











# Investigating Potential Therapeutics for Bacterial and Cancerous Diseases: Silver Nanoparticles as a Choice

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**Abstract:** Silver nanoparticles (AgNPs) have been identified as an efficient antibacterial representative capable of combating bacteria that cause infections. AgNPs have antibacterial properties against Gram-negative and Gram-positive bacteria and multidrug-resistant pathogens. AgNPs have various contemporary modes of action, and when used in conjunction with antibacterial agents such as chemical combinations or antibiotics, they have a synergic activity on bacteria pathogens. AgNPs have been extensively researched as components of sophisticated anti-cancer medications for improving cancer management in the clinic. The reduction of substances on silver ions often produces AgNPs. Living organisms and natural products, moreover, exceptional cascade for synthesizing AgNPs harbingers. AgNPs have properties that make them excellent for use in medical and healthcare goods, where they can effectively reduce the risk of developing infections. This review discusses the antibacterial and anti-cancer properties of AgNPs, and the benefits of using AgNPs as a novel, effective antibacterial combination with antibiotics, which will lessen the dosage required and avoid potential hazards.

**Keywords:** Silver nanoparticles; anti-bacterial; anticancer; multidrug-resistant; synthesis

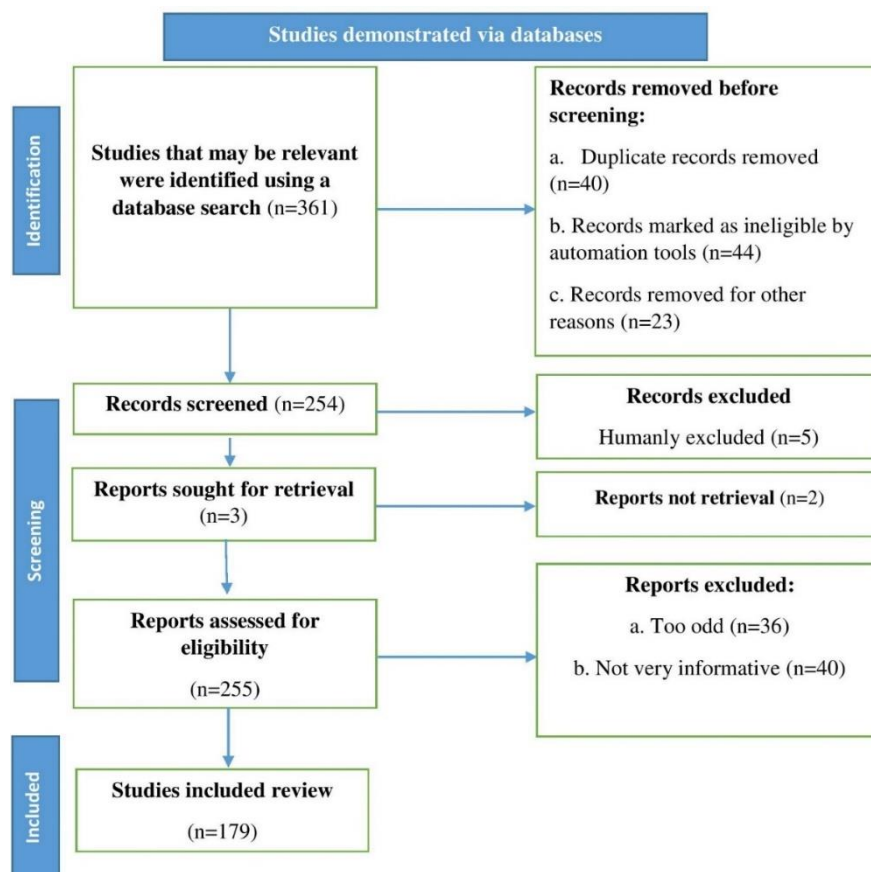
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## 1. Introduction

During the last few decades, nanotechnology has figured prominently in biomedical research, diagnostics, therapeutics, the manufacturing industry, scientific and technical investigations, and environmental regulation [1,2]. Nanomaterials, particles with the smallest dimension of less than 100 nm, have a dimension range of 1 nm to 100 nm. Silver, titanium, cerium, iron, gold, platinum, and thallium nanoparticles (NPs) are among the many types and

sizes of metal NPs [3]. Silver nanoparticles (AgNPs) are considered the most commonly studied metal NPs for a variety of scientific implementations due to their unique physicochemical characteristics, such as outstanding surface plasmon resonance, excellent surface area to volume ratio, ease of conjugation with numerous types of binding sites to obtain the desired personalized properties, toxic metabolites against pathogens, efficient anti-tumor activity related to cancer cells, catalytic applications, and so on [4]. AgNPs and nanocomposites are obvious among noble metal NPs and nanocomposites due to their enormous chances and various applications in the textile and food manufacturing divisions, water purification plants, contamination regulators, biomedical/therapeutics applications (anti-cancer, antimicrobial, antiangiogenic, contrast agents in diagnostic medical imaging, and diagnostic examinations in biological systems for the identification of diseases [5–7]. Several biological processes can convert inorganic metal ions into metal NPs by reducing their proteins and metabolites, such as manufacturing AgNPs. These environmental approaches could be used for chemical and physical AgNP synthesis [8–11].

Additionally, AgNPs made utilizing biologic methods usually have a consistent chemical arrangement and few shortcomings [12]. These AgNPs are efficient against a wide range of cancer cell kinds. AgNPs can cause cancer cell cytotoxicity by adjusting their shape, reducing their viability, and having a significant role in tumor control due to their antibacterial activity [13,14].



**Figure 1.** A flowchart illustrating the steps required for choosing published data for the current study is shown; n = the number of literature reports.

This review highlights some silver nanostructures with their significant antibacterial and anti-cancer actions and a plethora of purposes so that readers can recognize the significance and benefit of AgNPs and their possibility in novel science or nanotechnology purposes.

## 2. Methodology

The following databases were used: PubMed, Scopus, and Web of Science. Nanotechnology, silver nanoparticles, and antibacterial and anti-cancer terminology were used. Until 2022, English research reports, review articles, and original research articles were chosen and examined. According to Page *et al.*'s [15] guidelines, an algorithm that followed the flowchart in Fig. 1 and included all the processes and requirements for selecting the necessary literature was utilized.

## 3. Properties of Silver Nanoparticles

AgNPs have specific physicochemical properties that make it convenient for them to enter cells and associate with biomolecules efficiently within the cells or on their surface [16]. Following are some of the critical properties of AgNPs.

### 3.1. Shape and Size.

Hemocompatibility should be considered when selecting a restorative material to manage particular disease types, such as cancer [17]. Using AgNPs of specific form and size, Kwon *et al.* [18] reduced membrane damage such as potassium efflux, hemolysis, protein leakage, membrane fragility, and cell shape changes. As a result, the high anti-cancer properties of AgNPs are a significant contributor to mitochondrial respiratory chain disruption, which results in the free radicals manufacture and disturbance of adenosine triphosphate (ATP) synthesis, which tends to result in nucleic acid damage [19].

AgNPs' size and shape can be affected by many factors, including pH of the solution, product intensity, temperature, capping agent molar ratio to the forerunner, and dropping agents [20]. Depending on the synthesis procedures, various particle sizes and forms can be generated [21]. For instance, in the fungal production of *Fusarium acuminatum*, spherical AgNPs on the scale of 5–40 nm (regular size 13 nm) were detected [22]. By reducing the temperature to 10°C, the particle size was raised to 80–100 nm [23]. Low reaction temperatures have been shown to enhance the development of two-dimensional nanostructures [24]. As proven by the work on *Nelumbo nucifera*, plant-based green synthesis may yield a variety of sizes and forms of AgNPs. On average, particle sizes ranged from 25 to 80 nm, averaging 45 nm [25]. Vilchis-Nestor *et al.* [26] discovered more sphere-shaped and greater AgNPs when the content of *Camellia sinensis* (green tea) extracts rose in ambient settings.

### 3.2. Optical Properties.

Because of surface plasmon resonance, AgNPs can make more successful light contacts than organic or inorganic chromophores. The synergistic relationship is due to the confinement of a significant density of participating electrons to the nanometer scale associated with the mean accessible channel and the dielectric function's unusual sampling rate in metallic silver. Surface plasmon resonance (SPR)-related properties emerge from the interaction of these two variables. The frequency and resonance strength are controlled by the size and shape of the NPs [27]. Noninvasive techniques like dark-field microscopy can analyze AgNP monitoring and cellular uptake because of their different optical properties [28]. AgNPs can have their optical properties changed to produce intriguing outcomes. For example, the absorption spectra of AgNPs can be adjusted to the near-infrared region by carefully optimizing the synthesis

conditions. This eliminates tissue auto-fluorescence interference, giving NPs much hope for tumor-targeting [29].

### 3.3. Electrical Properties.

The zeta potential of AgNPs in a colloidal suspension is an essential electrical property controlled by the NPs' size. AgNPs have a high surface electrical charge and a significant absolute zeta potential, which can cause a substantial electrostatic repulsion among particles, preventing aggregation [30]. Accumulation must be avoided if particle expansion from the Nanoscale to the microscale is prevented. A high zeta potential of almost -25 mV has provided a robust, sufficient power barrier for nano suspension stability. For instance, Sankar *et al.* [31] discovered that the biosynthesis of AgNPs from *Origanum vulgare* with a standard size of  $136 \pm 10.09$  nm has a zeta capacity of about  $-26 \pm 0.77$  mV.

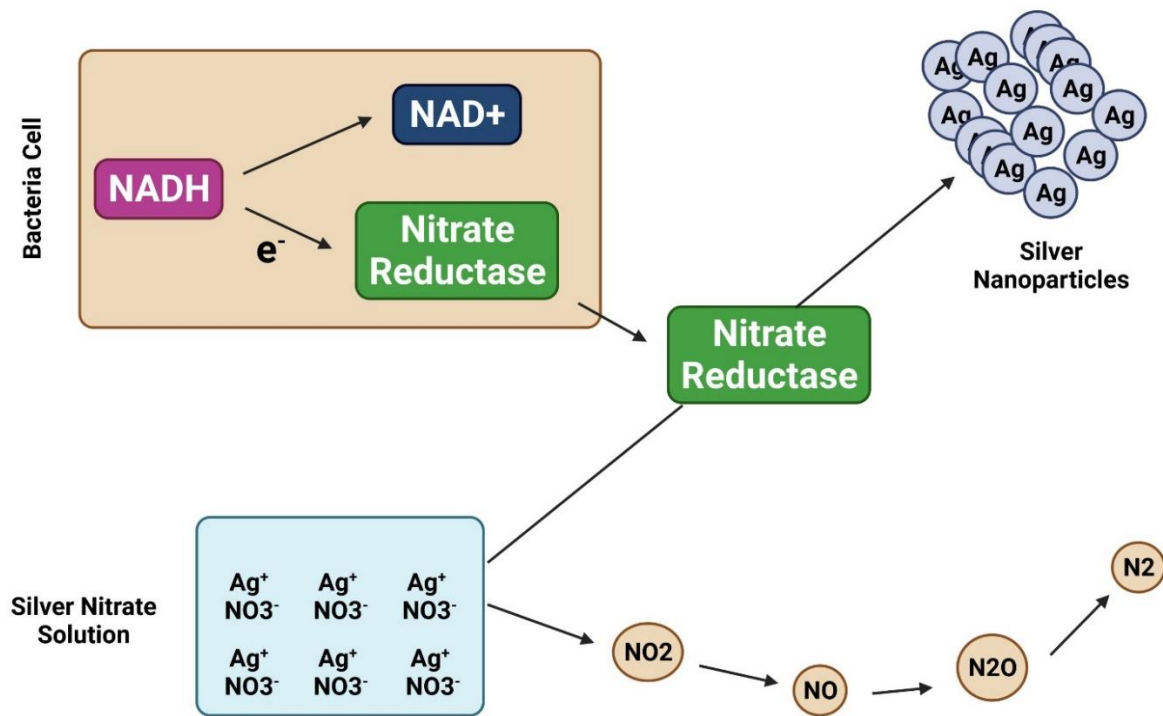
## 4. Silver Nanoparticles Biologic Synthesis

AgNPs' synthesis, both "top-down" and "bottom-up" techniques, can be used [32]. In a top-down technique, particles of an acceptable precursor material are reduced in size until NPs are created. Top-down approaches include ball milling and laser ablation, among other physical and chemical processes [33]. However, surface flaws on the NPs are a crucial shortcoming of top-down techniques [34]. On the other hand, a bottom-up technique makes NPs by mixing minor objects [35]. Sol-gel techniques are examples of bottom-up procedures that are chemical or biological [36]. The drawbacks of most AgNPs synthesis processes utilizing chemical and physical approaches involve high costs, and possibly dangerous ingredients, creating environmental and biological risks. Because harmful chemicals are absorbed into the NPs' surfaces, chemically synthesized NPs are frequently undesirable for therapeutic properties. For practical reasons, industrial AgNPs must be managed and must be cost-effective. As a result, alternative synthetic technologies that are environmentally and economically viable are desired. The search for such approaches has led to the study of biologic synthesis procedures [37]. In the biological synthesis of AgNPs, molecules found in live microbes (bacteria and fungi) function as reducing and stabilizing agents [38].

### 4.1. Bacterial synthesis of silver nanoparticles.

NPs can be produced either extracellularly or intracellularly. The NADH-dependent nitrate reductase-mediated silver ion ( $\text{Ag}^+$ ) decreased by many bacteria species, including *Streptomyces* spp. A broadly used extracellular method for AgNPs generation by bacteria is LK3 and *Bacillus licheniformis*. The nitrate ions ( $\text{NO}_3^-$ ) in silver nitrate ( $\text{AgNO}_3$ ) are reduced to nitrite ( $\text{NO}_2^-$ ) by taking two protons, generating two electrons, and water during the reduction process. Elemental silver ( $\text{Ag}^0$ ) is produced when electrons released are reassigned to the  $\text{Ag}^+$  ion during this process. Electron transport channels could be used in this strategy [39,40] (Fig. 2).

Nevertheless, enzymes aren't necessarily required for bacterial AgNP production. *Lactobacillus* A09, for instance, was found to have non-enzymatic intracellular production when  $\text{Ag}^+$  was reduced on the bacterial surface [41]. The cell walls of Gram-positive bacteria, including *Lactobacillus* A09, have a lot of anionic surface groups. These organizations may produce  $\text{Ag}^+$  biosorption sites [42].



**Figure 2.** Extracellular bacterial enzymatic biosynthesis of AgNPs.

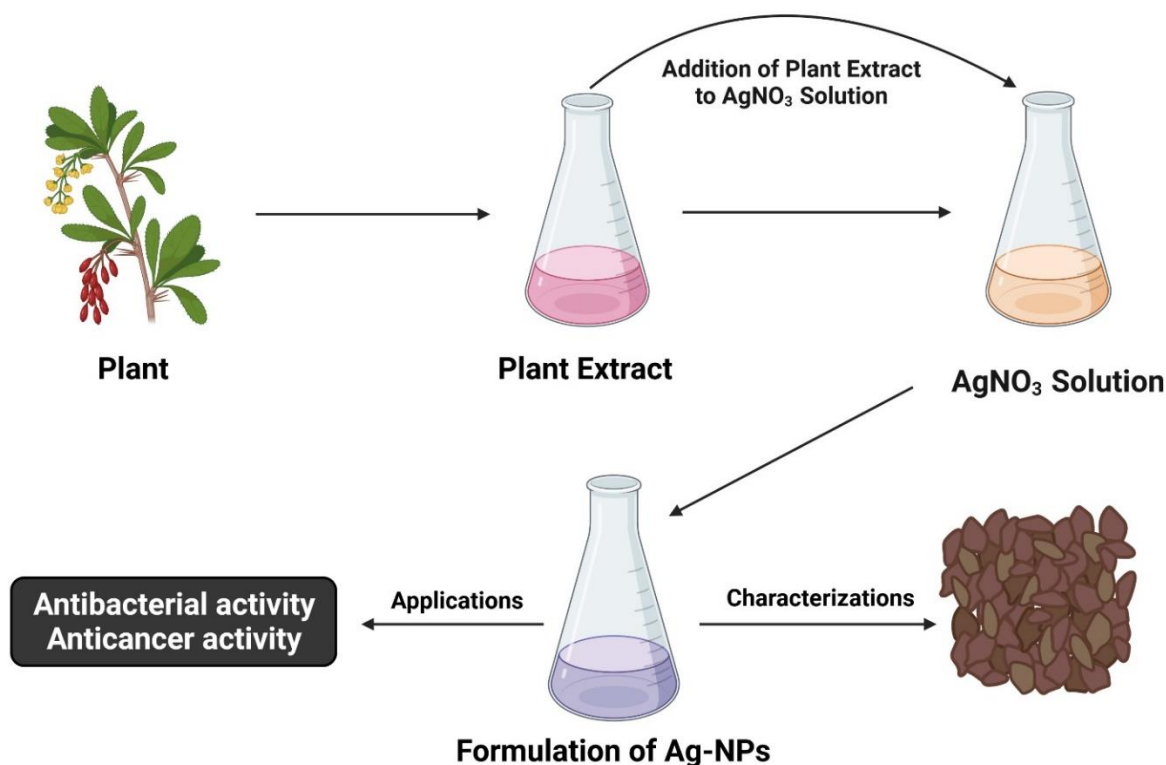
Following the adsorption of Ag<sup>+</sup> on *Lactobacillus* A09, the pH of the surrounding medium fell somewhat, indicating an interaction among proton and silver ion attachment to negatively charged regions [43]. Increased pH can cause the monosaccharide rings in bacteria's cell walls to be exposed and oxidized, resulting in open-chain aldehydes. Protons are separated from protonated anionic functional groups (–RH) simultaneously. Ag<sup>+</sup> ions can be converted to elemental Ag<sup>0</sup> using the two electrons released when alcohol forms an aldehyde group [44].

#### 4.2. Silver Nanoparticles Plant Synthesis.

Plant cuttings have been utilized to make AgNPs, and their accessibility, low toxicity, and safety make them exceptional in synthetic bacterial processes. Silver ions can be reduced dramatically by a broad range of extracted phytochemicals. Compared to microbial fermentation [45], lowering can be accomplished rapidly since this method minimizes the need for prolonged sterile cell culture under regulated circumstances [46]. Plant extracts can manufacture AgNPs as a reducing and stabilizing agent. Plant extract techniques can also reduce other metallic NPs [47]. The characteristics of AgNPs are determined by natural sources [48]. Depending on the plant source, specific phytonutrients and combinations of phytonutrients in extracts vary significantly. As a result, the properties of AgNPs can be controlled by altering the isolated structure [49]. The specific process of AgNPs synthesis differs due to the diversity of phytonutrients. The diminution of Ag<sup>+</sup> ions by certain practical classes is the principal mechanism. *Punica granatum* peel extract, for instance, includes flavonoids. The hydroxyl (–OH) groups in all these compounds allow Ag<sup>+</sup> ions to be reduced, heading to the production of AgNPs [50]. Not all phytonutrients discovered in plant extracts can reduce other active chemicals after Ag<sup>+</sup> ion reduction. Only a few phytonutrients are linked to removing other substances [49].

Nevertheless, only flavonoids were found to be involved in the production of AgNPs [51]. This subject of study is challenged by the broad diversity of phytonutrients found in

various plants participating in AgNP production. Phytoconstituents that act as organic reducing agents are found in many plants, potentially affecting bacterial and cancer cells (Fig. 3) [52].



**Figure 3.** Plant production of AgNPs, and their applications on bacterial and cancer cells.

## 5. Antibacterial Properties

One of AgNPs' biological features that have been carefully explored is antimicrobial activity [53], which has a wide range of antibacterial characteristics. AgNPs can penetrate bacterial cell walls, triggering changes in cell membrane structure and potentially cell death [54]. Anti-proliferative activity is the most effective in a minimal inhibitory concentration (MIC) experiment, as evaluated by bacterial growth inhibition on plate cultures. Plate agar, liquid LB medium, or a migration assay is commonly used to test Gram-positive and Gram-negative bacteria. Adding alcoholic petal extract of *Rosa indica* or AgNO<sub>3</sub> solutions to MIC assays against various bacterial strains and human pathogenic bacteria like *Streptococcus mutants* (MTCC-896), *Enterococcus faecalis* (MTCC-439) (Gram-positive), *E. coli* (MTCC-40), and *Klebsiella pneumonia* (MTCC-740) (Gram-negative) significantly reduced [55]. The high absorptivity of AgNPs across the bacterial wall and plasma membranes was hypothesized as the mechanism responsible for reducing proliferative capability seen during MIC testing [55]. The cytotoxic activity of biologically generated NPs was boosted when mixed with AgNO<sub>3</sub> solutions [55].

In contrast, adding Ag<sup>+</sup> ions to the culture medium inhibited the formation of biofilms by bacteria during growth. In biological studies, AgNPs were found to prevent biofilm generation in Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and Gram-negative (*Shigella sonnei* and *Pseudomonas aeruginosa*) bacteria [56]. Other pathogens were suppressed by AgNPs produced from *Artemisia vulgaris* leaf extract, including *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Haemophilus influenzae* [57]. AgNPs act together with plasma membranes, and spreading Ag<sup>+</sup> ions into the cytoplasm disturbed bacterial membrane and mesosome respiratory systems, as well as ion

exchange activities, and prevented sulfur-containing protein synthesis on ribosomes [57]. These action methods demonstrate AgNP's antibacterial properties and suggest they could be used as anti-pathogenic drugs to stop microbes from multiplying (Table 1) [58].

**Table 1.** AgNPs' mechanisms of action compared to bacteria and biofilms are described in detail.

Bacteria	Mechanism of Action	References
<i>Acinetobacter baumannii</i>	Modification of cell wall and cytoplasm.	[59,60]
<i>Enterococcus faecalis</i>	Variation of the cell wall and cytoplasm.	[61,62]
<i>Klebsiella pneumoniae</i>	Alteration of membrane	[63,64]
<i>Listeria monocytogenes</i>	Morphological changes, disconnection of the cytoplasmic membrane from the cell wall, plasmolysis	[65]
<i>Micrococcus luteus</i>	Alteration of membrane	[63]
Nitrifying bacteria	inhibits respiratory activity	[66]
<i>Proteus mirabilis</i>	Change of cell wall and cytoplasm.	[67,68]
<i>Staphylococcus epidermidis</i>	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes destruction, modification of intracellular ATP levels	[69,70]
<i>Salmonella typhi</i>	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes destruction, an adaptation of intracellular ATP levels	[71–73]
<i>Vibrio cholerae</i>	Alteration of membrane permeability and respiration	[71]

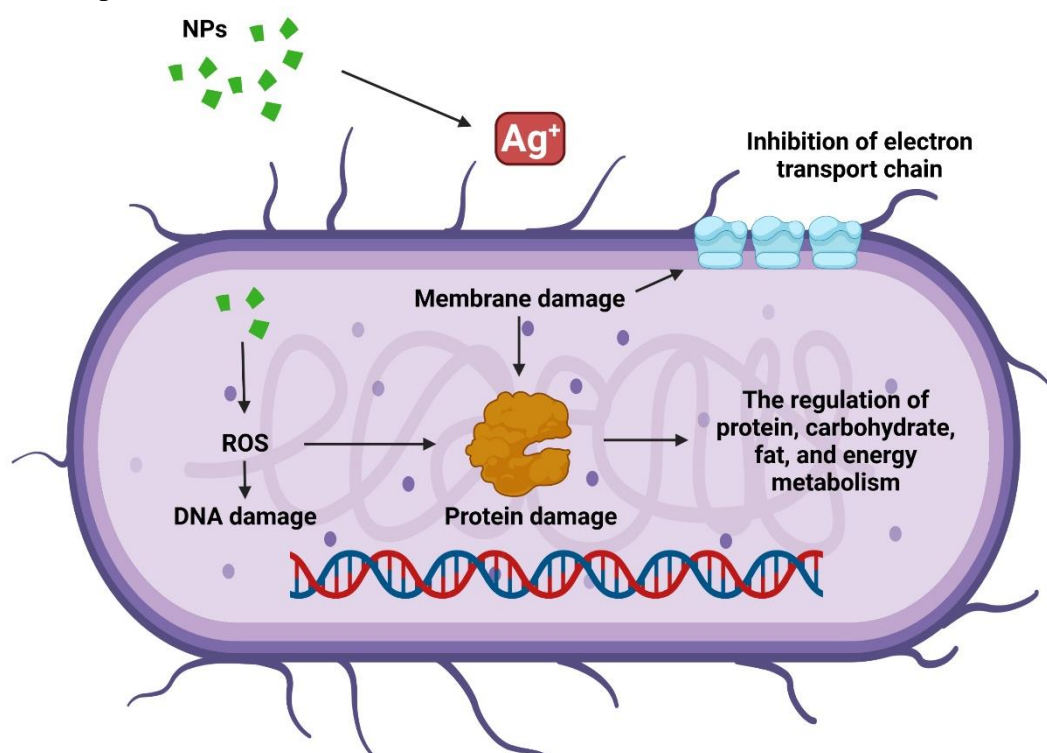
### 5.1. Silver nanoparticles' antibacterial action.

The AgNPs' antibacterial activities have been established compared to numerous Gram-positive and Gram-negative bacteria [74]. Their precise action mode, a growth inhibitory or bactericidal movement, is still unknown. Present investigational evidence supports various strategies considering AgNP's physicochemical properties, causing intracellular components to change directly [75]. The literature now supports three systems [76–78] by which AgNPs exert their antibacterial activity, described together or separately. The first hypothesis proposes that AgNPs act at the membrane level by penetrating the outer membrane and storing it in the inner membrane. Their adhesion to the cell causes membrane destabilization and damage and generates cellular content leakage and demise [79,80]. In addition, AgNPs have been demonstrated to attach to sulfur-containing proteins in bacteria's cell walls, causing structural damage and cell wall rupture. The second mode of action recommends that NPs can not only split and pass the cellular membranes, altering their framework and penetrability but also pass through the membrane, where AgNPs are thought to have an affinity for sulfur or phosphorus groups existing in intracellular content like modifying their structural and functional characteristics. Reactive oxygen species (ROS) and free radicals are formed when ROS and free radicals react with thiol groups in enzymes, causing the apoptotic process. Silver ions spreading from NPs are hypothesized to occur with the other two methods attributable to their size and charge. These can interact with biological structures, affecting metabolic routes, membranes, and genetic material [81–83].

### 5.2. Antibacterial mechanism of biosynthesized AgNPs.

*Olex scandens* leaf extract is a decreasing and stabilizing/capping agent to make AgNPs. The intensity/volume of the reducing agent is affected by the color, shape, size, and stability of AgNPs (here, *Olex scandens* extract). To get the best AgNPs, we ran a series of

reactions in a 5 mL solution with various conc. Of Olax leaves extract (150-750  $\mu\text{L}$ ) and constant attention of  $\text{AgNO}_3$ . This green chemistry approach to AgNP synthesis is a simple, efficient, cost-effective, and environmentally friendly technique that requires no specialist equipment, vacuum, catalyst, or template. Lastly, the response was held according to the typical circumstances in the water (universally accepted solvent). The bacteria were cultured in LB (Luria Bertani) broth media in a shaker at 37 degrees Celsius (spinning at 80 rpm). The growth inhibition of bacteria treated for up to 7 hours with biosynthesized AgNPs was measured using a multimode spectrophotometer (Varioskan). The antibacterial activity of b-Ag-NPs was determined using the spread plate technique dose-dependent (3-30 M) by counting colony-forming units (CFU) (Fig. 4). To evaluate the antibacterial activity of b-Ag-NPs against Gram-negative *E. coli*, the inhibitory zone was determined using the agar disc diffusion technique. A scanning electron microscope was used to study the interaction of AgNPs with *E. coli* (Hitachi S-3000 N, Japan) [84].



**Figure 4.** Silver nanoparticles' mechanisms of action in bacterial cells.

The antibacterial potential of AgNPs was tested using the usual disk distribution technique versus five distinct foodborne bacteria (*B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 49444, *E. coli* ATCC 43890, and *S. typhimurium* ATCC 43174) [85]. The bacteria were acquired from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) and preserved on nutrient agar media (Difco, Becton, Dickinson and Company, Sparks Glencoe, MD, USA). AgNPs were colloiddally dissolved in 5% dimethyl sulfoxide (DMSO, 1000 g/mL) and sonicated for 15 minutes at 30°C before usage. Filter paper disks with 50 g of AgNPs per disk were utilized in the experiment. Positive controls included five g/disk of common antibiotics, kanamycin, and rifampicin, whereas the negative control was 5% DMSO. Overnight cultures of examined bacteria were diluted to  $10^7$  colony-forming units for the assay. The thickness of inhibition zones was defined following 24 hours of incubation at 37°C to try the antibacterial activity of the AgNPs. The MIC and least bactericidal concentration of AgNPs were determined using a two-fold serial dilution technique. Several



concentrations of AgNPs (100–3.12 g/mL) were used for the MIC test. The pathogen was then introduced to each tube in a ten  $\mu$ L volume. This strategy was used to investigate all of the pathogens. The tubes were combined well and incubated overnight at 37°C in a shaker incubator. The MIC was defined by finding the lowest possible concentration of AgNPs that did not induce test organisms to proliferate. The MIC and higher concentrations were then spread out on nutrient agar (NA) plates and incubated for 24 hours at 37°C [86,87].

### 5.3 AgNPs' antibacterial applications in healthcare.

Health and medicine are the industries that have invested the most in NP-based antibacterial technology research and development [88–90]. Because of their biocompatibility and simplicity of functionalization, AgNPs can be utilized to impart bactericidal capabilities to a wide range of products. One of the most common applications of AgNPs is facial masks to improve their defensive qualities. In under 24 hours, Y. Li *et al.* [91] silver nitrate and titanium dioxide NPs were used to create a face mask that reduced *E. coli* by up to 100%. In a twin study, a commonly produced cover was processed with AgNPs solutions at 50 and 100 ppm, leading to masks with integrated NPs that suppressed *E. coli* and *S. aureus* development [92]. These findings are fascinating because AgNPs-enhanced face masks could help prevent diseases in hospitals, where dangerous bacteria are frequent. A recent study explored the possibility of disinfecting surgical masks with a disinfectant containing AgNPs as an active ingredient [93].

Catheters coated with AgNPs were also studied to determine whether they help prevent infection and maintain sterility. According to a study, catheters containing NPs prevented *E. coli*, *S. aureus*, and *Candida albicans* from growing and creating biofilms for at least 72 hours. They dramatically delayed the *S. aureus* CFU growth of the *P. aeruginosa* strain. According to this study, utilizing coated catheters for up to 10 days in animal models had no harmful consequences. The researchers discovered that up to 84 percent of the NP coating remained covered during the test. This technology could increase the antibacterial activity and safety of NP-coated catheters [94]. Wu *et al.* [95] polydopamine-based AgNPs were used to adorn catheters with AgNPs. In viability studies employing MC3T3 E1 cells, the yielding exhibited efficient antibacterial effectiveness against *S. aureus* and good biocompatibility at defined doses (osteoblastic cell line from mouse).

Researchers proposed a catheter encapsulated with AgNPs that demonstrate an antibacterial effect against harmful microbes that induce urinary infections, particularly *Bacillus sp.*, *E. coli*, and *C. albicans*, using an environmentally friendly alternative to generate these NPs [96]. These discoveries pave the path for improved catheters that can prevent infections caused by the device's long-term use in hospitalized patients. To avoid undesirable consequences, more studies into biocompatible doses and AgNP release rates from catheters. Nonetheless, economically viable products, including ON-Q Silver Soaker<sup>TM</sup>, SilverlineR, and AgTive, which use a variety of impregnated catheters, are now available in various nations. Silver and its NPs have been studied for their healing and antibacterial properties, resulting in several wound dressings that are both healing and antibacterial. Tian *et al.* [97] used an AgNP solution to treat superficial wounds and found that it assisted in wound healing and skin regeneration. AgNPs' regulatory action on pro-inflammatory cytokines is suggested to cause this effect. AgNPs are also recommended to help the regeneration process move along faster by lowering the duration of inflammation [98]. Investigating this property led to a topical AgNP solution for cutaneous lesions such as burns. The result was an antimicrobial mixture with no

harmful side effects and could accelerate wound healing [99]. It's also being examined if AgNPs coupled with organic compounds have more vital antibacterial characteristics. For example, a hydrogel containing AgNPs produced in situ was created utilizing lignin and polyvinyl alcohol.

After 10 hours of treatment, the produced product has outstanding antibacterial activity against *E. coli* and *S. aureus*, killing practically all germs. This formulation showed good biocompatibility in studies with L929 cells (a murine fibroblast cell line) [100]. A methanolic seed extract of *Pongamia pinnata* was employed in another study to create a hydrophilic AgNPs gel. After 18 days in animal models, the wound in the gel-treated group was more minor than in the non-treated group after 30 days. Meanwhile, in vitro tests on hazardous bacteria revealed that the gel might stop *E. coli* and *P. aeruginosa* from growing [101]. The products generated in the experiments above show a more substantial capability to enhance healing and speed skin regeneration in damaged regions, revealing the exceptional abilities of AgNPs in wound products. These findings demonstrate that AgNPs contribute antibacterial effects to the last goods and support using AgNPs in hydrogels, catheters, and medical fabrics [75].

## 6. Anti-cancer Properties of Silver Nanoparticles

Bactericides, chemotherapeutic drugs, and AgNPs are effective. Double-strand DNA breakage, oxidative stress, and chromosomal instability can cause cell death. Although larger particles (~100 nm) have a more substantial influence than smaller particles (~10 nm), smaller particles are more cytotoxic because they may quickly penetrate the cell and localize to the nucleus. Silver nanoparticles can also affect the function of p-glycoprotein, which is responsible for drug transport across the cell membrane. As a result, increased or decreased activity of this protein promotes multidrug resistance by removing or blocking medicines from entering the cell [102].

### 6.1. Anti-cancer Activity of AgNPs.

Despite considerable side effects and systemic toxicity, various anti-cancer medicines are currently on the market as medicinal alternatives to administer cancer death rates [103]. The discovery of new therapeutic and anti-cancer materials to battle malignant tumors is exciting in nanomedicine [104,105]. Nanobiotechnology-based cancer therapies are precise since they reduce invasive [106,107]. Metallic NPs are an exciting stage for possible cancer diagnostics and treatment attributable to their distinctive qualities of high diffusion and objective specificity for diagnostics and therapies [108,109]. Peptides, monoclonal antibodies, DNA/RNA, and/or tumor markers could be attached to metallic NPs to target cancer cell surface proteins or receptors [110,111]. AgNPs have thus demonstrated potential anti-cancer action in vivo [112]. Combination cancer therapy reduces chemotherapy side effects, lowers effective doses, and induces cellular self-protection from harmful substances [113]. Mixtures of new medications and NPs with previously recognized chemicals are still being researched for many elements of traditional therapy. The search for more effective drug administration protocols leads to modifying existing techniques and pharmacological agents in combination with natural, unconventional substances. Metal-based AgNPs have shown novel applicability in photodynamic treatment [114,115] in a range of cancer cell lines [116], such as squamous carcinoma SCC-25 cells [117]. In squamous carcinoma cells, the alkaloid berberine was studied as an anti-proliferative and pro-apoptotic agent alone [118,119] or combined with

AgNPs, which boosted its anti-cancer activities [117]. AgNPs have long been employed as an aseptic or preservative agent because of their antibacterial qualities. Still, they can also be used to create novel nanomaterials with potential applications in regenerative medication [120]. Metal NPs, for example, should be thoroughly studied for various applications, especially as they are easily administered by living organisms [58].

### 6.2. Anti-cancer mechanism of biosynthesized AgNPs.

Their size and shape can define AgNPs' cytotoxicity. Human lung epithelial A549 cells were reported to have probable deadly effects on AgNPs with 100–160 nm diameters, lengths of 1.5–25  $\mu$ m, and spherical shapes (30 nm) [31]. NPs are protected at low concentrations but can be dangerous at higher doses. Cell inhibition typically rises dose-dependent when exposed to varying NP concentrations. AgNPs have different dose effects in various formulations, affecting cytotoxicity or boosting anti-cancer potential. Numerous investigations have demonstrated that biosynthetic AgNPs have anti-cancer effects [121].

On the other hand, biosynthesized AgNPs are cytotoxic in some cases [122,123]. Angiogenesis is the development of different blood vessels from the present ones. Angiogenesis can result in wound treatment and the production of granulation tissue [124]. According to Folkman, forming new blood vessels causes solid tumor growth [125]. Both activator and inhibitor molecules influence this mechanism, which is crucial for cancer development and dissemination. Rendering to this notion, blood flow is required for tumor expansion. The new blood arteries support tumor development by supplying cancer cells with oxygen and nutrients, allowing them to penetrate and spread throughout the body [126]. Because of their ability to inhibit angiogenesis, several AgNP methods have been used to treat cancer [127].

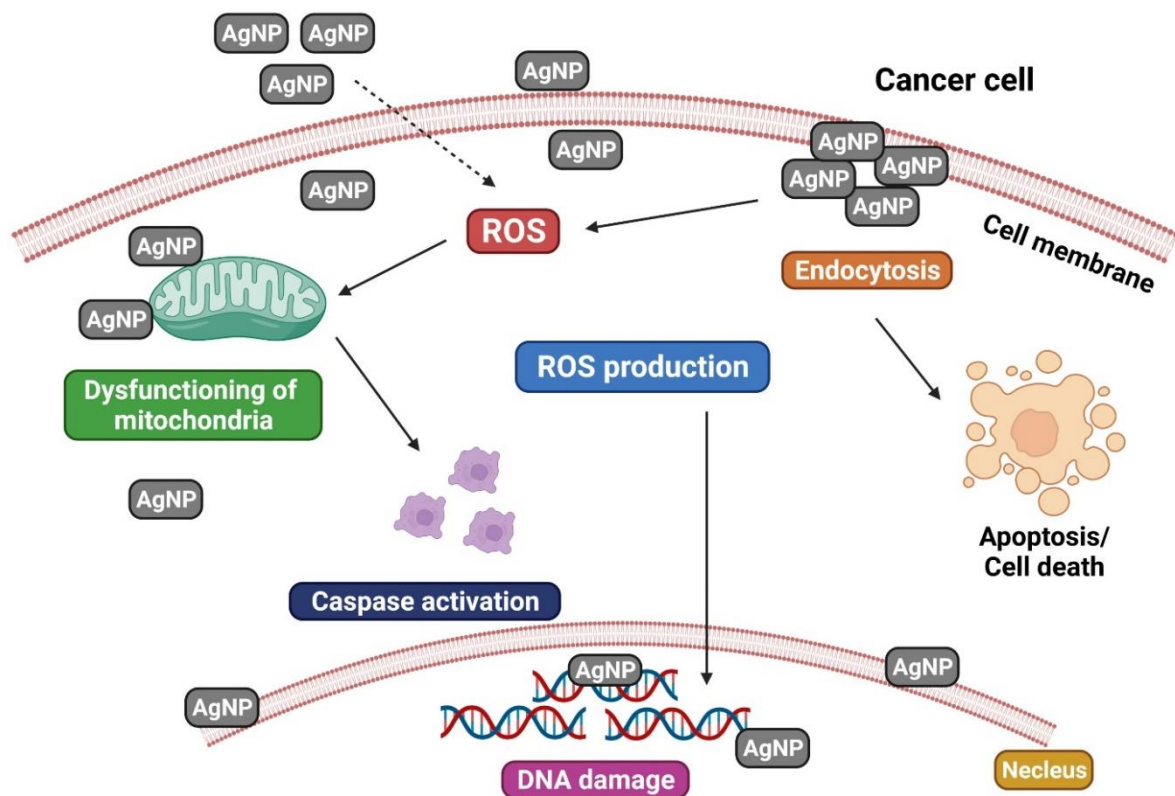


Figure 5: Mechanistic role of silver nanoparticles in cancer cells.

The cell cycle is a complicated sequence of signaling channels that divide, reproduce, and expand. This phenomenon is significant in the progression of cancer. However, this regulatory mechanism fails in cancer due to genetic abnormalities, resulting in uncontrolled cell development (Fig. 5). The main checkpoints for cell cycle arrest are DNA synthesis (S), Gap2/mitosis (G2/M), Gap1 (G0/G1), and subG1. Green-synthesized AgNPs have been proven to halt the cell cycle in the sub-G1 phase in multiple investigations. Chang *et al.* [128,129] discovered that curcumin-treated cancer cells were in the sub-G1 stage, explaining the link between apoptosis and malignant cells in the sub-G1 phase. Another study found a relationship between increasing cancer cell populations in the sub-G1 phase and caspase-3 production, a pro-apoptotic protease [130].

### 6.3. Phyto-nanotechnology and cancer.

In contrast to current cancer treatment procedures, new emerging technology uses cost-effective medicinal agents derived from natural resources, such as plants [131]. The use of medicinal plants has opened up new avenues and possibilities in the treatment of cancer. They provide new compounds through phytochemical screening and aid in developing innovative cancer treatments, such as the green production of AgNPs [132]. There has been a hypothesis modification in cancer therapy procedures and approaches recently. Improvement in medicinal plant research and nanotechnology has resulted in several innovative cancer therapy techniques [133]. The convergence of nanotechnology and plants for green nanoparticle manufacturing has piqued the interest of cancer investigators [134,135]. These green may overcome the limitations and problems of standard diagnosis and treatment methods produced by nanoparticles [136].

Cancer cells differ from typical healthful cells in blood vessel proliferation (angiogenesis), reduced capillary permeability, and the lymphatic drainage system. The changed microenvironment of malignant cells offers a platform for nanotechnologists to develop a suitable nano-drug with a selective advantage for precisely targeting cancer cells [137]. Presently, research studies focus on producing anti-cancer medications and developing a tool to target cancer cells precisely and accurately with optimum drug concentration and fewer side effects [138]. Cancer nanobiotechnology promises to enhance cancer recognition, diagnosis, and therapy [139]. AgNPs are currently being studied extensively for cancer diagnosis and treatment. Greenly manufactured AgNPs with phytochemical coatings have higher biological activity than chemically synthesized AgNPs. Besides their potential utility in cancer treatment, green-manufactured AgNPs have been found to have antibacterial, antileishmanial, antiviral, wound treatment, directed drug delivery, antitubercular, and antidiabetic properties [140,141].

Plant-mediated AgNPs have been shown to have anti-cancer properties in various cancer cell lines [84,142,143]. Nanoparticles have a site-specific and targeted effect that improves therapeutic efficacy by moving across impermeable membranes and combating immune system responses, making them ideal for cancer treatment [144]. Green AgNPs are effective against various cancers, including breast cancer, colon adenocarcinoma, Ehrlich ascites carcinoma, and liver cancer. Applying green-produced AgNPs at different intensities shows the remarkable anti-cancer potential against lung, liver, cervical, and carcinoma cancer. However, more research is required into the anti-cancer apparatus of green-produced AgNPs. In human HCT116 colon cancer cells, AgNPs produced from mint extract showed significant activity. These green-synthesized AgNPs slowed cell division in the G1 phase, indicating that

AgNPs can regulate the cell cycle and limit proliferation [145]. AgNPs synthesized from *Piper nigrum* leaf extract have been demonstrated effective against MCF-7 and Hella cancer cell lines [145]. When green-produced AgNPs were applied to MCF-7, they showed a change in morphological characteristics.

The integrity of the plasma membrane was significantly disrupted, and cell proliferation was inhibited. Furthermore, AgNPs treatment caused cytoplasm shrinkage and cell aggregation compared to healthy cells. Anti-cancer activity of AgNPs made from *Prosopis cineraria* and *Coriandrum sativum* against MCF-7 cancer cells was found [146]. Greenly produced AgNPs have demonstrated promising toxicity against A549 (human lung carcinoma cell) compared to non-cancerous lung cells, indicating that AgNPs can cause toxicity in target cells. This site-specific toxicity could be owing to cancer cells' acidic pH [147]. Zureberek *et al.* also found evidence that AgNPs' captivating ability affects cell viability. They looked into the state of respiration in AgNPs-induced oxidative stress. Because glucose is a critical energy source for cancer cells, researchers linked glucose availability to nanoparticle toxicity. The generation of H<sub>2</sub>O<sub>2</sub> by AgNPs application was found in a dose-dependent manner [148].

Furthermore, AgNPs caused less toxicity in cells with low glucose availability than in cells with high glucose accessibility. This conclusion recommended a lack of glucose, controlling ROS building and AgNP-induced toxicity [149]. AgNPs have a significant tumor-killing action in osteosarcoma cancer cells with a tumor-suppressive deficit. According to Kovacs *et al.*, AgNPs caused apoptosis in U2Os (wild-type p53) and SOAS-2 cells with p53 loss, indicating that they have the chemotherapeutic possible [150]. In a relative investigation, AgNPs produced from extracts of 30 medicinal plants showed significant cytotoxicity against lung cancer cells. Incubation of AgNPs in culture conditions with bovine serum albumin resulted in increased toxicity because it standardized protein corona exchanges [151]. AgNPs made from tamarind fruit shells caused programmed cell death in human breast cancer cells. Increased ROS production developed in mitochondrial impairment and DNA disturbance, which led to anti-cancer activity in a dose-dependent manner [152]. AgNPs made from *Nepeta deflersiana* were found to have high cytotoxicity against human cervical cancer cells in another study. AgNPs caused oxidative stress, which resulted in mitochondrial dysfunction and cell cycle arrest, exposing it to tumor cell death [153]. AgNPs derived from lotus extract were cytotoxic to cancer cells from the liver, stomach, and prostate [154]. AgNPs made from *Crataegus microphylla* fruit also significantly harmed gastric cancer cells [155]. Kanipandian and Kannan observed that the experience nano-silver produced from cotton leaf caused lung cancer cells to die in a mitochondrial-dependent manner [156].

Separately from their influences on cellular and subcellular constructions, AgNPs also decrease tumor angiogenesis, which is responsible for altered growth factor production and endothelial cell relocation and production [157]. AgNPs inhibited the transcription of hypoxia-inducible factor-1 (HIF-1) and promoted the expression of vascular endothelial growth factor-A (VEGF-A) in breast cancer, causing apoptosis. AgNPs have been shown to have an antiangiogenic effect by preventing tube formation in normal endothelial cells [158]. Similarly, when AgNPs are inoculated in the chorioallantoic membrane, they increase the production of caspase-3 and caspase-8 genes, leading to cellular death. AgNPs made from red amaranth decreased the number and length of blood vessels in breast cancer cells and showed cytotoxicity [159]. Besides their outstanding anti-cancer properties, considerable attention was paid to creating and testing innovative silver-based nanotechnology for enhanced chemotherapy and radiation. A conducted research in this regard examined the photothermal-induced cytotoxicity

of silver-gold nanoparticles coated with dopamine and subjected to near-infrared radiation against colon cancer cells. Nanoparticles promoted photothermal treatment (PTT) via apoptotic and necrotic pathways, according to an *in vitro* and *in vivo* investigation [160].

He *et al.* studied aggregation-induced emissions in multifunctional core-shell nanosystems and molecules based on AgNPs cores. The radiotherapy was greatly improved, and the PTT and photoacoustic impact were adjusted, thanks to this complicated nanosystem. They were also more prominent in tomography imaging as contrast and fluorescent agents [161]. Doxorubicin, a chemotherapeutic medication, is incorporated into silver/magnetite nanoparticles with a PEG layer and folic acid. Doxorubicin has been shown to have remarkable potential for the PTT of cervical cancer. Aside from their chemotherapeutic and photothermal effects, this nanosystem also demonstrated excellent differentiation abilities for cancer cells and imaging properties due to fluorescence and magnetic resonance [162]. Laser irradiation of a nanosystem based on Methotrexate coupled graphene oxide (GO) and AgNPs resulted in a dual chemotherapeutic and photothermal effect, producing high cytotoxicity against malignant cells [163]. Similar findings were obtained using 5-fluorouracil-loaded silver-gold nanoparticles covered with mesoporous SiO<sub>2</sub> [164,165].

#### 6.4. Application of anti-cancer AgNPs in healthcare.

Endothelial cells, IMR-90 lung fibroblasts, U251 glioblastoma cells, and MDA-MB-231 breast cancer cells have been thoroughly investigated for their anti-cancer potential [165,166]. AgNPs are effective delivery systems for anti-tumor drugs [167]. AgNPs address these issues by reducing side effects and improving cancer treatment efficacy. One of their differentiating attributes is their capability to bypass multiple biological obstacles and deliver drugs to areas. Combining green AgNP production and targeted anti-cancer medicine delivery to tumor tissues offers a potential cancer therapy method. AgNPs are plasmonic structures with a high capacity for scattering and absorbing light in specific locations. AgNPs-derived scattered photons can be employed for imaging after selective absorption into cancerous cells. Silver has long been used in dentistry as a component of amalgam fillings, which repair missing teeth. Although AgNPs are effective in dentistry, their toxicity in biological systems renders them unsuitable choices [168]. The malaria parasite (*Plasmodium falciparum*) and its vector have been proven susceptible to AgNPs [169].

In oral, cutaneous, and inhalational exposures, AgNP bioavailability is low, although it varies depending on particle size, dose, and soluble fraction. Park and colleagues [170] reported that rats' bioavailability was 1.2 and 4.2 percent after a single oral dosage of citrate-coated 7.9 nm AgNPs at 1 and 10 mg/kg, respectively. AgNPs can be ionized in the stomach after oral exposure to yield Ag<sup>+</sup>. The short stomach residence duration (10–240 minutes) contributes to disintegration. The complex produces H<sup>+</sup> and GS-Ag, eventually converted into Ag-GSH complexes, present during the body. On the other hand, the biliary system is the main route for AgNPs to be eliminated [171,172].

## 7. Silver Nanoparticle Synthesis' Biological Methods Advantages

In prior investigations, microorganisms, including algae, bacteria, yeast, and fungi, were employed to produce AgNPs. Numerous plant extracts have appeared as intriguing new assets for manufacturing non-toxic and safe NPs [173,174]. Biosynthesis utilizes less energy and has a lower environmental impact than typical chemical synthesis procedures.

Furthermore, because of the superior specificity of biomolecules participating in the biosynthesis manner, precise control of AgNP size and form may be possible, which is critical for optimal applications. Plants are a more sustainable and renewable source of AgNP than microorganisms, requiring costly microbial culture maintenance and more time for synthesis. Plant extracts make AgNPs, which offer several advantages, including being inexpensive, energy-efficient, and cost-efficient; generating more robust work environments and populations; and conserving human health and the ecosystem with less waste and better goods [47].

Furthermore, a previous study has discovered that plant extracts are more capable of bio-reduction than microbial cultures [175]. Based on the type of bacteria utilized in manufacturing, waste products from the microbial-based approach are expected to damage the environment. As a result, pollution from plant-mediated synthesis has a lower impact on the environment. The biosynthetic process using plant extracts is a viable methodology and an excellent substitute for standard chemical and physical NP production and microbial methods due to the above advantages and superior features over other approaches [176].

## **8. Concluding Remarks and Future Perspectives**

AgNPs are currently widely employed as a potent antimicrobial with excellent antibacterial characteristics. They answer several requirements for novel antimicrobial technologies to be effective, including antibacterial performance, quick action, and low cytotoxicity [177]. To avoid exposing microorganisms to sublethal NP doses, NPs used against bacteria must be regulated and monitored—the most significant advantages of combining NPs and antibiotics. AgNPs reduce the number of antibiotics and NPs needed to offer effective antibacterial action against a wide range of bacteria while lowering the risk of adverse effects [178]. Finally, they have antibacterial activity against various bacteria [74,179]. However, a comprehensive study on biocompatibility of living species should be conducted. Multiple effects seen after treatment with AgNPs suggest that metal-based NPs could be helpful in bio-nanotechnology, molecular medicine, and anti-cancer therapy. Because AgNPs affect the cell cycle, prevent cancer cell propagation, create oxidative stress, and trigger programmed cell death, they are potential anti-cancer therapeutics (apoptosis). They also defend against bacterial, fungal, and viral infections. Antimicrobial protection is desirable during chemo- and radiation because cancer patients' immune systems are compromised. In conclusion, more AgNPs research is required for novel discoveries and better AgNP properties.

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### **Conflicts of Interest**

The authors declare no conflict of interest.

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