

Green Synthesis of Antibacterial Nanoparticles from Aloe Vera: A Study of Molecular Interactions through Docking

Heba Alshater

Menoufia University Hospital

Sabreen M. El-Gamasy

sabreen_mohamed2008@yahoo.com

El-Menoufia University

Samar E. AbdEl-Razek

National Liver Institute, Menoufia University

Article

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Abstract

Utilizing both exploratory and computational strategies, this work analyzes the synthesis of nanoparticles utilizing Aloe vera extricate and surveys their antibacterial qualities. Within the green blend of nanoparticles, aloe vera, which is well-known for its inexhaustible phytochemical substance, acts as a natural stabilizing and decreasing agent. The estimate, shape and crystalline structure of the synthesized AgNPs from Aloe vera extricate were affirmed by characterization utilizing UV-Vis spectroscopy, FTIR spectroscopy, X-ray diffraction and transmission electron microscopy. Antibacterial movement was assessed against a range of bacterial species (*E. coli*, *Salmonella* spp, *S. aureus*, *Bacillus* spp.) appearing notable inhibition zones that are suggestive of antibacterial viability. Comes about appeared *Staphylococcus aureus* with a most extreme zone breadth of 26mm. Moreover, we appeared atomic docking studies to investigate the intelligent between AgNPs and the Omicron receptor-binding theme (RBM). The conceivable associations between the nanoparticles and bacterial targets were too clarified by atomic docking tests, which shed light on the instruments behind the antibacterial movement of the particles. Concurring to the discoveries, nanoparticles made from aloe vera display empowering antibacterial properties, demonstrating that they may discover utilize in therapeutic and natural domain.

Introduction

Antibiotic-resistant organisms, particularly those resistant to second- or third-line broad-spectrum antimicrobials, show a primary around the world risk to open wellbeing and the tertiary healthcare framework due to the rise of mortality rate and related wellbeing complications.¹ Over 700,000 individuals each year may absent as a result of antimicrobials' inadequacy to combat rapidly changing, resistant bacterial strains, states the 2019 WHO report. Consequently, this raises the chance of restorative issues and uncomplimentary comes about for immunocompromised individuals, cancer patients and others^{2,3}. These challenges highlight the basic need for continuous observing in medical situations and the conception of inventive approaches to development the control of multidrug-resistant (MDR) organisms, which show troublesome and protracted treatment issues. The consideration to metallic nanostructures and nanocomplexes has essentially raised recently⁴. As a imminent antifungal sedate, studies have set up that silver nanoparticles show fabulous bactericidal properties that labor against both Gram-positive and Gram-negative microscopic organisms, including multiresistant strains⁵. The union of AgNPs is regularly accomplished via physical strategies such as dissipation, condensation and laser removal⁶ or by chemical diminishment by implies of inorganic and natural lessening agents^{7,8}. In spite of their proficiency, these characteristic approaches can be unsafe, which underlines the need for more reliable alternatives^{5,6}. Eco-friendly strategies are getting to be increasingly communal, as green synthesis through microbes, enzymes and plant extracts^{7,9-13}. Other than, biogenic synthesis, primarily utilizing AgNPs have been proposed as a substitution for chemical methods since it is steadier and more economical⁶. Various plants, both restorative and non-medical, such as *Azadirachta indica*^{7,8}, *Citrus medica*, *Tagetes lemmonii*, *Tarenna asiatica*¹¹, *Rosa canina*¹⁴ and *Syzygium cumini*¹³ have as of now been applied within the synthesis and stabilization of biogenic metallic AgNPs. The antibacterial and

anticancer proficiencies of silver nanoparticles, alongside their imminent utilizations in heavy metal rummaging and catalysis against azo colors, put them at the bleeding edge of consider among other metallic nanoparticles.^{15,16} Ag is greatly destructive to microorganisms and proficiently eradicates them when it is in its metallic state. AgNPs coupling and conjugation incredibly change silver's bactericidal qualities, regularly since of the NPs' high surface area-to-volume proportion, which boosts their interactions and availability to target microorganisms^{15,17–20}. Diverse shapes, such as circular, cubic, rectangular, polyhedron, or indeed combined shapes, are shown by these nanoparticles. The green synthesis handle utilizes ethanolic fluid extracts inferred from different plants. Aloe vera may be a plant valued for its therapeutic properties since old times. Its leaves contain mucilaginous transparent gel inside the parenchyma's thin-walled cells and collenchyma. Typically gel, known as A. vera gel, is broadly utilized within the frame of extricates, juice and powder²¹. Recognized as safe, these Aloe vera-based items are commonly incorporated into nourishment, dietary supplements, and Ayurvedic drugs²¹. The internal clear gel found in its leaves is composed primarily of water (99%), beside glucomannans, amino acids, lipids, sterols and vitamins. Other than, Aloe vera contains 75% bioactive compounds, as vitamins, chemicals, minerals, sugars, lignin, salicylic acids and amino acids^{22,23}. A few thinks about have appeared that it has anti-inflammatory, antibacterial, antioxidant, antiviral, anti-ulcer, wound recuperating, lipid-lowering, antidiabetic, anticancer activities and antihypertensive, properties^{21–26}. These therapeutic benefits have been utilized in various commercial applications²⁷.

In this work, we have created a speedy and economical handle for blend AgNPs from silver nitrate by utilizing an extricate from Aloe vera takes off extricate. Furthermore, a comprehensive investigation of the synthesized tests was conducted utilizing ultraviolet-visible (UV-visible) spectroscopy, X-ray diffraction (XRD) spectroscopy, and scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to evaluate the impact of the synthesis conditions on the abdicate, morphology and steadiness of the NPs. Besides, the potential application of the integrated Ag NPs in anti-bacterial field was explored. In expansion, we explored the intelligent between AgNPs and the Omicron receptor-binding theme (RBM) utilizing atomic docking experiments. The findings recommended that AgNPs have the capacity to connect to the RBM, which seem clarify their antiviral properties.

Materials and methods

Aloe vera was collected from Menofia governorate (Egypt). Silver nitrate (AgNO_3) and ammonia solution (25%) were purchased from El-Gomhoria chemical company, Egypt. All the reagents were of the best grade available and used without further purification. All the preparations were done by using distilled water. For microbial studies, the sterilized water was used.

Preparation of Aloe vera Plant Extract

A fully grown Aloe vera leaf was taken after washing thoroughly with water and removing the green skin and cutting them into small pieces, and finally, Aloe vera gel was ground to form a colloidal solution. The

above colloidal solution was filtered and this solution was used for further analysis.

Synthesis of silver nanoparticles

About 5 ml of 10 mM AgNO₃ solution was taken into a 50-ml flask containing about 5 ml Aloe vera plant extract and finally diluted with 1% ammonia solution to adjust the pH of the medium. The reaction mixture was allowed to stand at room temperature and the appearance of orange yellow color indicated the formation of AgNPs ²⁸.

Antibacterial activity

The antimicrobial susceptibility testing was performed using the standard disc diffusion method according to Clinical and Laboratory Standards Institute guidelines. Mueller-Hinton agar plates were prepared and inoculated with standardized bacterial suspensions equivalent to 0.5 McFarland turbidity standard. Test discs were impregnated with the respective Aloe Vera-AgNO₃ solutions and placed on the bacterial lawns using sterile technique. The plates were incubated at 37°C for 24 hours under aerobic conditions, after which zone of inhibition diameters were measured using calipers and recorded in millimeters ²⁹.

Test Formulations

The experimental design incorporated multiple concentrations of Aloe Vera extract combined with Silver Nitrate to evaluate dose-dependent antimicrobial responses. The preparation involved systematic dilution of Aloe Vera extract in standardized volumes to ensure consistent testing conditions across all experimental groups.

Bacterial Test Organisms

The antimicrobial evaluation was conducted against a panel of clinically significant bacterial strains representing both gram-positive and gram-negative organisms. These organisms were selected based on their clinical relevance and representation of different bacterial cell wall structures and resistance patterns.

Molecular docking

The Materials Studio software (DMol3) is used to optimize the geometry of the AgNPs surface. The protein data bank (PDB ID: 8SPI – strain XBB.1.5) supplied the crystal structure of the chimeric omicron RBD main protease at 3.6 Å resolution in order to examine the connection between AgNPs and the omicron receptor binding motif (RBM). After that, this structure was coupled with AgNPs to examine Bio-

AgNPs' antiviral properties³⁰. Before beginning the docking investigation, we prepared the protein by removing Hetatm and minimizing energy using SPDBV-Swiss-PDB Viewer. Then, docking calculations were performed using the Lamarckian genetic algorithms (LGA) in AutoDock 4.2. The grid settings were configured as 10 36 5 with a 1 Å spacing. The AgNPs were allowed complete freedom to explore the whole 8SPI region in order to determine the optimal binding sites on the 8SPI molecule. This process was performed 100 times for each ligand, and the average affinity score that was found was selected. The results were visualized using Discovery Studio Visualizer v17.2.0.16349³¹.

Results and discussion

3.1. Biosynthesis and characterization of silver nanoparticles and UV-visible spectra.

Visual perception is the beginning stage of silver nanoparticles characterization. When Aloe vera extricate was added to a 1 mM of AgNO₃ solution within the dark, as seen in (Fig. 1), the solution's color changed from light yellow to dark brown, inside the primary 60 minutes, in any case the decrease response wasn't wrapped up until 4–6 hours after brooding, meaning the creation of AgNPs. Concurring to reports^{32,33} the actuation of AgNPs' surface plasmon vibrations is what causes this color move. Taking after visual perception, UV-Vis spectrophotometry, FTIR spectroscopy, XRD examinations and Transmission electron microscope were utilized for advance characterization of AgNPs. Maximum absorption peak bands at 425 nm were identified within the UV-visible spectra of AgNPs interceded by A. vera as seen in (Fig. 2(a,b)). The AgNPs are for the most part known to show assimilation most extreme top at 400–500 nm due to the surface plasmon resonance³⁴.

FTIR spectroscopy

FTIR analysis was utilized to find potential biomolecules in the extract of A. vera that could be in charge of converting Ag⁺ into Ag⁰. The FTIR spectra of A. vera extract and phytofabricated AgNPs are shown in (Fig. 3(a,b)). The A. vera extract spectrum's bandwidth between 3200 and 3500 cm⁻¹ is indicative of the N-H stretching of primary aliphatic amines or can be supported by vibrations of the O-H bond in phenols and alcohols which serves as reducing agent³⁵.

As well as, the stretching vibrations of secondary amines or their salts (NH³⁺) are signified by the peaks at 2916, 2860 and 1587 cm⁻¹. The aromatic frame's double bonds are represented by the assignments at 2121 and 1795 cm⁻¹. The absorption peaks at 1732 cm⁻¹ are attributed to C = O of the aldehyde. Peaks at 1245 and 1016 cm⁻¹ are attributed by functional groups of A. vera metabolites and represent C–O stretching from alcohol, carboxylic acid, or aldehyde groups. In addition to the loss of N-H stretching vibration peaks at 2860 cm⁻¹–2900 cm⁻¹, the absorption band corresponding to N–H bending vibrations at 1587 cm⁻¹ is missing from the spectrum of green synthesized AgNPs. This absence is concluded to the protein molecule served as the most likely reducing agent during the AgNP

synthesis process. Aromatic groups peaks in the extract are assigned from 700 cm⁻¹ to 900 cm⁻¹. Thus, according to the FTIR study analysis, bioactive components from *A. vera* extract likely stabilized AgNPs by forming a layer on top of them (biological capping) that stopped agglomeration.

XRD analysis

AgNPs' crystalline nature was verified by XRD analysis and the pattern exhibited Bragg's reflections, which stand for Ag's face-centered cubic structure (Fig. 4(a,b)). The highly crystalline nature of Ag crystals was confirmed by the XRD pattern, which displayed diffraction peaks at 27.90, 64.77° and 76.39° that are indexed to the (111), (200), (220) and (311) Bragg's reflection of their face-centered cubic structure ³⁶.

Transmission Electron Microscope

The TEM study (Fig. 5(a,b)) revealed details regarding the intended AgNPs mediated by *A. vera* size and shape. With a spherical shape and an average size of 25–50 nm, the generated nanocomposite was shown to be capped by *A. vera* extract without aggregating.

Antimicrobial Efficacy of Aloe Vera-Silver Nitrate Combinations

Zone of Inhibition Measurements

The disc diffusion assays revealed significant antimicrobial activity across all test concentrations and bacterial species, with notable variations in susceptibility patterns and dose-response relationships. The zone of inhibition measurements demonstrated clear evidence of antimicrobial efficacy, with distinct patterns emerging for different bacterial species and treatment concentrations (Figs. 6–9)

Bacterial species	Solvent(S)	Control©	S1(3g/50g)	S2(5g/50g)	S3(10g/50g)
E. coli	0	8	12	15	18
Salmonella spp.	0	10	16	20	24
S. aureus	0	9	18	22	26
Bacillus spp.	0	11	15	19	28

Antimicrobial Mechanisms and Zone Formation

The observed zone arrangement comes about from the synergistic combination of numerous bioactive components working through complementary mechanisms. Aloe Vera extricate contains various antimicrobial compounds including a loin, aloe-emodin, polysaccharides and different phenolic

compounds that can disturb bacterial cell films and interfere with cellular metabolism. When combined with Silver Nitrate, the silver particles give extra antimicrobial activity through multiple pathways counting layer disturbance, protein denaturation and DNA interference.

The zone diameter estimations given quantitative evidence of diffusion-dependent antimicrobial action, where bigger zones show more prominent compound entrance and supported antimicrobial concentrations within the agar medium. The consistent zone formation over all treatment concentrations proposes stable compound discharge and effective dissemination characteristics suitable for topical and surface applications.

Concentration-Dependent Efficacy and Optimization

The clear dose-dependent relationship observed in this consider demonstrated that antimicrobial viability can be efficiently optimized through cautious concentration adjustment. The progression from 3g to 10g of Aloe Vera per 50g arrangement consistently produced zone diameter increments extending from 33–87% depending on bacterial species, recommending that higher concentrations give improved antimicrobial movement without apparent saturation impacts within the tried range.

The S3 detailing (10g/50g) represented the optimal concentration tried, accomplishing the largest restraint zones over all bacterial species whereas maintaining practical detailing parameters. This concentration gives zone diameters comparable to numerous standard antimicrobial agents, recommending potential for clinical application development. The cost-benefit analysis of concentration increments appears diminishing returns over the S2 level for some organisms, demonstrating that S2 or S3 concentrations may represent ideal formulation targets for distinctive applications.

Comparative Antimicrobial Efficacy

When compared to ordinary antimicrobial agents, the zone diameters accomplished by the AloeVera-AgNO₃ combinations drop inside clinically significant ranges. Standard antimicrobial plates regularly deliver zones extending from 15–30 mm for susceptible organisms, setting the S2 and S3 definitions inside this therapeutic extend. The advantage of this natural-metallic hybrid framework lies in its multi-target mechanism of activity, which diminishes the probability of resistance advancement compared to single-target manufactured antimicrobials.

The sustained antimicrobial activity illustrated through consistent zone arrangement suggests potential advantages in sustained-release applications where drawn out antimicrobial activity is required. The characteristic root of the essential active component combined with the demonstrated antimicrobial adequacy of silver creates a formulation suitable for applications where characteristic antimicrobials are favored or required.

Clinical and Industrial Applications

The evaluated antimicrobial activity illustrated through zone diameter estimations upheld different potential applications over clinical and industrial sectors. The zone diameters achieved proposed

adequacy appropriate for wound care applications, where antimicrobial activity must be maintained over amplified periods whereas supporting tissue mending forms. The broad-spectrum action demonstrated by reliable zone arrangement over gram-positive and gram-negative microbes makes this formulation reasonable for common antimicrobial applications.

Topical antimicrobial preparations might benefit from the measured antimicrobial activity levels, especially in applications requiring natural or reduced-synthetic details. The evaluated dose-response relationship empowers precise detailing adjustment for particular antimicrobial necessities, permitting customization for diverse application needs extending from mild antiseptic activity to strong antimicrobial.

Molecular docking analysis

A powerful computational strategy for comprehending the relationship between AgNPs and the Omicron receptor-binding theme (RBM) is atomic docking considers. OmpC (PDB ID: 2J1N), the osmoporin of *Escherichia coli*, was chosen as the docking target since its unraveled crystal structure at 2.0 Å... determination gives precise atomic detail for its 16 stranded β^2 barrel and pore constriction zone, with ~ 74% of the pore lining residues preserved relative to OmpF, counting numerous polar and charged residues³⁷. Theoretical models anticipate that these moderated, ionizable amino acids constitute the electrostatic and hydrogen bonding environment basic for attraction and stabilization of little metal clusters. Experimentally, silver in nanoparticulate or cluster frame is known to disturb layers, increment porousness, create receptive oxygen species, and tie to sulfur or oxygen benefactor atoms in proteins, reliable with docking intelligent close charged or polar side chains³⁸. In our docking comes about, the Ag₄₄ cluster docked with favorable vitality ($E_{\text{place}} \sim 11.99$), unassuming RMSD (~ 1.96 Å...), and shaped contacts with residues interior the pore lumen (e.g. Gln, Tyr, Trp) that can offer both electrostatic and van der Waals interactions. These discoveries align with the exploratory antimicrobial assays, in which higher concentrations of silver lead to expanded inhibition zones, recommending that binding at or close the porin™ choking likely disables its permeability, contributing to bacterial restraint (Fig. 10).

Conclusion

This work has developed a rapid and environmentally responsible process for creating AgNPs from silver nitrate by using Aloe Vera leaves extract. UV visible spectra, FTIR, XRD and TEM used to characterize the AgNPs. According to the XRD data, the AgNPs have an average crystallite size of 29.55 ± 3.25 nm and TEM results revealed a spherical shape and an average size of 25–50 nm, the generated nanocomposite was shown to be capped by A. vera extract without aggregating. The comprehensive quantitative evaluation of Aloe Vera-Silver Nitrate combinations revealed significant antimicrobial efficacy against clinically relevant bacterial pathogens, with zone diameters ranging from 12–28 mm. The optimal S3 concentration (10g Aloe Vera per 50g solution) provides the most consistent and effective antimicrobial activity across all tested bacterial species, achieving zone diameters comparable to standard antimicrobial agents. This concentration represents a practical balance between antimicrobial efficacy and material utilization, making it suitable for further development and clinical evaluation studies.

Molecular docking experiments were used to further investigate the relationships between AgNPs and the Omicron receptor binding motif (RBM) and the inhibition of virus attachment to host receptors (the cellular receptor Angiotensin-Converting Enzyme 2 human ACE2 (hACE2)).

Declarations

Author Contribution

The authors' contributions to this paper are as follows. Heba Alshater , sabreen M El-Gamasy and Samar E. AbdEl-Razek were responsible for the idea of the research, outlining the dataset, characterizing the chemical problem, writing the related sections, collected and reviewing related works and contributing to the basics and background section and reviewing the paper. All authors thoroughly reviewed and approved the final paper.

Data Availability

"Data is provided within the manuscript files

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Figures

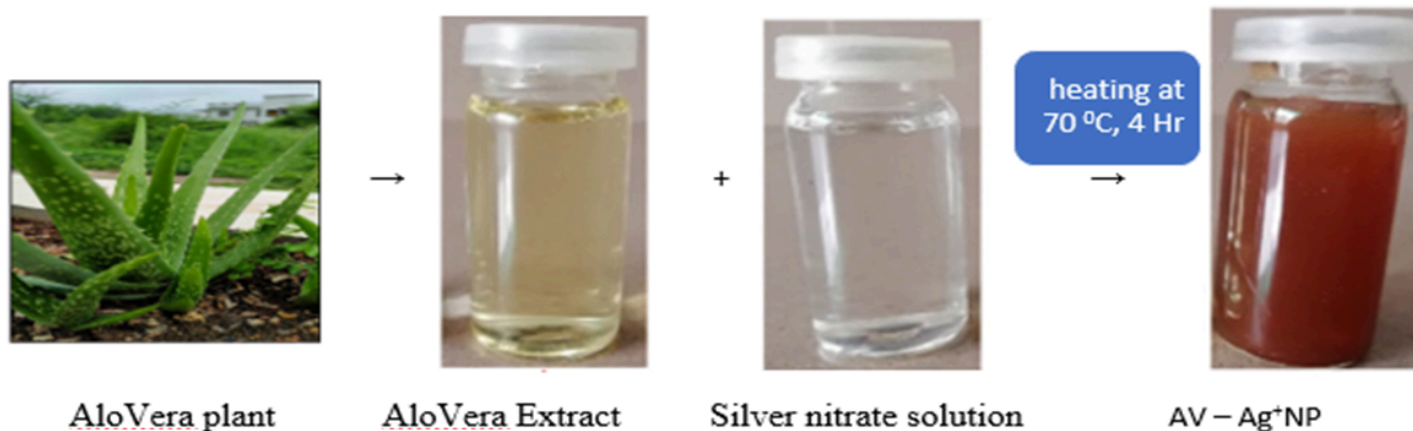


Figure 1

Change of color of solution before (a) and after (b) the phytofabrication process of silver nanoparticles with Aloe vera extract

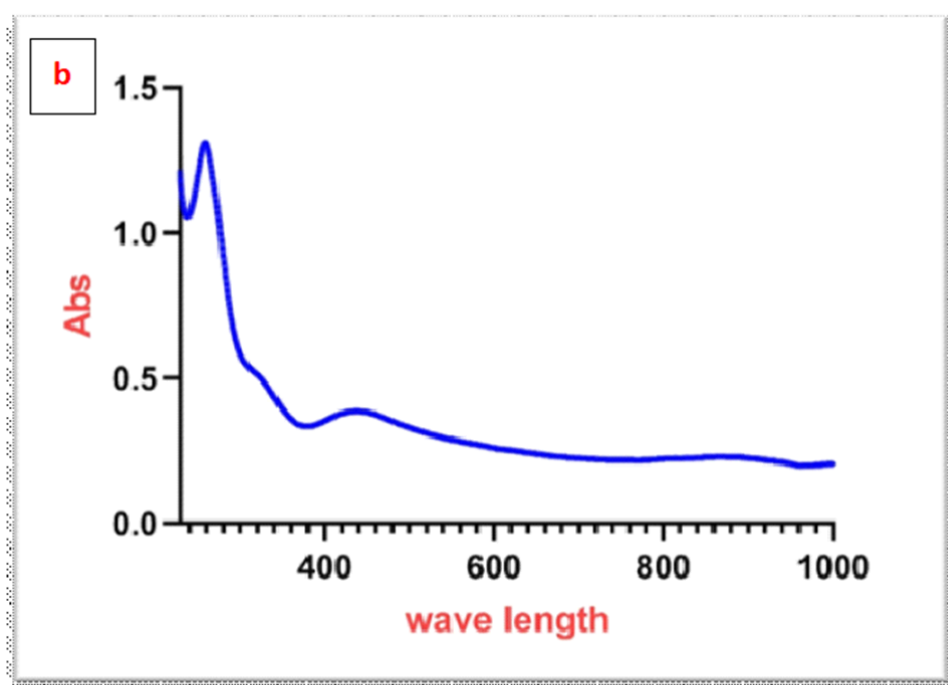
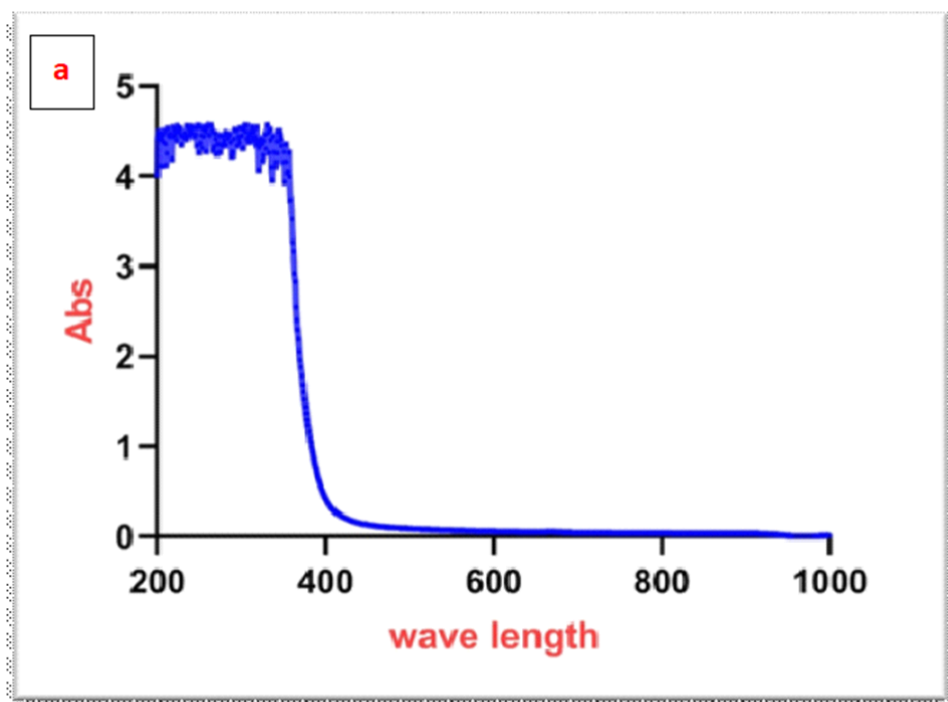


Figure 2

Ultraviolet–visible spectra of a) Aloe vera extract, b) green synthesized AgNPs.

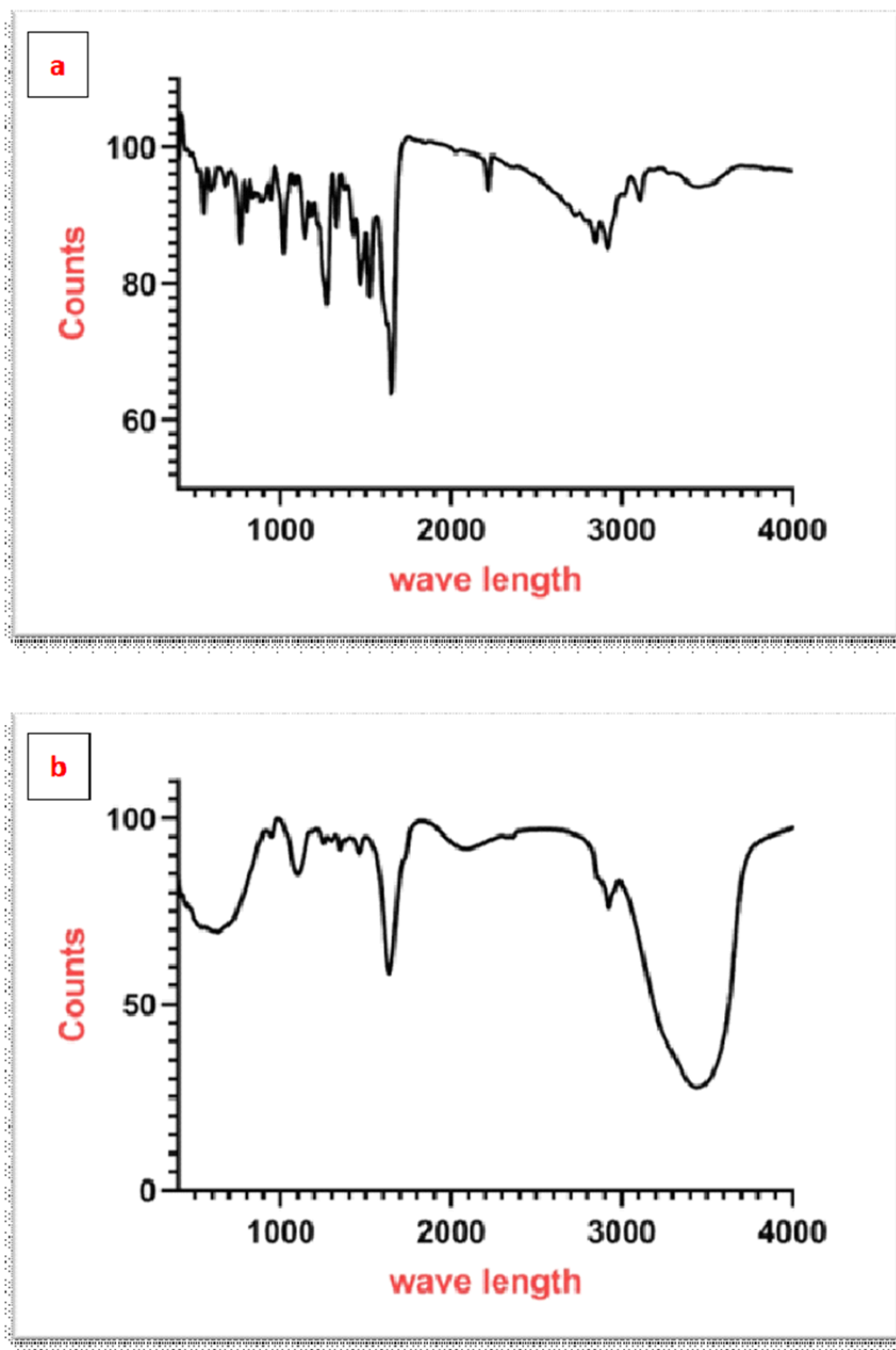


Figure 3

FTIR spectra of a) Aloe vera extract, b) green synthesized AgNPs.

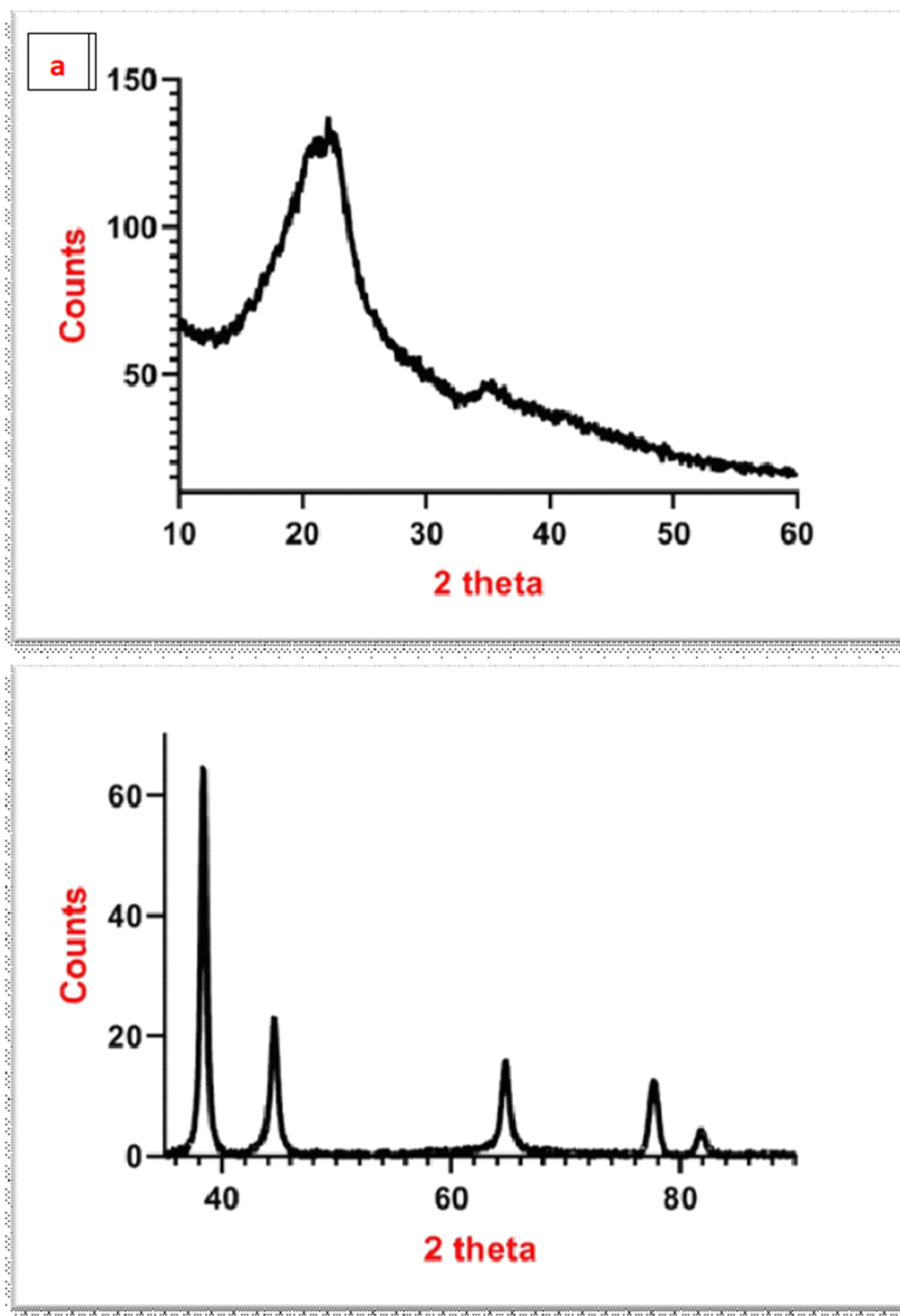


Figure 4

XRD pattern of a) Aloe vera extract, b) green synthesized AgNPs.

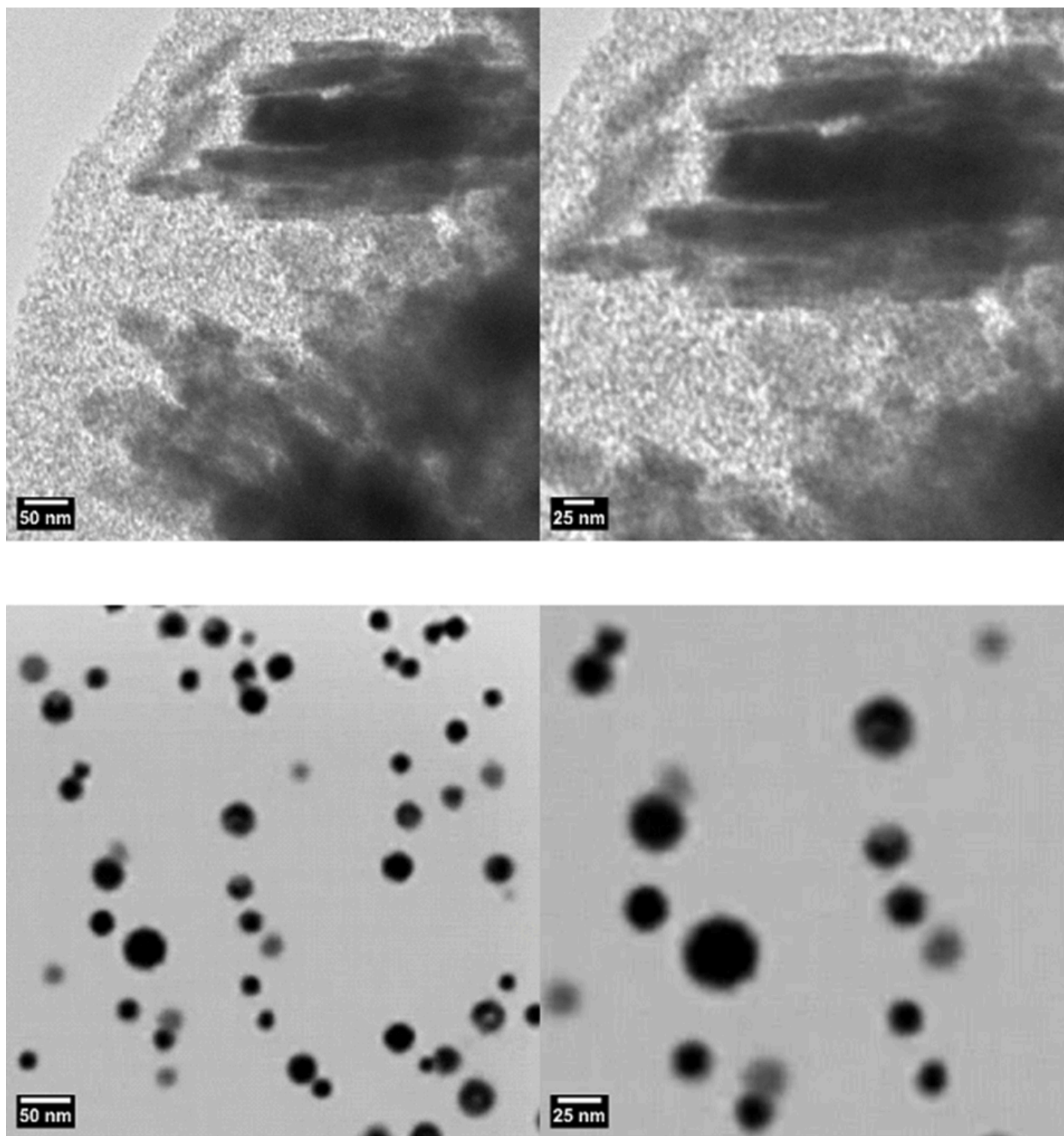


Figure 5

TEM images of a) Aloe vera extract, b) green synthesized AgNPs

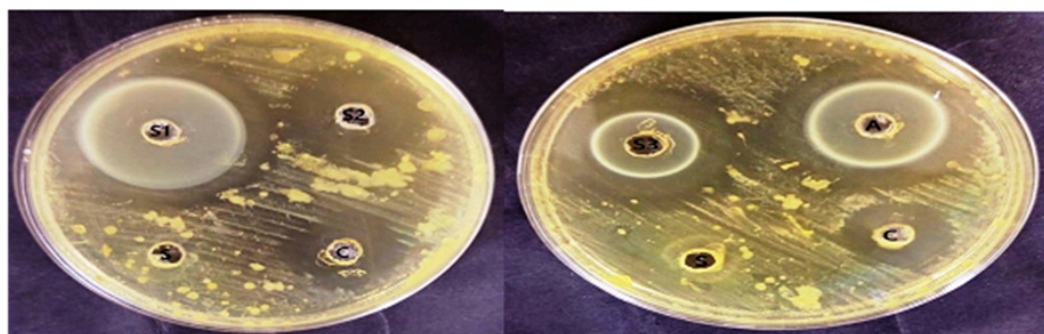
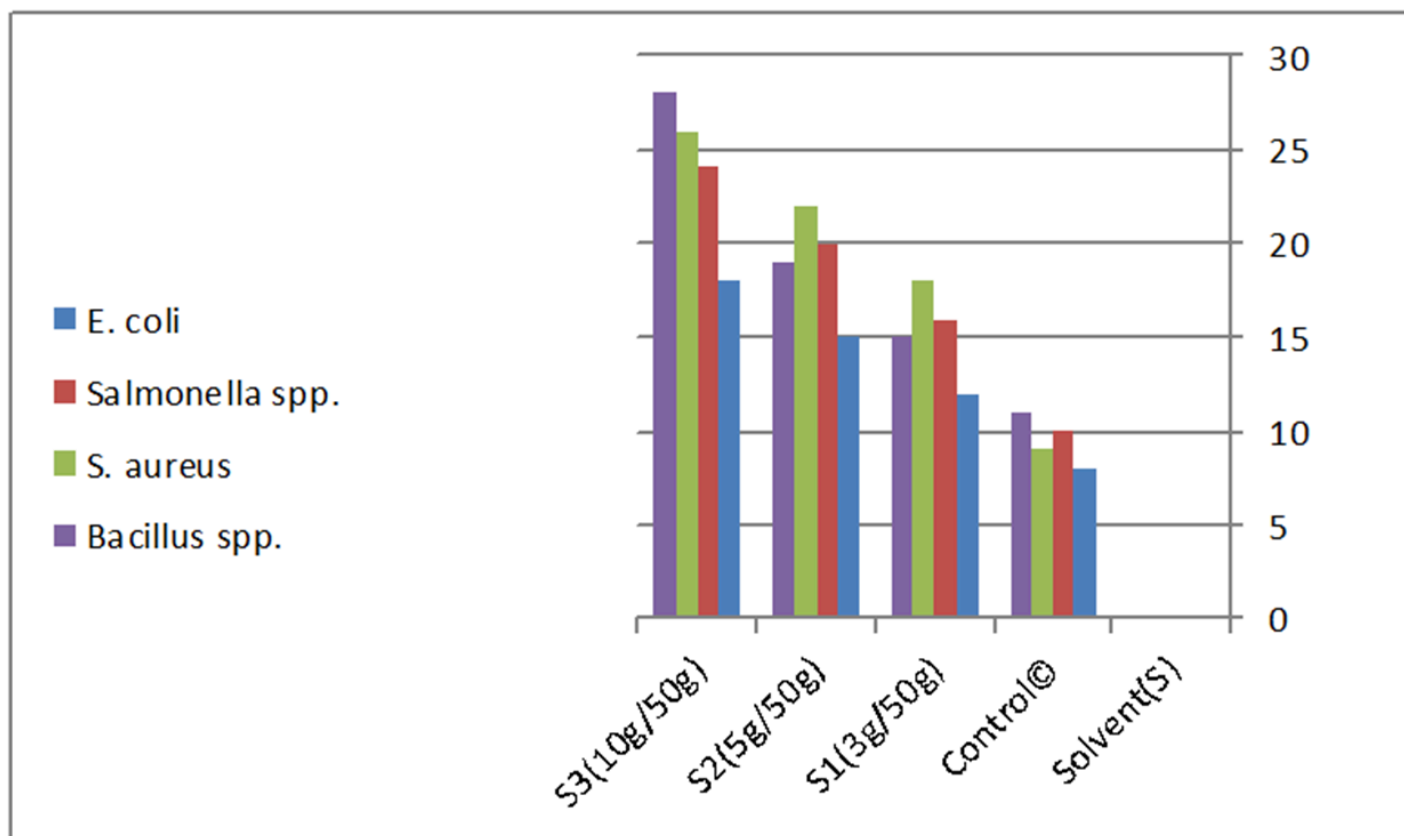


Figure 6

(Antimicrobial evaluation of extract and AgNPs against Escherichia coli)

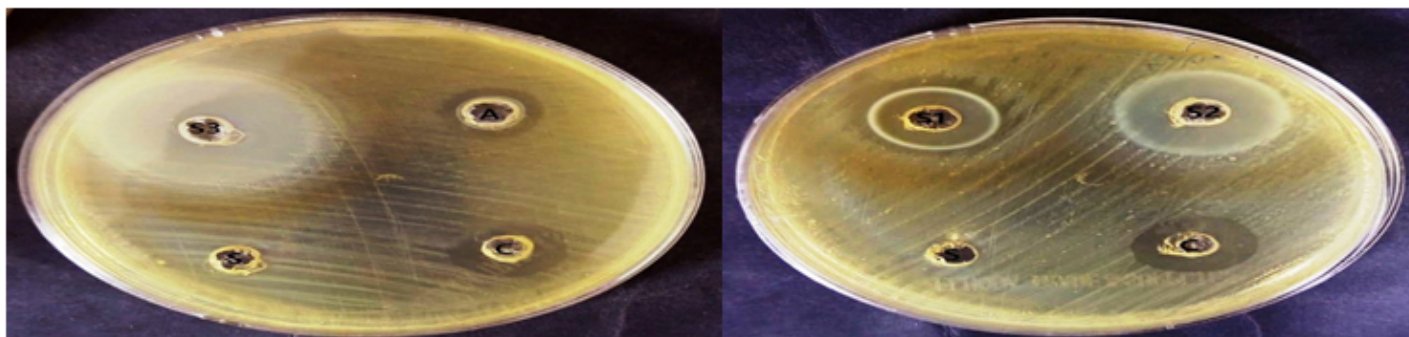


Figure 7

(Antimicrobial evaluation of extract and AgNPs against Salmonella species)

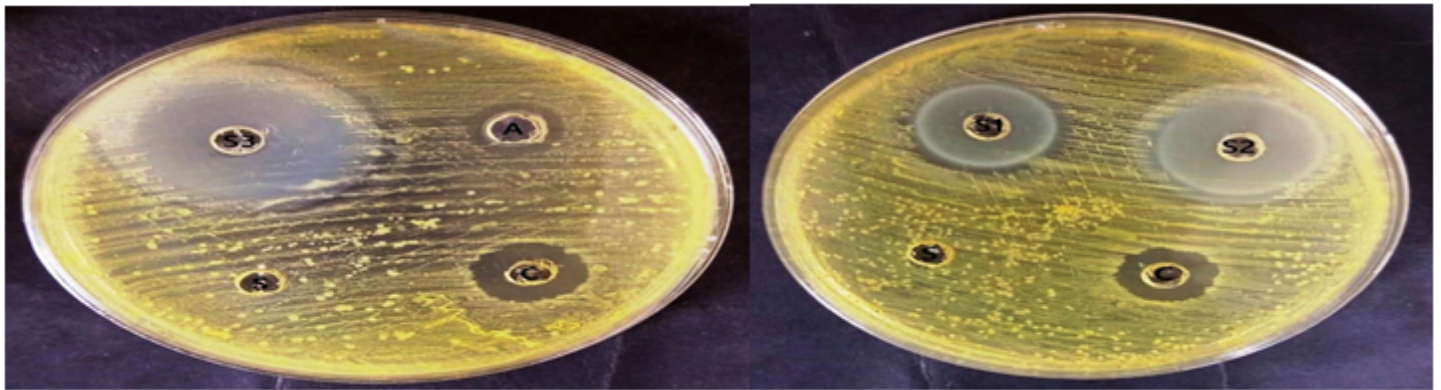


Figure 8

(Antimicrobial evaluation of extract and AgNPs against Staphylococcus aureus)

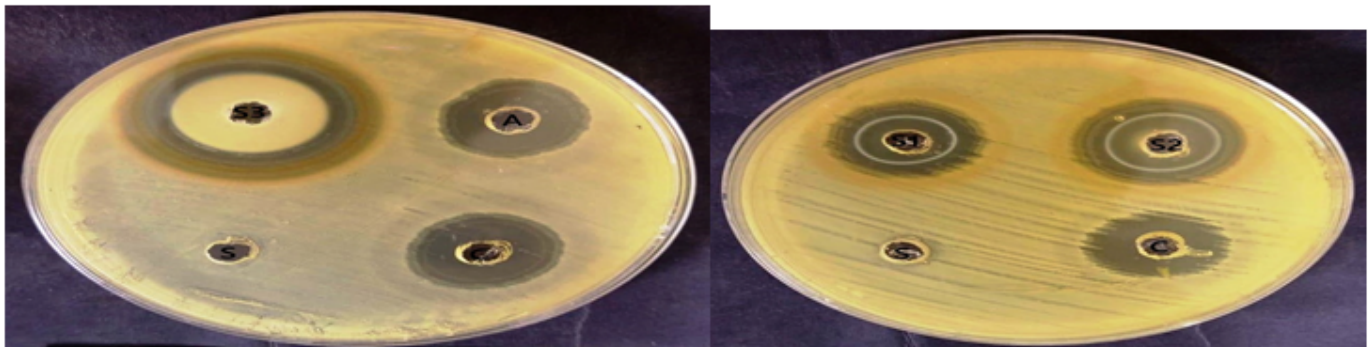


Figure 9

(Antimicrobial evaluation of extract and AgNPs against Bacillus species)

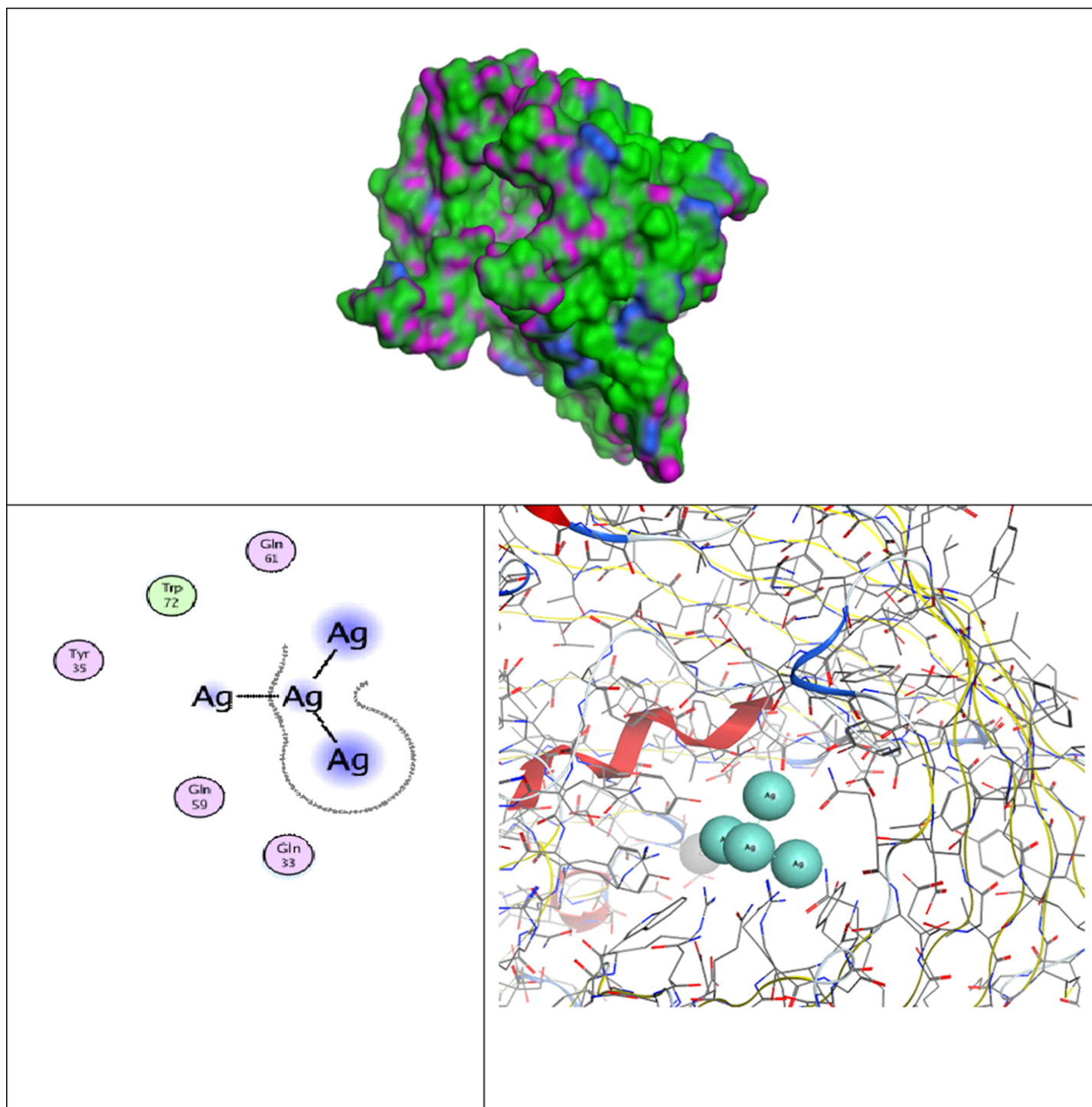


Figure 10

(C) Surface view: The molecular surface of *E. coli* OmpC (PDB ID: 2J1N); (A and B) 2-D and 3D Ag₄ cluster interaction map with the outer membrane porin OmpC (PDB ID: 2J1N), respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GA.png