Original Article

Evaluation of Silver Nanoparticles Interaction with Colistin and Antibiotics on Bacterial Isolates from Patients with Diarrhea

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Abstract

Background: Microbial resistance to antibiotics is one of the most critical health risks in our current era, and the development of these antibiotics through their association with agents that support inhibition and are safe for health is one of the recent tendencies in developing these antibiotics and thus eliminating severe diseases that threaten human health and safety. **Objectives:** This research aimed to determine the Silver nanoparticles (AgNPs) singly or with Colistin and Some antibiotics against the bacterial isolates from patients with diarrhea. **Materials and Methods:** The AgNPs were biosynthesized using the Cinnamon bark extract and characterized using the change in solution color, Ultra Violet–Visible spectroscopy (UV–VIS), Transmission Electronic Microscope (TEM) and X-ray Diffraction (XRD) techniques, and determine its ability singly or with Colistin peptide combinations with some antibiotics against bacterial isolates *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Enterococcus faecalis* isolates from patients with diarrhea. **Results:** The AgNPs characterization was revealed through the color change from pale to deep-brown as well as the absorption peaks appeared at 428 nm using the UV–VIS spectroscopy, which is evidence of the formation of AgNPs, as well as the TEM technique showed the shape of the AgNPs at an average size of 22.8 nm. XRD examination was found to clarify the crystalline properties. The AgNPs and Colistin showed antimicrobial activity by determining the minimum inhibitory concentration and impregnating them with the tested antibiotic discs that were ineffective against bacterial isolates. It was found that there was a partial synergism between AgNPs and Colistin with ceftriaxone and ciprofloxacin for *E. coli* and *S. typhimurium* and with vancomycin and tetracycline for *S. aureus* and *E. faecalis*. **Conclusions:** It was concluded that the synergy between AgNPs and Colistin was clear against all isolates used in the study.

Keywords: Antibiotic resistance, bacteria, colistin, diarrhea, silver nanoparticles

Introduction

Diarrhea is described as an increase in bowel motions to more than three times per day or a decrease in the quantity of stool firmness (to the point where it is liquid or semi-liquid), both of which are accompanied by clinical symptoms such as fever, vomiting, nausea, and stomach discomfort.[1] Statistics issued in 2019 indicated that diarrhea is one of the leading causes of death in more than 500,000 cases worldwide among children under five.[2] The main causes of diarrhea include parasites, viruses, and bacteria. Acute and chronic diarrhea are the two categories of diarrhea based on the time of day. Acute diarrhea passes in <14 days. It is brought on by a viral or bacterial infection in the stomach. The duration of chronic diarrhea exceeds 4 weeks. Numerous things may cause it, such as an infection caused by bacteria, viruses, or parasites or a problem with the intestinal tract's functioning components.[3,4] Bacterial infection-related diarrhea is one of the global health problems that cause infectious diarrhea, especially in developing countries.

Antibiotic resistance has become a major issue in global health as a result of the misuse and overuse of antibiotics, leading to a decrease in effectiveness against bacteria responsible for causing illness,[5] Furthermore, one of the

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factors contributing to the creation of bacterial strains resistant to several antibiotics is the use of antibiotics in the manufacturing of animal and agricultural feed.[6] According to the World Health Organization, antimicrobial-resistant bacteria have resulted in the deaths of approximately 541,000 individuals globally, with 133,000 fatalities occurring in Europe.[7] Finding new therapeutic alternatives other than antibiotics has become an urgent need due to the decreased efforts of most pharmaceutical companies in developing and producing new generations of antibiotics. Developing antibiotics is a complex and expensive process that requires an extended period for approval for their use. Additionally, some antibiotics face restrictions to reduce the emergence of resistance against them.[8]

Among the contemporary tools used in the biomedical sciences are nanomaterials, particularly silver nanoparticles (AgNPs), which help combat various bacterial, viral, and fungal infections and have anti-inflammatory properties.[9] AgNPs may be manufactured using a variety of physical, chemical, and biological processes.[10] The green synthesis technique is widely regarded as a very effective approach for the environmentally conscious manufacturing of nanomaterials. The methodology used many biological sources, including bacteria, fungus, and different plant components, to get extracts rich in diverse active substances such as aliphatic compounds, phenols, alcohols, proteins, and flavone compounds.[11] This research used an extract derived from the bark of Cinnamon verum, an aromatic plant known for its diverse range of physiologically active chemicals. These compounds have been shown to possess the potential to decrease silver ions and play a crucial role in preserving the integrity and stability of silver nanoparticles during the production process.[12]

Colistin is a pharmaceutical agent used for the treatment of bacterial infections in instances when medical professionals meet highly pathogenic bacteria that exhibit resistance to conventional antibiotics. The discovery of this phenomenon occurred in 1949, and its use as a treatment for bacterial diseases began in 1959. However, the use of this substance was terminated throughout the 1980s due to the adverse physiological effects it induces on the nerves and kidneys.[13] It is classified as a member of a collection of non-ribosomal cyclic peptides that are generated by Bacillus organisms belonging to the genus *Paenibacillus polymyxa*.[14] In the case of Gram-negative bacteria, the interaction between colistin and the negatively charged lipid A lipopolysaccharides leads to disruptions and impairments in the permeability of the outer cell wall surface.[15] The interaction between colistin and teichoic acid, which is found in the cell walls of Gram-positive bacteria, indicates that these acids play a significant part in the mechanism of action of polymyxin-associated antimicrobials. Therefore, this characteristic enhances the efficacy of other antimicrobial agents in eradicating bacterial pathogens.[16]

Materials and Methods

Sample collection and bacterial isolation

A total of 255 stool samples were obtained from patients of both genders, aged between six months and 72 years, who were hospitalized to Ramadi Teaching Hospital and Ramadi Women's and Children's Hospital. These samples were collected using stool containers specifically designed for patients experiencing diarrhea. The collection period spanned from November 21, 2021 to October 5, 2022. The bacterial infection was detected using microscopic analysis, wherein a portion of the sample was transferred onto a glass slide using a sterile wooden stick and combined with droplets of neutral physiological saline solution. The specimens were cultured on Blood and MacConkey agar. Selective culture media such as Salmonella Shigella agar (S.S. agar), Xylose lysine deoxycholate agar (XLD), Mannitol salt agar, and Pfizer Selective Enterococcus agar were utilized, along with diagnostic biochemical tests like (IMVIC), Urease, Catalase, Oxidase, Kligler, and Coagulase tests, to culture the resulting bacteria. Confirmatory testing was done using the Vitek 2 Compact to identify the specific type of bacteria.

Sensitivity of bacteria to various antibiotics

The Kirby–Bauer disk diffusion technique was used by spreading a bacterial suspension titrated with (1.5×10^8 CFU/mL) MacFarland's standard turbidity constant with a sterile cotton swab onto a petri dish of Mueller–Hinton agar culture medium.[17] Eight antibiotics (Mast-Group, Liverpool, UK) were tested, which included Ampicillin (10 µg), Ceftriaxone (30 µg), Gentamycin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Trimethoprim (5 µg), Tetracycline (30 µg), Erythromycin (15 µg), and Vancomycin (30 µg).

Green biosynthesis of silver nanoparticles

The experiment was conducted in a controlled setting when the illumination conditions were intentionally diminished. It added 10 mL of Cinnamomum verum extract to 90 mL of silver nitrate solution (1 mM) at 60°C, as depicted in Figure 1. The addition was conducted slowly and gradually, with the mixture being continuously stirred for 1 h. The visual observation of a dark brown color within the mixture serves as a visual indicator of the successful synthesis of silver nanoparticles.[18] Various diagnostic tests were performed using diverse methodologies, including UV–visible spectroscopy, FT-IR, XRD, and Transmission Electronic Microscope (TEM).

Synergistic efficacy of AgNPs and colistin with antibiotics

The MIC of silver nanoparticles (AgNPs) and colistin was evaluated by employing a 96-well microtiter plate and the dye resazurin. The operational mechanism of this dye is predicated on the ability of living cells to reduce the blue dye, leading to a conversion into a reddish-pink hue as a

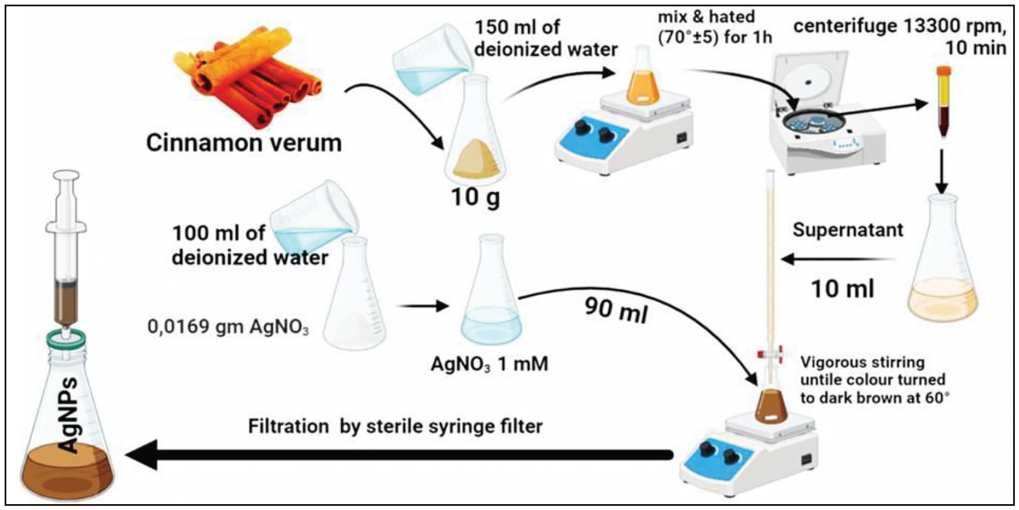

Figure 1: Schematic diagram depicting the biosynthesis process of AgNPs

consequence of resorufin production.[19] Furthermore, the potential synergistic effects of AgNPs and colistin with ineffective antibiotics were assessed By applying the following equation to the FICI value:

$$FICI=\frac{\text{Growth inhibition of Ab+inhibition of AgNPs or Colistin}}{\text{Combinations growth inhibition}}$$

FICI considered synergy when FICI ≤ 0.5, partial synergy when 0.5 < FICI, additive when FICI = 1, effective when 1 < FICI < 4, and antagonism 1 > 4.[20,21]

The equation below was applied to a 96-well microtiter plate indicator of the color change of resazurin stain in a checkerboard assay to estimate the synergistic state between colistin and AgNPs.[22]

$$FICI=\frac{\text{MIC of colistin in combination}}{\text{MIC of Colistin alone}}+\frac{\text{MIC of Ag-NPs in combination}}{\text{MIC of Ag-NPs alone}}$$

Ethical approval

The research proposal and its corresponding implementation strategies were formally submitted to the Scientific Research Ethics Committee at Anbar University and approved by Ref: 22, Data: May 24, 2022. Furthermore, the researchers received agreement from the study participants or their legal guardians in the case of individuals under 18.

Results

Identification of bacteria

After collecting a total of 255 stool samples obtained from diarrheal patients and diagnosed by selective media, biochemical tests, and Vitek 2 system, Out of the total 120 samples analyzed, 47.06% were determined to be caused by bacterial infections. These infections were found to be spread in different proportions, with *Escherichia coli* accounting for the highest percentage at 53.33%. The remaining bacterial isolates, including *Salmonella typhimurium*, *Enterococcus faecalis*, and *Staphylococcus aureus*, were recorded at percentages of 11.67%, 10.83%, and 6.67%, respectively.

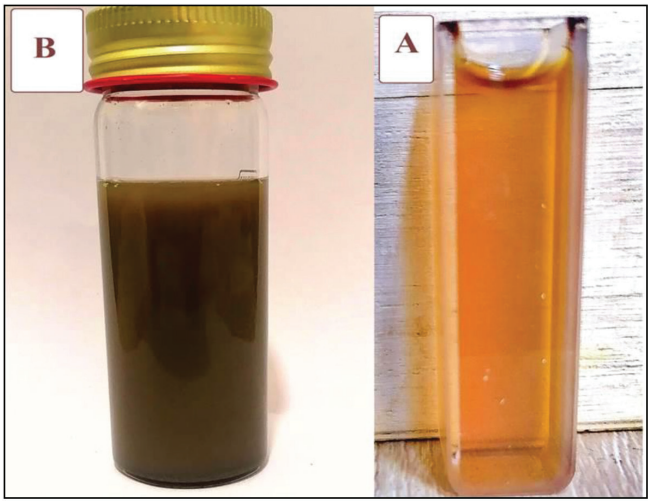

Figure 2: (a) Cinnamon verum extract, (b) AgNPs deep brown

Biosynthesis of silver nanoparticles

The efficacy of silver ion-reducing bio composites in cinnamon bark extract was demonstrated by the precise color change of a colorless $AgNO_3$ solution to a dark brown color, indicating the conversion of Ag^+ ions into Nano silver Ag^0. This outcome, depicted in Figure 2, highlights the successful green biosynthesis process.[23]

UV–Vis spectroscopy

AgNP synthesis was confirmed by UV–Vis spectroscopy analysis on both the cinnamon plant extract and the

nano solution. The resulting standard curves of UV absorption, depicted in Figure 3, indicated the presence of two peaks at 220, 312, and 429 nm for the synthesized silver nanoparticles, which corresponded to the synthesized silver nanoparticles. The test results show concurrence with prior research, wherein the bark of the cinnamon plant was employed as a reducing agent for metallic silver ions.[12]

FT-IR

The results of the FT-IR technique, as depicted in Figure 4, demonstrated the spectral range of 400–4000 cm^{-1} for silver nanoparticles that were reduced using cinnamon bark extract. The analysis revealed the presence of numerous active biomolecules, with phenols being represented by the carboxyl group (−OH) at 3587 cm^{-1} and the carbonyl group (C=O) at 1747 cm^{-1}. Furthermore, the frequency range of 1577 cm^{-1} indicated the existence of aromatic compounds (C–C), while the wave number of 1539 cm^{-1} suggested the presence of organic nitro compounds. Additionally, the fingerprinting region exhibited the occurrence of various organic compounds, including aliphatic amine (C–N) groups, (C–N) groups, and the chemical bond (C–O) associated with amino groups.[24]

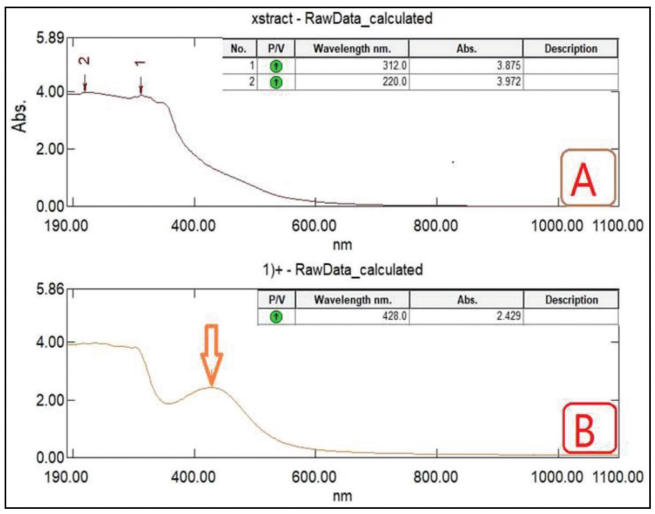

Figure 3: UV–visible spectroscopy, (a) Cinnamon verum extract, (b) AgNPs

X-ray diffraction (XRD)

The X-ray diffraction (XRD) analysis of the nanoparticles provides a precise assessment of their crystalline structural characteristics. The examination revealed the presence of four distinct peaks (at 38.45°, 44.65°, 64.90°, and 77.80°) within the 2 theta angular range, which corresponds to the crystallographic plane labeled as Ag—Card number [00-004-0783]. The observed peaks in Figure 5 indicate a cubic shape for the nanoparticles.[25]

Based on the data provided in Table 1, the application of Scherrer's equation yielded an average crystalline size of 20.35 nm for the silver nanoparticles.

Transmission electron microscope (TEM)

In Figure 6, TEM images display a surface model of AgNPs with a mainly spherical shape. The observed AgNPs possess varying sizes, ranging from 8.13 to 54.11 nm. with an average of 22.81.

The results obtained from Figure 7 of the energy dispersive spectroscopy test revealed the identification and quantification of chemical elements and minerals, along with an estimation of their relative abundance. Specifically, the analysis indicated the presence of the chemical element silver, which accounted for 94.6% of the total weight percentage. The highest intensity peak in the obtained spectrum was observed at an energy level of 3 KeV. In contrast, the presence of the oxygen element was observed at a relatively modest proportion of 5.4%.

Investigation of antibiotic resistance of bacterial isolates

The resistance patterns of the bacterial isolates analyzed for the selected antibiotics commonly associated with bacterial-induced diarrheal disease are presented in Table 2. The results indicate that there was a presence of ampicillin resistance in *E. coli, Salmonella* spp., and *Enterococcus* spp., with resistance rates of 76.6%, 71.4%, and 69.2%, respectively. As previously stated, the isolates demonstrated differing degrees of resistance to Ceftriaxone, with resistance proportions recorded at 51.6%, 35.7%, and 46.2%, respectively. The isolates of *E. coli*, *Salmonella* species, and *Staphylococcus* species showed relatively low levels of resistance to Gentamicin,

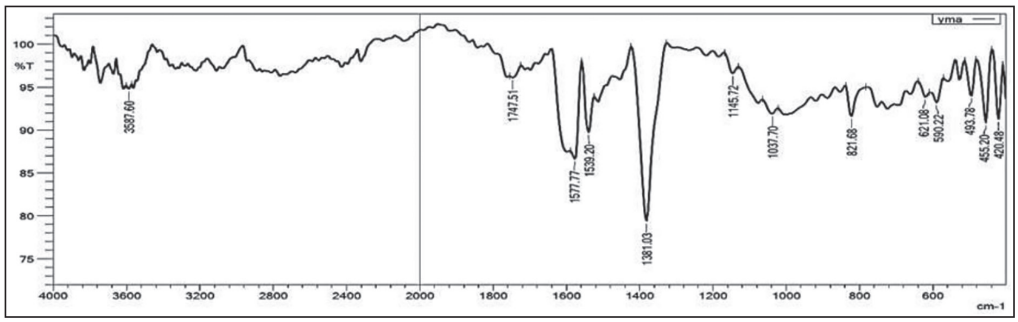

Figure 4: FT-IR for AgNPs

with resistance percentages of 15.6%, 7.1%, and 37.5%, respectively. The resistance rates for *E. coli* and *Salmonella* spp. were found to be relatively low for Amikacin, at 4.7% and 0%, respectively. Variation in resistance to ciprofloxacin was observed across different species, with around 50% of the isolates, specifically *Salmonella* and *Staphylococcus* spp., displaying resistance to this particular antibiotic. On the other hand, the rates of resistance for *E. coli* and *Enterococcus* spp. were found to be 29.7% and 23%, respectively. The species chosen for this investigation exhibited a high level of resistance to Trimethoprim, with recorded percentages of 61%, 71.4%, and 62.5% for *E. coli*, *Salmonella*, and *Staphylococcus* spp., respectively. The resistance rates to tetracycline varied between 57.1% and 50% among multiple bacterial species, except for *Enterococcus* spp., which displayed a resistance rate of 38.5%.

Two types of Gram-positive bacteria, specifically *Staphylococcus* and *Enterococcus* species, were also recorded. The observed resistance rates for erythromycin were 87.5% and 76.5% for the respective species, whereas the resistance rates for vancomycin were 37.5% and 23%.

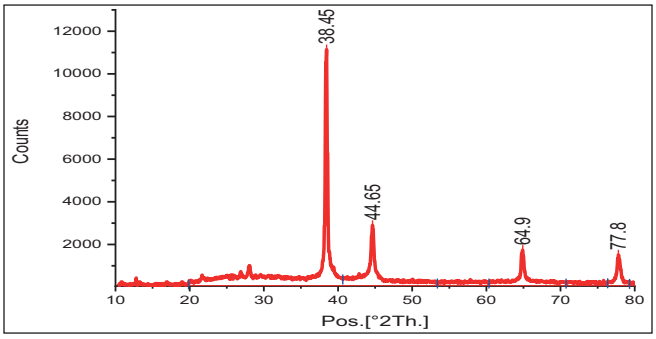

Figure 5: XRD pattern AgNPs

Table 1: Average of crystalline size by Scherrer's equation

Peak position	FWHM	Size of AgNPs
38.45	0.35256	23.85
44.6501	0.46261	18.55
64.9043	0.44877	20.97
77.8021	0.56605	18.02
Average		20.3475

Antimicrobial activity of AgNPs and colistin

According to their respective MIC values, Table 3 shows the efficacy of AgNPs and colistin on the chosen bacterial isolates. Subsequently, these concentrations are utilized to determine the inhibitory zone diameter, as depicted in Figures 8 and 9.

The MIC for both *E. coli* and *S. typhimurium* was shown to be 5 µg/mL, resulting in a halo with a diameter of 12 mm. Similarly, the MIC for *S. aureus* and *E. faecalis* was found to be 10 µg/mL, leading to a halo with a diameter of 15 mm.

Table 3 presents the findings indicating the efficacy of colistin against *E. coli* and *S. typhimurium*, as demonstrated by MIC values of 2 and 8 µg/mL and the corresponding inhibition zones of 16 and 17 mm, respectively. Nevertheless, it should be noted that both *S. aureus* and *E. faecalis* exhibit reduced susceptibility to colistin, as seen by MICs of 64 and 32 µg/mL and inhibition zones of 11 and 12 mm, respectively.

Synergistic efficacy of AgNPs and colistin with antibiotics

Tables 4 and 5 presents the observed synergistic effect between AgNPs and colistin when combined with antibiotic discs that were previously assessed and shown to be ineffective against the bacterial isolates. As depicted in Figure 10 and Table 4

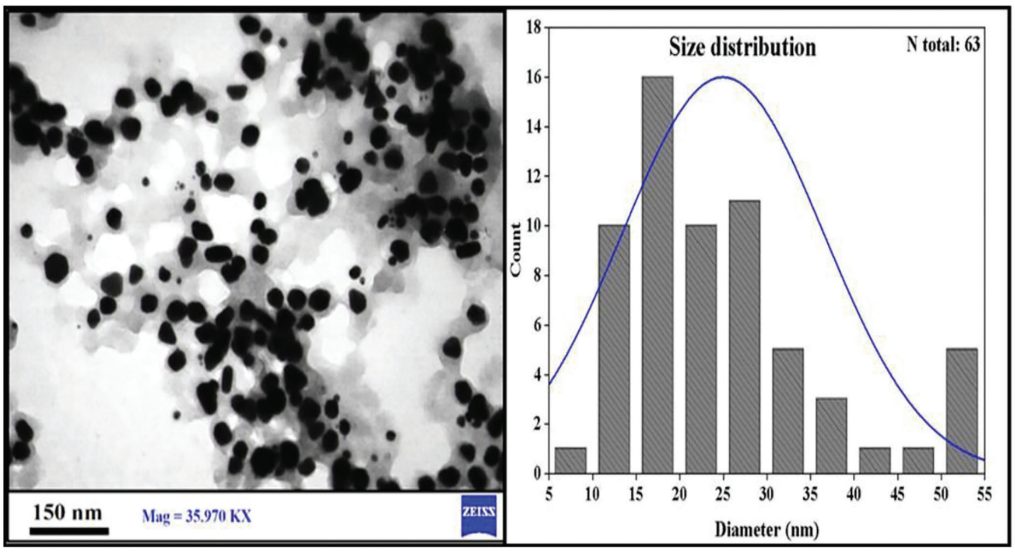

Figure 6: TEM of AgNPs and size distribution

shows that a partial synergistic effect was observed when AgNPs and colistin were combined with Ceftriaxone and Ciprofloxacin. *For E. coli*, the FICI was (0.74, 0.6) and 0.86 for combining AgNPs with ceftriaxone for *S. typhimurium*. However, no explicit synergistic effect was observed with the other antibiotics. FICI values of 0.86 and 0.85 showed that colistin and ceftriaxone demonstrated a partial synergy against *E. coli* and *S. typhimurium*. Furthermore, it was observed that the combination of colistin and ciprofloxacin exhibited partial synergy, as indicated by (FICI) value of 0.94, when tested against *E. coli*.

The results presented in Table 5 demonstrate the interaction between vancomycin and tetracycline in the presence of AgNPs, specifically in the partial synergy mode. The FICI values obtained for *S. aureus* were 0.8 and 0.52, while for *E. faecalis*, they were 0.86 and 0.78. In contrast, the remaining antibiotics did not exhibit any evident instances of synergy. Also, a study found that colistin and tetracycline worked well against *S. aureus*, with a FICI of 0.63. On the other hand, the combination of vancomycin, tetracycline, and colistin had a partial synergistic effect on *E. faecalis*. This was shown by a FICI value of 0.91.

Synergistic efficacy of AgNPs and colistin with antibiotics

The findings depicted in Table 6 and Figure 11 illustrate a distinct, synergistic effect between colistin and AgNPs.

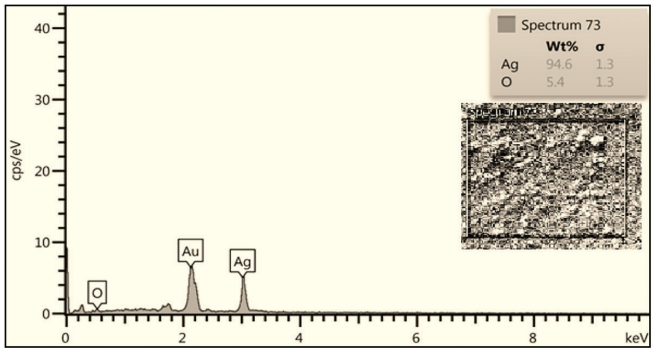

Figure 7: EDS test for prepared AgNPs

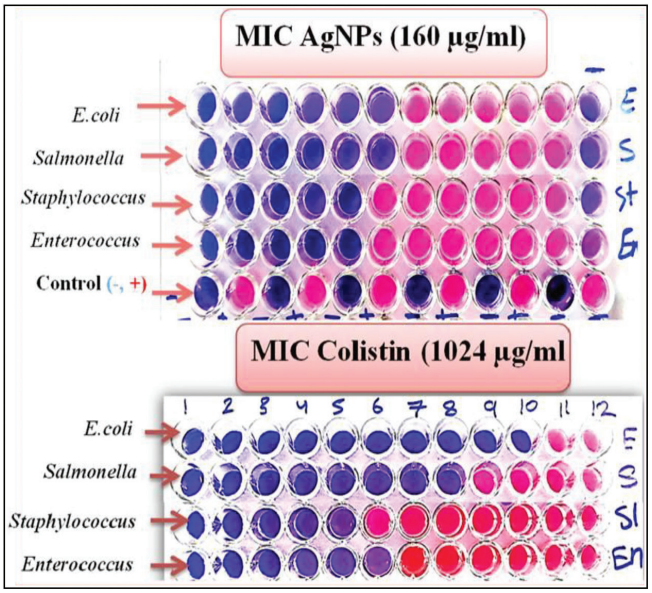

Figure 8: MIC for AgNPs and colistin

Table 2: Percentages of antibiotic resistance

Antibiotics	*Escherichia coli* (64)	*Salmonella* spp.[14]	*Staphylococcus* spp.[8]	*Enterococcus* spp.[13]
	R (%)	*R* (%)	*R* (%)	*R* (%)
Ampicillin	49 (76.6%)	10 (71.4%)	–	9 (69.2%)
Ceftriaxone	33 (51.6%)	5 (35.7%)	–	6 (46.2%)
Gentamicin	10 (15.6%)	1 (7.1%)	3 (37.5%)	–
Amikacin	3 (4.7%)	0 (0%)	–	–
Ciprofloxacin	19 (29.7%)	7 (50%)	4 (50%)	3 (23%)
Trimethoprim	39 (61%)	10 (71.4%)	5 (62.5%)	–
Tetracycline	36 (56.2%)	8 (57.1%)	4 (50%)	5 (38.5%)
Erythromycin	–	–	7 (87.5%)	10 (76.5%)
Vancomycin	–	–	3 (37.5%)	3 (23%)

Table 3: Antimicrobial activity of AgNPs and colistin

Bacteria	AgNPs		Colistin	
	MIC (µg/mL)	IZ (Mm)	MIC (µg/mL)	IZ (Mm)
Escherichia coli	5	12	2	16
Salmonella typhimurium	5	12	8	17
Staphylococcus aureus	10	15	64	12
Enterococcus faecalis	10	15	32	11

MIC = minimum inhibitory concentration, µg/mL = microgram/mL, IZ = inhibition zone, mm = millimeter

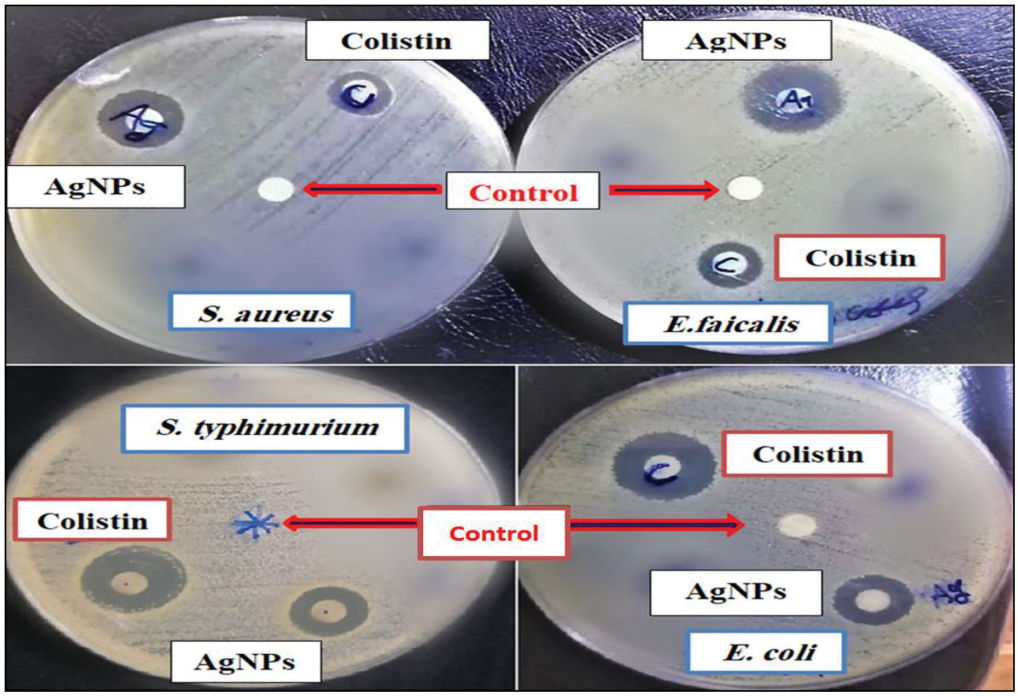

Figure 9: Inhibition zone of colistin and AgNPs

Table 4: The synergistic effectiveness of AgNPs and colistin in combination with antibiotics against *E. coli* and *S. typhimurium*

Treatments	AM	CTR	GM	AK	CIP	TM	TE
Escherichia coli							
Ab only	0	8	–	–	0	5	–
Ab+ AgNPs	12	27	–	–	18	13	–
FICI	1	0.74	–	–	0.6	1.3	–
Effect	Additive	P. synergy	–	–	P. synergy	Non	–
E. coli							
Ab only	0	8	–	–	0	5	–
Ab+ Colistin	11	28	–	–	17	11	–
FICI	1.45	0.86	–	–	0.94	1.9	–
Effect	Non	P. synergy	–	–	P. synergy	Non	–
Salmonella typhymurium							
Ab only	0	7	10	–	11	5	0
Ab+ AgNPs	12	22	20	–	17	13	12
FICI	1	0.86	1.1	–	1.35	1.3	1
Effect	Additive	P. synergy	Non	–	Non	Non	Non
Salmonella typhymurium							
Ab only	0	7	10	–	11	5	0
Ab+ Colistin	12	28	18	–	22	12	11
FICI	1.41	0.85	1.5	–	1.3	1.8	1.54
Effect	Non	P. synergy	Non	–	Non	Non	Non

AM = ampicillin, CTR = ceftriaxone, GM = gentamycin, AK = amikacin, CIP = ciprofloxacin, TM = trimethoprim, TE = tetracycline

FICI values obtained for the bacterial strains *E. coli, S. typhimurium, S. aureus*, and *E. faecalis* were 0.18, 0.37, 0.25, and 0.5, respectively.

Discussion

The biosynthesis of nanoparticles has garnered significant interest among researchers due to their lack of harmful ingredients, rendering the resulting nanomaterials suitable for use as therapeutic agents and economically viable. Biological extracts serve the dual purpose of acting as reducing agents and ensuring the stability of the resultant nanomaterials.[26] Cinnamon verum, an aromatherapeutic substance, has been recognized for its efficacy against bacteria, fungus, and oxidative stress. This can be attributed to the presence of the phenylpropanoid cinnamaldehyde

Table 5: The synergistic effectiveness of AgNPs and colistin in combination with antibiotics against *S. aureus* and *E. faecalis*

Treatments	AM	VA	E	TE	CIP	TM
Staphylococcus aureus						
Ab only	14	8	0	0	–	5
Ab+ AgNPs	20	26	7	29	–	15
FICI	1.45	0.8	1.74	0.52	–	1.33
Effect	Non	P. synergy	Non	P. synergy	–	Non
Staphylococcus aureus						
Ab only	14	8	0	0	–	5
Ab+ Colistin	18	18	10	19	–	12
FICI4.	1.44	1.1	1.2	0.63	–	1.41
Effect	Non	Non	Non	P. synergy	–	Non
Enterococcus faecalis						
Ab only	–	10	0	11	15	16
Ab+ AgNPs	–	29	8	33	20	18
FICI	–	0.86	1.87	0.78	1.5	1.72
Effect	–	P. synergy	Non	P. synergy	Non	Non
Enterococcus faecalis						
Ab only	–	10	0	11	15	16
Ab+ Colistin	–	24	9	24	26	18
FICI	–	0.91	1.2	0.91	1	1.5
Effect	–	P. synergy	Non	P. synergy	Non	Non

AM = ampicillin, VA = vancomycin, E = erythromycin, TE = tetracycline, CIP = ciprofloxacin, TM = trimethoprim

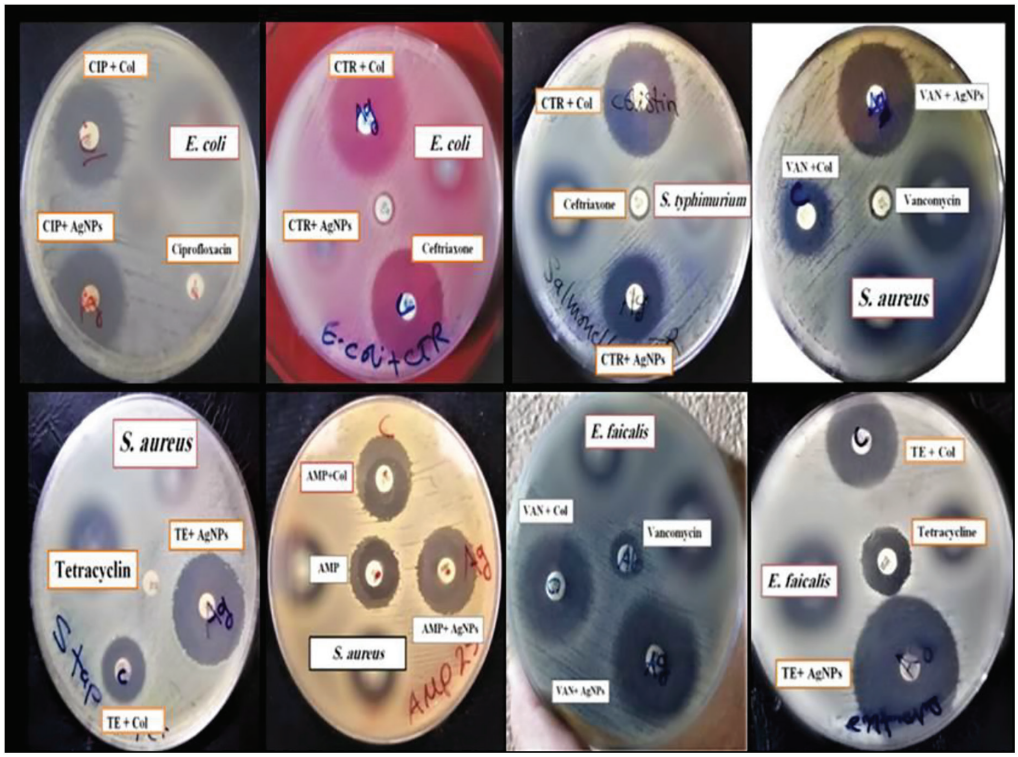

Figure 10: Synegism pattern of colistin ang AgNPs with antibiotics

and the reductive properties of $AgNO_3$, which facilitate the synthesis of AgNPs.[27]

In general, it has been observed that the antimicrobial activity of AgNPs is more pronounced against gram-negative bacteria compared to gram-positive bacteria. This observation is based on the determination of MIC values and the measurement of growth inhibition halo diameter. The differential susceptibility can be attributed to the structural disparity in the cell wall composition. Gram-positive bacteria possess an outer layer of peptidoglycan with a thickness of

approximately 30 nm, whereas gram-negative bacteria have a thinner peptidoglycan layer of about 4 nm.[28] The differential effectiveness of silver nanoparticles (AgNPs) synthesized using cinnamon bark extract, in comparison to those obtained from alternative biological origins (such as plants or microorganisms), can be ascribed to the complex interaction of several elements. The shape and size of nanoparticles are significant characteristics that have a major impact, as toxicity levels demonstrate an inverse correlation with particle size. There is a positive correlation between the effectiveness of granules and their smaller size, whereby smaller granules exhibit a higher effectiveness rate. Moreover, granules exhibiting a spherical morphology or a close approximation exhibit an augmented surface area, enhancing the interaction probability between the nanoparticles' most extensive surface region and cellular membranes. As a result, these granules, which are spherical or spherical-like in shape, demonstrate superior efficacy in comparison to other manufactured shapes that exhibit transparency.[29] TEM analysis exhibited a discernible circular morphology, while XRD investigation indicated the presence of a cubic crystalline structure. Furthermore, the application of the Scherrer's equation yielded relatively tiny crystal sizes. The findings from the EDX examinations also revealed a notable prevalence of biosynthesized silver nanoparticles, which can be related to biomolecules' effectiveness in reducing silver nitrate. The FT-IR results revealed the presence of Biomolecules in conjunction with the nanomaterials, serving as coating agents to maintain the stability and dispersion of the nanomaterials, hence preventing their agglomeration. The inclusion of Lipopolysaccharides with negative charges in the cellular architecture of Gram-negative bacteria facilitates the possibility of electrostatic interaction with colistin, which exhibits a positive charge. This electrostatic interaction contributes to the enhancement of bacterial wall permeability and the subsequent disruption of the glycolipid structure of the cell wall.[30] Moreover, the elevated quantities required to impact the membranes of gram-positive cells can be related to the depletion of their cell walls, specifically Lipopolysaccharides. Colistin can decrease the positive charge on the cellular walls of bacteria by facilitating the leakage of ions through the penetration of bacterial wall components and its high permeability rate.[31] The observed synergistic interaction between AgNPs and ceftriaxone can be attributed to

Table 6: The synergistic effect of combining colistin and AgNPs

Treatments	*E. coli*	*S. typhimurium*	*S. aureus*	*E. faecalis*
Colistin alone	2	8	64	32
Synergistic colistin	0.25	1	8	8
AgNP alone	5	5	10	10
Synergistic of AgNP	0.31	1.25	1.25	1.25
FICI	0.18	0.37	0.25	0.5
Effect	Synergy	Synergy	Synergy	Synergy

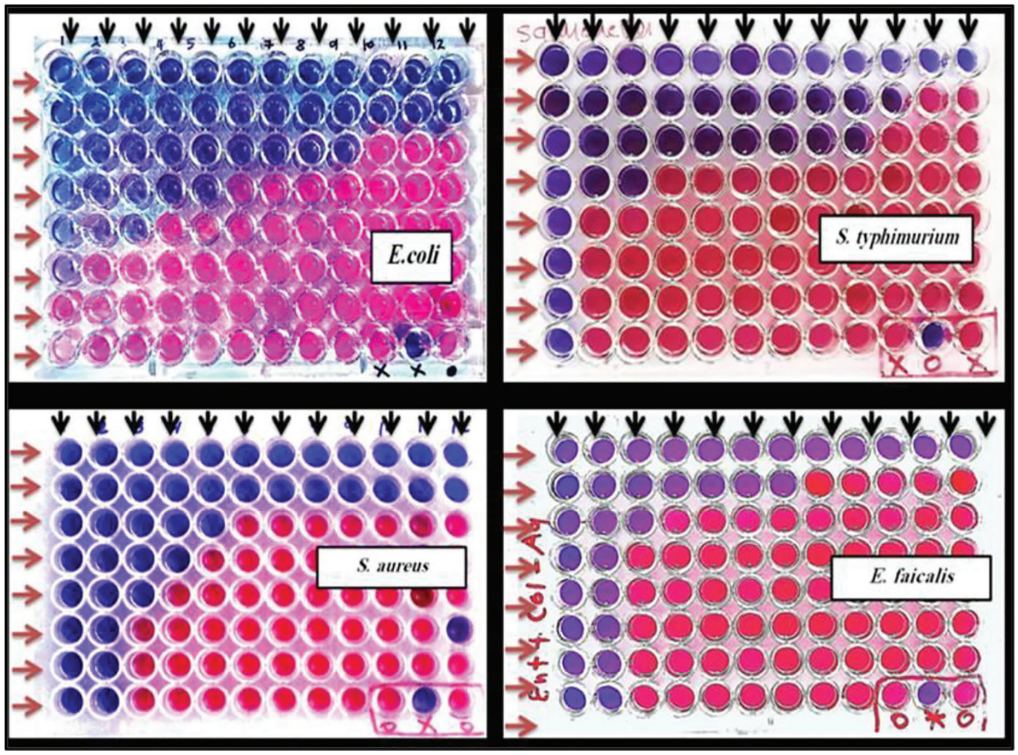

Figure 11: Checkerboard assy for colistin and AgNPs

the inhibitory effect of AgNPs on bacterial synthesis of extended-spectrum β-lactamases. This makes it possible for ceftriaxone to target and break up the cell wall structure without being affected by enzyme blockers that could make the antibiotic less effective.[32] The efficacy of AgNPs is attributed to their capacity to adhere to the cellular wall and subsequently disrupt the bacterial cell membrane. This disruption is achieved by stimulating cells to release reactive oxygen species (ROS) from free radicals. These ROS exhibit toxic properties against bacteria and contribute to the degradation of essential biomaterials. Various critical components can be found within the cellular environment, including DNA, proteins, and lipids.[33]

The synergistic activity of colistin with certain antibiotics can be attributed to its capacity to disrupt the outer membrane of the cell wall, penetrate the inner membrane, and disrupt the phospholipid bilayer.[34] This process enhances the ability of ceftriaxone to interact with the peptidoglycan and carry out its therapeutic function. Moreover, elevated levels of colistin with gram-positive bacteria induce a disruption in the bacterial membrane as a result of its interaction with Teichoic acid. This interaction further triggers the activation of other antibiotics, leading to the eradication of bacterial pathogens.[16]

The observed synergistic interaction between colistin and AgNPs, evident in Figure 11 may be attributed to their efficacy in targeting the bacterial cell wall or to the complementary action of AgNPs in conjunction with colistin against Gram-negative Enterobacteriaceae strains harboring the *mcr_1* genes, which encode MCR-1 enzymes that confer resistance to colistin. Silver nanoparticles AgNPs exhibit the ability to attach to the enzyme's active site, hence impeding its ability to inhibit colistin. Consequently, this interaction expands the range of colistin's effectiveness in targeting bacterial membranes, as enzyme-degrading inhibitors no longer hinder it.[35] AgNPs also contribute to the release of the silver ion Ag^{+1}. Its interaction with the negatively charged cell wall changes the structural shape of the cell wall, which helps the penetration of colistin with its better interaction with the negatively charged Teichoic acid available in the walls of pathogens positive for the Gram stain, supported by its positive charge, which contributes to Increased cell membrane permeability and leakage of cellular contents.[36,37]

Conclusions

The information presented led to the observation that the production of silver nanoparticles could be effectively achieved using a green biological technique that included cinnamon bark extract. Colistin and AgNPs have both shown antibacterial properties by successfully preventing the growth of germs. Moreover, they have shown synergistic benefits in combination with ceftriaxone, ciprofloxacin, vancomycin, and tetracycline, among other antibiotics. It has been shown that the effectiveness of colistin against Gram-negative bacteria is greater than that of Gram-positive bacteria.

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Conflicts of interest

There are no conflicts of interest.

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