

# Antioxidant and Antibacterial Activity of Potato Peel Derived Bimetallic Ag/Cu Nanoparticles

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**Abstract:** The application of plant extracts in the biogenic synthesis of nanoparticles has gained recognition for its cost-effective and environmental features and its potential for large-scale manufacturing. Among metallic nanoparticles, silver and copper are highly efficient against various pathogens but exceptionally biocompatible and easy to incorporate into medicinal applications. This investigation focuses on the use of *Solanum tuberosum* peel extract (commonly known as potato peel), a commonly generated domestic biowaste that acts as both a reducing agent and a capping agent in the formation of AgCu bimetallic nanoparticles. UV–visible spectrometry unveiled a distinct peak for AgCu nanoparticles at 234 nm and 384 nm. Subsequently, synthesized nanoparticles were employed to assess antioxidant scavenging, dye degrading, and antibacterial properties. Findings demonstrated heightened antioxidant efficacy, 4-nitrophenol dye degradation, and antibacterial activity against *Streptococcus anginosus*, *Bacillus subtilis*, and *Escherichia coli*. AgCu nanoparticles exhibited an elevated scavenging activity of 78.54% at 500 µg/mL. Maximum degradation for 4-nitrophenol dye, reaching 94.36%, occurred in 5 hours. Notably, *B. subtilis* displayed the largest inhibition zone (12mm) at a nanoparticle concentration of 1000 µg/mL. Consequently, this research underscores an inventive strategy for transforming routinely produced bio waste into a functional material, exemplified by AgCu bimetallic nanoparticles, offering substantial antioxidant, antibacterial, and catalytic properties.

**Keywords:** biowaste utilization; bimetallic; antioxidant; catalytic activity; antibacterial.

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## 1. Introduction

Nanoparticles are those particles that have a size ranging from 1 to 100 nm, emphasizing their significant potential in several sectors, including environmental and medicine. This study focuses on modifying matter at the atomic and molecular levels [1]. Nanoparticles exhibit distinct characteristics, exhibiting enhanced chemical and biological activity, enzymatic reactivity, permeability, catalytic behavior, and quantum features compared to bigger particles of the same composition [2]. Significantly, at the nanoscale, there exists the ability to influence and alter molecular structures [3]. With less need for chemical reagents,

precursors, and catalysts, biosynthesis of nanoparticles is an eco-friendly and sustainable technique. Biological agents include a variety of secondary metabolites that are thought to be reducing, capping, and stabilizing agents, such as flavonoids, terpenoids, enzymes, alkaloids, antioxidants, fungal metabolites, and algal compounds [4]. Metallic nanoparticles, such as monometallic, bimetallic, and trimetallic nanoparticles, have garnered interest for their significant biological capabilities and distinctive characteristics related to size, shape, and distribution variations. Due to their tiny size, Nanoparticles have a greater surface-to-volume ratio, leading to improved biological efficiency [5-7]. There is an increasing demand to create eco-friendly techniques for producing nanoparticles, using harmless substances and sustainable resources to minimize adverse consequences in medicinal uses. Scientists are employing eco-friendly techniques to synthesize silver nanoparticles by utilizing biological substances [8]. A study by Dilbar *in silico*. [9] synthesized silver nanoparticles (Sn-AgNPs) derived from medicinal *Salvia nubicola*, which had effective antibacterial activity against *R. solanacearum*, the causative agent of tomato bacterial wilt. These methods also enable the production of bimetallic nanoparticles consisting of silver and other metals. Naturally occurring plants will undoubtedly yield the added benefit of stable metal nanoparticles [10]. Plant phytochemicals being included in nanoparticle manufacturing exemplifies a novel connection between plant science and nanotechnology.

Bimetallic nanoparticles are composed of two different metallic elements, exhibiting a combination of characteristics derived from the presence of each unique metal. These nanoparticles may exhibit unique properties not present in the individual metals, making them very fascinating for use in catalysis, biomedical sciences, biosensors, and optoelectronics. The unique characteristics of bimetallic nanoparticles may be easily modified by changing the ratio of the two metals in their composition. Remarkably, the overall qualities and results of combining two monometallic nanoparticles to make a bimetallic nanoparticle were astounding. This was especially evident in the study of Idris *in silico*. [11] a combination of iron oxide (FeO) and silver (Ag), demonstrated more than 63.87% antioxidant activity at a concentration of 1000 µg/mL. Similarly, more than 85% of methyl red, 93% of phenol red, and 78% of eosin yellow dyes were degraded in one hour by the catalytic activity demonstrated by silver-zinc oxide (Ag-Zn) bimetallic nanoparticles, which was synthesized using domestic waste of kiwi peel extract [12]. Bimetallic or trimetallic nanoparticles can be produced via chemical, biological, or physical processes. The physical approach has the benefit of keeping the chemical composition constant, but the drawback is that the particle size varies. On the other hand, chemical synthesis, although useful in generating the intended nanoparticles, typically uses chemicals in the manufacturing process that, if released into the environment, may be hazardous to human and animal health [13]. In our previous investigation [14], we utilized biowaste from potato peels as an alternative source because of its recyclability, reusability, and affordability for the formation of silver nanoparticles, revealing a distinctive AgNP peak at 471 nm possessing a potential antimicrobial action.

With the growing global interest in household trash creation, there is a heightened emphasis on studies on food waste recovery. Municipal garbage derived from plants and vegetables, such as plant bark and vegetable peels, frequently has a higher concentration of bioactive compounds, such as phenolics, compared to edible parts. This suggests the capacity to extract several bioactive compounds from it. The present work is centered on the biosynthetic production of AgCu nanoparticles and their applications. Plant-based composites are used to create stable synthetic AgCu nanoparticles, which are regarded as eco-friendly due

to their minimum utilization of hazardous chemicals [15-17]. Potatoes (*Solanum tuberosum*), a widely eaten crop, generate substantial waste in the form of their skins. Potato peels, which have traditionally been utilized as animal feed, possess a range of chemicals that exhibit antibacterial, antiseptic, anti-inflammatory, and antioxidant activities. Gallic acid, a phenolic acid present in the skin of potatoes, is one of the bioactive compounds responsible for its antioxidant properties [18]. The primary chemical constituents responsible for the antibacterial effects of potato peel extract are caffeic acid, chlorogenic acid, and neochlorogenic acid [19]. Moreover, the substantial concentration of lipids and other bioactive constituents in potatoes facilitates the production of eco-friendly nanoparticles for human use [20]. In this study, the bioreduction approach for the synthesis of stable AgCu bimetallic NPs using aqueous potato peel extract was explored with a prime focus on comparing and studying applications of nanoparticles in antioxidant, antibacterial, and catalytic activity. Moreover, it may pave the way for further research based on these findings.

## 2. Materials and Methods

### 2.1. Chemicals required.

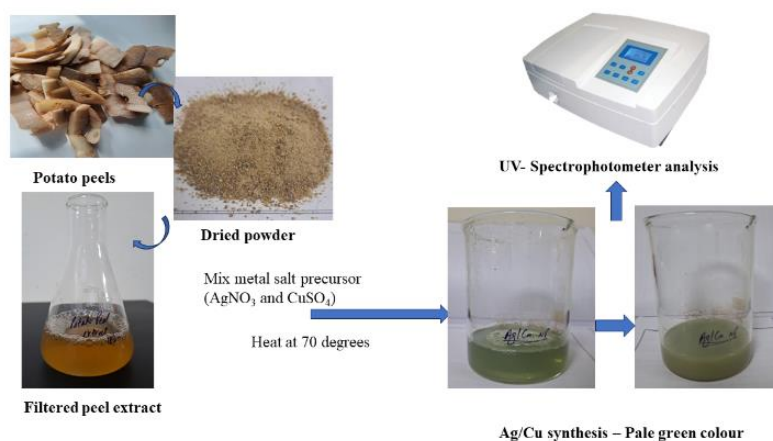
Potato, peel extract, silver nitrate ( $\text{AgNO}_3$ ), and copper sulfate ( $\text{CuSO}_4$ ) are used as metal bases. Sodium borohydride ( $\text{NaBH}_4$ ), ascorbic acid, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), ethanol, and 4-nitrophenol dye, double distilled water.

### 2.2. Peel extract preparation.

The potatoes were acquired from a market in Delhi, India, and were cleansed meticulously using deionized distilled water. The peels were thereafter sliced with a peeler and cleansed with a detergent and double-distilled water. Subsequently, they were positioned on a piece of paper and subjected to a hot-air oven for 30 minutes to decrease their moisture content. The peel undergoes a process of becoming fragile and may be effortlessly pulverized using a blender. The peel extraction was conducted per the previously published work [21]. A quantity of 5 grams of peel powder was measured and combined with 100 mL of distilled water. The mixture was then subjected to boiling for a duration of 30 minutes in a hot water bath maintained at a temperature of  $70^\circ\text{C}$ . The extract was filtered using Whatman Filter Paper No. 1. The provided sample comprised bioactive chemicals that functioned as reducing and stabilizing agents. A comprehensive phytochemical investigation was conducted to identify the specific bioactive compounds found in the extract derived from the peel.

### 2.3. Fabrication of Ag/Cu nanoparticles.

AgCu nanoparticles were synthesized using a methodology similar to the one described by Idris *in silico*. [22], with few modifications. First, we mixed solutions of  $\text{AgNO}_3$  and  $\text{CuSO}_4$ , both at a concentration of 5mM. Then, in a ratio of 1:1, we added potato peel extract solution to salt solution in equal amounts while warming it up to  $80^\circ\text{C}$ . Through successive co-reduction of silver nanoparticles and then copper nanoparticles, it was performed. In about 30 minutes, we noticed the change in color from light greenish to pale green (Figure 1).



**Figure 1.** Ag-Cu bimetallic nanoparticles synthesis.

#### 2.4. UV analysis.

The preliminary UV–vis analysis was conducted within the 200-700 nm wavelength range using a Shimadzu UV-1800 spectrophotometer manufactured in Japan. The resulting absorbance spectrum exhibits absorbance values shown on the y-axis in proportion to the relevant wavelengths on the x-axis.

#### 2.5. DPPH scavenging activity.

Antioxidant properties of the bimetallic nanoparticles were evaluated by the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test, employing L-ascorbic acid as the reference standard for comparison. The DPPH reagent was prepared by dissolving 11.8 mg of DPPH in ethanol to create a solution with a concentration of 0.3 mM (100 mL). Simultaneously, a solution of ascorbic acid at a concentration of 1 mg/mL was generated by dissolving 100 mg of ascorbic acid in 100 mL of ethanol. The solution was subsequently diluted to various concentrations (10, 50, 100, 150, 200, 500  $\mu\text{g}/\text{mL}$ ) for the test. Simultaneously, different quantities (10, 50, 100, 150, 200, 500  $\mu\text{g}/\text{mL}$ ) of the synthesized nanoparticles were also produced in ethanol. 3 mL of the normal ascorbic acid solution and the solution of synthesized bimetallic nanoparticles were each combined with 1 mL of the DPPH solution in separate containers. The combination was subjected to a 30-minute incubation period in the absence of light. The solutions were incubated, and then their absorbance was measured at a wavelength of 517 nm using a spectrophotometer. A positive control was created by diluting 1 mL of DPPH with ethanol to a total volume of 4 mL. The percentage of inhibition was determined using a standard DPPH activity formula.

#### 2.6. Dye degradation activity.

In order to assess the efficacy of Ag-Cu bimetallic nanoparticles as catalysts, an experiment was performed in an aqueous environment to reduce and decompose 4-nitrophenol (4-NP) dye. Using a conventional procedure, a solution containing 30 mM NaBH<sub>4</sub>, 1mg/mL Ag/Cu NP, and 1 mM 4-NP dye. 1 mL of NaBH<sub>4</sub> was combined with 1 ml of the 4-NP stock solution in separate tubes. The capacity increased to 9 ml with the addition of 7 mL of water. Subsequently, 1 mL of Ag/Cu was added to achieve a final volume of 10 mL. Spectrophotometer measurements (200-600 nm) in a room-light environment after a duration of 5 hours were conducted. The decline in peak signifies the catalytic efficiency of the

nanoparticles. The percentage of degradation of the dye solution was determined by measuring the absorbance values obtained at the maximum wavelength of 400nm for 4-nitrophenol, both before and after the catalytic degradation process.

### 2.7. Antibacterial activity.

The agar-well diffusion method was utilized to assess the antibacterial characteristics of the biosynthesized Ag/Cu NPs by examining three cultures. A combined volume of 500 mL of N.A. and N.B. was prepared, with 100 mL of N.B. being necessary for bacterial growth. Bacterial cultures were transferred from stocks stored at 4°C using a loop and incubated overnight in a shaker incubator at 37°C and 800 rpm. 100 µL of each bacterial culture was evenly spread onto nutrient agar plates using sterile swabs. This study utilized a solution containing Ag/Cu nanoparticles at a concentration of 1000 µg/mL, which was added to the wells on the bacterial spread plates using the agar-well diffusion method. After being incubated overnight at a temperature of 37°C, the areas where the growth of bacteria was inhibited were measured in mm. The positive control comprised streptomycin, whereas the negative control comprised a plant extract and a metal salt solution. The experiment was conducted three times for each pathogen.

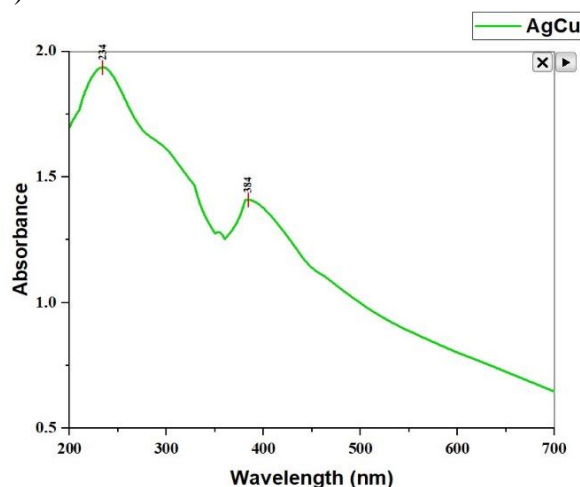
### 2.8. Statistical analysis.

Each experiment was replicated thrice, presenting the findings as the mean value plus or minus the standard deviation. Significance from a statistical perspective was acknowledged at a threshold of ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. UV-Vis evaluation.

Upon adding the plant extract to Ag and Cu salts and subsequent heating at a temperature of 70°C, the color of the combination underwent a transformation from light brown to off-white. The color of the synthesized AgCu NPs was used to characterize the surface plasmon resonance (SPR).



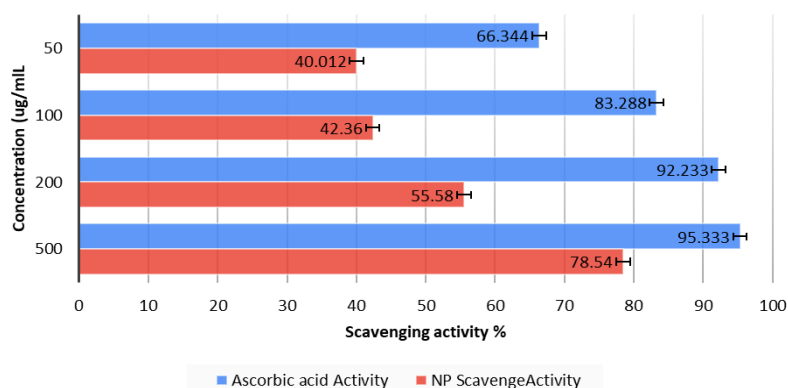
**Figure 2.** UV analysis of AgCu bimetallic nanoparticles.

Spectrum data was collected using a UV-Vis spectrophotometer, which indicated the conversion of Ag and Cu ions into silver and copper nanoparticles by reduction. AgNPs

exhibited absorption within the wavelength range of 350–400 nm, whereas CuNPs displayed absorption within the 200-300 nm range. AgCu nanoparticles were observed to display absorption peaks at wavelengths of 234 nm and 384 nm (Figure 2). The UV-Vis spectra have verified the presence of two peaks that fall within the wavelength range of AgCu nanoparticles that were previously reported [22]. The peak shows the presence of excited free electrons in the metal during the synthesis, which corresponds to surface plasmon resonance (SPR).

### 3.2. Antioxidant activity.

Oxygen-reactive species, which are oxygen-containing molecular entities, demonstrate exceptional reactivity and possess the capability to exist autonomously. Inherent to the human body, they are produced by metabolic processes (oxidation) or exposure to radiation. These molecular entities provide a potential hazard for causing harm to cells, DNA, and proteins. Contrary to popular misconception, free radicals are not necessarily detrimental; they can sometimes have beneficial functions. Conversely, antioxidants are compounds that can prevent damage done by free radicals to cells and other biological components. This occurs when the antioxidant transfers an electron to the free radicals, neutralizing and stabilizing them, thereby preventing possible harm to biological and cellular constituents. This process is referred to as antioxidant activity. Dietary supplements, spices, herbs, and fortified meals contain natural and manufactured antioxidants, such as vitamins E and C, beta-carotene, and polyphenols. Prior research [23,24] reports the use of bimetallic nanoparticles as antioxidants. The use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis is commonly acknowledged as a means to evaluate the antioxidant capacity of a material. DPPH, a persistent radical with a profound violet color, undergoes a color alteration upon exposure to an antioxidant. This transformation occurs when the antioxidant transfers an electron, counteracting the free radical and resulting in the disappearance of the violet color. The extent of the color alteration serves as an indicator of the antioxidant efficacy of the drug. The experiment is usually performed using a spectrophotometer set at a wavelength of 517 nm. It detects the absorbance of the sample, and a decrease in absorbance indicates the scavenging activity of the antioxidant.



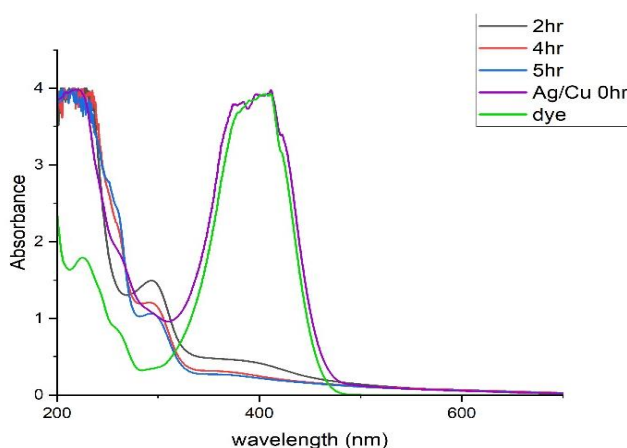
**Figure 3.** Antioxidant activity of AgCu nanoparticles.

This study aimed to evaluate the antioxidant capacity of AgCu bimetallic nanoparticles synthesized using green methods and compare it to the scavenging ability of the standard antioxidant, ascorbic acid. The bimetallic nanoparticles composed of silver (Ag) and copper (Cu) exhibited enhanced DPPH scavenging activity, which was shown to increase proportionally with the concentration of the nanoparticles. As a result, these nanoparticles exhibited an increased scavenging activity of 78.54% at a concentration of 500µg/mL (Figure 3). Prior research has recorded the antioxidant capabilities of AgCu bimetallic nanoparticles,

as reported by Alavi *in silico*. [25]. The scavenging activity of Ag nanoparticles and Cu nanoparticles was found to be 37% and 31%, respectively, at a concentration of 500  $\mu\text{g/mL}$ . In contrast, Idris *in silico*. Documented the production of Ag-CuO bimetallic nanoparticles that exhibited a scavenging activity of 63.87% at a concentration of 500  $\mu\text{g/mL}$  [22].

### 3.3. Dye degradation activity.

Dyes are colored compounds used to give vibrant and attractive colors to various materials, including paper, plastic, and textiles. They are commonly derived from living organisms or artificially produced. Nevertheless, the extensive use of this industry leads to a significant release of waste into the ecosystem, which presents potential risks to both the environment and human health due to the challenges in breaking down some dyes and their propensity to cause cancer. Hence, there are apprehensions over these colors' disposal and ecological consequences. Current efforts are concentrated on developing strong, long-lasting, and eco-friendly methods for treating and breaking down dyes to reduce their impact on the environment. Biogenic nanoparticles have a distinct function in this particular scenario. Bimetallic nanoparticles have been utilized as nano-catalysts in the presence of  $\text{NaBH}_4$  to break down and disassemble 4-nitrophenol. The increased surface area of bimetallic nanoparticles is responsible for aiding the breakdown and electron transfer from  $\text{NaBH}_4$  to the dye molecule, resulting in reduction and degradation. It is important to emphasize that  $\text{NaBH}_4$  alone cannot effectively degrade dyes because of differences in redox potential [26,27]. Bimetallic nanoparticles, known for their significant surface area, are considered the primary contenders for nano-catalysts in degrading dyes [28]. The effectiveness of the AgCu nano-catalyst in breaking down the 4-nitrophenol dye when  $\text{NaBH}_4$  was present was evaluated (Figure 4). The AgCu bimetallic nanoparticles obtained a degradation rate of 90.79% for 4-nitrophenol, converting it to 4-aminophenol during a 5 hours timeframe when  $\text{NaBH}_4$  was present. Nazir *in silico*. Synthesized Ag-Cu bimetallic nanoparticles using *Seidlitzia stocksii* stem and investigated their photocatalytic efficiency in degrading methylene blue [29]. The nanoparticles achieved a 44% degradation of methylene blue after 70 minutes of exposure to light. In a study by Alam *in silico*., Co-Mn nanoparticles were employed as an adsorbent for methyl violet adsorption under optimized conditions: pH 9, adsorbent dosage of 0.01 g, and 30 minutes of contact time [30]. In another investigation by Baste *in silico*. [31], methylene blue dye was remarkably degraded by 94.43% when Ag@CuO NPs were at a greater concentration, i.e., 20 mg/100 mL.



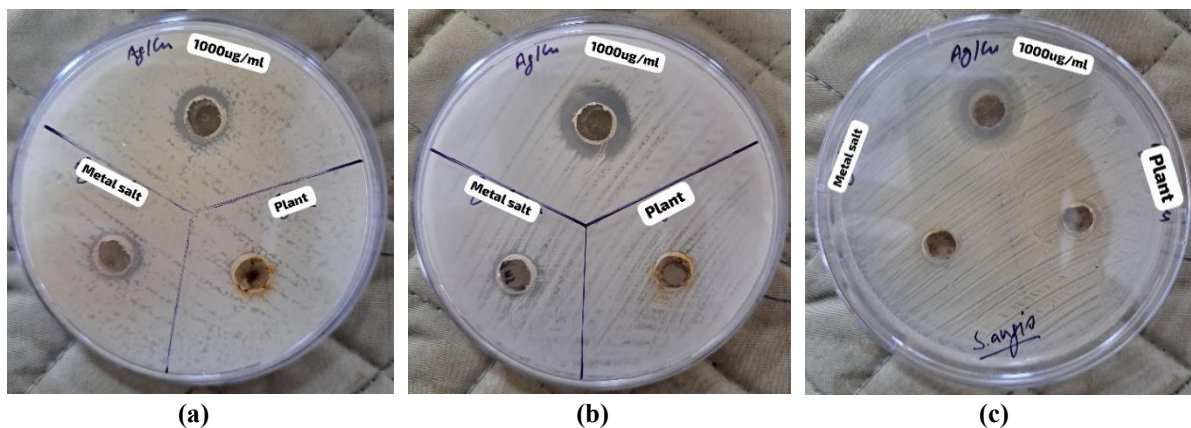
**Figure 4.** 4-Nitrophenol degradation of Ag/Cu nanoparticles.

3.4. Antibacterial activity.

Bimetallic nanoparticles have attracted a lot of attention because of their special synergistic effects and enhanced antibacterial properties [32], offering a novel approach to treating antimicrobial resistance [33,34]. The antimicrobial effectiveness of AgCu bimetallic nanoparticles, synthesized by biological means, was evaluated against two gram-positive bacteria, specifically *S. angiosus* and *B. subtilis*, as well as one gram-negative bacteria, *E. coli*. The antibacterial efficacy was assessed by applying the agar well diffusion technique, utilizing varied doses of AgCu nanoparticles against various bacterial strains. A solution containing silver and copper ions in water produced a dispersed circular pattern on all types of bacteria. The AgCu bimetallic nanoparticles showed notable superiority in inhibiting *B. subtilis* (12 mm) compared to *S.angiosus* (10 mm) and *E. coli* (6 mm) (Table 1 and Figure 5). Furthermore, the bactericidal impact of streptomycin was demonstrated, with a range of 28-32 mm, against the bacterial pathogens. *Citrus sinensis* peel extract-derived silver nanoparticles were found to have potential antibacterial activity, and the range of inhibition zone was 7-16 mm against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [35]. Another investigation examined the antibacterial efficacy of Ag–Cu obtained from the leaf extract of *Alternanthera sessilis* (Linn.) against *E. coli*, *B. subtilis*, and *S. aureus* [36]. The largest area of inhibition was reported against *B. subtilis*, measuring precisely at 12 mm. Thirumoorthy *in silico*. [37] used the good diffusion method to demonstrate the green-produced Ag–Cu NPs' strong antibacterial activity against gram-positive (*S. aureus*) and gram-negative (*P. aeruginosa*) pathogens.

**Table 1.** Antimicrobial properties of silver/copper nanoparticles.

Pathogen	Zone of inhibition (mm)			Concentration of Ag/Cu NPs 1000 µg/ml
	Streptomycin (Positive control)	Ag/Cu salt 1mM (negative)	Potato peel extract (negative)	
	<i>Streptococcus anginosus</i>	30 ± 0.577	0	0
<i>Bacillus subtilis</i>	32 ± 0.252	4 ± 0.342	0	12 ± 0.321
<i>Escherichia coli</i>	28 ± 0.572	3 ± 0.523	0	6 ± 0.125



**Figure 5.** Antibacterial zone of inhibition activity of AgCu nanoparticles (a) *Escherichia coli*; (b) *Bacillus subtilis*; (c) *Streptococcus anginosus*.

4. Conclusions

Employing environmentally friendly techniques for nanoparticle synthesis mitigates the release of harmful by-products into the surroundings. Additionally, utilizing waste materials of organic origin can reduce the cost of producing nanoparticles, offering a dual benefit of cost-efficiency and environmental remediation. The pursuit of creating nanoparticles using biological waste proved to be promising. In this paper, potato peel extract was harnessed as a

dual-functioning agent, acting as both the reducer and capping agent in forming AgCu bimetallic nanoparticles. These synthesized nanoparticles showcased notable activities in antioxidant, antibacterial, and catalytic domains. AgCu NPs exhibited an impressive scavenging activity of 78.54% at a 500 µg/ml concentration. The most significant antibacterial impact was observed against *B. subtilis*, coupled with 94.36% dye degradation capabilities against 4-nitrophenol in 5 hours. This research underscores the environmentally friendly biosynthesis of AgCu nanoparticles and its versatile applications in diverse fields.

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## Conflicts of Interest

The authors declare no conflict of interest.

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