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Antibacterial Activity of Silica Nanoparticles Synthesized from Licorice Root Extract against *Streptococcus mutans*

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Abstract

One hundred and twenty dental caries samples were collected during the period between September 2023 and January 2024. *Streptococcus mutans* were isolated and diagnosed based on cultural, microscopic characteristics, and biochemical tests such as IMViC and the Remel RapID STR system. Obtained results showed that 64 isolates (53.4%) belonged to the genus *Streptococci* and that 40 isolates (62.5%) of this genus belonged to the species *Streptococcus mutans*, while 56 isolates (46.6%) of the total 120 isolates did not belong to the genus Non-Streptococci. Silica nanoparticles (SiO2 NPs) was prepared using the aqueous extract of Glycyrrhiza glabra (licorice) root extract and characterized using UV-Visible spectra, X-ray diffraction (XRD), and FESEM. SiO2 NPs showed absorbance peak at 497 nm. The XRD pattern showed that the strong four intense peaks indicate crystalline nature and face centered cubic structure of silica nanoparticles. Field emission scanning electron microscope (FESEM) was used to study the morphology of the SiO2 NPs. It exhibited a spherical shape with diameters of 46 to 77 nm. The obtained results of antibacterial activity showed that the prepared SiO2 NPs were effective against the isolated *Streptococcus mutans*, as the minimum inhibitory concentration (MIC) value was 12.5 units/mL, and the minimum bactericidal concentration (MBC) value was 25 units/mL.

Keywords: Silica Nanoparticles, dental caries, *Streptococcus mutans*, Glycyrrhiza.

الفعالية الضد بكتيرية لجسيمات السيليكا النانوية المصنعة من مستخلص جذور عرق السوس ضد بكتريا العقدّية الطافرة

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الخلاصة

جمعت 120 عينة من إلتهابات تسّوس الأسنان للمدة بين أيلول 2023 وكانون الثاني 2024. عزلت بكتريا *Streptococcus mutans* من اصابات تسوس الأسنان وشخصت بالأعتماد على الصفات الزرعية والمجهرية والخصائص الكيموحيوية مثل اختبارات الأندول، ، المثيل الأحمر، فوكاس بروسكاور، استهلاك السترات، والتشخيص باعتماد Remel RapID STR system . أوضحت نتائج التشخيص البكتيري أنّ 64 عزلة (53.4%) تنتمي الى جنس المكورات المسبحيَّة Streptococci وأنّ 40 عزلة (62.5%) من عزلات هذا الجنس تنتمي الى النوع *Streptococcus mutans*، بينما كانت 56 عزلة (46.6%) من مجموع العزلات المائة والعشرين لا تنتمي الى جنس المكورات المسبحيَّة Non-Streptococci . حُضّرت جسيمات السيليكا النانوية باستعمال المستخلص المائي لجذور نبات عرق السوس وتَمّ تشخيصها بوساطة تقنيات تحليل الطيف المرئي للأشعة فوق البنفسجية، تقنية حيود الاشعة السينية X-ray diffraction (XRD) ، تقنية المجهر الالكتروني الماسح عالي الدقة Field Emission Scanning Electron Microscopy (FESEM). أظهرت جسيمات السيليكا النانوية ذروة الامتصاص عند 497 نانومتر. أظهر نمط XRD أن القمم الأربع تشير إلى الطبيعة البلورية لجسيمات السيليكا النانوية. تم استخدام المجهر الإلكتروني الماسح بالانبعاث الميداني (FESEM) لدراسة مورفولوجيا جسيمات السيليكا النانوية. وقد أظهرت شكلاً كروياً بأقطار تتراوح بين 46 إلى 77 نانومتر. أظهرت نتائج الفعالية المضادة للبكتيريا التي تم الحصول عليها أن جسيمات السيليكا النانوية المحضرة كانت فعالة ضد بكتريا *Streptococcus mutans* المعزولة، حيث كانت قيمة الحد الأدنى للتركيز المثبط 12.5 وحدة / مل، وكانت قيمة الحد الأدنى للتركيز القاتل 25 وحدة / مل.

1. Introduction

Dental caries is a widespread infectious disease caused by the complex interaction between different microorganisms within the oral cavity. The primary bacteria responsible for dental caries is Streptococcus mutants, which are known for their ability to produce lactic acid through the fermentation of sugars, resulting in a decrease in pH and destruction of tooth enamel minerals. These bacteria's virulence is increased by their ability to form biofilms, which provide a protective environment that facilitates bacterial colonization and resistance to antimicrobial agents. Other bacteria, including Lactobacillus, were isolated from dental caries cases, albeit at lower frequencies than S. mutans [1, 2]. Licorice, scientifically known as Glycyrrhiza, is a genus of perennial herbs belonging to the family Fabaceae. It includes more than 30 species distributed widely worldwide, including the regions of the Mediterranean, central-southern Russia, Asia, Turkey, Iraq, and Iran regions [3]. The roots and rhizomes of these plants are the primary medicinal parts used in traditional and modern medicine [4]. The plant contains several bioactive compounds, including glycyrrhizin, licoricidin, and glycyrrhetinic acid, contributing to its therapeutic benefits [5]. Studies demonstrated that licorice extract, whether as mouthwash, gel, or other dental products, effectively reduces the number of bacteria in the oral cavity [6]. The ethanolic extract of the plant showed effectiveness similar to chlorhexidine and superior results to fluoride mouthwashes [7, 8]. The presence of bioactive compounds such as phenols, flavonoids, and saponins in licorice extract contributes to its antimicrobial and antioxidant activities, making it a valuable agent in the pharmaceutical and food industries for promoting oral health [9].

Using plant extracts to synthesize nanoparticles provides numerous benefits, making them a promising alternative to traditional chemical and physical synthesis methods. One of the primary benefits is its eco-friendliness and cost-effectiveness, as it eliminates the need for hazardous chemicals and high energy consumption, which are common in traditional methods [10]. This green synthesis approach not only reduces environmental pollution but also produces biocompatible nanoparticles with improved biological properties, making them suitable for various biomedical utilizations, including as antimicrobial, anticancer, and anti-inflammatory agents, and enhancing drug effectiveness [11, 12]. Licorice extract, derived from Glycyrrhiza glabra plants, has shown great potential in many nanotechnology applications due to its diverse therapeutic properties and ability to act as a reducing and stabilizing agent in nanoparticle synthesis. One important use is the green synthesis of metal nanoparticles like Gold, Silver, and Zinc. In this process, licorice extract acts as an environmentally friendly reducing agent, creating nanoparticles with antibacterial, antioxidant, and anticancer properties [13-15]. Furthermore, Nanoparticles synthesized using licorice plant extract show great potential in combating biofilms, complex structures formed by bacteria to adhere to surfaces [16, 17]. The antibacterial potency of these NPs is increased by their ability to disrupt biofilm formation and prevent the growth of pathogenic bacteria such as *Streptococcus mutans* and Mirabilis protein [15].

The study aimed to evaluate the antimicrobial activity of green synthesized silica nanoparticles using the aqueous extract of Glycyrrhiza glabra (licorice) root extract against *Streptococcus mutans* isolated from dental caries.

2. Materials and Methods

2.1 Sampling

One hundred and twenty dental caries samples were collected during the period between September 2023 and January 2024. Samples were collected from the teeth caries areas of patients representing different age groups (7- 65 years) visiting the Specialized Dental Center and private dental clinics in Ramadi.

2.2 Isolation and identification of Streptococcus mutans

The collected samples were grown on selective Mitis Salivarius Agar media supplemented with Bacitracin and grown at 37°C for 24 hours. *Streptococcus mutans* isolates were identified based on the morphological characteristics of the bacterial colonies such as their size, shape, color, and elevation in addition to microscopic characteristics of the bacterial cells after staining with Gram stain, and biochemical tests. The final identification was confirmed using the Remel diagnostic system which uses enzyme technology and carbohydrates fermentation to identify the bacteria.

2.3 Preparation of licorice aqueous extract

A 50g weight of ground licorice was placed in a 1-litre flask, and 500 mL of distilled water was added. Next, it was placed in a water bath with continuous shaking for 45 minutes. After that, it was filtered with filter paper, and the filtrate was collected and stored at four °C until used [18].

2.4 Preparation of silica nanoparticle solution

Silica nanoparticles were prepared using silica nitrate and licorice extract. 2 g of silica nitrate was added to 100 mL of distilled water and shaken well until dissolved. Then, six mL of the licorice aqueous extract solution was added to four mL of silica nitrate solution and mixed at 40 °C for two hours until the color began to change to yellow, as preliminary evidence of the formation of silica nanoparticles. Synthesized silica nanoparticles were characterized using UV-Visible spectra, X-ray diffraction (XRD), and FESEM [19].

2.5 Antibacterial activity of prepared silica nanoparticles

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the prepared silica nanoparticles were determined using the half-dilution method in liquid media [20]. A half-dilution series was generated by preparing 5 mL of nutrient broth media containing 10-100 µg/mL of SiO2 NPs. A half-dilution series was generated by preparing 5 mL of nutrient broth media containing 10–100 µg/mL of SiO2 NPs. Each tube was inoculated with 25µl of the bacterial suspension containing 1.5 x 108 CFU/mL and incubated at 37°C for 24 hours. After incubation, MIC was determined as the minimum concentration of SiO2 NPs that inhibited bacterial growth, while MBC was determined as the minimum concentration of SiO2 NPs that destroyed bacteria.

3. Results and Discussion

3.1 Isolation and identification of Streptococcus mutans

One hundred and twenty dental caries samples were collected during the period between September 2023 and January 2024. The results of bacterial diagnosis showed that 64 isolates (53.4%) belonged to the genus Streptococci and that 40 isolates (62.5%) of this genus belonged to the species Streptococcus mutans, while 56 isolates (46.6%) of the total 120 isolates did not belong to the genus Non-Streptococci.

Results of culturing bacteria in Mitis Salivarius Agar medium showed that 40 bacterial isolates (positive for Gram stain) were obtained, producing circular, blue-colored colonies with a mucous consistency (Figure 1). The microscopic examination results of the samples also showed that they were spherical bacteria (coccus) that formed short and long chains (streptococci). The results of the biochemical tests of the S. mutans isolates showed that these isolates were negative for the catalase test, which indicates their inability to oxidize hydrogen peroxide H2O2 to oxygen O2 and water H2O, and negative for the oxidase test, as they are facultative anaerobic bacteria that require the presence of 5% CO2. The results of the biochemical tests were consistent with the international identification characteristics of this bacteria.

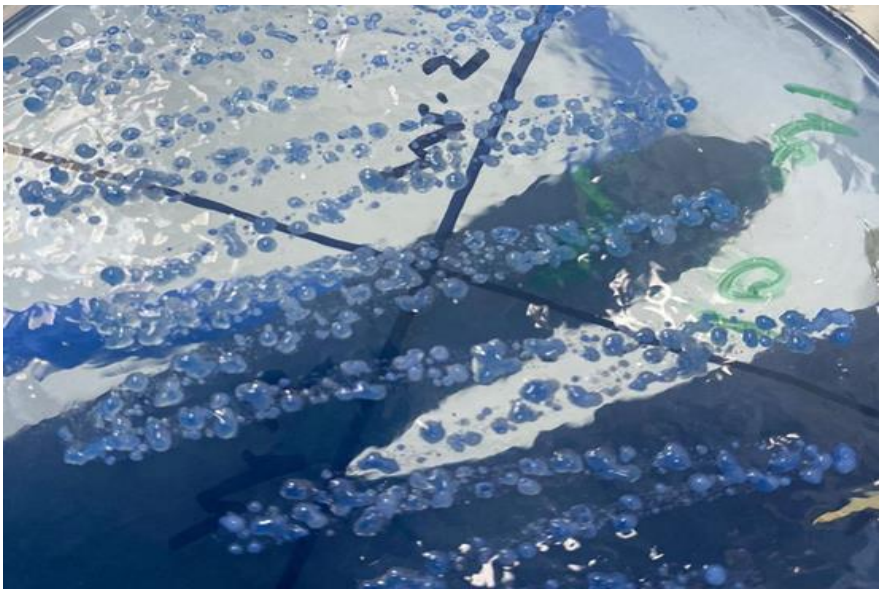

Image - 1 Colonies of *Streptococcus mutans* on Mitis Salivarius Agar

3.2 Preparation of silica nanoparticle solution

UV-Vis spectroscopy was used to investigate the optical absorption properties of SiO2 NPs produced by the biosynthesis using licorice extract. The optical characterization of the NPs provided information about physical properties such as absorbance and band gap energy. Plotting the experimental absorbance data is a common method to measure the optical band gap of NPs.

Figure 2 illustrates the UV–Vis absorption spectra of SiO2 NPs out in the range of 250–500 nm to show the absorption peak of silica nanoparticles which is in the range of 290 to 350 nm. For UV–Vis absorption spectrum, a plot of optical absorbance as a function of the wavelength is shown in Figure 2. The strong absorption of SiO2 photocatalyst is located in the UV region. At the end the maximum absorbance was observed at 390 nm which indicates formation of silicon dioxide nanoparticles. The absorption in the visible range directly affects

the perceived color of the chemicals involved and in this electromagnetic spectrum, molecules undergo electronic transition.

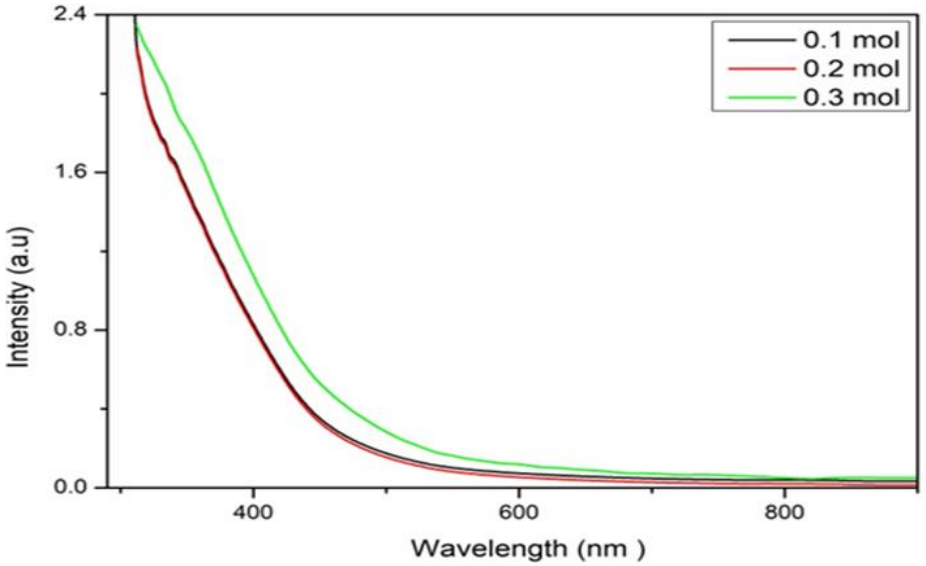

Figure -1 UV–VIS absorbance spectra of SiO_2 NPs

From spectra the it was observed that, the peaks are exposing the formation of silica nanoparticles have dimensions in nm range and the reflection from (100), (110), (102), (111), (200) and (201) planes, at 2h values of 20.861, 36.550, 39.470, 40.296, 42.457, and 45.800 for the sample. From the XRD pattern, it is confirmed that the material formed has hexagonal crystal structure and is primitive lattice with lattice parameters a = b = 4.913 Å and c = 5.405 Å. The XRD pattern ensures formation of silica nanoparticles which are in agreement with JCPDS Card 85–0335. The JCPDS indicates that the material formed is SiO2 (Quartz) nanoparticles (Figure 3).

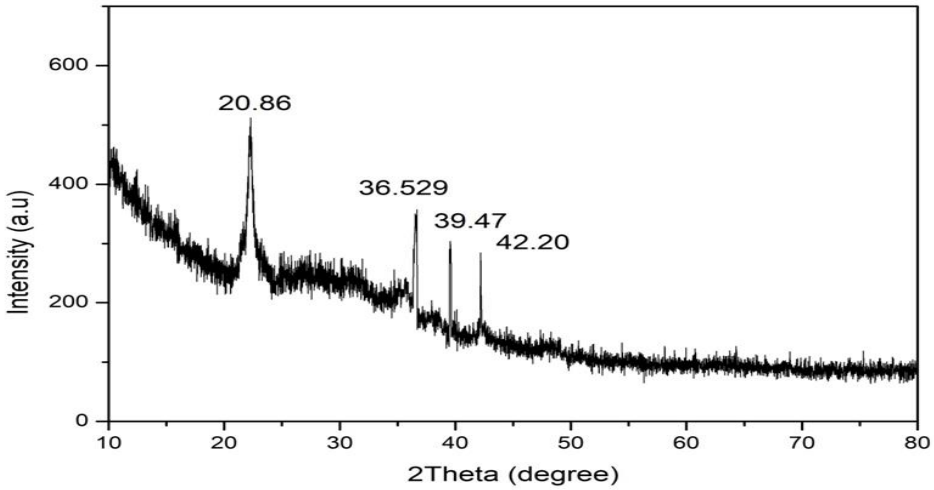

Figure -2 The XRD patterns for SiO_2 NPs using biosynthesis

FSEM Silica nanoparticles structural morphology was analyzed using FSEM, and it gives significant information regarding the shape, growth mechanism and size. Figure 4 show that the SiO2 NPs with average diameter in the range of 26-34 nm with spherical shape. The image illsultered that came from small particles.

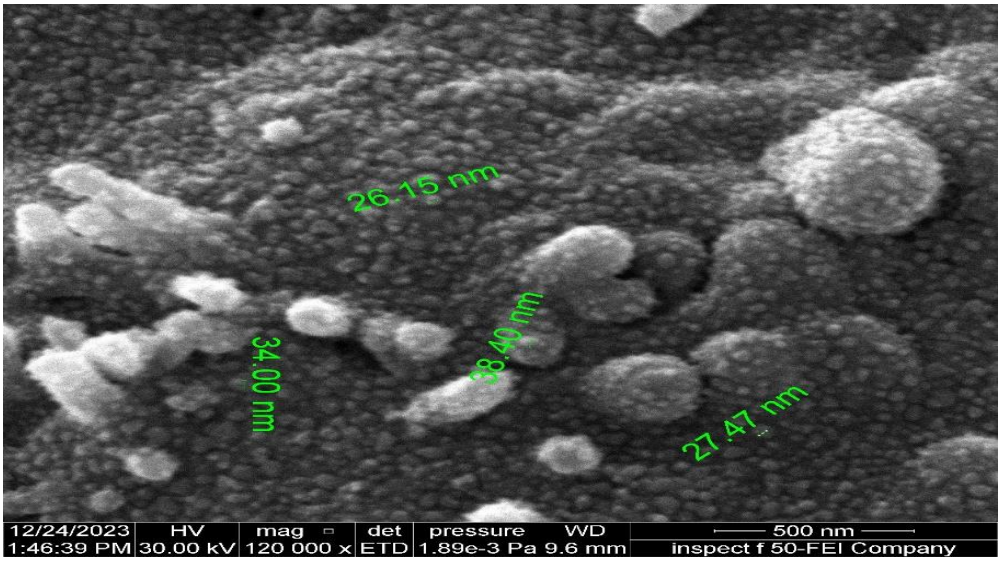

Image -2 FESEM image of SiO_2 NPs prepared plasma jet

3.3 Antibacterial activity of prepared silica nanoparticles

The results showed that the prepared silica nanoparticles were effective against the isolated bacteria Streptococcus mutans, as the MIC value was 12.5 units/mL, and the MBC value was 25 units/mL.

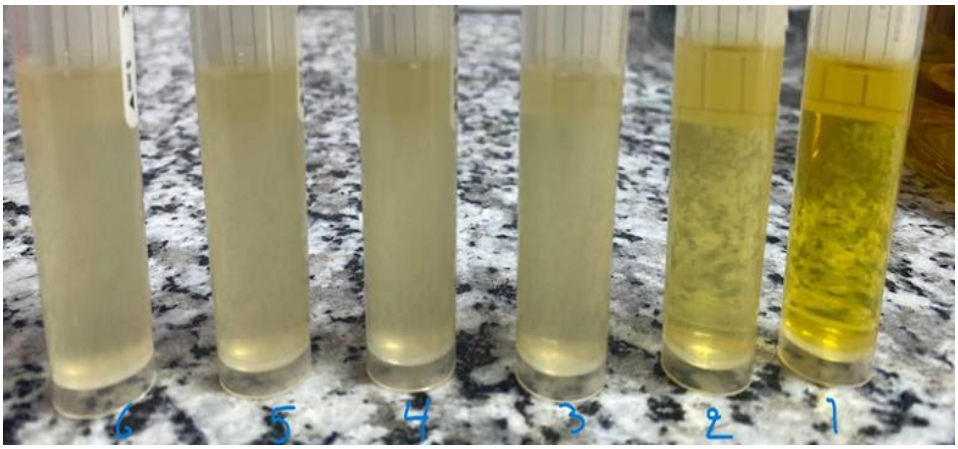

Image -3 MIC results

Metal nanoparticles can affect microbial cells by inducing oxidative stress and inducing damage to the plasma membrane or proteins and DNA. In addition, metal nanoparticles can be attached to other nanostructures and used as carriers for antibiotics, improving the range of potential applications [21]. The antibacterial activity of silicon oxide nanoparticles is attributed to their small size and large surface area, which helps in the accumulation of large numbers of them on the surface of bacterial cells, which leads to an increase in their toxicity. Consequently, they may change the properties of the plasma membrane, especially affecting its permeability and the respiratory enzymes present in it. Nanoparticles may cause DNA damage and the release of toxic ions, which leads to cell death [22].

4. Conclusion

Silica nanoparticles were successfully prepared using licorice root extract by environmentally friendly biosynthesis as stabilizing and reducing agents. The synthesized SiO2 NPs were identified using multiple techniques. The anti-cariogenic activity of the prepared SiO2 NPs against *Streptococcus mutans* demonstrated the presence of bioactive groups in the silica particles made from aqueous extract of licorice plants.

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