





Biogenic synthesis of sulfur nanoparticles using saffron flower extract and its synergistic antimicrobial activity

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Original Research

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Abstract:

Sulfur nanoparticles were successfully prepared by Thiourea ($\text{CH}_4\text{N}_2\text{S}$) and saffron crocus flower extract at 70 °C. (XRD), UV-Vis Fourier Transform Infrared (FT-IR), and Scanning Electron Microscopy (SEM) were used to study the sulfur nanoparticles that resulted. From XRD was average particle size of about 21.5 nm, “the size of the nanoparticles can be modified by changing the amount of saffron crocus flower extract”. (SEM) was used to determine the surface characteristics of the particles, and “the average size of the sulphur particles was found to be 19.32 nm”. The optical band gap value of the obtained semiconductor nanoparticles (SNPs) have been determined to be 4 eV, the observation suggests the existence of a blue shift. The Fourier Transform Infrared (FT-IR) spectrum of the synthesized sulfur nanoparticles demonstrates the presence of characteristic peaks associated with sulfur, specifically at (563, 601, 636 and 756) cm^{-1} . Additionally, the plant-mediated synthesis of these nanoparticles has exhibited antimicrobial activity against both bacterial and fungal pathogens.

Keywords: Sulfur; Nanoparticles; Green synthesis; Saffron crocus plant; Antifungal; Bacterial Activity

1. Introduction

Recently, scientists have discovered the enormous potential offered by nanotechnology, and since then, it has become a sovereign force in modern times. Certainly, nanotechnology has become very popular given the enormous benefits and possibilities it can offer humans. Materials on the nanometer scale, Its dimensions change from (1 – 100) nm, exhibit quite different properties from those that exist on a wider scale and therefore acquire new selective biological, physical, and chemical properties that make them valuable in nanotechnology fields and find extensive applications in different fields [1–5]. Metal sulphide has various applications in areas such as Medical and agriculture [6, 7]. Sulfur is also an ingredient in some of the most widely used fertilizers in agriculture, such as calcium sulfate, which improves the quality of phosphorus and nitrogen nutrients [8]. Accordingly, nanosulphur molecules (S-NPs) produce important applications in a variety of new techniques, such as batteries of lithium-sulphate, insecticides, fungicides, carbon-tube

modification, gas sensors, catalysts, and their applications in the treatment of cancer using neutron families [9, 10]. In addition, nanosulphur particles play a prominent role in areas such as biological research, where they are used to develop antibacterial products, pharmaceutical products, and products that show anti-tumor and antioxidant activity and assist in healing processes [11, 12].

So far, many different methods have been used for the manufacture of nanosulphur particles (S-NPs), including the microemulsifier method, the method with the help of the surfactant, the solvent-free method, the sedimentation method, the electrochemical method, the method with the assistance of the egg crust membrane, the chemical deposition method in the liquid phase, the heating of sulphur powder with polyethylene glycol PEG-600, and, finally, the oversaturated solvent method [13, 14]. The concept of “green nanotechnology” has become more common thanks to sustainable efforts in synthesis, at appropriate prices, with higher security, without negative impact on the environment, and because of the simplicity of protocols [15]. Plants have the

advantage of being the preferred source for the synthesis of nanomolecules thanks to their stability and diversity in the shapes and sizes that enable them to form them. In addition, plants contribute to the formation of nanoparticles supported by plant extracts, providing excellent reduction and stabilization factors [16]. Saffron is a substance that helps neutralize harmful free radicals because it's rich in antioxidants, a fact that's closely linked to many diseases, such as cancer.

A study has found that saffron and its compounds inhibit the growth of tumors while preserving healthy cells and also selectively eliminate cancer cells. [17]. The main objective of this research is to manufacture nanosulphur molecules (SNPs) using green chemical methods and study their impact on the growth of bacteria and fungi.

2. Experimental part

2.1 Preparation of plant leaf extract

It starts by washing the saffron with (DW)distilled water several times to remove any impurities, and then it dries to remove any remaining moisture. In the grinding mill, the dried flower is grinded into a soft powder. One gram of saffron boiling flower for 10 minutes in a glass with 100 mL of sterile distilled water. The solution forms a red color after boiling and is left to cool at the surrounding temperature. To dispose of heavy biomaterials, the water extract of the flower was filtered using Whatman No. 1 filter paper and fired by centrifuge at 1200 cycles per minute for 5 minutes. The water extract shall be kept at room temperature until it is used to produce nanosulphur particles in this work.

2.2 Preparation of S-NPs

Sulfur nanoparticles (SNPs) were prepared as follows in a typical reaction: 0.076 g of thiourea ($\text{SC}(\text{NH}_2)_2$) was dissolved in 100 mL of distilled water for 30 min at 70 °C with stirring. 5 mL of saffron flower extract was added to the 1 mL thiourea solution five times for 5 min, and then 0.25 mL of sodium hydroxide (0.1 M) was added, ensuring a constant temperature to obtain a homogeneous

sulfur nanoparticle solution. The final result was a pale yellow solution with a concentration of 0.1 M. Figure 1 illustrates the green structure of the sulfur nanoparticles. This is consistent with the technological approach of the Ref. [18, 19].

2.3 Characterization Techniques for S-NPs

Various known methods are employed for the analysis of nanoparticles (NPs). These methods involve a range of analytical tools, including [UV, XRD, (FT-IR)] spectroscopy, and (SEM). SNP properties may be investigated using these techniques, which can assist resolve a variety of parameters such as particle size, crystallinity, and morphology, also it studied antibacterial and antifungal tests.

3. Results and discussion

Figure 2 illustrate the XRD analysis of sulfur nps formed in green synthesis method. The 2θ vertex at 23.04°, 25.98°, 27.86°, 28.89°, 31° and 47.08°, attributed to the crystal planes of sulfur at 222, 313, 040, 044, 515, respectively. It was found that the sulfur nanoparticles were crystalline, as the exact position of the peaks intensities was in excellent agreement with the standard single-phase patterns of sulfur. (JCPDS 4:8-0247). No further development has been found, which means that the single-mile pure Sulphur phase has been prepared under these experimental conditions [20–22]. The average crystalline size is estimated at 21.5 nm. Surface morphology examined using SEM examination. Precursors have resulted in the synthesis of nanoparticles of various sizes and morphologies, as illustrated in figure 3 with two distinct magnifications of 1 μ and 200 nm. There are particles with smaller sizes and more homogenous shape, “the average size of the sulfur particles was found to be 19.32 nm”, this finding is consistent with XRD diffraction. The UV-Vis spectrometers of S-NPs are shown in figure 4 (a). The peak acute absorption detected at 374 nm indicates the formation of S-NPs using green synthesis technology. No additional peak was detected in the spectrum, indicating good purity for S-NPs produced using Saffron.



Figure 1. Green synthesis of S NPs.

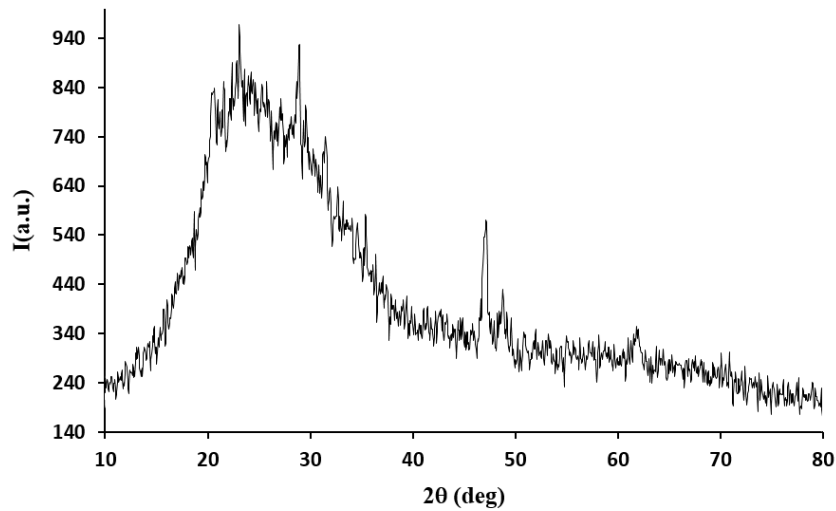


Figure 2. XRD diffraction of sulfur nanostructure.

The blue shift in the absorption area clearly shows the existence of the NPs QC feature [23, 24]. The particle range gap increases, resulting in a lower wavelength of the absorption edge as a result of reduced particle size due to quantitative

confinement. The visual band gap was determined by 4 eV through the absorption spectrometer, as shown in figure 4 (b). Figure 5 shows the FTIR spectrum of S-NPs. The results of

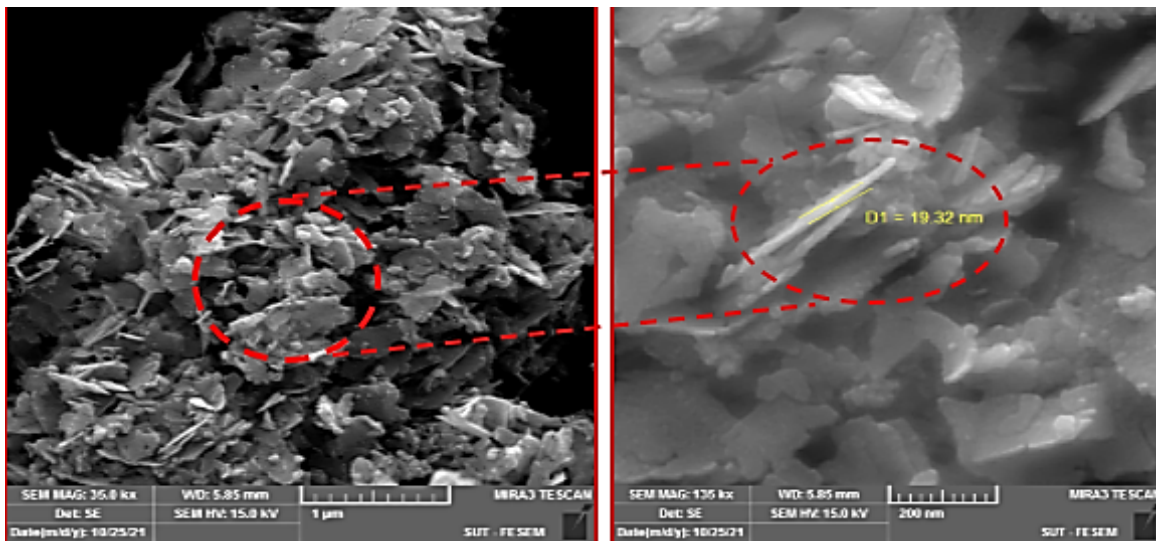


Figure 3. SEM image of sulfur nanostructure.

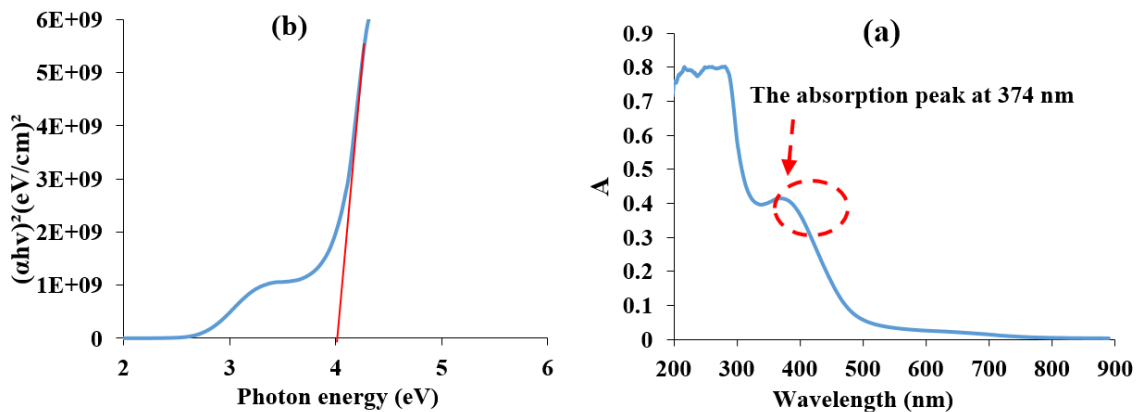


Figure 4. (a) UV-Visible absorption spectra for S nanoparticles.(b) Tauc plot represents the energy band gap of S-NPs.

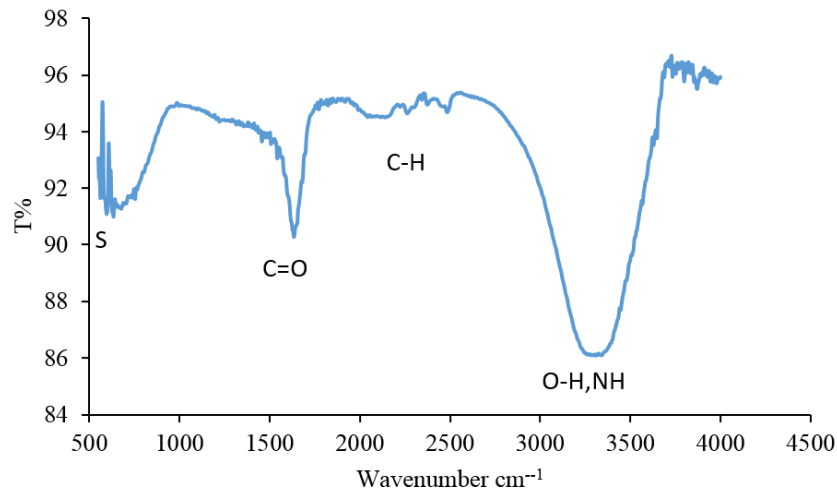


Figure 5. FTIR for S-NPs.

the analysis of the infrared hyperspectral FT-IR show that there is a significant absorption tape at 3380 cm^{-1} that can be attributed to the presence of amineo (-NH) and hydroxyl (-OH) groups associated with hydrogen (-H) in alcohol and phenols. In addition, the symmetrical and symmetrical extension of the petal functional groups -CH, CH_2 and CH_3 to the high sucking caps at (2268 , 2380 , and 2484) cm^{-1} respectively, For the first amid and the second amid, the ranges of amid carbonyl at 1636 and 1666 cm^{-1} indicate the presence of the carbonel group. When comparing the results with the standard sulphur, the FT-IR spectrometer for sulphur nanoparticles reveals all single-mile sulfate peaks at 563 cm^{-1} , 601 cm^{-1} , 636 cm^{-1} and 756 cm^{-1} . In addition, the FT-IR spectrometers of the produced sulphur nanoparticle indicate that there is a new chemical link on the surface, indicating that the carbonate residues of amino acids in the derived carbide protein can interact with the

sulphur nanoparticle and act as a stabilizing and dispersing agent, thus preventing its mass [25–27].

SNPs' antifungal and antibacterial characteristics were identified and studied using pathogenic organisms such as *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*. The strains were cultured in LB broth for 24 hours at $37\text{ }^\circ\text{C}$ (bacteria) or $30\text{ }^\circ\text{C}$ (*Candida albicans*) before being dispersed onto LB agar plates with a sterilised glass diffuser. Sterile paper discs (6 mm in diameter) were then placed on the inoculation plates, and the number of S-NPs on each disc was observed. Their antibacterial and antifungal activity was assessed by determining the inhibitory area formed throughout the discs. Figures 6 and 7 reveal that plant-mediated SNP production has substantial efficacy against bacteria and fungus.

The papers explain the travel paths of SNPs. Nanoparticles

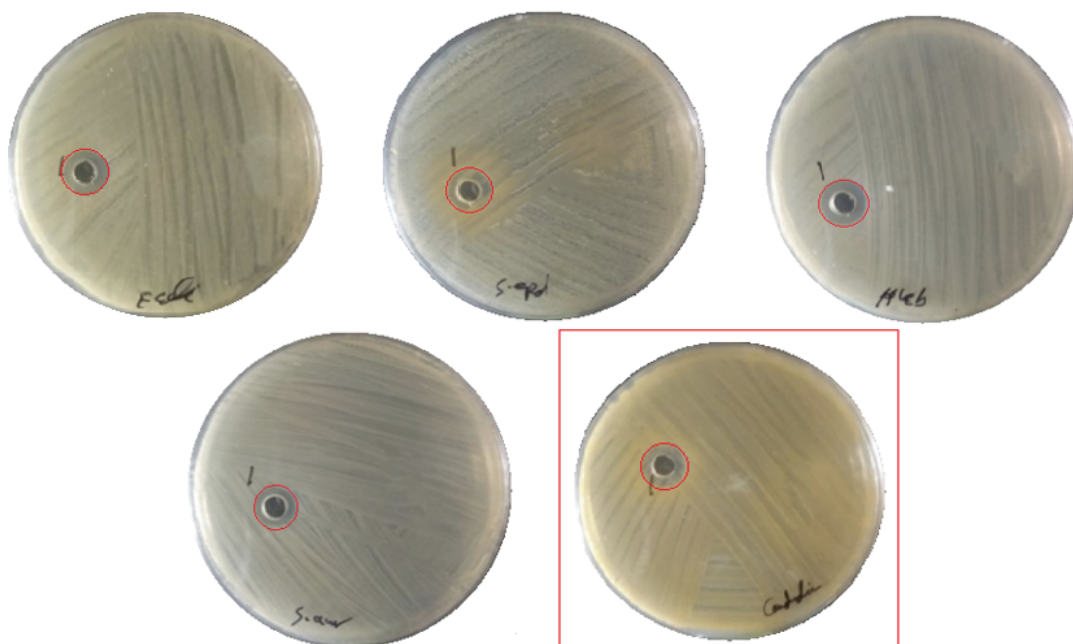


Figure 6. Antibacterial and antifungal activity of S-NPs.

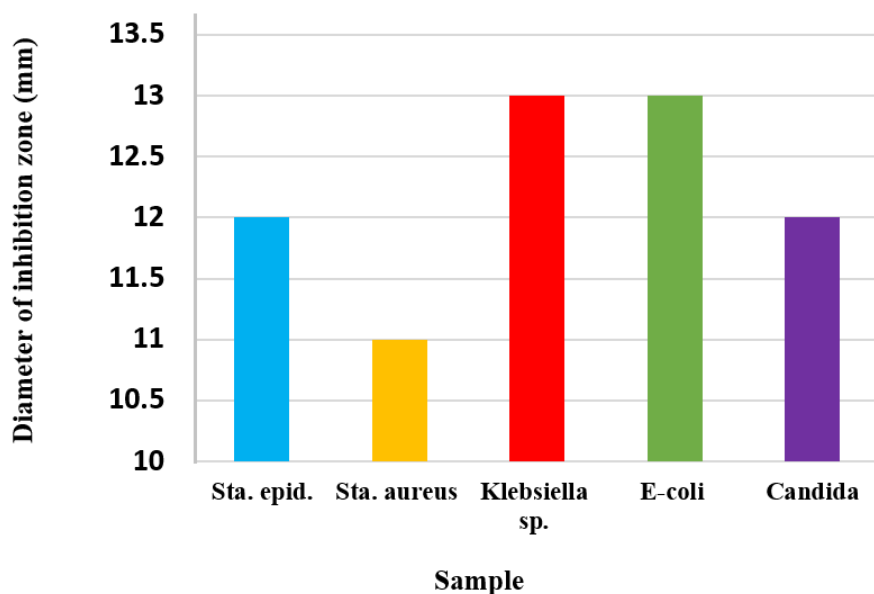


Figure 7. Diameter of inhibition zone of S-NPs.

enter cells by diffusion and endocytosis, interfering with mitochondrial function and increasing the release of reactive oxygen species (ROS) and ions (S-2). These ions can travel across the membrane to DNA, triggering nuclear damage and cell death [28, 29].

The specific method by which nanoparticles exert antibacterial and antifungal action is yet unclear. Three major pathways have been postulated thus far: (1) breakdown of cell walls and membranes, (2) intracellular penetration and injury, and (3) oxidative stress. This is prevalent in the scientific process of reference [25, 30]. It should also be noted that the practice approach takes precedence in the inhibitory capacity of the substance, as this approach is dependent on the type of extract and the concentrations of the substances used, with results being more pronounced in the degree of inhibition of microorganisms and fungi compared to the reference [31, 32].

4. Conclusion

The present study shows a simple, effective, and environmentally friendly preparation of sulphur particles (S-NPs) using Saffron and SC (NH₂) extraction. The formation of sulphur particles has been confirmed using several characterization techniques such as XRD, SEM, UV-Vis, and infrared spectroscopy (FTIR). UV-Vis showed a peak absorption at the wavelength of 374 nm for biosulphur particles produced. FTIR spectrometers revealed 563, 636, and 756 cm⁻¹, which is consistent with the effect of sulfur vibration. The FESEM survey revealed that the size of sulphur particles was 19.32 nm. The band gap in the optical range of sulphur was estimated at about 4 eV, indicating a blue transformation. The study also showed that the sulphur particles produced have a simple antibacterial effect on bacteria and fungi, This method is considered a sustainable development program with high economic quality and low toxicity because it does not leave difficult-to-remove solids or toxic vapors.

Authors Contribution

Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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