



## Silver-Decorated 2D Nanomaterials for Rapid and Broad-Spectrum Antibacterial Applications

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#### Authors' Contribution

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### ABSTRACT

Nanomaterials based on silver have become the focus of attention because of their excellent bactericidal effects and low chance of resistance occurring. Two-dimensional (2D) nanomaterials with silver decoration were prepared in this study and evaluated based on their speedy and universal antibacterial activity. X-ray diffraction (XRD), Fourier-transform infrared spectrophotometry (FTIR), and UV-Visible spectra were used to perform structural and physicochemical characterization of the silver nanoparticles to verify the success of the formation and stabilization of the material on the 2D substrate. The XRD pattern indicated that the crystal structure of metallic silver was face-centered cubic crystal and the FTIR analysis indicated that functional groups that anchored the nanoparticles were present. The spectroscopy UV Vis observed a typical surface plasmon resonance peak at 420 nm, which reflected that the silver nanoparticles were well dispersed. The discovery of nanocomposite synthesized was tested against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* by disc diffusion. The findings showed that they have a potent concentration-related antibacterial effect, and the silver-decorated 2D nanomaterials were the promising candidates to the development of the advanced antimicrobial applications.

## 1. INTRODUCTION

The quick development of antibiotic-resistant microorganisms has emerged as one of the most critical health issues in the world in the recent decades[1]. The proliferation and frequent uncontrolled use of the traditional antibiotics have contributed to the rapid development of the resistant strains of bacteria significantly decreasing the efficacy of the traditional antimicrobial treatment[2]. The consequence has been the increased prevalence of both Gram-positive and Gram-negative bacterial infection that is becoming more difficult to manage, resulting in increased morbidity, mortality, and healthcare expenses on a global scale[3]. This is a worrying trend that has necessitated the dire need of other antimicrobial interventions that can efficiently counter the resistance of pathogens and reduce the emergence of even more resistance. In this regard, nanotechnology has become a potential solution to the creation of new

multifunctional and highly efficient antimicrobial materials[4].

Silver nanoparticles are one of such nanomaterials that have received a lot of attention due to their proven antimicrobial effect and their ability to be effective against a large spectrum of microorganisms[5]. Silver nanoparticles demonstrate high levels of antibacterial activity by various pathways which include release of silver ions, destabilization of bacterial cell membranes and production of reactive oxygen species that cause oxidative stress on cells of microbes[6]. In contrast to the traditional antibiotics, which normally have a specific biochemical pathway, the silver nanoparticles may act simultaneously on several cellular functions thus lowering the chances of development of bacterial resistance. Nevertheless, the ability to flock in aqueous solutions is among the key issues of silver nanoparticles that decrease the available active surface and constrain their antimicrobial properties[7].

The solution to these shortcomings has been the recent research aimed at combining silver nanoparticles with two-dimensional (2D) nanomaterials, including graphene, graphene oxide, transition metal dichalcogenides, and MXenes[8]. The 2D materials have distinct structural and physicochemical characteristics such as high surface area, great mechanical strength, and chemical stability[9]. Their stratified structure gives them a perfect foundation of metal nanoparticles, and they can be dispersed uniformly and avoided aggregation[10]. Moreover, 2D materials have high surface to volume ratio that increases contact between the nanocomposite and bacterial cells and this can greatly improve antimicrobial action[11]. The action of combining silver nanoparticles with 2D nanosheets thus forms synergistic system that has the capacity to provide additional antibacterial effect by combining both physical and chemical interactions[12].

Although silver-decorated 2D nanomaterials have a promising future, there are a number of issues that have not been resolved yet in full understanding and optical properties of these nanoparticles with regard to antibacterial effects[13]. The synthesis of such nanocomposites or the fundamental antibacterial activity of these nanocomposites has been the primary concern of many studies reported in the past, and very little attention has been paid to systematic study of their structural properties, surface chemistry and optical properties with regard to their antimicrobial action[14]. Particularly, there is a necessity to create the well-dispersed system of silver nanoparticles-2D nanomaterials that might be able to deliver quick and efficient antibacterial effect against Gram-positive and Gram-negative bacteria. Also, the detailed interpretation of the role played by the synergistic effect between silver nanoparticles and 2D substrates towards the development of a broad-spectrum antimicrobial effect is a gap in research[15].

Thus, the current research work seeks to review and explore silver-decorated 2D nanomaterials as a promising antibacterial surface. To confirm the structural, chemical, and optical characteristics of the synthesized nanocomposite, several analytical methods including X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-Vis) spectroscopy were used to analyse the obtained nanocomposite. Moreover, the antibacterial of the developed nanomaterials was tested against typical Gram-positive and Gram-negative bacteria types according to the disc diffusion technique. The primary aim of this study is to show the synergetic impact of silver nanoparticles and 2D nanosheets on the antibacterial activity and investigate the future perspective of the given nanocomposite as the promising alternative in the quick and broad-spectrum use in antimicrobial activity.

## 2. LITERATURE REVIEW

Nanotechnology has come out as an influential instrument in the construct of superior antimicrobial materials that can deal with the increasing issue of antibiotic resistance[16]. Silver nanoparticles are among other types of nanomaterials that have received a lot of attention, owing to their high antibacterial efficiency as well as their efficacy against a large variety of microorganisms[17]. Silver nanoparticles have antimicrobial activity achieved in

various ways such as release of silver ions, perturbation of bacterial cell membranes and the formation of reactive oxygen species which causes oxidative stress in microbial cells. These processes enable the silver nanoparticles to activate several cellular processes at a time, and hence are effective against Gram-positive and Gram-negative bacteria and less prone to progression to resistance. Other researchers have proven that silver nanoparticles have high bactericidal effects on pathogens *Escherichia coli*, *Staphylococcus aureus*, and other microorganisms of clinical significance[18].

Although silver nanoparticles have a good antibacterial property, there are some limitations to the use of this product in practice, especially the propensity of the particles to aggregate in water. The process of aggregation minimizes the effective surface area of the nanoparticles and restricts their contact with bacteria cells, which has the ultimate effect of reducing their antimicrobial efficacy. In an attempt to overcome such shortcomings, scientists have looked at using nanocomposite systems whereby silver nanoparticles are immobilized on some supporting material that inhibits aggregation and also enhances the stability of the nanoparticle. In this respect, graphene oxide has been of particular interest in terms of broader surface area, high chemical resistance and availability of oxygen-related functional groups that may serve as nucleation point of metal nanoparticles. Such characteristics can enable the application of graphene-based materials to develop convenient platforms of uniform dispersion of silver nanoparticles so that their antibacterial activity can be advanced[19].

Graphene oxide and other two-dimensional materials also have their own antibacterial effects. The high edge density and surface area of graphene-based sheets allow for high interactions between the sheets and bacterial membranes. This results in the disruption of the membranes and the inhibition of bacterial metabolism. Graphene oxide has also shown that it induces oxidative stress in bacterial membranes by generating reactive oxygen species. This further enhances the antibacterial effects of graphene oxide. Graphene oxide nanocomposites containing silver nanoparticles have shown synergistic effects against bacterial membranes. This is because both materials work together to disrupt bacterial membranes and interfere with essential bacterial functions. Research has shown that graphene oxide nanocomposites containing silver nanoparticles have a much higher antibacterial effect compared to the individual effects of graphene oxide and silver nanoparticles[20].

Some other researchers have also examined the synthesis of silver-decorated graphene-based nanocomposites for their antibacterial properties. In this regard, previous studies have synthesized graphene oxide-silver nanocomposites where silver nanoparticles are uniformly dispersed on the surface of graphene sheets. The synthesized nanocomposites have also exhibited excellent antibacterial properties against both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria, indicating their possible applications in biomedical fields, including wound care, coatings, and drug delivery systems. The good dispersion of silver nanoparticles on graphene oxide not

only improves the antibacterial properties of nanocomposites but also helps in stabilizing the nanoparticles by avoiding their aggregation[21].

Recent research has also focused on more intricate nanocomposite materials that incorporate graphene oxide, silica nanoparticles, and silver nanoparticles to further boost antibacterial activity. For example, nanocomposites that incorporate graphene oxide as a support material for nano silica with silver nanoparticles have been found to display extremely potent antibacterial activity. The potent antibacterial activity of these nanocomposites is largely due to the synergistic effect of the high-surface-area support materials and the inherent antibacterial activity of silver nanoparticles[22].

Although great progress has been achieved in the development of silver-based nanocomposites for antimicrobial applications, further work is necessary to design nanocomposites that exhibit better stability, homogeneity in nanoparticle dispersion, and better antibacterial efficiency. In addition, the development of silver-decorated two-dimensional nanomaterials that are better engineered for surface interactions and nanoparticle dispersion is still a developing area. Elucidating the correlation between the structural characteristics of these nanocomposites and their antibacterial efficiency is a key requirement for furthering their application in biomedical and environmental fields.

### 3. MATERIALS AND METHODS

Silver nitrate ( $\text{AgNO}_3$ ), sodium borohydride ( $\text{NaBH}_4$ ), and other analytical-grade reagents were purchased from Sigma-Aldrich (USA) without further purification. The 2D nanomaterial precursor (e.g., GO) was obtained commercially or synthesized according to a previously published procedure. Nutrient agar and nutrient broth used to perform antibacterial activity were purchased from Oxoid (UK). De-ionized water was used to perform all the experiments.

#### 3.1 Materials

#### 3.2 Synthesis of 2D Nanomaterials

The 2D nanomaterial substrate was synthesized via a modified hydrothermal process. To be specific, the precursor was first dispersed into deionized water and underwent ultrasonic treatment for 1 h to ensure a homogeneous solution. The solution was then placed in a Teflon-lined stainless-steel autoclave and underwent hydrothermal treatment at 180 °C for 12 h. After cooling to room temperature, the product was rinsed several times using deionized water and ethanol to remove any possible impurities. The 2D nanosheets were collected via centrifugation and dried at 60 °C via a vacuum oven.

#### a. Decoration of 2D Nanomaterials with Silver Nanoparticles

Silver nanoparticles were deposited onto the surface of the 2D nanosheets through a chemical reduction method. In a typical synthesis, the prepared 2D nanomaterials were dispersed in deionized water under ultrasonic agitation to form a stable suspension. A calculated amount of silver nitrate solution was then added to the suspension under continuous stirring. Subsequently, freshly prepared

sodium borohydride solution was slowly introduced as a reducing agent to reduce  $\text{Ag}^+$  ions to metallic silver nanoparticles. The reaction mixture was stirred for 2 h at room temperature to ensure uniform deposition of silver nanoparticles on the surface of the 2D material. The resulting silver-decorated nanocomposite was collected by centrifugation, washed several times with deionized water and ethanol, and dried under vacuum.

#### b. Characterization of Nanomaterials

The morphology and surface characteristics of the synthesized nanomaterials were studied using a Scanning Electron Microscopy (SEM). The structural characteristics were studied using X-ray Diffraction (XRD). Fourier Transform Infrared Spectroscopy (FTIR) was employed for identifying the functional groups and assessing the chemical interaction between the silver nanoparticles and the 2D nanomaterial surface. UV-Vis spectroscopy was carried out for assessing the optical properties and confirming the synthesis of Ag nanoparticles using surface plasmon resonance peaks.

### 3.5 Antibacterial Activity Evaluation

#### 3.5.1 Bacterial Strains

The antibacterial activity of the synthesized nanocomposites was examined against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The test organisms were obtained from the microbiology laboratory culture collection and were preserved on nutrient agar at 4 °C.

#### 3.5.2 Agar Well Diffusion Assay

The antibacterial activity of the synthesized materials was first assessed using the agar well diffusion method. Fresh bacterial cultures were grown overnight at 37 °C in nutrient broth. The bacterial suspension was adjusted to approximately  $10^8$  CFU/mL and evenly spread on nutrient agar plates. Wells of approximately 6 mm diameter were punched into the agar using a sterile cork borer. Different concentrations of the nanomaterial suspension were added to the wells. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the inhibition zones around the wells.

#### 3.5.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the silver-coated nanomaterials was tested by a broth microdilution method. Diluted solutions of the nanomaterials in a nutrient broth medium were added to the bacterial cultures. The samples were then incubated for 24 hours at 37 °C. The growth of the bacteria was observed by measuring the optical density at 600 nm using a UV-Visible spectrophotometer. The MIC was described as the lowest concentration of the nanomaterials that completely inhibited the growth of the bacteria.

#### c. Statistical Analysis

All experiments were done in triplicates. The results were presented as mean  $\pm$  SD. The statistical analysis was performed using appropriate statistical software. The differences between groups were considered statistically significant at  $p < 0.05$ .

**Table 3.1**

Summary of materials, synthesis protocol, characterization techniques, and antibacterial evaluation methods.

Category	Sub-Category	Details / Specifications
Materials	Chemicals	Silver nitrate ( $\text{AgNO}_3$ ), Sodium borohydride ( $\text{NaBH}_4$ ) – Sigma-Aldrich, USA
	2D Substrate	Graphene oxide nanosheets (Lab-prepared)
	Microbiology Media	Nutrient agar and nutrient broth – Oxoid, UK
Synthesis	Solvent	Deionized water
	2D Nanomaterial Preparation	<ul style="list-style-type: none"> <li>Method: Modified hydrothermal method</li> <li>Conditions: Ultrasonication (1 h), Autoclave at <math>180^\circ\text{C}</math> (12 h)</li> <li>Post-treatment: Washed (water), dried at <math>60^\circ\text{C}</math> under vacuum</li> </ul>
	Silver Nanoparticle Decoration	<ul style="list-style-type: none"> <li>Method: Chemical reduction</li> <li>Reducing Agent: <math>\text{NaBH}_4</math></li> <li>Process: 2D suspension + <math>\text{AgNO}_3</math> + <math>\text{NaBH}_4</math>, stirred for 2 h (RT), centrifuged, washed, vacuum dried</li> </ul>
Characterization	Morphology	Scanning Electron Microscopy (SEM)
	Crystal Structure	X-ray Diffraction (XRD) – Cu-K $\alpha$ radiation
	Surface Chemistry	Fourier Transform Infrared Spectroscopy (FTIR)
	Optical Properties	UV-Visible Spectroscopy (SPR peak monitored)
Antibacterial Assay	Test Strains	<ul style="list-style-type: none"> <li><i>Escherichia coli</i> (Gram-negative)</li> <li><i>Staphylococcus aureus</i> (Gram-positive)</li> </ul>
	Agar Well Diffusion	<ul style="list-style-type: none"> <li>Inoculum: <math>\sim 10^8</math> CFU/mL</li> <li>Well Size: 6 mm diameter</li> <li>Incubation: <math>37^\circ\text{C}</math> for 24 h</li> <li>Measurement: Zone of Inhibition (ZOI) in mm</li> </ul>
	Minimum Inhibitory Concentration (MIC)	<ul style="list-style-type: none"> <li>Method: Broth microdilution</li> <li>Monitoring: Optical density at 600 nm</li> <li>Definition: Lowest concentration with no visible growth</li> </ul>
Data Analysis	Statistical Treatment	Triplicate experiments, Mean $\pm$ SD, significance at $p < 0.05$

## 4. RESULTS AND DISCUSSION

### 4.1 Scanning Electron Microscopy (SEM)

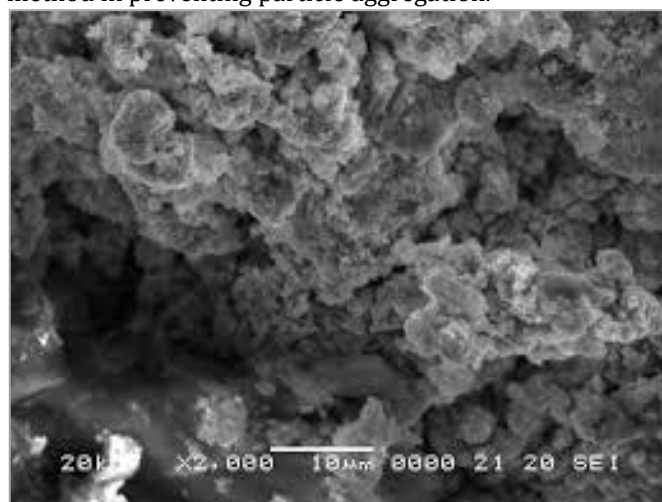
The (SEM) analysis of the Silver Decorated 2D nanomaterials offers a direct visual confirmation of the morphology of the composite material as well as the successful integration of the metallic nanoparticles onto the 2D substrate. The micrograph indicates a layered sheet-like morphology characteristic of the 2D nanomaterial substrate. This substrate is utilized as a high-surface-area material. The nanosheets contain a number of small bright spherical features that indicate the presence of the silver nanoparticles. The SEM micrograph indicates that the synthesis method utilized for the material has been successful in providing a uniform distribution of the silver nanoparticles. This is important as it has prevented the aggregation of the silver nanoparticles that is typically a hindrance to the effectiveness of the silver-based

material. This arrangement is important as it allows the silver to be accessible to the environment.

The particular morphology observed in the SEM image is the physical basis of this "rapid and broad-spectrum" antibacterial property of the material. The high surface area/volume ratio of the 2D sheets ensures that there is maximum contact between the Ag-coated platform and the bacterial membrane. This close proximity ensures that there is a focused delivery of  $\text{Ag}^+$  ions directly onto the bacterial cell walls of *E. coli* and *S. aureus* pathogens. In addition, the presence of uniformly dispersed nanoparticles observed in the micrograph ensures that there is a uniform delivery of these  $\text{Ag}^+$  ions over the entire surface, thereby creating ROS that causes total cell death of the bacteria.

**Figure 4.1**

Scanning electron microscopy (SEM) image of the synthesized silver-decorated 2D nanocomposite. The micrograph reveals the layered structure of the 2D substrate with spherical silver nanoparticles uniformly anchored onto the surface. The uniform dispersion confirms the effectiveness of the chemical reduction method in preventing particle aggregation.



### 4.2 Structural Analysis by X-Ray Diffraction

The X-ray diffraction pattern of the silver-decorated 2D nanomaterials confirms the successful formation of the silver nanoparticles on the substrate. From the diffractogram, it can be observed that there are four distinct characteristic peaks appearing at  $2\theta$  values of around  $38.1^\circ$ ,  $44.3^\circ$ ,  $64.5^\circ$ , and  $77.5^\circ$ . These diffraction peaks are associated with the (111), (200), (220), and (311) planes of the face-centered cubic (FCC) unit cell of the silver nanoparticles. The diffraction peaks are matched to the standard reference pattern of silver (PDF Card No. 04-0783). The sharp diffraction peaks indicate a high degree of crystallinity. This suggests the formation of well-ordered metallic silver nanoparticles on the substrate rather than an amorphous phase.

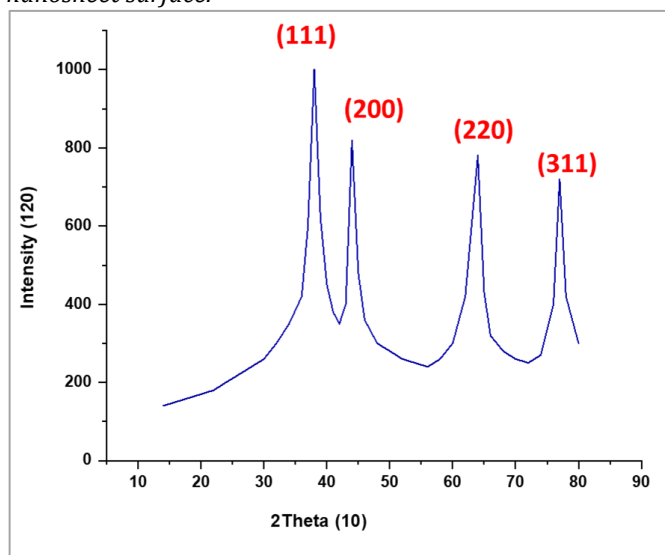
Besides the identification of the phase, the peak broadening observed in the XRD pattern can provide information on the nanoscale size of the silver crystallites. This is due to the inverse relationship between the size of the crystallites and the width of the peak, as given by the Scherrer equation. The observed peak broadening thus confirms the formation of nanoscale silver particles rather

than bulk metallic silver. The intensity of the (111) peak observed in the XRD pattern is significant for antibacterial activity since the silver crystallites have higher surface energy and atomic density on the (111) plane. This can thus enhance the efficient release of silver ions ( $\text{Ag}^+$ ), which are significant in the antibacterial activity. The silver ions interact with the cell membranes of the bacteria, thus interfering with the membrane integrity and the essential cell functions. Additionally, the XRD pattern can provide information on the nature of the 2D nanomaterial support. The relatively broad and low-intensity diffraction observed at the lower  $2\theta$  values ( $10^\circ$  to  $25^\circ$ ) indicates the good exfoliation of the 2D nanosheets. This shows the absence of long-range stacking order.

This feature reveals that a significant surface area platform is provided for anchoring the silver nanoparticles. The lack of strong peaks for the substrates in comparison with the strong peaks for the silver nanoparticles further reveals that the 2D nanomaterial is acting as a supporting platform for the uniform distribution of the silver nanoparticles and preventing their agglomeration, thus forming a synergistic effect with the highly crystalline silver nanoparticles for enhancing the antibacterial properties of the synthesized nanocomposite for a wide variety of pathogenic microorganisms.

**Figure 4.2**

*X-ray diffraction pattern of silver-decorated 2D nanomaterials showing characteristic diffraction peaks at  $2\theta \approx 38^\circ, 44^\circ, 64^\circ,$  and  $77^\circ$ , corresponding to the (111), (200), (220), and (311) planes of crystalline silver, confirming successful deposition of Ag nanoparticles on the nanosheet surface.*



### 4.3 Surface Chemistry and Functional Group Analysis

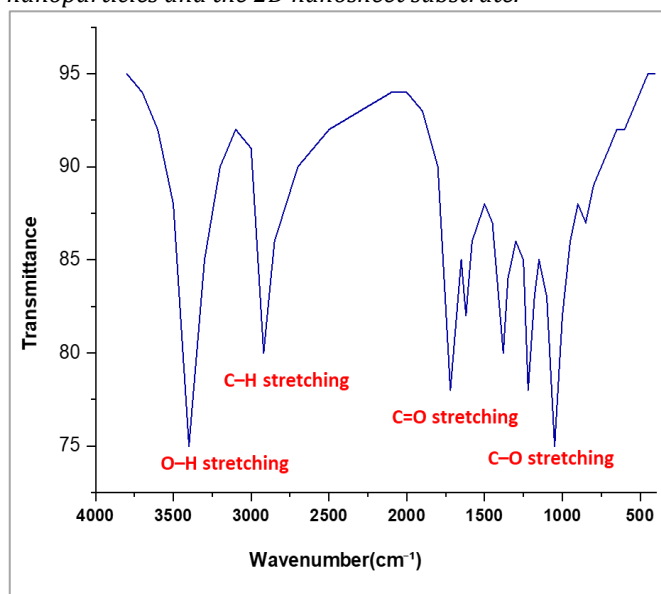
In addition, the Fourier Transform Infrared (FTIR) spectrum of the Ag-decorated 2D nanomaterial is useful in providing information on the surface chemistry and successful functionalization of the composite material. The broad peak in the spectrum observed at  $3400\text{ cm}^{-1}$  is ascribed to O–H stretching vibrations, which are characteristic of hydroxyl groups present in the 2D nanomaterial or adsorbed moisture on its surface. The

peak observed in the spectrum at  $2920\text{ cm}^{-1}$  corresponds to C–H stretching vibrations, thus confirming the presence of organic components in the 2D nanomaterial structure. In addition, a strong peak in the spectrum observed at  $1720\text{ cm}^{-1}$  corresponds to C=O stretching vibrations, which are characteristic of carbonyl or carboxyl functional groups present on the surface of the 2D nanosheets. Another strong peak in the spectrum observed at  $1050\text{ cm}^{-1}$  corresponds to C–O stretching vibrations, thus confirming the presence of these oxygen-containing functional groups on the surface of the 2D nanomaterial, which play a crucial role as active nucleation sites for the attachment of Ag nanoparticles on its surface.

The surface chemistry observed from the FTIR spectrum is linked to the antibacterial property of the nanocomposite material. The availability of a high number of oxygen-containing functional groups on the material's surface improves the hydrophilicity and dispersion stability of the material in aqueous media. This allows for a better interaction between the material and the bacterial cell membranes. In addition, these functional groups on the material's surface may be used for the controlled release of silver ions ( $\text{Ag}^+$ ) from the nanoparticles that are anchored on the material's surface. This controlled release of silver ions from the nanoparticles, along with the high surface area provided by the 2D nanosheets, improves the oxidative stress experienced by the bacterial cell membranes. This leads to the death of the bacterial cells by damaging the membranes and proteins in the bacterial cells. Therefore, the interaction between the surface chemistry of the functionalized 2D nanomaterial and the silver nanoparticles anchored on the material's surface plays a significant role in the high antibacterial property observed from the synthesized nanocomposite material.

**Figure 4.3**

*FTIR spectrum of silver-decorated 2D nanomaterials showing characteristic absorption bands at  $\sim 3400\text{ cm}^{-1}$  (O–H stretching),  $2920\text{ cm}^{-1}$  (C–H stretching),  $1720\text{ cm}^{-1}$  (C=O stretching), and  $1050\text{--}1220\text{ cm}^{-1}$  corresponding to C–O functional groups, confirming the presence of surface functional groups and interaction between silver nanoparticles and the 2D nanosheet substrate.*



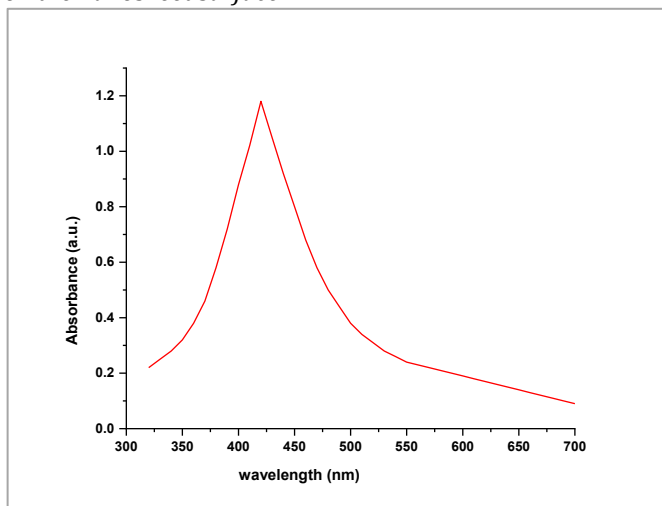
#### 4.4 Optical Properties

The optical evidence for the formation of nanoparticles on the surface of the 2D nanomaterials is provided by the Ultraviolet-Visible (UV-Vis) absorption spectrum of the silver-decorated 2D nanomaterials. The UV-Vis spectrum shows a sharp peak at 420 nm, which is a signature of the surface plasmon resonance of the nanoparticles formed on the surface of the 2D nanomaterials. The surface plasmon resonance of the nanoparticles is the oscillation of the free electrons present on the surface of the nanoparticles due to the effect of the incident light. The sharp peak observed in the UV-Vis spectrum indicates that the nanoparticles are well dispersed on the surface of the 2D nanosheets without any aggregation.

It is worth noting that the position and intensity of the SPR peak are directly linked with the antibacterial properties of the nanocomposite. The high absorbance in the range of 420 nm is a clear indication of a substantial amount of metallic silver, which serves as a source for the gradual release of  $\text{Ag}^+$  ions. These ions are a crucial element in the antibacterial properties of nanocomposites, as they interact with the membranes of bacterial cells, causing interference with their fundamental biological processes. In addition, the interaction between the Ag nanoparticles and the 2D nanomaterial substrate ensures that the size of these nanoparticles remains minimal, preventing their aggregation and thus increasing their surface area for interaction with bacterial cells, leading to the production of ROS, which interferes with their membranes and biological processes, ultimately causing their death. Consequently, the combined effect of Ag nanoparticles and the 2D nanomaterial framework results in a rapid effect on a wide range of both Gram-positive and Gram-negative bacteria.

**Figure 4.5**

UV-Visible absorption spectrum of the synthesized silver-decorated 2D nanomaterials showing a characteristic surface plasmon resonance peak at approximately 420 nm, confirming the successful formation of silver nanoparticles on the nanosheet surface.



#### 4.5 Antibacterial Activity of Silver-Decorated 2D Nanomaterials

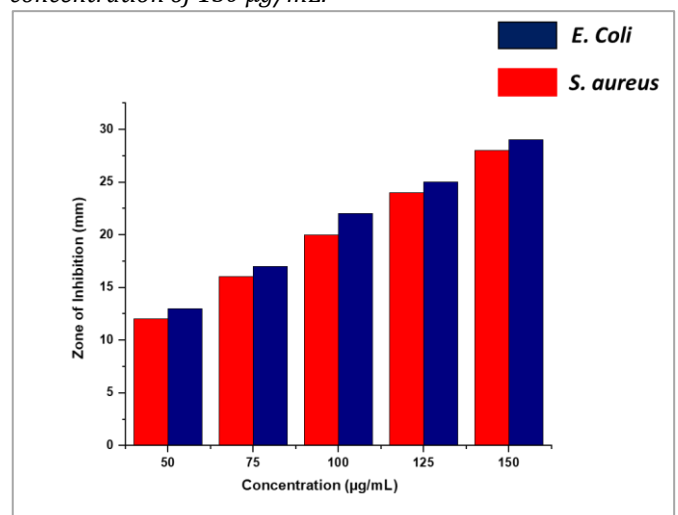
The antibacterial property of the silver-decorated 2D nanomaterials was tested by using the disc diffusion

method. In this method, the zone of inhibition (ZOI) was used to determine the effectiveness of the nanomaterials against the selected bacterial strains. For this study, two strains of bacteria were selected: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). It was observed that the zone of inhibition increased with the increase in the concentration of the nanomaterial from 50  $\mu\text{g}/\text{mL}$  to 150  $\mu\text{g}/\text{mL}$ . For *Escherichia coli*, the zone of inhibition increased from 13 mm to 29 mm, whereas for *Staphylococcus aureus*, the zone of inhibition increased from 12 mm to 28 mm. This increasing trend clearly indicates that the synthesized nanocomposite has shown strong broad-spectrum antibacterial property by inhibiting the growth of both Gram-negative and Gram-positive bacteria.

From the results, it is observed that the nanomaterial showed slightly higher antibacterial activity against *E. coli* than *S. aureus* at all concentrations. This is due to differences in the structure of the cell wall of these two microorganisms. *E. coli* is a Gram-negative bacterium, whereas *S. aureus* is a Gram-positive bacterium. The cell wall of Gram-negative bacteria is thin, allowing  $\text{Ag}^+$  ions and ROS generated by the nanomaterial to easily penetrate the bacterial cell membrane, resulting in effective inhibition of bacterial growth. On the other hand, Gram-positive bacteria have a thicker peptidoglycan layer that acts as a protective layer against various external agents. However, it is observed that even though *S. aureus* is a Gram-positive bacterium, a larger inhibition zone was observed against this bacterium when compared to *E. coli* even at the lowest concentrations of the nanocomposite material synthesized. This is due to the rapid antibacterial property of the nanocomposite material synthesized in this study. The increased rate of antibacterial performance of this nanocomposite material is due to its large surface area, which allows for effective contact between the nanomaterial and the bacterial cell membrane, resulting in the release of  $\text{Ag}^+$  ions that are effective against bacteria.

**Figure 4.6**

Comparative evaluation of the antibacterial activity of synthesized silver-decorated nanocomposites. The bar chart displays the zones of inhibition (ZOI) for *E. coli* (blue) and *S. aureus* (red). *E. coli* exhibits higher susceptibility than *S. aureus*, with the largest inhibition observed at a concentration of 150  $\mu\text{g}/\text{mL}$ .



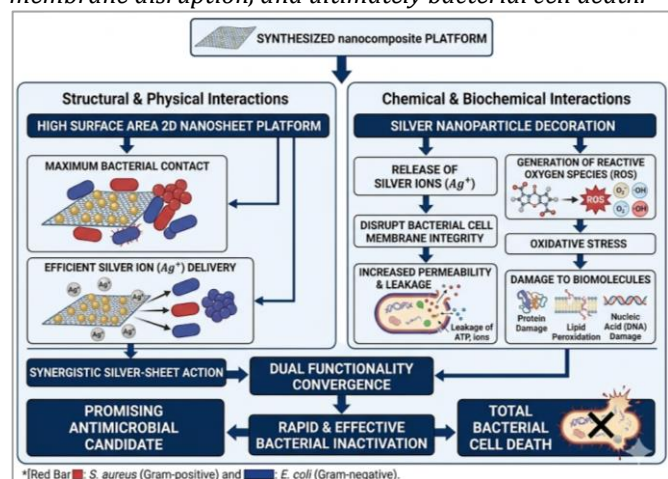
#### 4.6 Proposed Antibacterial Mechanism

The schematic diagram above demonstrates the antibacterial mechanism of the silver-decorated 2D nanocomposite material, showing how it acts against bacteria via structural, physical, chemical, and biochemical mechanisms. The structural mechanism is based on the 2D nanocomposite material's ability to act as an effective platform for maximizing contact with target microbial cells such as *E. coli* and *S. aureus*. The physical interaction of the 2D nanocomposite material with microbial cells is based on the physical interaction of the 2D nanocomposite material with microbial cells, which enables the trapping of microbial cells. This is a synergistic effect with the chemical mechanism of the material.

At the biochemical level, the gradual release of silver ions ( $Ag^+$ ) is coupled with the simultaneous enhancement of the generation of reactive oxygen species (ROS), including superoxide radicals and hydroxyl radicals. The released  $Ag^+$  ions react with the bacterial cell membrane, causing disruption of membrane integrity and increasing its permeability. This leads to the leakage of important cell components, including ATP, proteins, and intracellular ions. In addition, oxidative stress induced by ROS causes extensive damage to important biomolecules, including protein denaturation, peroxidation of the cell membrane's lipids, and fragmentation of nucleic acids including DNA. The cumulative effects of membrane disruption and oxidative damage cause death of the bacterial cells. In conclusion, the cooperative action of the 2D nanomaterial platform and the silver nanoparticles leads to rapid antibacterial activity, indicating its strong potential for various applications in combating pathogens.

**Figure 4.7**

*Proposed synergistic antibacterial mechanism illustrating the combined effects of physical contact between the silver-decorated 2D nanosheets and bacterial cells, the release of silver ions ( $Ag^+$ ), and the generation of reactive oxygen species (ROS), which together induce oxidative stress, membrane disruption, and ultimately bacterial cell death.*



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