amoxicillin rate (100%) , The antibiotic Penicillin is rate (87%) , The antibiotic Ampicilin is rate(60%), The antibioticVancomycin is rate(33%), The antibiotic Tetracycline is rate (47%) , The antibiotic Metronidazole is rate (100%), , The antibiotic Cefotaxiame is rate(80%) The remarkable variability shown by *Strep. mutans* isolates in their resistance to antibiotics and their uses is due to the diversity in resistance mechanisms through the use of the beta-lactam enzyme that inhibits these antibiotics or through a change in the binding sites of penicillin-binding proteins(23), Understanding these mechanisms is critical for developing innovative therapeutic strategies and addressing the growing challenges associated with antibiotic resistance in healthcare settings. The overuse of antibiotics poses a significant challenge in terms of treating infections and selecting the most appropriate antimicrobial therapy.

In current study anti-biofilm effect of plant thymus extract on 2 isolation of *S.mutans* (one Isolation dental ,one root caries), the result of biofilm producers was measured in response to concantraction (A=control ,B=6.25 mg/ml ,C=12.5 mg /ml ,D=25mg /ml ,E=50 mg/ml ,F=100mg /ml) by using tissue culture plates and crystal violet staining method , It showed significant inhibition of biofilms. reduced dramatically dilution increased, as illustrated in (figure 4) and (table 3), in the treated with different concentrations of thymus plant demonstrating a concentration-dependent decrease in biofilm thymus plant can prevent

biofilm development by inhibiting bacterial adhesion in microtiter plate method when the (p-value) less than 0.05, The consistent with researcher with(24).

The study anti-biofilm effect of gold nanoparticals (Au NPs) extract on 2 isolation of *S.mutans* (one Isolation dental ,one root caries), the result of biofilm producers was measured in response to concantraction (A=control ,B=6.25 mg/ml ,C=12.5 mg /ml ,D=25mg /ml ,E=50 mg/ml ,F=100mg /ml) by using tissue culture plates and crystal violet staining method , It showed significant inhibition of biofilms. reduced dramatically dilution increased, as illustrated in (figure 5) and (table 4), The results showed that the effect of the membranes decreased with increasing dilution of the concentrations. Also, the gold nanoparticles appeared higher than the plant extract, due to its high effectiveness and its compounds. The consistent with researcher with(25).

The study anti-biofilm effect of gold nanoparticals with thymus plants (Au P.E-NPs) extract on 2 isolation of *S.mutans* (one Isolation dental ,one root caries), the result of biofilm producers was measured in response to concantraction (A=control ,B=6.25 mg/ml ,C=12.5 mg /ml ,D=25mg /ml ,E=50 mg/ml ,F=100mg /ml) by using tissue culture plates and crystal violet staining method , It showed significant inhibition of biofilms. reduced dramatically dilution increased, as illustrated in (figure 6) and (table 5), The results showed that the effect of the membranes decreased with increasing dilution of the concentrations.. In our study, the lowest possible concentration was used that had a significant effect on inhibiting bacterial growth or as an anti-biofilm this result Compatible with , In vitro studies ,more reports have suggested the use of low over the high concentrations . Higher concentrations caused extensive agglomeration, whereas not observed with lower concentrations, indicating that low concentrations are prone to a more homogenous distribution of the NPs in the polymer . The uniform particle dispersion and impregnation in the matrix is crucial to avoid the development of stress concentration areas, impairing the mechanical properties of the resin. When the data are analyzed in terms of bacterial eradication, it is evident that finer surfaces polished with silica nanoparticles are easier to remove S. mutans bacteria from than rougher surfaces. This may be useful in shielding teeth against cariogenic bacteria-induced damage. The results of this study show promise for usage as additive constituents in toothpastes, mouthwashes, gels, varnishes, resin composites, etc. that comply with(26)

Conclusions

This study underscores the therapeutic potential of gold nanoparticles, thymus plant ,gold in mitigating biofilm-associated infections caused by S. mutans. Given the rising antibiotic resistance, AuNPs present a viable alternative for dental applications, warranting further in vivo and clinical investigations**.**

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