

Effect of Sulphur Nanoparticles Biosynthesized From *Bacillus Coagulans* Extract Against *S. aureus* Isolated From Dermatitis Patients in Iraqi Hospitals

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(Submitted: 16 January 2024 – Revised version received: 08 February 2024 – Accepted: 03 March 2024 – Published Online: 26 April 2024)

Abstract

Objective: The goal is to biosynthesize sulphur nanoparticles (Sulphur NPs) from *Bacillus coagulans* extract and test its bioactivity against *S. aureus* gram positive bacteri that was isolated from dermatitis patients.

Methods: 125 specimens were taken from patients of dermatitis attended two Baghdad hospitals. Among 125 specimens, only 32 of isolates were identified as *S. aureus*. Five *S. aureus* isolates biofilm forming (strong) and multi drug resistant (MDR) were selected. Fourteen types of antibiotics from different classes were used and the results showed different patterns of resistance. Congo red agar (CRA) and microtiter plate method (MPT) were used to estimate the ability of MDR isolates of *S. aureus* to create biofilms. A series of characterization techniques, including UV-vis, FTIR, FE-SEM, EDS, XRD and TEM analysis, have been carried out to characterize the biosynthesized sulfur NPs. The antimicrobial, antibiofilm and antioxidant activities of sulfur NPs were estimated.

Results: Sulfur NPs biosynthesized exhibited antimicrobial activity against examined bacteria with inhibition zone ranged between 12 to 20, 18 to 23 and 22 to 26mm at 100, 200 and 300 µg/ml, respectively. Only five strong-biofilm producers and MDR of *S. aureus* (A5, A8, A9, A11 and A29) were used to assess the ability of sulfur NPs as anti-biofilm agent. No significant differences ($P > 0.05$) in inhibition rate of all strain isolates (A5, A8, A9, A11 and A29) for all concentrations of Sulfur NPs with $P = 0.886$.

Conclusions: The antioxidant activity of sulfur NPs were decreased into 81.6, 80.3, 75.2 and 68.9% with using concentrations of 400, 300, 200 and 100 ug/ml, respectively, also, nanoparticles had the potential to scavenge DPPH free radicals.

Keywords: *S. aureus*, sulfur nanoparticles, antibiofilm, antioxidant, *B. coagulans*

Introduction

Staphylococcus aureus is one of the most prevalent pathogens and is an invasive pathogen causes infections of the skin, soft tissues, and bloodstream, the bacterial ability to produce various enzymes and toxins that aid in invasion and tissue damage, such as hemolysin toxin, is one of the most significant virulence factors that contribute to the rise in *S. aureus* infection rates. This ability allows the bacteria to evade the body's defense mechanisms and subsequently cause infection.¹ Also, the World Health Organization (WHO) has designated *Staphylococcus* MRSA as one of the nine germs of global concern because of its high level of antibiotic resistance.²

Failure to treat the infection increases the likelihood that biofilms may form, which create a favorable environment. Resistance to dietary deprivation improves host immunity and antibiotic evasion. The possibility that biofilms could facilitate the spread of genes for antibiotic resistance and contribute to the formation of adaptive mutations is extremely concerning, however, the haphazard application of antibiotics results in *S. aureus* stains that are resistant to the drugs, a serious worry that has raised the need for carefully considered substitute therapies.³

The evolution of nano-biotechnology has led to many novel antibacterial solutions. Due to their small dimension, nanoparticles are perfect for biological antibacterial actions, sulphur is known to exhibit a wide range of biological activities, including antibacterial, antioxidant, anticancer, and radical-scavenging capabilities, along with its abundance of chemically varied organic and inorganic compounds.⁴ Sulphur

NPs, have been shown to demonstrate significant bioactivity against harmful bacteria, such as *S. aureus*. A promising alternative approach to treating this bacterium and its biofilm is therapeutic treatment based on nanoparticles. Sulphur NPs, have been shown to demonstrate significant bioactivity against harmful bacteria, such as *S. aureus*,⁵ *B. coagulans* has been classified as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration.⁶ In past studies, the characterization and identification of nano-inorganic particles were investigated in order to evaluate the potential of *B. coagulans* strains for the creation of nanoparticles.⁷ *B. coagulans* bacteria were used for the synthesis of NPs as stabilizing and bio-reducing agents.⁸

Thus, the goal of the current study is to synthesize the sulfur nanoparticles via green biosynthesis, characterize, them and evaluate the antioxidant, antibacterial, and antibiofilm properties of sulphur NPs.

Materials and Methods

Collection of Pathogenic Specimens

125 samples were collected from dermatitis patients, which were obtained from two hospitals in Baghdad. These samples were all obtained using swabs, stored in transportable medium, and then brought into a lab for testing.

Isolation and Diagnosis of *S. aureus* Isolates

All specimens were streaked on blood agar and Mannitol salt agar (MSA) plates and incubated for 24 hours at 37°C in order

to isolate *S. aureus*. Several tests, such as microscopic inspection, biochemical analyses (catalase, oxidase, and coagulase), and the VITEK-2 system, were used to identify the cultivated isolates.

Antibiotic Susceptibility Test

The susceptibility of isolates were determined using antimicrobial agents. Erythromycin (E; 15 µg), Trimethoprim-sulfamethoxazole (COT; 1.25/23.75 µg), Tetracycline (TE; 30 µg), Oxacillin (OX; 10 µg), Penicillin (P; 10 µg), Vancomycin (VA; 5 µg), Chloramphenicol (C; 30 µg), Gentamycin (GEN; 10 µg), Rifampicin (RIF; 5 µg), Azithromycin (AZM; 15 µg), Levofloxacin (LE; 5 µg), Norfloxacin (NOR; 10 µg), Ciprofloxacin (CIP; 5 µg), and Cefoxitin (CX; 30 µg), in accordance with System of CLSI 2022.⁹

Detection of Biofilm Production

Two methods, including Congo red agar (CRA) and microtiter plate method (MPT) were utilized to detect whether MDR isolates of *S. aureus* create biofilms or not as described by Babapour E, et al.¹⁰

Biosynthesis of Sulphur NPs Using *B. coagulans* Extract

In order to create a *B. coagulans* supernatant, the bacteria was cultured for 24 hours at 37°C in brain heart infusion broth. Following the incubation period, the precipitate was extracted from the colloidal suspension by centrifuging it for 15 minutes at 10,000 rpm. Sulphur NPs were made by filtering the supernatant via filter paper. A mortar and pestle were used to finely grind the powdered sulphur. 50 cc of sodium sulphide (1M) was placed in a flask along with the ground sulphur powder. To dissolve the sulphur powder, the reaction mixture was heated to 100°C while being continuously stirred. The creation of sodium polysulfide solution was indicated by the solution's colour changing from pale yellow to reddish-orange. Following constant stirring, the resultant sodium polysulfide solution was combined with *B. coagulans* bacterial extract in 1:4 ratio. Next, concentrated H₂SO₄ was added dropwise to cause the precipitation of sulphur NPs. After centrifuging the suspended sulphur NPs for 45 minutes at 4000 rpm, any remaining biological materials were repeatedly rinsed away with sterile deionized water. The resulting sulphur nanoparticles were dried at 50°C in a hot air oven.

Characterization of Biosynthesized Sulphur NPs

For characterization of sulphur NPs, some technologies were utilized, including UV-visible spectroscopy analysis, field Emission scanning electron microscope (FE-SEM) analysis, energy dispersive (EDS) X-Ray spectroscopy analysis, X-ray diffraction (XRD) analysis transmission electron microscopy (TEM) analysis and FTIR (Fourier Transform Infrared) analysis.

Antibacterial Activities of Sulphur NPs

Using agar well diffusion methods, the antibacterial properties of sulphur NPs were examined against biofilm-forming bacteria and multidrug-resistant MDR bacteria. Five *S. aureus* isolates from cutaneous infections were examined. Five milliliters of Brain Heart Infusion Broth (BHIB) were used to suspend five new colonies, which were then incubated for

eighteen hours at 37°C. In order to achieve an optical density that meets the 0.5 McFarland requirements, which is 1.5 X 10⁸ cells/mL, the turbidity produced by the growing culture was calibrated using broth that has been cleansed sterilized cotton swabs were treated with the fluids using the Wells diffusion agar method. The dipping cotton swabs stained the whole surface of a Mueller Hinton Agar tray. Subsequently, three sterile cork wells were created in each dish, and 150 µl of sulphur NPs at three distinct concentrations (100, 200, and 300 µg/ml) were added. After that, petri dishes were stored at 37°C for a whole day. To evaluate the antibacterial action, the diameter of the growth inhibition regions in millimeters was determined.

Anti-Biofilm Effect by Sulphur NPs

The in vitro antibiofilm activity was evaluated using the 96-well micro-titer plate technique. 100 µL of brain heart infusion BHI broth medium containing 1% glucose and 100 µL of biosynthetic sulphur NPs were placed in the first well micro-titer plate. Next, a range of diluted concentrations were created (1024, 512, 256, 128, 64, 32, 16, 8, 4, and 2 µg/ml). Until the last well of the micro-titer plate was employed as a control, 100 µL of the diluted concentration was applied to each well to verify the growth of biofilm by bacteria and the suppression of biofilm formation by sulphur nanoparticles. Next, 10 µL of isolated *S. aureus* bacterium that has been cultured for the past 24 hours is added to each well of the micro-titer plate.

Consequently, there will be 110 µL of mixing suspensions in each micro-titer plate well. The micro-titer plates are incubated for a full day at 37°C. The contents of each well were withdrawn, and 0.2 mL of phosphate buffer saline (PBS, pH 7.2) was used three times to wash away any free-floating "planktonic" bacteria. To eliminate free-floating "planktonic" bacteria, repeat the washing process three times. Sodium acetate (2% w/v) was used to fix created biofilms, and crystal violet dye (0.1% w/v) was used to colour them. After using sterile deionized water to remove any remaining discoloration, the plates were allowed to air dry. Following drying, 200 µL of 95% (v/v) ethanol alcohol was applied to the wells. An ELISA reader tuned at 570 nm was used to calculate the optical densities (OD). were made during the test three times. Bacterial adhesion to the wells and biofilm formation were shown by optical densities. The proportion of bacterial biofilm suppression by sulphur NPs concentration was determined using the following optical density (OD) equation:¹¹

$$\text{Biofilm Inhibition \%} = \frac{\text{OD of Cells Treated with SNPs}}{\text{OD of Non - Treated cells (Control)}} \times 100$$

Antioxidant Activity of Sulphur NPs

Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, the extracts' capacity to scavenge free radicals was evaluated. The DPPH solution (0.006% w/v) was made in methanol. The plates are 96-well. A final volume of 200 µl was achieved by adding 100 µl of sulphur NPs (100, 200, 300, and 400 µg/ml) to each well's DPPH solution, 200 µl of methanol to the blank well, and 200 µl of freshly made DPPH solution to the control well. The amount of discolouration was measured

at 517 nm following 30 minutes of incubation in the dark. DPPH solution served as the control. The proportion of DPPH free radical scavenging was calculated using the following formula:

$$\text{DPPH scavenging impact (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A1 is the absorbance in the presence of the sulfur NPs and A0 is the absorbance of the control.¹²

Results

Identification of Bacterial Isolates

Based on the results, 32 (25.6%) of isolates among 125 samples were identified as *S. aureus* using different examinations, in addition to VITEK2 system. Also, the selected *B. coagulans* isolate was identified using different examinations and confirmed using VITEK system, which was later used to biosynthesize sulphur NPs.

Antibiotic Susceptibility Test for *S. aureus* Isolates

Fourteen types of antibiotics from different classes were used, including Tetracyclines (Tetracycline; TET), Phenicol (chloramphenicol; CHL), Fluoroquinolones (levofloxacin; LVX and norfloxacin; NOR), Aminoglycosides (Gentamicin; GEN), Ansamycins (rifampin; RIF), Macrolides (erythromycin; ERY and azithromycin; AZM), Penicillins (penicillin; PEN and oxacillin; OXA), Glycopeptides (vancomycin; VAN), Cepheims (Cefoxitin; FOX) and folate pathway antiagonists (Trimethoprim-sulfamethoxazole; SXT). According to results, all isolates were resistant to each of Penicillin, Oxacillin and cefoxitin, 22 isolate (68.75%) resistant to Azithromycin, 19 isolate (53.12%) resistant to Erythromycin, while all 32 isolates (100%) were sensitive to trimethoprim-sulfamethoxazole and chloramphenicol, 27 isolate (90.63%) were sensitive to vancomycin, 31 (86.88%) were sensitive to Rifampin, 22 isolate (75%) sensitive to gentamicin, 26 isolate (81.25%) sensitive to Levofloxacin, and 25 (78.13%), 24 (81.25%), 27 (83.37%) of them were sensitive

to Norfloxacin, Tetracycline and ciprofloxacin, respectively. (Figure 1) shows 32 isolates of *S. aureus* and the numbers of antibiotics resistant to them.

Detection of Biofilm Formation

Congo Red Agar (Qualitative)

By using this method, the results were separated into three reference strains based on the amount of black pigment that could be produced. These strains are classified as positive (strong and moderate) biofilm-producing and negative (weak and non) biofilm-producing. This method was used to give quantitative results about the ability of *S. aureus* to produce biofilm. The crystal violet test on microtiter plates has been used extensively to investigate biofilm generation in bacterial isolates due to its high accuracy, even though the CRA method is a good phenotypic detectable method for biofilm.¹³

Detection of Biofilm Formation by Microtiter Plate Method (Quantitative)

The microtiter plate method was used to test the isolates of *S. aureus* for their ability to form biofilm. The potential of 32 isolates to form biofilm was assessed by comparing the O.D. values of stained attached bacterial cells. Figure 2 shows the distribution of *S. aureus* isolates according to biofilm formation.

Biosynthesis of Sulphur NPs Using Extracts of *B. coagulans*

B. coagulans extract was used in the biosynthesis of sulphur NPs. After adding extract to sodium polysulfide as a base material at pH 7 and extract concentration: sodium polysulfide (4:1), using cell free supernatant for extract, the extract showed extracellular biosynthesis, the reaction mixture's colour changed to a yellowish white.

Characterization of Biosynthesized Sulphur NPs

For characterization, different techniques, including UV-vis, FTIR, FE-SEM, EDS, XRD and TEM analysis, were performed, as following:

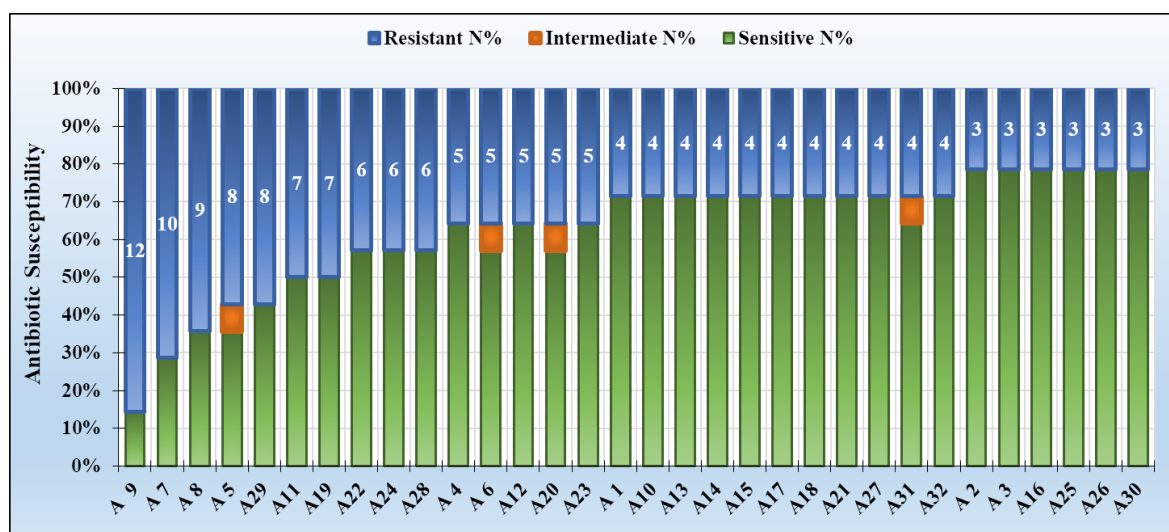


Fig. 1 Isolates of *S. aureus* bacteria and numbers of antibiotics resistant to them using an antibiotic susceptibility test.

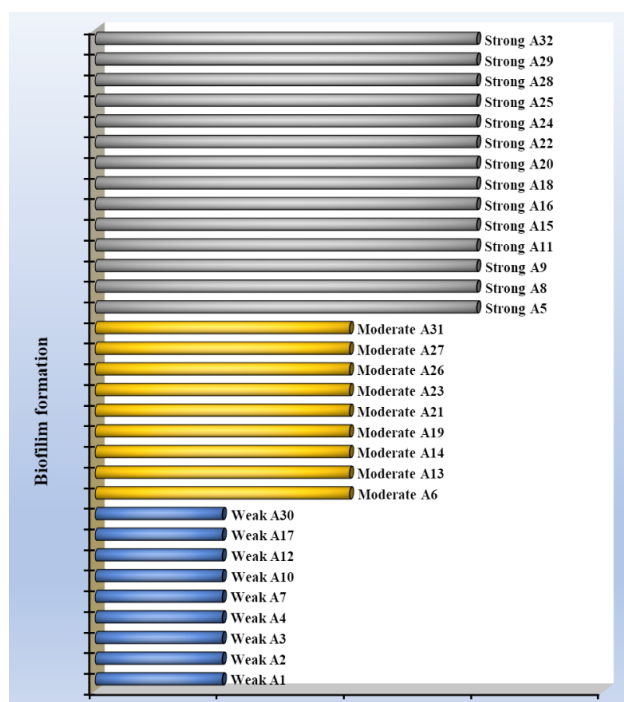


Fig. 2 Distribution of *S. aureus* isolates according biofilm formation via Microtiter plate technique.

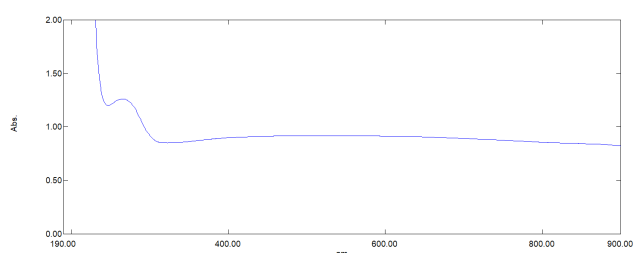


Fig. 3 UV-visible spectra of sulphur NPs by *B. coagulans*.

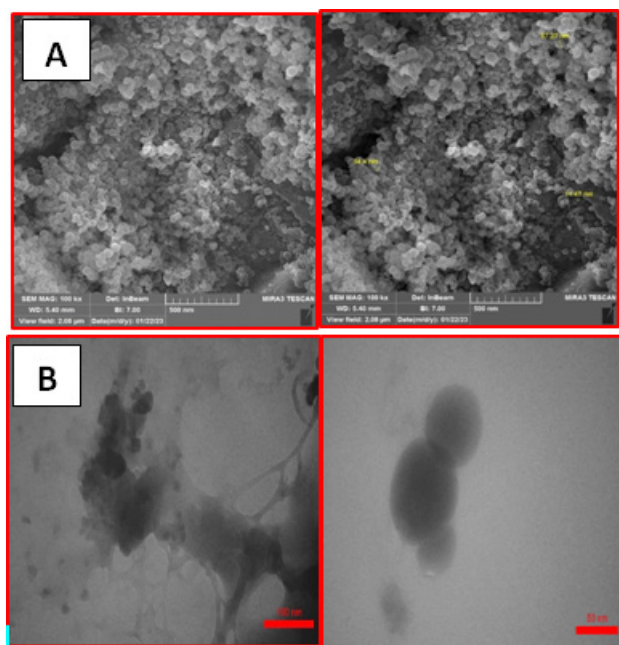


Fig. 4 (A) FE-SEM images, (B) TEM image of of Sulphur NPs synthesized by *B. coagulans*.

UV-Visible Spectroscopy Analysis

It is important method for determining the stability and production of SNPs is UV-vis spectroscopy. The absorbance spectrum of SNPs was displayed in the (Figure 3).

Field Emission Scanning Electron Microscope (FE-SEM) Analysis

The sulphur nanoparticles was described as depicted in (Figure 4A) FE-SEM pictures. The FE-SEM image demonstrates that the sulphur NPs are uniformly distributed and mostly spherical, with a small number of exceptions that are ellipsoidal in shape. The nanoparticles' average diameter is roughly 35.38 nm. It's possible that the smaller sulphur NPs aggregated to form the larger ones.

Transmission Electron Microscopy (TEM) Analysis

The TEM image of the sulphur NPs was shown in (Figure 4B). Images show that the nanoparticles are less than 100 nm in size and have a spherical or semi-spherical form. This reaffirms that particles arise at the nanoscale.

Energy Dispersive (EDS) X-Ray Spectroscopy Analysis

Sulphur NPs are subjected to elemental analysis or chemical characterization using the EDS analytical method. The purity and chemical makeup of the sulphur NPs that were biosynthesized were disclosed by EDS. By analyzing the optical absorption peaks of the constituent elements, Energy Dispersive Spectroscopy was utilized to estimate the existence of nanoparticles. The percentage of elements that make up sulphur and oxygen was revealed by elemental analysis, as the insert figure illustrates. Furthermore, the measurement's Au element appearance was ascribed to the supports that were applied to the substrate. The creation of sulphur is confirmed by the data, and no other contaminants are present, (Figure 5A).

X-ray Diffraction (XRD) Analysis

The XRD diffraction of sulphur NPs produced by *B. coagulans* is shown in (Figure 5B). The principal sulphur peaks were observed in accordance with the usual sulphur particle diffraction pattern (JCPD). The primary peaks are the same when compared to sulphur, but the strength of the peak is higher. Additionally, new peaks at 2theta = 42.84 and 47.99 are identified, which are ascribed to the sulphur crystal planes at 319 and 515, respectively. The Joint Committee on Powder Diffraction standard (JCPDS No. 08247) and these values are in agreement. Table 1 showed the XRD diffraction findings summarised.

Fourier Transform Infrared (FTIR) Analysis

Figure 5C shows the FTIR of spectra of the synthesized sulphur NPs by *B. coagulans*. It observed n that the same main peaks are present in sulphur. The peak at 3440 cm^{-1} and 1575 cm^{-1} due to O-H vibration and C=O vibration. While the peaks at 1429 cm^{-1} and 1118 cm^{-1} are assigned to the methylene scissoring vibration from the proteins and the vibration mode of C-O. The band at 1045 cm^{-1} assigned to the C-O stretching vibrations of alcohols. The vibration at 647 cm^{-1} and 467 cm^{-1} cans be assigned to bending modes of aromatic compounds and the sulphur NPs.¹⁴

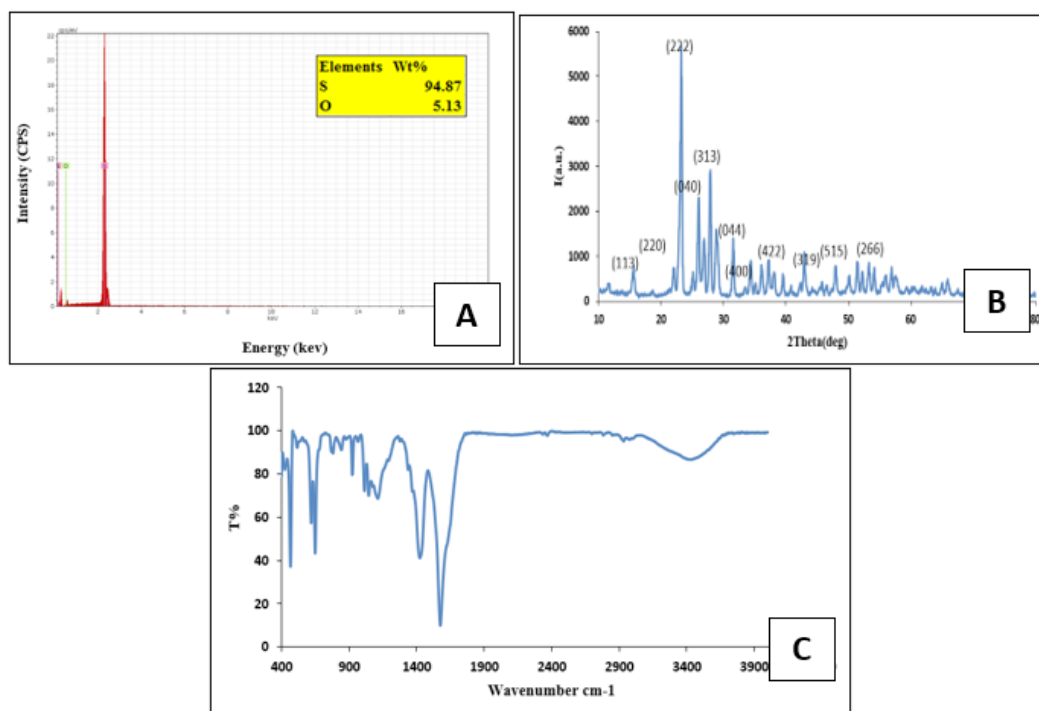


Fig. 5 (A) EDS, (B) XRD, (C) FTIR of Sulphur NPs synthesized by *B. coagulans*.

Table 1. Summary of results XRD diffraction

Sample	2Theta (deg)	D (Å)	FWHM (deg)	C.S (nm)	Hkl
Sulphur nanoparticles	15.74	5.73	0.15	53.60	113
	22.14	3.91	0.15	54.10	220
	23.39	3.84	0.14	57.43	222
	25.29	3.44	0.14	58.30	040
	27.89	3.21	0.16	51.28	313
	31.74	2.84	0.16	51.74	044
	34.54	2.62	0.14	59.56	400
	37.39	2.42	0.20	42.03	422
	42.84	2.14	0.18	47.51	319
	47.99	1.90	0.24	36.30	515
	51.46	1.75	0.16	55.22	266

Table 2. Antibacterial activity of sulphur NPs biosynthesized by *B. coagulans* extract against MDR bacterial isolates of *S. aureus*

Tested bacteria <i>S. aureus</i>	Inhibition zone (mm)		
	100 ug/ml	200 ug/ml	300 ug/ml
A29	18	22	25
A8	14	19	23
A9	12	18	22
A11	18	21	25
A5	20	23	26

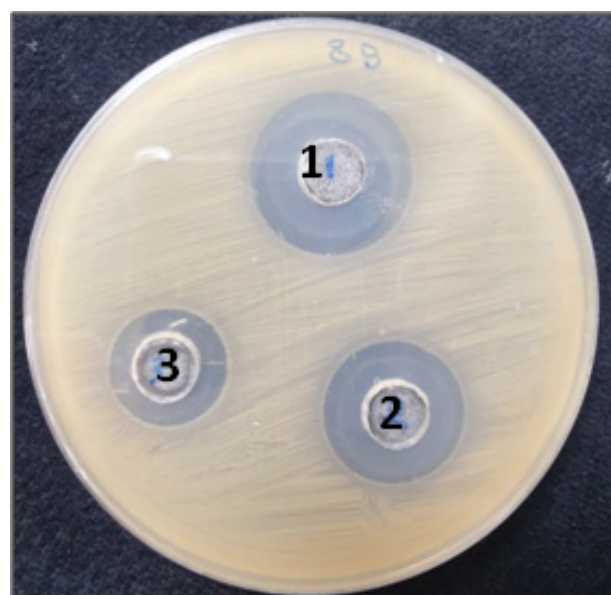


Fig. 6 The antimicrobial activity of Sulphur NPs biosynthesized by *B. coagulans* against *S. aureus* A8 under concentration of (1:300 µg/ml, 2:200 µg/ml, 3:100 µg/ml).

Antibacterial Activity of Sulphur NPs

The study showed that all biogenic NPs synthesized by *B. coagulans* extract have an inhibitory growth effect against five bio-film producers and MDR bacterial isolates of *S. aureus* (A5, A8, A9, A11 and A29). The agar well diffusion method was primary method which used for detecting the antibacterial activity of biogenic NPs. sulphur NPs with different concentrations (100, 200 and 300 µg/ml). According to the results in (Table 2 and Figure 6), sulphur NPs exhibited antimicrobial

Table 3. Anti-biofilm activity of sulphur NPs against *S. aureus* isolates

S.NPs synthesis	Concentration	Inhibition % (killing rate %)					Mean \pm SE	P-value
		A29	A8	A5	A11	A9		
<i>B. coagulans</i>	1024	93.5	93.5	92.0	96.8	87.6	92.7 \pm 1.5	0.886 ns
	512	89.8	92.7	89.9	95.6	83.2	90.2 \pm 2.1	
	256	86.7	80.6	83.3	92.4	80.7	84.7 \pm 2.2	
	128	80.3	76.2	74.9	86.4	70.3	77.6 \pm 2.7	
	64	74.3	74.9	40.3	49.2	44.6	56.6 \pm 7.5	
	32	67.3	71.0	39.6	32.5	39.9	50.1 \pm 7.9	
	16	32.7	30.5	27.0	28.9	28.3	29.5 \pm 1	
	8	27.7	23.9	18.1	27.2	11.9	21.7 \pm 3	
	4	14.0	21.6	7.7	25.8	5.7	15 \pm 3.9	
2	8.6	7.2	1.4	1.0	3.0	4.3 \pm 1.6		
P-value				0.0001				

activity against examined bacteria with inhibition zone ranged between 12 to 20, 18 to 23 and 22 to 26 mm at 100, 200 and 300 μ g/ml, respectively. All of bacterial isolates studied were inhibited by Sulphur NPs at different doses (100, 200 and 300 μ g/ml). The largest inhibition zone of Sulphur NPs was (26 mm) against *S. aureus* A5 by a concentration of 300 μ g/ml, whereas the lowest inhibition zone was (12 mm) against *S. aureus* A9 at the concentration of 100 μ g/ml.

Anti-Biofilm Activity of Sulphur NPs

Only five strong-biofilm producers and MDR of *S. aureus* (A5, A8, A9, A11 and A29) were used to assess the ability of sulphur NPs as anti-biofilm agent to inhibit biofilm. The antibiofilm efficacy of Sulphur NPs, generated by extract of *B. coagulans* was investigated using micro titter plate technique with varied doses of sulphur NPs (1024, 512, 256, 128, 64, 32, 16, 8, 4 and 2 μ g/ml) versus *S. aureus* strains (A5, A8, A9, A11 and A29). The results of statistical analysis in the (Table 3) show no significant differences ($P > 0.05$) in inhibition rate of all strain isolates (A5, A8, A9, A11 and A29) for all concentrations of sulphur NPs with $P = 0.886$. Furthermore, the effects of different concentrations of sulphur NPs indicated that same isolates to significant ($P < 0.05$) increase in inhibition rate (activity as antibiofilm) proportional with elevated of Sulphur NPs concentrations, where was the mean \pm SE inhibition rate at 1024 μ g/ml about 92.7 \pm 1.5 compared with decreased sulphur NPs concentrations at 2 μ g/ml up to 4.3 \pm 1.6 mm, with $P = 0.0001$. The antibiofilm effectiveness results differ depending on the bacterial strains.

Antioxidant Activity of Sulphur NPs

As shown in (Figure 7), the antioxidant activity of sulphur NPs were decreased into 81.6, 80.3, 75.2 and 68.9% with using concentrations of 400, 300, 200 and 100 μ g/ml, respectively. The results demonstrated that nanoparticles had the potential to scavenge DPPH free radicals.

Discussion

Numerous processes, including multidrug efflux pumps, DNA modification, cellular membrane change, and the activation of

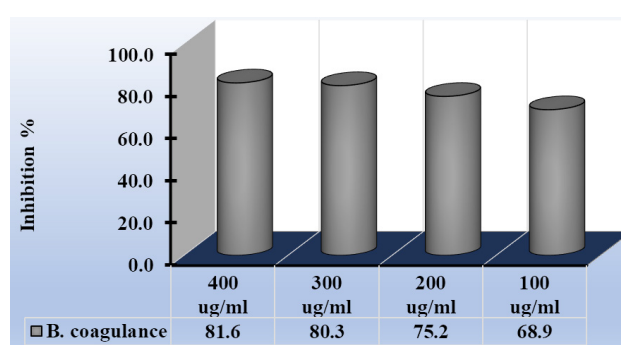


Fig. 7 Antioxidant activity of sulphur NPs synthesized by extract of *B. coagulans* and influence of different concentrations.

certain enzymes that may break down medicines intracellularly, are used by bacteria to build drug resistance. Because they are unable to reach an appropriate therapeutic level intracellularly, conventional antibiotics are therefore unable to treat resistant microorganisms.¹² He effectiveness and utility of these new antibiotics are still being investigated in clinical studies, despite the fact that they are being examined as therapy alternatives to get beyond the constantly changing resistance mechanisms.¹⁵ Therefore, it was necessary to use alternatives to antibiotics to treat resistant bacteria.

Strains of *S. aureus* are growing increasingly resistant to medications due to their capacity to stick to surfaces and create biofilms, biofilms play a significant role in the emergence of antibiotic resistance through two main mechanisms: firstly, by impeding the entry of drugs into the bacterium due to the formidable barrier formed by the biofilm and its protective matrix; and secondly, by inducing physiological alterations in the bacteria within the biofilms, including a substantial reduction in their growth rate, resembling a dormant state.¹⁶

It is hypothesized that variations in metabolic activity among bacterial cells within different layers of biofilms, as well as drug resistance in cells embedded within the biofilm, are factors contributing to the varying levels of antibiotic resistance. Additionally, the compact structure of biofilms and the presence of a matrix create a hypoxic environment in the deeper layers, which further contributes to the limited

effectiveness of antibiotics. As a result, bacteria can develop a resistant phenotype by modifying drug targets that are crucial for antibiotic activity.¹⁷

In the extracellular fabrication of NPs, microbes are grown in suitable media. The broth containing microbial cells is centrifuged, and the supernatant containing microbial enzymes is then used for the synthesis of NPs, the supernatant containing reductase enzymes is allowed to react with the metal ions in a separate vessel. The bioreduction of metal ions in a cell-free supernatant results in the formation of NPs.¹⁸ There could be no study reported the role of *B. coagulans* in biosynthesis of Sulfur NPs. However, supernatant of *B. coagulans* was used to biosynthesize copper oxide nanoparticles, iron oxide NPs, zinc oxide nanoparticles.¹⁹

The supernatant of *B. coagulans* bacteria contain diverse biomolecules, such as enzymes, vitamins, polysaccharides, and amino acids, that reduce metal ions to NPs, and this method is a green and ecologically friendly way, this bacterial species was favored in industrial many types of enzyme production, lactic acid fermenter, bacteriocin secreted as well as spore forming and these characteristics are important in the diagnostic of these bacteria, supernatant of bacteria *B. coagulans* was used as stabilizing and capping agent.¹⁹

Sulphur NPs are characterized by UV-vis absorption measurements at wavelengths between 190 and 900 nm. A single peak at 250–300 nm indicates that the NPs were successfully formed.⁴ The existence of the protein that envelops the nanoparticles may be the cause of certain particles' tendency to clump together when their sizes are marginally greater than 100 nm. According to both TEM and FESEM statistics, sulphur has a propensity to aggregate.²⁰

Consequently, sulphur NPs biosynthesized from the aforementioned extracts exhibited concentration-dependent antibacterial properties. Sulphur NPs' activity rose as their concentration increased, in accordance with Rai M, et al.²¹ The evaluation of the antibacterial activity of sulphur NPs clearly takes important factors into account. The bioavailability and velocity of diffusion of nanoparticles in two different mediums, agar and broth, may vary depending on the organism.²⁰ Sulphur NPs have strong antibacterial action against *S. aureus* with an inhibition zone ranging from 13.0 to 14.7 mm.⁵

E. coli and *P. aeruginosa* did not exhibit any activity against sulphur NPs even at high concentrations of 800 µg/mL. Sulphur NPs; on the other hand, shown strong antibacterial activity against Gram-positive bacteria (*S. aureus*) with a diameter of 5.7nm.²⁰ The authors clarified that the barrier that the outer membrane of Gram-negative bacteria forms serves as a defense against sulphur NPs This barrier prevents or limits sulphur NPs from entering bacterial cells and may even encourage the nanoparticles to self-aggregate.

The current study has focused on understanding the processes underlying the antibacterial activities of various metal nanoparticles. The following could be involved in potential mechanisms: (i) Negatively charged sulphur nanoparticles can interact intensely with biological target molecules on the surface of cells, such as proteins and enzymes, to produce gaps and promote cellular component leakage. (ii) Sulphur NPs are thought to function similarly to silver nanoparticles in that they release sulphur ions, which then react with -SH groups to create reactive oxygen species (ROS) stress, which denaturates proteins and fats; (iii) There is yet another way that H₂S can

be produced, which is when unknown molecules like NADH reduce sulphur NPs. Furthermore, it's possible that sulphur NPs will interact with DNA, causing it to break down and eventually cause cellular death.²²

It has been discovered that sulphur NPs have inhibitory effects on *S. aureus* biofilm development. *S. aureus* is a prevalent bacteria linked to a number of human diseases. The efficiency of sulphur NPs in preventing the formation of biofilms is attributed to their special characteristics, which include their small size, large surface area, and chemical reactivity.²³ Over the course of 48 hours of exposure, sulphur NPs were found to suppress the production of *S. aureus* biofilms; however, *P. aeruginosa* biofilms were not inhibited by the nanoparticles.²⁴

The interactions between nanoparticles (NPs) and biofilms are governed by three basically sequential mechanisms: NP mobility within the biofilm, NP attachment to the outer portion of the biofilm surface, and NP transport to the interface between the biofilm and fluid.²⁵ The physicochemical characteristics of biofilm components—such as size, shape, surface charge, hydrophobicity, and functional groups determine how they interact with NPs. These characteristics can be found on the surface of the bacteria as well as in the EPS matrix.²⁶

As concentrations of nanoparticles rise, so does their ability to scavenge DPPH free radicals, in sulphur NPs, the maximum inhibition was observed at a concentration of 400 µg/ml, while the lowest inhibition was observed at a concentration of 100 µg/ml. In addition to scavenging free radicals, antioxidants also prevent the production of new free radicals, when it comes to oxidative stress, free radicals have a strong bactericidal effect. In addition to the membrane being damaged, biological macromolecules that encourage cell death, such as proteins, lipids, DNA enzymes, and RNA, have also caused harm. The inhibition titer differs throughout nanoparticle types because of an electron that is provided and accepted by DPPH. It has been reported that at a dosage of 5 mg/kg, the sulphur NPs had noteworthy anticancer and antioxidant characteristics, as demonstrated by an increase in SOD, GPx, and TAC production relative to the positive control group.²⁷

Conclusion

- This study revealed the possibility of using *B. coagulans* extract for the first time in the production of sulfur NPs.
- Sulfur NPs can be used as antimicrobial and biofilm candidates and antioxidant agents.
- This can opens new horizons in studying the use of other bacterial extracts for the biosynthesis of sulfur NPs.

Acknowledgments

The authors thanks Mustansiriyah University (www.Mustansiriyah.com) for the support to complete this study.

Funding

Self-funded.

Declaration of Interest

No conflict of interest. ■

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