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GREEN SYNTHESIS OF SULFUR NANOPARTICLES USING ZIZIPHUS-SPINA TREE LEAVES AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

The main goal of this research was to use an environmentally friendly, simple, and sustainable method to produce sulfur nanoparticles using the aqueous extract of Ziziphus-spina tree leaves and hydrochloric acid. The prepared sulfur nanoparticles were characterized using various techniques, including X-ray diffraction (XRD), infrared spectroscopy (FT-IR), scanning electron microscope (SEM), transmission electron microscope (TEM), and atomic force microscopy (AFM). XRD results showed that their average diameter was 9.26 nm and the sulfur nanoparticles were well-crystalline and highly pure. SEM images revealed the formation of spherical particles. FT-IR analysis showed a sharp peak for sulfur nanoparticles at 460 cm^{-1}, indicating that their surface contained a new chemical bond. This shows that the carbonyl residue of amino acids found in the material extracted from the Ziziphus spina tree may be a means for linking it to sulfur molecules. This extract acts as a stabilizer for the synthesized sulfur nanoparticles. The antibacterial activity of the prepared SNPs was tested against 2 species each of gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria using the agar well diffusion method. The results show that the SNPs have antibacterial activity.

Keywords: SNPs, Ziziphus-spina leaf extract, Green synthesis, Antibacterial activity.

التحضير الأخضر لجسيمات الكبريت النانوية باستخدام أوراق شجرة الزيزفوس-سبينا ونشاطها المضاد للبكتيريا

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الخلاصة

الهدف الرئيسي من هذا البحث هو استخدام طريقة صديقة للبيئة ومستدامة لإنتاج جزيئات الكبريت النانوية. تم تحضير جزيئات الكبريت النانوية باستخدام المستخلص المائي لأوراق شجرة Ziziphus–spina وحامض الهيدروكلوريك بطريقة بسيطة وصديقة للبيئة. تم تشخيص جسيمات الكبريت النانوية المحضرة باستخدام تقنيات مختلفة، بما في ذلك حيود الأشعة السينية (XRD)، التحليل الطيفي للأشعة تحت الحمراء (FT–IR)، المجهر الإلكتروني الماسح (SEM)، المجهر الإلكتروني النافذ (TEM)، ومجهر القوة الذرية (AFM). أظهرت نتائج XRD أن متوسط قطرها كان 9.26 نانومتر. وكانت الجسيمات النانوية الكبريتية بلورية بشكل جيد ونقية للغاية. كشفت صور SEM عن تكوين جسيمات كروية. أظهر تحليل FT–IR قمة حادة لجسيمات الكبريت النانوية عند 460 سم$^{-1}$، مما يشير إلى أن سطح جسيمات الكبريت النانوية يحتوي على رابطة كيميائية جديدة. وتبين أن بقايا الكربونيل من الأحماض الأمينية الموجودة في المادة المستخرجة من شجرة Ziziphus spina قد تكون وسيلة لربطها بجزيئات الكبريت. يعمل هذا المستخلص كمثبت لجزيئات الكبريت النانوية المركبة. تم اختبار النشاط المضاد للبكتيريا ل SNPs المحضرة ضد نوعين من البكتيريا موجبة الغرام (Staphylococcus aureus، Staphylococcus epidermidis) ونوعين من البكتيريا سالبة الغرام (Escherichia coli و Klebsiella pneumonia) باستخدام طريقة انتشار طبق الاكار، وأظهرت النتائج أن SNPs لها نشاط مضاد للبكتريا.

كلمات مفتاحية: SNPs، مستخلص أوراق نبات Ziziphus–spina، توليف أخضر، نشاط مضاد للبكتيريا.

Introduction

Nanotechnology is a contemporary technology used in many industries, including agriculture (11). The prefix “nano” comes from the ancient Greek word “dwarf,” indicating small size. Nanotechnology is technically defined as the science and engineering of reproducible materials, or nanomaterials technology (9). The smallest unit of measurement in the metric system is the nanometer, which is a millionth of a millimeter (4). Substances with diameters varying from 1-100 nm are known as nanomaterials. Their unique properties are defined independently of their constituent materials. These properties make them applicable to a variety of fields in life and industrial technology, such as electronics, medical biology, solar energy processes, and catalysts (5). The Ziziphus spina is a well-known native tree found in most parts of Africa and the tropical and subtropical regions of the world. Historically, traditional medicine widely used Ziziphus plants to treat a wide range of diseases. The Ziziphus spina-christi could represent a valuable tree of great importance for many medicines in the future (3). Biofiltration processes (biological filters) are among the important technologies that include sulfur, as is the case in the fertilizer industry, the preparation of various medicines, rubber, and fiber industries, various types of pesticides and antimicrobials, and fumigation processes (13).

Sulfur nanoparticles (SNPs) have unique properties such as distinct nano sizes and large surface areas. These features enhance their appeal in pharmaceutical and carbon nanotube development, compared to bulk sulfur particles (BSPs) and diverse non-metallic nanoparticles (24). Several methods have been developed to manufacture nanoparticles, including the biological method which is considered easy, fast, cheap, and environmentally safe. In this method, chemical reactions (nanoparticle manufacturing reactions) are catalyzed using microorganisms (bacteria or fungi) and plant extracts. Particles produced this way have many applications in the medical, electronic, and energy fields (17). The use of plants as raw materials in the processes of manufacturing sulfur nanoparticles is one of the green technologies that reduces the risks of preparing sulfur nanoparticles and their toxic wastes that are harmful to the environment (8).

Various studies have demonstrated the feasibility of using plant extracts in the preparation of nanoparticles, including the production of SNPs using Psidium guajava tree leaf extract. The sulfur nanoparticles prepared in this way were characterized by techniques such as ultraviolet-visible spectroscopy, self-conversion instantaneous infrared spectroscopy, X-ray scanning electron microscopy, and high-resolution transmission electron microscopy. The aqueous extract of the Psidium guajava leaves acts as encapsulating and stabilizing agents for nanoparticle synthesis. Biosynthesized sulfur nanoparticles were tested for their antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* using well diffusion methods. Its antifungal activity against *Candida albicans* was also determined using the same method (7). This research highlights the use of aqueous extracts from the leaves of the Ziziphus-spina tree to prepare sulfur nanoparticles using an environmentally safe technique. To characterize the prepared compounds, several techniques were used, namely XRD to determine the crystalline structure, AFM to examine the surface topography, and SEM to examine their surface characteristics in terms of size, shape, and distribution of crystals.

Materials and Methods

Equipment and Materials: The main compounds used in this study included aqueous sodium thiosulfate ($Na_2S_2O_3.5H_2O$), hydrochloric acid (HCl), deionized water, DMSO, Mueller Hinton agar plate, and ethanol (C_2H_5OH). The diagnostic techniques included using infrared spectroscopy (FT-IR) according to the company Bruker, Germany, atomic force microscopy (AFM) (CSPM, USA), X-ray diffraction (XRD) (Inspect S50, Japan), scanning electron microscope (SEM) (Hitachi-S 4160, Japan) and transmission electron microscope (TEM) (JEM 1400 Plus – HC FC, Japan).

Ziziphus-spina tree leaves extract preparation: First, distilled water was used to wash the leaves collected from the Ziziphus-spina tree. The leaves were dried in an oven and then crushed using a mortar to obtain a powder. A sieve was used to remove any adhering particles, and the powder was stored in the shade until use. A 500-mL glass beaker was filled with 400 mL of deionized water and 20 grams of powdered ziziphus spina, boiled for thirty minutes, and allowed to cool at room temperature. The filtered solution was then stored in a refrigerator at 4° C (6).

Preparation of SNPs: One hundred milliliters of aqueous Ziziphus-spina leaf extract was mixed with 1.2 grams of sodium bisulfate ($Na_2S_2O_3.5H_2O$) and shaken at room temperature. The sodium bisulphate solution was then shaken and single drops of 10% hydrochloric acid added to facilitate even crystallization of the sulpur. Centrifugation was used to concentrate the resulting sulfur suspension particles for 20 min at room temperature at 1000 rpm. To eliminate any biological material, the sediment was cleaned repeatedly with distilled water and absolute ethanol. After removing the upper liquids, the product was vacuum dried at 60° C for 4 hours. The resulting color was light yellow (16).

Antibacterial activity of prepared SNPs: The antibacterial activity of the prepared SNPs was tested against 2 species each of gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria using the agar well diffusion method. As the initial stock solution prepared with low concentrations of SNPs did not produce any results, the concentration was increased to 4 mg of SNPs dissolved in 1 ml 10% (v/v) DMSO and filtered (0.45 µm Millipore). Briefly, 100 µl of the particular bacterial suspension was inoculated on Muller Hinton agar and swabbed. Six-mm wells were made in the agar using a sterile cork borer and then loaded with 25 µl of SNPs. Moreover, 10% v/v DMSO was used as a negative control. The plates were incubated at 37° C for 24 hrs. The antibacterial activity was identified by a clearing zone around the wells (22).

Results and Discussion

This research employed the green synthesis method which is considered a safe, environmentally friendly, and cost-effective approach compared to other methods. The SNPs were prepared using an aqueous extract of the Ziziphus-spina tree leaves and sodium thiosulfate. The formed particles were determined by XRD, FT-IR, SEM, TEM, and AFM techniques. Plant extracts have the ability to act as an easy-to-use, inexpensive, and environmentally friendly biomaterial to stabilize and distribute sulfur nanoparticles during their installation (14). This work is similar to that conducted by many researchers who also used the green method to prepare sulfur nanoparticles, but from different plant extracts. For instance, (15) prepared SNPs using the aqueous extract of basil leaves (Ocimum basilicum) and characterized the formed particles using UV-vis, FT-IR, TEM, and XRD techniques where the particle size was found to be 23 nm with a spherical shape and crystalline nature. On the other hand, (12) used the aqueous extract of garlic (Allium sativum) to prepare sulfur nanoparticles. Using FT-IR, XRD, and SEM-EDX spectroscopy, gSNP was described. The results show that gSNPs had an average particle size of approximately 54.73 nm, exhibited high purity, and had a spherical presence. Various sulfur particles were synthesized using Bengalensis leaves and characterized by XRD, FT-IR, SEM, HR-TEM, XED and AFM by (21). Transmission electron microscope images showed that the zero-valent sulfur nanoparticles were 2–15 nm in size and had irregular shapes with low agglomeration.

FT-IR spectrum: The analysis of the Ziziphus-spina tree leaves extract is displayed in Figure 1. The stretching vibrations of alcohol and phenol amino (-NH) and hydroxyl (-OH) groups are responsible for the strong and wide absorption bands observed at 3282 cm^{-1}. The asymmetric and symmetric stretching vibrations of $-CH_2$ and -CH3 functional groups of aliphatic compounds are responsible for the absorption peaks at 2919 cm^{-1} and 2852 cm^{-1}. The shoulder peak, located at 1729 cm^{-1}, is associated with the carboxyl groups' stretching vibrations. The carbonyl amide group in the first and second amides is what distinguishes the band at 1619 cm^{-1}. The protein methylene group's scissoring vibrations are responsible for the range at 1443 cm^{-1}. The vibrations of carboxylic acids and aromatic amines' C-N bonds result in the formation of a band at 1371 cm^{-1}. The band at 1033 cm^{-1} is related to the stretching vibrations of alcohols' second carbon dioxide. Compounds that are aromatic can be identified by the peaks located at 516 and 559 cm^{-1}. Throughout the synthesis process, these functional groups serve as agents for coating, distributing, and stabilizing S-NPs.

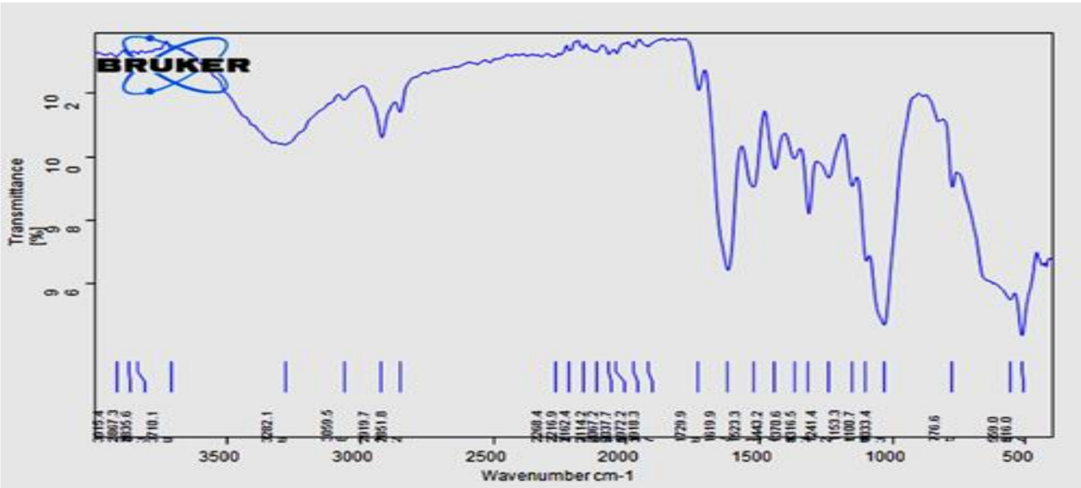


Figure 1: FT-IR analysis of Ziziphus-spina tree extract.

The FT-IR spectrum of the sulfur nanoparticles (S-NPs) in Figure 2 reveals the existence of a novel chemical bond on their surface. This implies that the application of seder leaf extract may aid in the dispersion and stability of sulfur nanoparticles. The process of stopping particle agglomeration was achieved by taking advantage of interactions between amino acid residues of the protein and sulfur nanoparticles. At 460 cm^{-1} a prominent peak appears in the FT-IR spectrum, clearly showing the effect of seder leaf extract on sulfur nanoparticles. The fruit extract of the Albizia julibrissin and sodium thiosulfate were used by (6) in preparing SNPs, and the results were consistent with this research. (16) Used citric acid and an aqueous extract of the leaves of the Melia azedrach plant. SEM, FT-IR, and XRD characterizations of the results demonstrated that the extract stabilizes the formation of the SNPs.

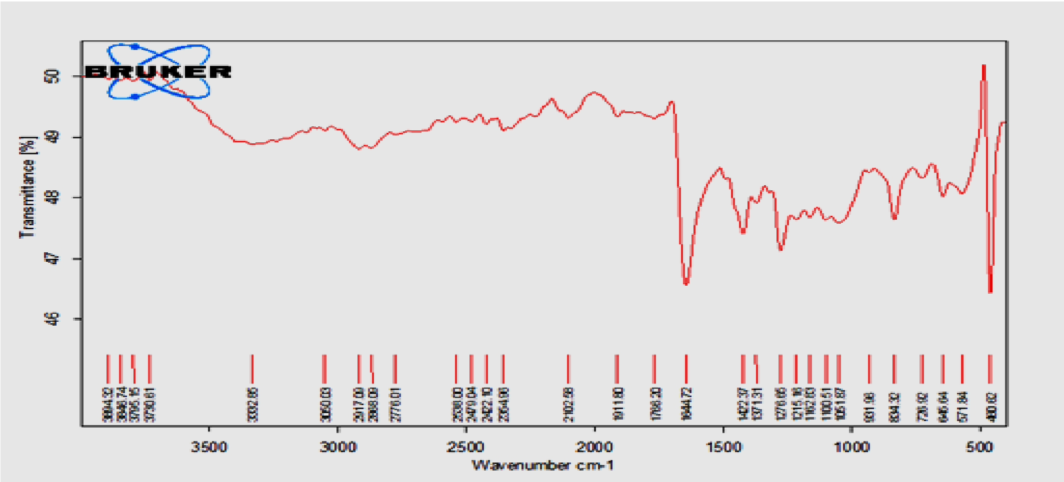


Figure 2: FT-IR analysis of synthesized sulfur nanoparticles.

Scanning electron microscopy: SEM is an imaging technique that projects a beam of electrons into the area to be studied. The electrons interact with the surface atoms, generating three types of rays, i.e., secondary electrons, back-scattered electrons, and X-rays, where the magnification range reaches 25-250,000 times to determine the surface (1). SEM pictures of sulfur nanoparticles produced in the Ziziphus spina tree leaves extract are displayed in Figure 3. These pictures demonstrate how the nanoparticles made using the method outlined are uniform in size and nearly spherical in shape. According to the SEM results the sulfur crystals agglomerated during processing, and the SNPs were approximately 97.65 nm in size.

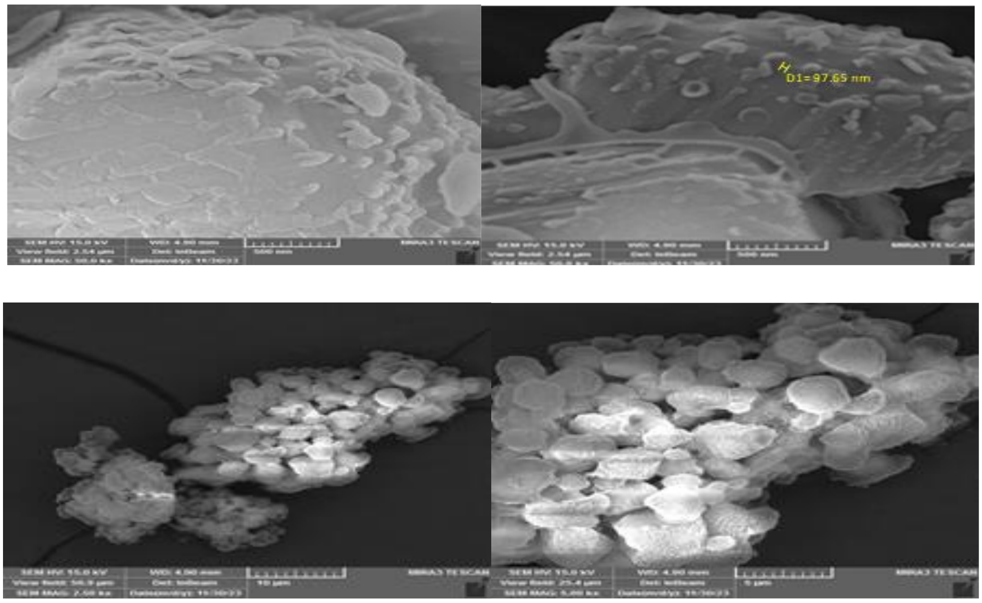


Figure 3: SEM pictures of sulfur nanoparticles in various contexts (500 nm, 10 µm, 5 µm).

Atomic force microscopy: Atomic force microscopy (AFM) was used to examine fine details of the surface topography and crystal structure of nanomaterials. The capacity to magnify an image up to a million times is what makes this microscope unique, and more capable of imaging fine details compared to other electron microscopes (10). Figure 4 shows two- and three-dimensional images of the surface of nano-sulfur particles prepared in the laboratory using the green method. As shown, the grain size of the prepared particles has a diameter of about 40 nanometers.

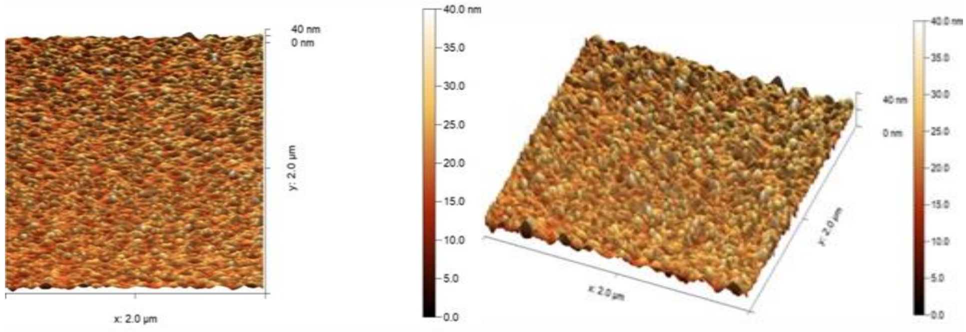


Figure 4: 2D and 3D AFM images of sulfur nanoparticles tested by the green synthesis method.

X-ray diffraction: The crystalline structure of the green-produced sulfur nanoparticles was ascertained through the use of XRD analysis. The positions of the peaks are seen at different angles when the X-rays were directed to the identified samples. The XRD data of the SNPs are displayed in Figure 5 beside the typical reference pattern for sulfur (JCPDS 4: 8-0247). The diffraction peaks at 113, 220, 222, 040, 313, 044, 422, 319, 515, and 266 were associated with the corresponding angles of 15.3°, 22.9°, 24.3°, 26.3°, 27.3°, 28.6°, 31.0°, 42.9°, 55.4°, and 65.1°, respectively. These results indicate and confirm that the sulfur nanoparticles exhibit a well-defined crystalline structure. The nanoparticle size was calculated using the Debye-Scherrer equation:

$$D = \frac{k\lambda}{\beta\ cos\theta}$$

Where λ is the X-ray wavelength, equal to 1.504 A°, (β) is the greater half of the full width maximum (FWHM) and (K) is the shape factor, which is equal to 0.94 (20). The size of the SNPs determined by the Debye-Scherrer equation was 9.26 nm.

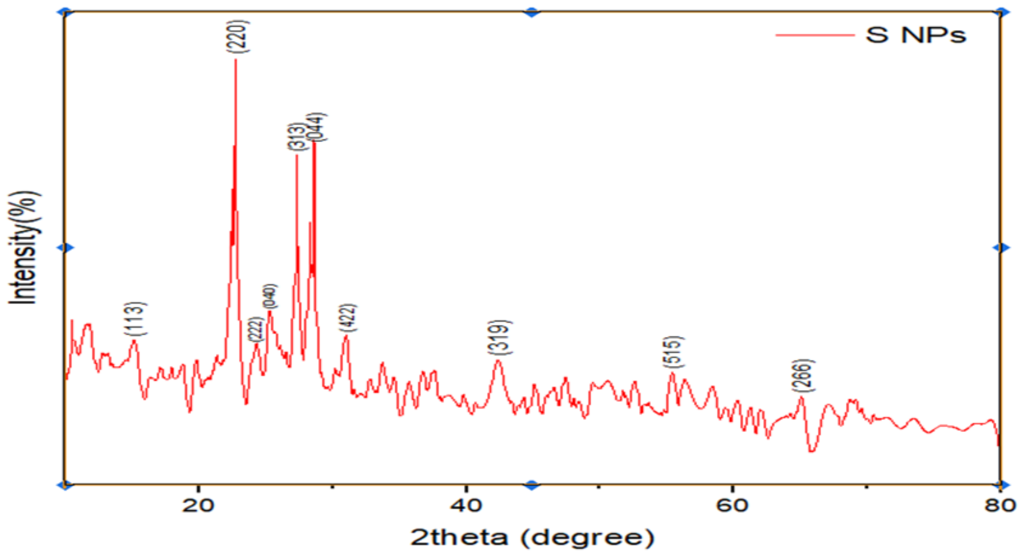


Figure 5: Synthetic sulfur nanoparticles' XRD pattern.

This is consistent with the findings of (2) who synthesized sulfur nanoparticles using an aqueous extract of *Rosmarinus officinalis* leaves and sodium thiosulphate. They also used techniques such as XRD, SEM, TEM, and FT-IR spectroscopy. The average size of the crystalline sulfur nanoparticles was 40 nm (2). The sulfur nanoparticles generated in this study utilizing the aqueous extract of Ziziphus spina tree leaves showed good correlation between the diffraction peaks of the sulfur nanoparticles synthesized by (2).

Transmission electron microscopy: TEM is a crucial instrument for use in researching layer growth and structure. Though it employs electrons rather than light, it operates on the same fundamental concepts as optical microscopy and produces much higher resolution images. Thus, TEM can reveal fine details of internal structures, even in instances as small as individual atoms (23). The morphology of the sulfur nanoparticles synthesized by the green method was analyzed using TEM (Figure 6) and showed mean sizes of 50 – 100 nm and spherical-like shapes. This again confirms the formation of lower size particles.

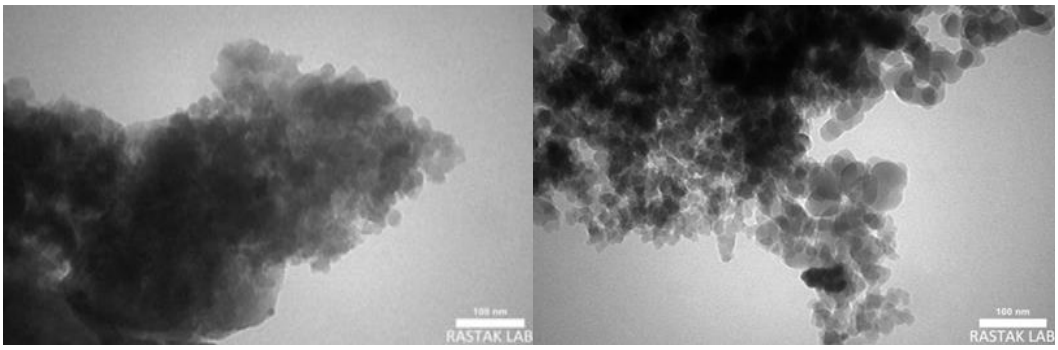
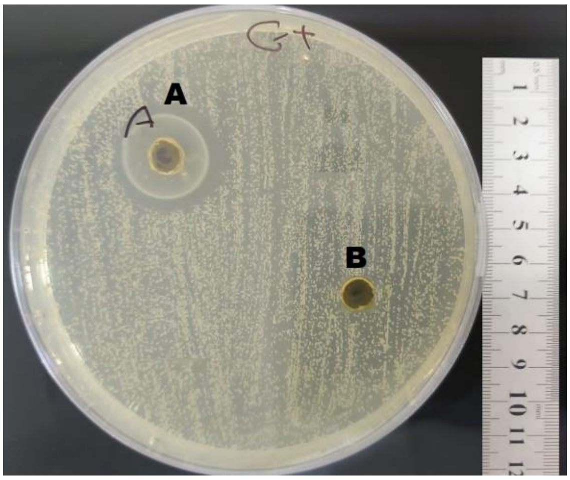
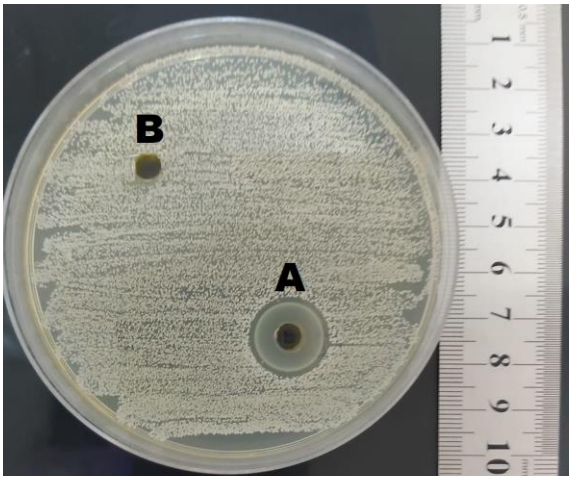


Figure 6: TEM micrographs of sulfur nanoparticles.

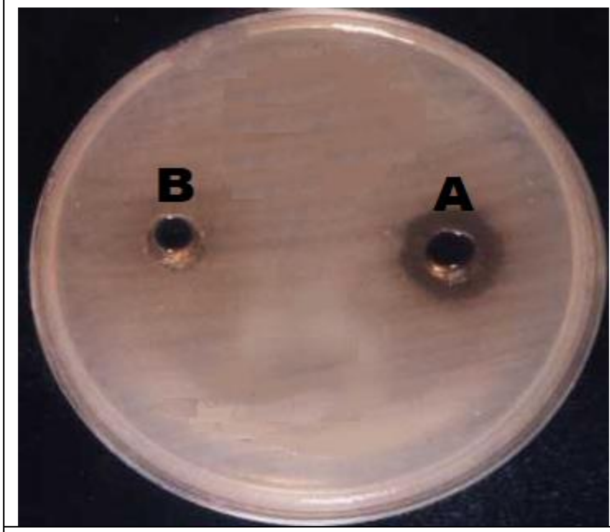
Antibacterial activity: The results show the prepared SNPs having antibacterial activity against gram-negative and gram-positive tested bacteria. Its activities varied according to the tested bacteria with inhibition zone diameters ranging from 7 mm to 22 mm. Meanwhile, the negative control of the 10% DMSO solvent did not inhibit all the bacteria tested. The highest inhibition zones were 22, 18, 11, and 7 mm against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli,* and *Klebsiella pneumonia*, respectively for the nanoparticles at the 4 mg/ml concentration (Figure 7). The synthesized SNPs appeared the best antibacterial agent against gram-positive bacteria and its strong activity against *Staphylococcus aureus* is of critical importance considering that it is an emerging human pathogen. The antibacterial mode of action of nanoparticles is generally described as relating to one of the following mechanisms: cell plasma membrane damage, generation of reactive oxygen species, and penetration of bacterial cytoplasmic membrane, as well as leading to intracellular effects such as interacting with deoxyribonucleic acid (DNA) and cell protein (18 and 19).



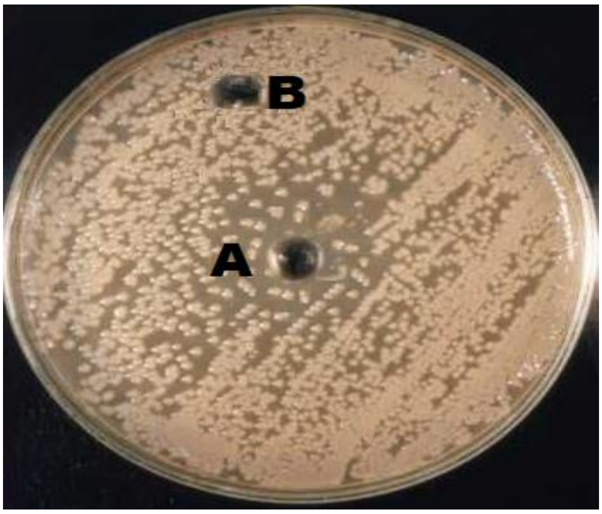
Staphylococcus aureus



Staphylococcus epidermidis



Escherichia coli



Klebsiella pneumonia

Figure 7: Antibacterial activity of SNPs against tested bacteria. A: 4 mg/ml; B: 10% DMSO.

Conclusions

Ziziphus-spina tree leaves were successfully used to prepare sulfur nanoparticles from the aqueous extract of its leaves. FT-IR analysis showed a strong peak at 460 cm^{-1}, indicating the formation of the nanoparticles. X-ray diffraction results displayed the presence of nanoparticles sized 9.26 nm, indicating their high crystalline purity, while transmission electron microscopy revealed their spherical shapes. Meanwhile, atomic force microscopy showed that the grain size of the prepared particles was approximately 40 nm. The prepared sulfur nanoparticles possessed antibacterial activity especially against Staphylococcus aureus.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

This work is the collaboration of three authors. Rand Malallah Hasan diagnosed the case for study, collected the samples, did the investigation, and wrote the original draft. M. M, Sirhan reviewed and edited the manuscript and supported it with resources for drafting. Hameed Khalid Ali analysed the data and reviewed the manuscript.

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Institutional Review Board Statement:

This study was conducted in accordance with the protocol authorized by the Ministry of Water Resources, Head of the Central Drinking Water, Ethics Committee, Syrian Arab Republic.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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