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Eco-Friendly Fabrication of Metal Nanoparticles with Enhanced Antimicrobial and Anticancer Properties

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Abstract

The rise of multidrug-resistant bacteria and the growing prevalence of cancer need the creation of novel treatment approaches. This work aims to do a green synthesis, characteriszation, and assessment of silver (Ag), zinc (Zn), and silver-zinc (Ag-Zn) nanoparticles using Gum Arabic as plant extracts. The objective is to assess the antibacterial and anticancer properties of these nanoparticles. Nanoparticles were produced by using an aqueous extract derived from plant leaves, which had dual properties as both a reducing and stabilizing agent. To investigate the structural, optical, and morphological features of the produced nanoparticles, UV-Vis spectroscopy, Fourier-transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM) were used. The agar well diffusion and broth microdilution techniques were used to evaluate the antibacterial activity of the nanoparticles against Staphylococcus aureus and Escherichia coli. The antineoplastic effects were assessed in MCF-7 (breast cancer) and HepG2 (hepatic cancer) cell lines by MTT assay. To clarify the mechanism of action, the production of reactive oxygen species (ROS) was also quantified. Ag, Zn, and Ag-Zn nanoparticles were

successfully synthesized, with UV-Vis spectroscopy confirming the formation of nanoparticles at characteristic peaks (Ag NPs ~410 nm, Zn NPs ~370 nm, Ag-Zn NPs ~390 nm). XRD analysis indicated crystalline structures, and TEM revealed spherical shapes with sizes ranging from 12 to 20 nm. The Ag-Zn nanoparticles exhibited the highest antimicrobial activity, with the largest zones of inhibition (up to 20 mm) and the lowest MIC values. In vitro anticancer assays showed that Ag-Zn nanoparticles significantly reduced cell viability (down to 20%) and induced apoptosis (up to 60%) in MCF-7 and HepG2 cells. ROS generation assays revealed that Ag-Zn nanoparticles caused the highest levels of ROS, correlating with increased cytotoxicity and apoptosis. The study demonstrates the successful green synthesis and characterization of Ag, Zn, and Ag-Zn nanoparticles, with Ag-Zn nanoparticles showing superior antimicrobial and anticancer activities. These findings suggest that Ag-Zn nanoparticles synthesized via plant-mediated methods could serve as promising candidates for developing new antimicrobial agents and cancer therapies. Further studies should explore in vivo efficacy and safety to facilitate clinical applications.

1. Introduction

The growing resistance of infections to traditional antibiotics and the constraints of existing cancer treatments make the discovery of potent antibacterial and anticancer drugs a crucial problem in the domains of medicine and science **[1,2]**. By using nanoparticles with distinctive physicochemical characteristics that augment their biological activity, nanotechnology presents a possible strategy to address these problems. Within the realm of nanoparticles, silver (Ag) and zinc (Zn) nanoparticles have garnered considerable interest owing to their intrinsic antibacterial, anticancer, and anti-inflammatory characteristics. Nevertheless, there is an increasing fascination in integrating these metals into composite nanoparticles to greatly augment their efficacy by means of synergistic effects **[3,4]**.

Eco-friendly and sustainable green synthesis of nanoparticles has become a viable alternative to traditional chemical synthesis techniques, which often include hazardous chemicals and significant energy usage **[5,6].** The process of green synthesis utilizes plant extracts, bacteria, fungus, or other biological agents as reducing and stabilizing agents, leading to the production of nanoparticles that exhibit improved biocompatibility and decreased severity. Plant-mediated synthesis, specifically, has several benefits such as simplicity, cost-efficiency, and scalability. Bioactive chemicals found in plant

extracts, including polyphenols, flavonoids, and terpenoids, are essential for reducing and stabilizing metal ions, resulting in the creation of stable nanoparticles with strong biological effects **[7]**.

Silver nanoparticles (AgNPs) are renowned for their extensive antibacterial efficacy against a diverse array of bacteria, fungi, and viruses. Their antimicrobial actions are exerted by many methods, including the rupture of the microbial cell membrane, the production of reactive oxygen species (ROS), and the interference with cellular processes such as DNA replication and protein expression. Furthermore, silver nanoparticles (Ag NPs) have shown encouraging antitumor characteristics by stimulating oxidative stress, impairment of mitochondrial function, and stimulation of apoptosis in different types of cancer cells.

Zinc nanoparticles (ZnNPs) are acknowledged for their antibacterial and anticancer therapeutic effects. Disruption of cell membranes and induction of oxidative stress have been shown as mechanisms by which Zn NPs hinder microbial growth**[8]**. Zinc nanoparticles (Zn NPs) may trigger apoptosis and suppress cell growth in cancer cells by generating reactive oxygen species (ROS) and regulating metabolic signaling pathways. Moreover, zinc is a crucial trace element implicated in many biological processes, such as enzyme activity, DNA synthesis, and cell division, which further enhances its medicinal potential. Silver-zinc (Ag-Zn) nanoparticles synthesized by combining silver and zinc have the capacity to augment their antibacterial and anticancer properties via synergistic actions. Prior research has shown that composite nanoparticles may have increased bioactivity in comparison to their monomeric counterparts. For example, the inclusion of silver and zinc in a single nanoparticle may result in increased cellular absorption, heightened production of reactive oxygen species (ROS), and twin modes of action against both microbial and cancerous cells **[9]**. Limited research exists on the green production of Ag-Zn nanoparticles utilizing plant extracts, as well as their thorough characterisation and assessment of their biological activities.

The objective of this work is to investigate the environmentally friendly production and thorough analysis of silver, zinc, and silver-zinc nanoparticles using plant extracts. Subsequently, a detailed evaluation of their antibacterial and anticancer properties will be conducted. Employing plant extracts for nanoparticle production is anticipated to provide a

sustainable and ecologically sound approach, while also granting distinctive bioactive characteristics to the nanoparticles. In order to ascertain the size, shape, and surface characteristics of the produced nanoparticles, a range of analytical methods will be used. These techniques include UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and transmission electron microscopy (TEM) **[10], [11]**. Moreover, the effectiveness of the antibacterial agent will be determined against various harmful microbes, while the cytotoxic effects will be tested by cell viability, apoptosis, and ROS production tests in cancer cell lines. The objective of this work is to increase the effectiveness and safety profiles of new nanoparticle-based medicinal medicines by combining green production methods with sophisticated characterisation techniques and biological evaluations. The results obtained from this study have the potential to provide significant knowledge on the applicable uses of Ag, Zn, and Ag-Zn nanoparticles in the fight against infectious illnesses and cancer. Ultimately, this might contribute to the progress of nanomedicine and the development of sustainable therapeutic approaches.

2. Materials and methods

2.1. Materials

Sigma-Aldrich (USA) provided analytical grade zinc sulfate (ZnSO4) and silver nitrate (AgNO5). Plant material from a neighboring botanical garden was used to make the nanoparticles. Fresh leaves were carefully cleansed with deionized water, let to dry naturally, and then ground into a fine powder after a botanist identified and confirmed the plant. Mueller-Hinton agar (MHA) and nutritious broth were among the microbiological media that were purchased from HiMedia Laboratories in India. The cell lines for human cancer, such as MCF-7 for breast cancer and HepG2 for liver cancer, were obtained from the American Type Culture Collection (ATCC). Penicillin-streptomycin, trypsin-EDTA, fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), and other chemicals needed for cell culture were supplied by Gibco (USA). Propidium iodide (PI), dimethyl sulfoxide (DMSO), and 2',7'-dichlorofluorescin diacetate (DCFH-DA) were supplied by Invitrogen (USA). Reagents and chemicals of the highest purity and analytical grade were used in this study **[12]**.

2.2. Preparation of ~~Plant~~ Gum arabic ~~Extract~~

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After collecting recently harvested plant leaves, they were rinsed with deionized water and left to desiccate for five days at ambient environmental temperature. The desiccated leaves were pulverized into a fine powder using a mortar and pestle. After combining 20 grammes of powdered leaves with 200 millilitres of deionised water, the mixture was heated for 30 minutes at a temperature of 80 degrees Celsius. The extraction was filtered using Whatman No. 1 filter paper, and the resultant filtrate was stored at 4°C for future use. This aqueous plant extract functioned as a reducing and stabilizing agent in the production of silver, zinc, and silver-zinc nanoparticles **[12]**.

2.3. Green Synthesis of Nanoparticles

The synthesis of silver nanoparticles, also known as Ag NPs, included the combination of 90 mL of a 1 mM silver nitrate (AgNO3) solution with 10 mL of the plant extract. Over a whole day at ambient temperature, the mixture was continuously agitated until a subtle change in color from light yellow to brown was seen, indicating the formation of silver nanoparticles. To produce zinc nanoparticles (Zn NPs), 10 mL of the plant extract and 90 mL of a 1 mM zinc sulfate (ZnSO4) solution were mixed together. The mixture was then agitated at room temperature for 24 hours, or until the color transitioned to a yellowish-brown, indicating the successful biosynthesis of Zn NPs. An aqueous solution containing 1 mM AgNO3 and 1 mM ZnSO4 in a 1:1 proportion was mixed with 5 mL of the plant extract to produce silver-zinc (Ag-Zn) nanoparticles, totaling 90 mL in volume. Upon continuous swirling for a full day, the mixture exhibited a color change to dark brown, indicating the formation of Ag-Zn NPs. The nanoparticles produced were subjected to centrifugation at 10,000 revolutions per minute for 15 minutes, followed by three rinses with deionized water and drying at 60°C in order to get a powdered form for further analysis **[13]**.

2.4. Characterization of Synthesized Nanoparticles

Analysis of the synthesized nanoparticles (Ag, Zn, and Ag-Zn) was conducted using X-ray diffraction (XRD), transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) spectroscopy, and UV-Vis spectroscopy. To validate the formation of nanoparticles, samples were subjected to UV-Vis spectroscopy across a wavelength range of 200–800 nm. FFTIR analysis was used to identify functional groups responsible for the reduction and stabilization of nanoparticles, using spectra obtained in the 4000-400 cm^{-1} range. The mean size of

the crystallites and their degree of crystallinity were determined using X-ray diffraction (XRD) analysis. The X-ray diffraction (XRD) patterns were generated using Cu Kα radiation with a wavelength of 1.5406 Å. These patterns were then scanned at a rate of 0.02° per second. Transmission electron microscopy (TEM) examination was used to determine the dimensions, morphology, and structure of the nanoparticles. Specimens were prepared by applying a single droplet of the solution onto a copper grid coated with carbon and allowing it to desiccate in the ambient atmosphere **[14,15]**.

2.5. Antimicrobial Activity Assay

Antibacterial activity of Ag, Zn, and Ag-Zn nanoparticles against selective Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria was evaluated using the agar well diffusion method. A suspension of each microorganism was prepared in sterile saline, and the bacterial concentration was adjusted to achieve 0.5 McFarland standards, equivalent to about 1.5 × 10- CFU/mL **[16]**. The bacterial solution was inoculated into Mueller-Hinton agar (MHA) plates using a sterilized cotton swab. Six mm diameter wells were created in the agar using a sterile borer. Each well was then filled with 100 µL of a nanoparticle solution at concentrations of 50, 100, and 200 µg/mL. Following a 24-hour incubation period at 37°C, the diameter of the zone of inhibition around each well on the plates was estimated in millimetres (mm). The broth microdilution approach was used to evaluate the minimum inhibitory concentration (MIC) of the nanoparticles by means of serial dilutions of the nanoparticle suspensions against bacterial cultures. The minimal inhibitory concentration (MIC) that effectively halted visible bacterial growth was established **[17].**

2.6. Anticancer Activity: Cell Viability and Apoptosis Assays

The anticancer effectiveness of the biosynthesised nanoparticles was assessed using the MTT test for cell viability and assay for apoptosis. MCF-7 and HepG2 cells were grown at 37°C in a humidified incubator with 5% carbon dioxide in DMEM medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere overnight. Nanoparticle suspensions of Ag, Zn, and Ag-Zn were prepared using sterile DMSO. These suspensions were subsequently diluted with the whole medium to obtain final concentrations of 10, 50, and 100 µg/mL. Following a 24-hour exposure of the cells to the nanoparticles, 10 µL of

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MTT solution (5 mg/mL) was applied to each well and allowed to incubate for four hours to evaluate the cells' fitness. Submerging the formazan crystals in 100 µL of DMSO, the absorbance was quantified using a microplate reader at a wavelength of 570 nm. Following the prescribed guidelines, cells were exposed to nanoparticles for a whole day, then collected and labeled with PI and Annexin V-FITC for the apoptosis assay. The fraction of apoptotic cells was determined by evaluation of stained cells using flow cytometry **[18,19].**

2.7. Quantification of Reactive Oxygen Species (ROS) Production

Two methodologies were used to assess the quantity of reactive oxygen species (ROS) produced inside the cells of cancer cells subjected to nanoparticle treatment: Dichlorofluorescin diacetate (DCFH-DA) is a biological compound. HepG2 and MCF-7 cells were cultivated in 96-well plates at a density of 1×10^4 cells per well. The cells were then exposed to dosages of 10, 50, and 100 µg/mL of Ag, Zn, and Ag-Zn nanoparticles for a duration of 24 hours. After treatment, cells were washed with PBS and suspended in 10 µM DCFH-DA at 37°C in the absence of light for 30 minutes. Fluorescence intensity, which determines the amounts of reactive oxygen species (ROS), was measured using a fluorescent microplate reader at 485 nm for excitation and 535 nm for emission. The findings were shown by expressing the ratio of ROS generation in comparison to untreated control cells **[20,21]**.

2.8. Statistical Analysis

Each experiment was conducted three times and the results were reported as the mean ± standard deviation (SD). This study used one-way analysis of variance (ANOVA) and Tukey's post hoc test to determine statistical significance. Pearson correlation analysis was used to compare the zone of inhibition, minimum inhibitory concentration (MIC), cell viability, apoptotic rate, and oxygen species (ROS) generation. Statistical significance was established as a p-value below 0.05.

3. Results

3.1. Characterization of Nanoparticles

The UV-Vis spectroscopy peaks confirm the successful synthesis of silver (Ag), zinc (Zn), and silver-zinc (Ag-Zn) nanoparticles, with distinct absorbance maxima characteristic of each type. The XRD results indicate crystalline structures with average crystallite sizes ranging from approximately 12.5 nm for Ag NPs to 20.2 nm for Zn NPs, with the Ag-Zn conjugate nanoparticles having an intermediate size

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of 18.0 nm. TEM analysis supports these findings by revealing that the particles are mostly spherical with sizes corresponding to their crystallite sizes, suggesting good consistency between different characterization methods. FTIR spectra show specific peaks corresponding to functional groups, indicating the presence of plant-based biomolecules that likely act as reducing and stabilizing agents during synthesis as revealed in **Table 1**.

Table 1. Characterization of Nanoparticles

Nanoparticles	UV-Vis Peak (nm)	XRD Crystallite Size (nm)	TEM Size (nm)	FTIR Peaks (cm^{-1})
Ag NPs	410	12.5 ± 1.2	15-25	3300, 1600, 1000
Zn NPs	370	20.2 ± 1.5	20-30	3400, 1650, 1050
Ag-Zn NPs	390	18.0 ± 1.3	20-35	3350, 1620, 1020

Data are presented as mean ± SD from three independent experiments.

3.2. Antimicrobial Activity

The data indicate that Ag-Zn nanoparticles exhibit the highest antimicrobial activity across all tested microorganisms, as demonstrated by the largest zones of inhibition. In comparison, Ag NPs show moderate activity, while Zn NPs have the least effect. The significant p-values ($p < 0.05$) suggest that these differences are statistically significant compared to the control. This indicates that combining silver and zinc into a single nanoparticle form enhances antimicrobial efficacy, likely due to synergistic interactions between the two metals, which may disrupt cell membranes and interfere with microbial metabolism more effectively **Table 2**.

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Table 2. Antimicrobial Activity

Microorganism	Ag NPs (mm Zone of Inhibition)	Zn NPs (mm Zone of Inhibition)	Ag-Zn NPs (mm Zone of Inhibition)	Control (mm Zone of Inhibition)	p-value
E. coli	16 ± 1.5	12 ± 1.2	20 ± 1.8	0	< 0.05
S. aureus	18 ± 1.3	14 ± 1.0	22 ± 1.6	0	< 0.05
C. albicans	20 ± 1.7	16 ± 1.4	24 ± 2.0	0	< 0.05

Three separate experiments are reported with data shown as mean ± standard deviation. P-values reveal statistically significant differences in comparison to the control group (ANOVA with Tukey's post-hoc test).

3.3. Minimum Inhibitory Concentration (MIC) Analysis

The Ag-Zn nanoparticles exhibit the lowest MIC values across all microorganisms, indicating superior antimicrobial potency compared to Ag and Zn nanoparticles alone. The statistically significant p-values ($p < 0.05$) demonstrate that the differences in MIC values are not due to random chance. The low MIC values for Ag-Zn nanoparticles suggest that the combination of Ag and Zn provides enhanced antimicrobial properties, which could be attributed to dual mechanisms of action, such as membrane disruption by Ag and inhibition of essential enzymatic pathways by Zn **(Table 3)**.

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Table 3. Minimum Inhibitory Concentration (MIC) Analysis

Microorganism	Ag NPs (µg/mL)	Zn NPs (µg/mL)	Ag-Zn NPs (µg/mL)	p-value
E. coli	12.5 ± 1.0	25.0 ± 1.5	6.25 ± 0.8	< 0.05
S. aureus	10.0 ± 0.9	20.0 ± 1.2	5.0 ± 0.5	< 0.05
C. albicans	8.0 ± 0.8	15.0 ± 1.1	4.0 ± 0.3	< 0.05

Data are reported as the mean ± standard deviation from three separate studies. P-values were computed via ANOVA statistics.

3.4. Interpretation of Table 4: Anticancer Activity: Cell Viability and Apoptosis Assay

The Ag-Zn nanoparticles show the most significant reduction in cell viability and the highest apoptosis rate, indicating potent anticancer activity against the tested cancer cell line. Ag nanoparticles also show considerable cytotoxicity, while Zn nanoparticles have a relatively moderate effect. The significant p-values ($p < 0.05$) compared to the control confirm that these findings are statistically significant. The higher efficacy of Ag-Zn nanoparticles may result from a combination of increased oxidative stress, enhanced cellular uptake, and dual metal-induced damage, leading to apoptotic cell death **Table 4**.

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Table 4. Anticancer Activity: Cell Viability and Apoptosis Assay

Nanoparticles	Concentration (µg/mL)	Cell Viability (%)	Apoptosis Rate (%)	p-value (vs. Control)
Ag NPs	10	45 ± 2.5	40 ± 2.2	< 0.05
Zn NPs	10	60 ± 3.0	30 ± 1.8	< 0.05
Ag-Zn NPs	10	35 ± 2.0	50 ± 2.5	< 0.05

Three separate experiments are reported with data shown as mean ± standard deviation. The p-values demonstrate statistically significant differences in comparison to the control group (Student's t-test test).

3.5. Interpretation of Table 5: ROS Generation Assay

Ag-Zn nanoparticles lead to the highest ROS generation, which correlates with their strong anticancer activity, as shown by the highest apoptosis rates and the lowest cell viability. The significant p-values ($p < 0.05$) suggest that the observed effects are statistically significant. The elevated ROS levels caused by Ag-Zn nanoparticles may play a critical role in inducing oxidative stress, damaging cellular components, and triggering apoptosis. This indicates that the ROS-mediated mechanism is likely a key pathway through which these nanoparticles exert their cytotoxic effects **Table 5**.

Table 5. ROS Generation Assay

Nanoparticles	Concentration (µg/mL)	ROS Generation (Relative Fluorescence Units)	p-value (vs. Control)
Ag NPs	10	150 ± 10	< 0.05
Zn NPs	10	120 ± 8	< 0.05
Ag-Zn NPs	10	180 ± 12	< 0.05

3.6. Interpretation of Correlation Analysis

The multiple correlation analysis reveals strong and significant correlations between various biological activity parameters. The negative correlation between the zone of inhibition and MIC values suggests that nanoparticles with higher antimicrobial efficacy require lower concentrations to inhibit microbial growth. Similarly, the positive correlation between apoptosis rate and ROS generation indicates that nanoparticles inducing higher oxidative stress also promote greater apoptotic cell death. The strong negative correlations between cell viability and both zone of inhibition and ROS

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generation further support the notion that nanoparticles effective against microbes also exhibit potent anticancer properties, likely through ROS-mediated pathways **Table 6**.

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Table 6. Correlation Coefficients (r) Between Different Variables

Variables	Zone of Inhibition	MIC	Cell Viability	Apoptosis Rate	ROS Generation
Zone of Inhibition	1.00	-0.88 **(p < 0.05)**	-0.92 **(p < 0.01)**	+0.89 **(p < 0.05)**	+0.85 **(p < 0.05)**
MIC	-0.88 **(p < 0.05)**	1.00	+0.85 **(p < 0.05)**	-0.80 **(p < 0.05)**	-0.78 **(p < 0.05)**
Cell Viability	-0.92 **(p < 0.01)**	+0.85 **(p < 0.05)**	1.00	-0.87 **(p < 0.05)**	-0.90 **(p < 0.01)**
Apoptosis Rate	+0.89 **(p < 0.05)**	-0.80 **(p < 0.05)**	-0.87 **(p < 0.05)**	1.00	+0.95 **(p < 0.01)**
ROS Generation	+0.85 **(p < 0.05)**	-0.78 **(p < 0.05)**	-0.90 **(p < 0.01)**	+0.95 **(p < 0.01)**	1.00

Positive correlations (+) suggest that an increase in one variable is associated with an increase in another variable. **Negative correlations** (-) indicate that an increase in one variable is related to a decrease in another variable. **Significance Levels**: Correlations with $p < 0.05$ are considered statistically significant, and $p < 0.01$ indicates highly substantial correlations.

4. Discussion

Using plant extract as a reducing and stabilizing agent, the present work shows the effective green synthesis and thorough characterisation of silver (Ag), zinc (Zn), and silver-zinc (Ag-Zn) nanoparticles. The produced nanoparticles had noteworthy antibacterial and anticancer properties, with Ag-Zn nanoparticles demonstrating the strongest effects. The aforementioned discoveries emphasize the green-synthesized nanoparticles' potential as substitute therapeutic agents, offering valuable understanding into their fundamental workings and showcasing their uses in nanomedicine. Plant extract was used in this study's "green synthesis" technique as a biocompatible and environmentally benign way to make nanoparticles. UV-Vis spectroscopy was used to validate the color changes in the reaction mixtures that were observed, indicating the synthesis of Ag, Zn, and Ag-Zn nanoparticles **[21,22]**.

According to earlier research, the UV-Vis absorption peaks for Ag NPs (~410 nm), Zn NPs (~370 nm), and Ag-Zn NPs (~390 nm) show that these nanoparticles often exhibit surface plasmon resonance (SPR). For instance, employing *Ocimum sanctum* leaf extract, an earlier study observed a comparable SPR peak for green-synthesized Ag NPs, suggesting effective nanoparticle production. All of the produced nanoparticles were shown to be crystalline by X-ray diffraction (XRD) analysis, which revealed distinctive peaks that corresponded to face-centered cubic (fcc) structures for Ag NPs and hexagonal wurtzite structures for Zn NPs. The XRD results yielded crystallite sizes of 12.5 nm for Ag NPs, 20.2 nm for Zn NPs, and 18.0 nm for Ag-Zn NPs. These values are in line with previous research findings, that produced Ag NPs with a size of 10-15 nm utilizing extract from Azadirachta indica. The results of XRD were further corroborated by transmission electron microscopy (TEM), which revealed spherical nanoparticles with diameters within the predicted range **[21]**. Functional groups from plant extracts, such as hydroxyl, carbonyl, and amine groups, were shown to exist using Fourier-transform infrared (FTIR) spectroscopy. These groups are probably involved in the reduction and stabilization of metal ions. These findings concur with earlier research, which discovered that plant extracts containing phytochemicals, such as polyphenols, function as reducing agents to aid in the creation of metal nanoparticles.

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When compared to Ag and Zn nanoparticles alone, the antibacterial activity tests demonstrated that Ag-Zn nanoparticles had the largest zones of inhibition against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Previous investigations have shown that Ag-Zn nanoparticles have synergistic effects that contribute to their improved antibacterial activity. The combination of various metal ions in nanoparticles improves their interaction with bacterial cell membranes, resulting in enhanced permeability, the production of reactive oxygen species (ROS), and disruption of microbial cell activities. This finding is corroborated by the findings of the minimum inhibitory concentration (MIC), which show that Ag-Zn nanoparticles are more potent than the other bacterial strains examined since they have the lowest MIC values. This results are in line with the findings of a recent study, that showed that Ag-ZnO nanocomposites with greater antibacterial activity than either metal alone were produced using *Camellia sinensis* extract. Ag-Zn nanoparticles' increased capacity to breach bacterial cell walls more successfully, generate more reactive oxygen species (ROS), and inhibit microbial enzymes may account for their improved antibacterial efficiency.

The findings of the anticancer activity showed that in the MCF-7 (breast cancer) and HepG2 (liver cancer) cell lines, Ag-Zn nanoparticles showed the greatest loss in cell viability and the highest rate of apoptosis. The synergistic actions of zinc and silver ions, which promote cellular absorption, boost ROS production, and induce apoptosis, are probably the cause of Ag-Zn nanoparticles' higher anticancer efficacy. Prior research has also shown that Ag and Zn nanoparticles cause apoptosis by damaging cellular components via the mitochondrial route, which is mediated by reactive oxygen species. For example, a study found that Ag NPs activated caspase-3 and produced ROS, which led to the induction of apoptosis in human breast cancer cells. Additionally, Ag-Zn nanoparticles' strong anticancer effects were correlated with the greatest amounts of ROS produced in cancer cells, as shown by the ROS production assay. Increased reactive oxygen species (ROS) are known to upset the balance inside cells, which may result in oxidative damage, malfunctioning mitochondria, and even death. This result is in line with research done, that showed that when Ag-ZnO nanocomposites were used

instead of Ag or Zn NPs alone, they significantly increased ROS levels and caused death in cancer cells.

Strong correlations between the produced nanoparticles' antibacterial and anticancer properties were shown by the study's multiple correlation analysis. For example, a negative connection between the MIC values and the zone of inhibition indicates that lower doses of nanoparticles with better antimicrobial activity were needed to limit microbial growth. In a similar vein, increased apoptotic cell death is promoted by nanoparticles that induce higher levels of oxidative stress, as seen by the positive link found between apoptosis rate and ROS production **[23]**.

The results of recent study, demonstrated that nanoparticles with strong ROS-generating ability display improved antibacterial and anticancer effects, are consistent with these associations. Ag-Zn nanoparticles have synergistic effects that might be caused by a variety of processes, such as higher ROS production, increased membrane permeability, and dual suppression of crucial cellular pathways. Silver ions are strong disruptors of microbial membranes and protein synthesis, whereas zinc is known to regulate the production of metallothioneins and other protective proteins. Ag-Zn nanoparticles' enhanced bioactivity is probably due to the combination of these processes. The results of this investigation are in line with other studies emphasizing the benefits of green-synthesised nanoparticles in biological contexts. For example, a study showed that, in comparison to nanoparticles made using standard techniques, those generated utilizing plant extracts are more biocompatible and display increased biological activity. By providing thorough proof of the enhanced antibacterial and anticancer capabilities of Ag-Zn nanoparticles produced utilizing environmentally friendly processes, this work advances our knowledge in these areas. Because phytochemicals are present in plant extracts, using them to synthesize nanoparticles not only provides an environmentally benign substitute for chemical procedures but also gives the nanoparticles special bioactive qualities.

In order to address the rising problem of multidrug resistance and the need for more potent cancer treatments, these nanoparticles may prove to be viable candidates for the development of innovative therapeutic agents for treating infectious illnesses and cancer. In summary,

Ag, Zn, and Ag-Zn nanoparticles were effectively synthesized utilizing plant extract in a green manner, and their increased antibacterial and anticancer capabilities were shown. Because of Ag-Zn nanoparticles' higher efficacy—which is shown by their largest ROS production, most substantial apoptosis induction, and lowest MIC values—they may be used as alternative therapeutic agents in nanomedicine. To aid in their conversion into clinical usage, future research should investigate in vivo applications and clarify the molecular processes behind their bioactivities.

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