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Characterization of Composite of Silver Nanoparticles with Local Bacterial Cellulose and Determine Its Inhibitory Effects against some Pathogenic Bacteria.

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ABSTRACT

Local isolates of Gluconobacter species produced bacterial cellulose. The fact that bacterial cellulose has no antibacterial action to stop microbe infections makes it an intriguing material for use in medicinal medications and industrial coatings. Bacterial cellulose was submerged in a silver nitrate solution to saturate it with silver nanoparticles, resulting in antibacterial action. The present research was conducted to investigate the antibacterial properties of a composite of local bacterial cellulose and silver nanoparticles against MDR Staphylococcus aureus and E. coli isolates. Overall the isolates were obtained from bacteriological unit in Baaqubah Teaching Hospita (different sources of foods). during the period from December 2024 to January 2025. The absorbed silver ion (Ag+) in the bacterial cellulose was later reduced to metallic silver nanoparticles (Ag0) with the help of purified bacterial cellulose. The optical absorption band was visible in silver nanoparticles at about 420 nm. Transmission electron microscopy was employed to analyze the silver nanoparticles' particle size distribution. X-ray diffraction also demonstrated the production of silver nanoparticles. Bacterial cellulose coupled with freeze-dried silver nanoparticles demonstrated potent antibacterial action against isolates of Staphylococcus aureus and pathogenic Escherichia coli. This research presents a green synthesis method for producing a new bio-composite that employs bacterial cellulose as a capping agent, demonstrating its efficacy as an antibacterial agent. The results demonstrated that the inhibition zone of Ag Nps and AgNps/BC against MDR S. aureus ranged between 14-16 mm and 16-20 mm in diameter, and that the silver nanoparticles acquired in the cellulose matrix showed antimicrobial effects on both resistant Gram-positive and resistant Gram-negative bacteria. in the same order, in contrast, the inhibition zone against MDR E. coli varied from 15–17 mm to 18–21 mm.

***Keywords*:** Silver Nanoparticales, Composite, Food, Bacterial cellulose, Pathogenic bacteria.

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Introduction

One of the most intriguing research topics is wound dressings; the materials are produced using various biological or chemical processes and are intended to promote improved wound healing. [1] Over the recent years, the biological method of polymer synthesis has gained important popularity among various techniques due to its simplicity and the typically high purity of the subsequent polymers. Among the diverse range of materials used for wound dressings, bacterial cellulose holds particular appeal. The molecular composition of bacterial cellulose is characterized by a complex three-dimensional microfiber network composed of chains of glucose molecules, and it possesses a remarkable capacity to retain water [2]. Additionally, during its highly inflated state, this polymer shields the wound from germs and other contaminants. This barrier serves to reduce the risk of infection, which is essential for the wound healing process. Moreover, This polymer can easily be sterilized and preserve the wound's temperature without changing its structural integrity. [3].

Cellulose is mainly synthesized by several types of plants and specific bacterial species via biological method. It is also present in the cell walls of certain types of algae and tunicates. Bacterial cellulose is certainly purer than plant cellulose. This polymer is easily produced by certain types of bacteria, which secrete pure cellulose as a part of their extracellular matrix. This polymer produced by bacteria is free from impurities such as lignin and hemicellulose, which are common in plant cellulose [4]. Some bacterial genera, Gram-positive and Gram-negative, capable of producing cellulose include Agrobacterium, Alcaligenes, Achromobacter, Aerobacter, Azotobacter, Rhizobium, Pseudomonas, Sarcina and Salmonella [5]. Moreover, Acetobacter and Gluconacetobacter are the two primary genera of acetic acid bacteria that produce a lot of cellulose film that is used in medicine. The bacterial cellulose film lacks inherent antibacterial properties, which is a disadvantage in its application in the medical field [6]. In order to enhance the effectiveness of this polymer, researchers are

now investigating the making of a novel iteration of bacterial cellulose that possesses antibacterial properties. Researchers successfully incorporated chemically produced silver nanoparticles (SNPs) into the bacterial cellulose film [7,8,9]. Throughout history, silver has been employed for its antibacterial properties. Currently, silver nanoparticles (SNPs) are being discovered as a promising antibacterial properties against a great number of infectious bacteria which includes *Bacillus subtilis*, *V. cholera*, *Escherichia coli*, *Staphylococcus aureu*s, *Syphilis typhus* and *Pseudomonas aeruginosa*, it is reserve and are employed in the treatment of infections that are resistant to several drugs [10, 11]. Contrary to regular silver metal, Silver nanoparticles (SNPs) are known for their strong antibacterial properties owing to their greater specific surface area, toxicity and reactivity of nanoparticles frequently depend on the relative surface area, and it suggests that the potential for toxicity of smaller nanoparticles may be greater [12]. Research has established that SNPs impact bacterial cells through various mechanisms, including interactions with DNA and the cell membrane [13]. It is commonly believed that when heavy metals interact with proteins, they combine with the thiol (SH) groups, inactivating the proteins as a result [14, 15]. The creation of sustainable (NPs) composite biomaterials, which use inexpensive and locally available resources to address the demand for eco-friendly products, is the study's most noteworthy contribution [16]. The focus of the current work was to synthesize bacterial cellulose in a reasonably inexpensive media and use it to embed and disperse AgNPs to preparing a composite use as dressing in burn and wound infection, This method, which hasn't been studied, was inexpensive, straightforward, and employed inexpensive chemicals.

Material and Methods

Production of Bacterial cellulose

Cellulose-producing microbes were extracted from diverse local vinegar samples. Hestrin–Schramm (HS) agar medium was used to hold a volume of 1 mL from each source that had been gradually diluted. For five days, all plates were allowed to grow at 30°C. In the static cultivation procedure, 90 mL of HS medium containing 106 CFU/mL, or 0.5 McFarland standard, was mixed with 10 mL of the bacterial culture. The mixture was then maintained at 30°C for a maximum of 10 days. The bacterial culture exhibiting the highest cellulose yield was chosen [17].

Purification of BC Membrane

The bacterial cellulose sheets were formed from the culture was collected and treated with a 1% sodium hydroxide solution through boiling for duration of 60 min to removal of medium and The bacterial cellulose membrane contains embedded microbial cells. After purification the membrane was rinsed with deionized water, and then repeated washing with deionized water for about 4 to 5 times until neutral pH. Afterward BC membrane was boiled and stored in deionized water in aseptic conditions at room temperature prior to additional investigation [18].

Biosynthesis of Ag Nanoparticles from and Impregnation (Ag) into bacterial cellulose membrane.

The bacterial cellulose membrane achieved initially was treated by immersing in (0.001 M aqueous) of the $AgNO_3$ with continuous mixing for 60 min., To ensure that the Ag+ ions fully adsorb into the bacterial cellulose matrix, it is then allowed to rest. After being treated with AgNO3, the BC membrane was rinsed with deionized water for five minutes and then reduced by dipping it in a 0.01 N NaOH solution for fifteen minutes. Darky bacterial cellulose membrane showed the formation of AgNPs in the BC composite [8,23,25]. After that, the bacterial cellulose membrane that had been impregnated with silver was dried for approximately two hours at 60°C in a Memmert oven. [19, 20]

Evaluation of silver nanoparticle characteristics

1. FTIR analysis

The Nicolet Avatar 360 IR instrument was used to gather data from Fourier transform infrared spectroscopy. The FTIR spectrum was acquired at wavelengths between 400 and 4000 cm-1.
[21].

2. Scanning Electron Microscope (SEM)

Before and after treatment with the AgNO3 solution, scanning electron microscopy was used to investigate the BC membrane. Using a vacuum sputter-coater, thin layers of BC and BC-AgNPs samples were coated with evaporated gold to increase the sample's conductivity and provide better pictures. An image of both samples was then recorded at 5 and 25 kV using a FE-SEM; FEI Quanta 450 F, USA [22].

3. **X-ray Diffraction (XRD) Analysis**

Cu Kα radiation (k = 1.5418Å) was used to record X-ray diffraction spectra (XRD) at 45 kV and 40 mA on a (PAN analytical) X-ray diffractometer. The diffraction data were gathered at 1◦/min with a scan angle range of 2, which is 5 to 50 degrees. For native cellulose, the crystallite index (CrI) was calculated using the Segal et al. 1959 method: CrI = (I200 – Iam)/I200 × 100%, where Iam is the intensity of the baseline obtained at 2θ = 18o and CrI is the
[23].

4. Antimicrobial activity of composite Ag Nps bacterial cellulose

- **Sources of pathogenic bacteria**

Two distinct pathogenic bacteria, (25) *E coli*, and (25) *S aureus* were obtained from bacteriological unit in Baaqubah Teaching hospitals (different sources of foods). The bacterial isolates were identified according to some classical biochemical

tests (IMVC, catalase, oxidase and coagulase, hemolysis and biofilm formation), Vitec 2 system was used for confirm the identification.

- **Antimicrobial susceptibility test**

All pathogenic isolates were assessed for antibiotic susceptibility using the disk diffusion method on Mueller-Hinton agar, following the recommendations of the Clinical and Laboratory Standards Institute.

- **Antimicrobial activity of composite Ag NPs bacterial cellulose**

The test was done by inoculating approximately 10^5-10^7 CFU/ml of the pathogenic bacteria on Muller Hinton agar and the BC / Ag NPs composites and pure BC without Ag NPs were cut into disc shapes (8 mm in diameter) and sterilized by autoclaving for15 minutes at 120 ºC. then the disc pressed on the surface of cultured media, subsequently incubated at 37°C for a duration of 24 hours. Using calipers, the diameter of the inhibitory zone was measured in millimeters. [23, 24].

Results and Discussion

Isolation of the Bacteria Producing Cellulose

A total of thirty-two distinct bacterial isolates which capable of producing BC membranes were isolated, were isolated from 50 samples of fermented fruit sources, particularly vinegar. Using phenotypic test, five of them were shown to have better BC membrane producing abilities, and their silver nanoparticle biosynthesis was investigated.

Identification of Phenotypes

Based on their phenotypic characteristics, all of the isolates were determined to be Gram-negative and to be Gluconacetobacter species bacteria that did not form spores.

Membrane bacterial cellulose purification

Purification of the BC membrane was accomplished. Figure 1 shows the pure BC membrane. The consistency, appearance, and stiffness of the BC samples did not alter as a result of repeated rewashing and boiling, according to the results.

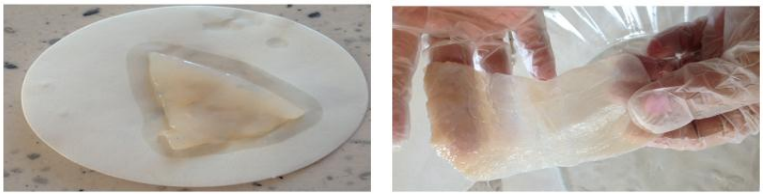

Figure1: purified bacterial cellelose membrane

Silver Nanoparticle Biosynthesis Impregnation in bacterial cellulose

The capacity of the sliver nanoparticles production in the recognized BC creating isolates was investigated. This stage involved the development of AgNPs as seen by the changing in the color of the membranes made by Gluconacetobacter bacteria from yellow to dark brown. Bacterial cellulose has a structure made up of many pores and three-dimensional networks. As a result, silver ions easily entered the bacterial cellulose through its pores when it was submerged in the aqueous $AgNO_3$. The absorbed Ag+ was likely connected to the bacterial cellulose microfibrils through electrostatic interactions, as it is anticipated that the electron-rich oxygen particles of polar hydroxyl and ether groups of BC will interact with electropositive transition metal cations. [25]. Immersion in a 0.01N NaOH solution was the next step to provide strong alkaline conditions and enable the BC to concurrently decrease Ag^+ to Ag^0. Generate AgNPs, which the BC membrane caps [26]. Following submersion in NaOH solution, the BC membrane developed a brownish hue as shown in Figure (2), signifying the AgNP production [27].

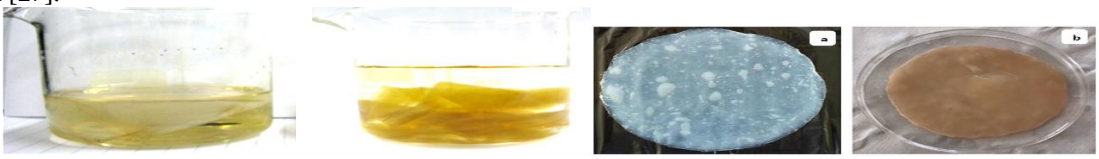

Figure 2: Bacterial cellulose: (a) reduced by NaOH solution and (b) immersed in $AgNO_3$ solution.

Characterizations of Ag Nanoparticles composite

Using Fourier Transform Infrared Spectroscopy (FTIP) to analyze functional groupings

The FTIR spectra of BC and AgNPs templated BC are displayed in (Figure 3) to identify the spectrum shift that indicates the functional groups involved in AgNP synthesis and binding within the BC matrix structure. Both the BC/AgNP and BC FTIR spectra showed the characteristic cellulose spectrum. The spectra distinguished approximately 3,200–3,400 cm−1, there was a lot of OH group visible in both graphs, which could be important for lowering Ag+ from $AgNO_3$ to Ag0 of sliver nanoparticles (AgNPs) and stabilizing sliver nanoparticles (AgNPs) in the bacterial cellulose matrix by serving as a capping agent. moreover, it has been demonstrated that hydroxyl groups can bind with metal ions[28, 29,30] . The shift of the aldehyde group's carbonyl group due to reducing Ag+ ions in the BC matrix was indicated by the BC/AgNP FTIR spectra, which changed from 2,792 to 2,804 cm−1. Furthermore, a spectrum corresponding to the carbonyl group's C=O was detected at 1714.432 cm−1. The oxidation of aldehyde and hydroxyl groups to generate carbonyl groups was one of the important

components in the creation of metal nanoparticles, as evidenced by these notable alterations in the FTIR spectrum. The stretching vibrations of CH and CH2 are attributed to two comparatively minor bands at 2900−3000 cm−1 and a strong band at 1554.45 cm−1, respectively [31, 32,33] . The band that emerged in the 1,700–1,600 cm−1 range indicated that AgNPs capped in biostructure had formed, based on previously published studies. According to previously published research, the band that appeared in the 1,800–1,700 cm−1 range suggested that AgNPs capped in biostructure had formed. The C−C and C−O stretching vibrations of the cellulose network give rise to a set of absorption peaks at the wavenumber region of 1000−800 cm−1. The fibril cellulose network exhibits comparatively weaker peaks at inferior wavenumbers. The BC/Ag composite's lack of any NO3-related vibrational band at 1410.23 cm−1 verifies that nitrates have been eliminated from the material[34,35,36].

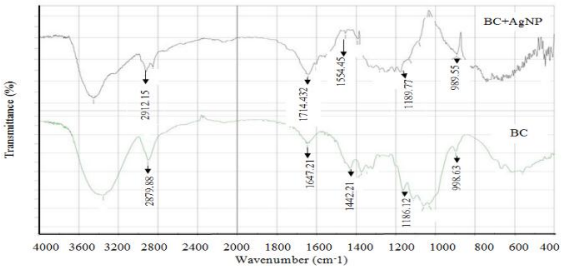

Figure 3: FTIR spectra of pure BC and AgNP/BC.

AgNP template BC's morphology:

The information about the morphological surface was obtained using SEM examination. As demonstrated in (Figure 4), pristine BC is composed of mostly 20−40 nm diameter cellulose micro fibrils networks, establishing a three dimensional web like structure. The three main components of the conventional BC network structure are porosity, compaction, and water tightness [37]. The morphology of BC has been demonstrated in all studies to be a homogeneous network of cellulose microfibrils, as seen in (Figure 4). A finely woven porous structure is formed by the distribution and interconnection of the nanoscale threads. This unique property facilitates the equal distribution of silver ions throughout the material and along the surfaces of the cellulose microfibrils as well as their diffusion into the spongy structure. Although they are not present in pure BC samples as shown in figure 4a, the AgNPs are clearly evident (Figure 4b) inside the BC sample either as individual spherical particles or aggregates (white dot). The spherical sliver nanoparticles twisted in the BC films depend on the surface crystallography and porosity [38, 39, 40].

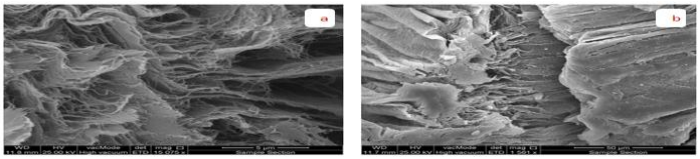

Figure 4: SEM image of: (a) pure BC and (b) AgNP/BC.

XRD analysis:

The XRD examination indicates the morphology, crystallinity index, crystal structure, and Ag content of the BC samples. From 10° to 80°, diffraction was evaluated at 2θ valuesThe evolution of broad angle XRD patterns recorded on pristine BC and AgNPs templated BC is revealed in (Figure 5). These diffraction peaks indicated the typical profile cellulose I pattern. Strong diffraction of BC indicated prominent peaks at 2θ that equal 14.49° which match Iα = 110 for triclinic structure and Iβ = 100 for monoclinic structure; 16.65° for Iα = 010 and Iβ = 110; and 22.11° for Iα = 110 and Iβ = 200 (41, 42). AgNPs were present in the BC matrix as evidenced by the diffraction peaks of AgNP/BC at 32.91°, 42.71°, 53.52°, and 69. 68°, which correlated to the peak plane values (111), (200), (220), and (311) of a face- centered cubic metallic Ag crystal correlated with a JCPDS card of Sliver (No. 4-783), (43,44) showing that AgNPs are present in the BC sample. The crystallinity index (CI) of these samples was 87.25% for BC templated with AgNPs and 90.11% for pure BC. These outcomes agreed with crystallinity index information for BC products from formerly published papers [45, 46] and demonstrated that the crystal structure of BC remained unaffected by the deposition of AgNPs.

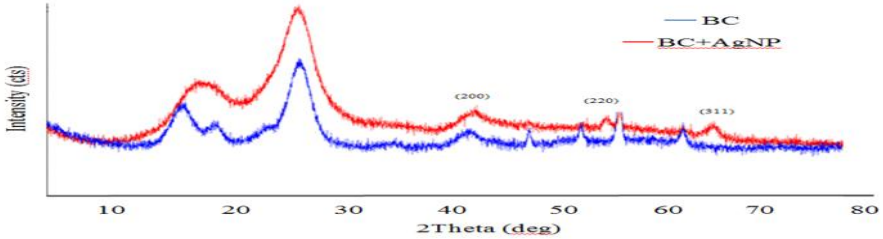

Figure 5: XRD graph of pure BC and AgNP/BC

Determination of antibacterial activity

Initially, the S. aureus isolates were cultured on Mannitol salt agar and Blood agar. While some isolates show gamma hemolysis, others produce obvious β-hemolysis around their colonies in blood agar. Table 1 shows the results, which show that oxidase is negative while catalase and coagulase are positive. The Vitek 2 System verified the identity of the recovered bacteria; nevertheless, the *E. Coli* isolates were catalase positive but oxidase and urease negative as shown in table 2.

Table (1) Identification of *S. aureus*

Biochemical tests Mannitol salt agar	*S. aureus* Yellow colonies
Coagulase test	(+)
Oxidase test	(-)
Catalase test	(+)

Table (2] Identification of *Escherichia coli*

Biochemical Tests	*Escherichia coli*
Catalase	(+ve)
Oxidase	(-ve)
Indole	(+ve)
Methyl red	(+ve)
Vogas-Proskauer	(-ve)
Citrate utilization	(-ve)
Kliger's iron	A/A ++
Urease production	(ve)

A 100% rate of multi-drug resistance (MDR, XDR, and PDR) to the tested antibiotics was seen in all isolates (S. aureus) in the current investigation. Twenty-five S. aureus isolates were examined for the prevalence of pan-drug resistance (PDR), extended drug resistance (XDR), and multi-drug resistance (MDR). Isolates that exhibited resistance to at least three of the antimicrobial categories this study looked at were categorized as MDR isolates. An isolate is said to have extensive drug resistance (XDR) if it is resistant to all but one or two classes. All seven investigated antibiotic classes do not affect PDR isolates. [31]. Figure 4 illustrates our findings, which showed that 82% of the isolates were verified as MDR, 14% as XDR, and 4% as PDR. The widespread and careless use of antibiotics in treatment, especially for S. aureus bacteria, has led to the development of antibiotic resistance as a significant issue.

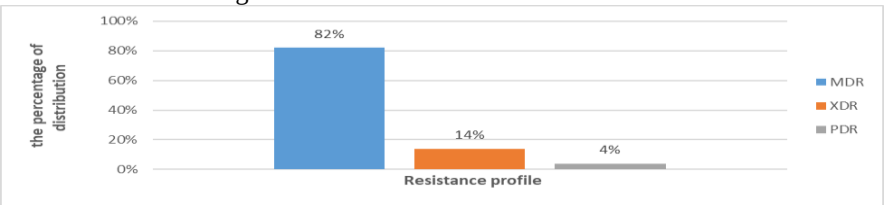

Figure (4): percentage of S. aureus that is pan-drug-resistant (PDR), extended-drug- resistant (XDR), and multidrug-resistant (MDR).

The current study's 25 E. Coli isolates exhibited a 100% multi-drug resistance (MDR, XDR) degree of resistance to the tested antibiotics. Results presented that one isolate was resistant to 2 antibiotic groups and another isolate which had resistance to 4 antibiotic groups, and 5 antibiotic groups as well as the results presented some isolates resist to 6,7 and 8 antibiotic groups as appeared in table 3.

Table 3 Resistance pattern for *E. coli* isolates

No. &(%) of isolates		No. of antibiotic resisted	Resistance type
7	(28%)	3	MDR
4	(16%)	4	XDR
5	(20%)	5	XDR
7	(28%)	6	XDR
6	(24%)	7	XDR
1	(4%)	8	XDR

The antibacterial effectiveness of biosynthesized AgNPs embedded in bacterial cellulose against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria was evaluated using an inverted disk-diffusion method to address the low solubility of cellulose compounds. The inhibition zone of Ag Nps and AgNps/BC against MDR S. aureus was demonstrated and the diameter of inhibition zones was ranged between 14-16 mm, and 16-20 mm. respectively, while the inhibition zone ranged between 15-17mm and 18-21 against the MDR *E. coli* respectively as seen in table 4 and 5.

Table (4): Antibacterial activity of AgNps and AgNps/BC against MDR *S. aureus* isolates

No.	AgNps	AgNps/ BC
S1	14 mm	17 mm
S2	15 mm	20 mm
S3	17 mm	19 mm
S4	16 mm	16 mm
S5	12 mm	16 mm

Table (5): Antibacterial activity of AgNps and AgNps/BC against MDR *E. coli* isolates

No.	AgNps	AgNps/ BC
E1	15 mm	18 mm
E2	17 mm	21 mm
E3	17 mm	20 mm
E4	15 mm	18 mm
E5	15 mm	18 mm

Antibiotic Microbial Resistance (AMR) is a significant worldwide issue as, if left unchecked, it poses a serious risk to public health. New antimicrobial agents are always needed to combat AMR bacteria, and their effectiveness has to be evaluated. Natural cellulose, particularly that made by regional isolates of Gluconbacter species, seems to be a suitable covering agent for the environmentally friendly production of silver nanoparticles. Using bacterial cellulose as a capping agent, we present a green synthesis approach to create a new bio composite and show that this material is an effective antibacterial agent. The cellulose matrix contained silver nanoparticles that exhibited antibacterial action against both resistant Gram-positive and resistant Gram-negative bacteria. Additionally, to get around the limited solubility of cellulose compounds, an inverted disk-diffusion technology was used. This new silver nanoparticle-cellulose bio polymer, which was created using an environmentally friendly process, has the potential to be used in the creation of biomedical devices and treatments in the future.

Conclusion

The fabrication of silver nanoparticles that mimic the bacterial cellulose membrane and the effects of the membrane's microstructure as a potential material for industrial and antibacterial applications have been studied. Bacterial cellulose was utilized to create the silver nanoparticle templates in a simple and eco-friendly manner. This study demonstrates that, with the aid of reduction in NaOH solution (0.01N), Ag ions in AgNO3 solution may be efficiently integrated into BC membrane as AgNPs. FTIR, SEM, and XRD analyses were used to verify the presence of AgNPs in the BC membrane. Additionally, the presence of AgNPs in the Bacterial cellulose membrane indicated their antibacterial activity against *Staphylococcus. aureus* and *Escherichia. coli*, which is crucial for regulatory dangerous bacteria connected to a variety of human illnesses .Lastly, the static cultivation process used here to produce BC from the Gluconacetobacter species grown in HS broth medium and used as a template to create AgNPs offers an easy-to-use and inexpensive way to create biomaterial with antibacterial and industrial uses.

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توصيف مركب جزيئات الفضة النانوية مع السليلوز البكتيري المحلي وتحديد تأثيراته التثبيطية ضد بعض البكتيريا المسببة للأمراض.

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الخلاصة
تم إنتاج السليلوز البكتيري من العزلات المحلية لبكتيريا *Gluconobacter spp*. تعتبر خصائص السليلوز البكتيري مادة مثيرة للاهتمام لاستخدامها في الأدوية الصيدلانية وفي التغليف الصناعي، السليلوز البكتيري ليس له نشاط مضاد للميكروبات ومنع عدوى الميكروبات. ولتحقيق النشاط المضاد للميكروبات، تم تشبع جزيئات الفضة النانوية في السليلوز البكتيري عن طريق غمر السليلوز البكتيري في محلول نترات الفضة. أجريت الدراسة الحالية لفحص التضاد البكتيري لمركب السليلوز البكتيري المحلي وجزيئات الفضة النانوية ضد من المكورات العنقودية الذهبية *MDR* و من بكتيريا *E. coli*. حيث تم الحصول على العزلات من الوحدة البكتريولوجية في مستشفى بعقوبة التعليمي (مصادر مختلفة من الأطعمة). خلال الفترة من تشرين الأول2024 إلى كانون الثاني 2025. تم استخدام السليلوز

البكتيري المنقى لاختزال أيون الفضة الممتص (*Ag+*) داخل السليلوز البكتيري إلى جسيمات الفضة النانوية المعدنية (*Ag0*) ،أظهرت الجسيمات النانوية الفضية نطاق الامتصاص البصري حوالي 420 نانومتر حجم جسيمات الفضة النانوية بعد فحصها بواسطة المجهر الإلكتروني النافذ. كما تم إظهار تكوين جسيمات الفضة النانوية من خلال حيود الأشعة السينية. أظهر السليلوز البكتيري المختلط بجسيمات الفضة النانوية نشاطًا قويا مضادًا للميكروبات ضد عزلات الإشريكية القولونية والمكورات العنقودية الذهبية. هدفت الدراسة الحالية بوصف طريقة التخلق الأخضر لإنتاج مركب حيوي جديد، باستخدام السليلوز البكتيري وإثبات أن هذه المادة هي عامل فعال مضاد للميكروبات. تم الحصول على جسيمات الفضة النانوية المتداخلة مع اغشية السليلوز وكان لها نشاط مضاد للميكروبات ضد البكتيريا إيجابية الجرام المقاومة وكذلك البكتيريا سالبة الجرام المقاومة؛ أظهرت النتائج أن منطقة التثبيط لـ *Ag Nps* و *AgNps/BC* ضد *MDR S. aureus* وتراوحت أقطار مناطق التثبيط بين 14-16 ملم، و16-20 ملم. على التوالي، في حين تراوحت منطقة التثبيط بين 15-17 ملم و18-21 ضد بكتيريا الإشريكية القولونية المقاومة للأدوية على التوالي.

الكلمات المفتاحية: جزيئات الفضة النانوية، المركبات، الغذاء، السليلوز البكتيري، البكتيريا المسببة للأمراض.