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Therapeutic evaluation of plant-mediated Ag/Cu nanoparticles: Insights into antioxidant, cytotoxic, and genotoxic responses

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This study investigates the biological potential of *Seidlitzia stocksii*-mediated Ag/Cu bimetallic nanoparticles (NPs), synthesized using plant extract as a natural reducing and stabilizing agent. The aqueous stem extract of *S. stocksii* (SSAE) was evaluated for its phytochemical composition, revealing a total phenolic content (TPC) of 47.91 mg GAE/g and a total flavonoid content (TFC) of 40.78 mg QE/g at the highest tested concentration. The biosynthesized Ag/Cu bimetallic nanoparticles were assessed for their antioxidant, antibacterial, and membrane-stabilizing potential. The NPs were further evaluated for their anti-cancerous potential against HepG2 carcinoma cells, while their mutagenic properties were examined using the Ames test. Antioxidant analysis demonstrated a dose-dependent increase in activity, with DPPH radical scavenging reaching 47.32% and reducing power measured at 38.76%. Antibacterial efficacy was tested against *Escherichia coli* and *Bacillus subtilis*, showing a concentration-dependent increase in the zone of inhibition (ZOI). Among the tested strains, *E. coli* showed the highest susceptibility, with a ZOI ranging from 12 to 23 mm. Hemolytic analysis revealed that erythrocyte lysis remained below the ASTM threshold, with 4.76% hemolysis observed at 120 mg/mL. Cytotoxic evaluation via MTT assays on HepG2 cells indicated 86.87% cell viability, supporting NPs' potential anticancer activity. Genotoxicity assessment using *S. typhimurium* strains TA98 and TA100 revealed no significant increase in revertant colony count, confirming the non-mutagenic nature of NPs. Collectively, these findings indicate that *S. stocksii*-mediated Ag/Cu-NPs exhibit multifunctional biological activities, establishing these NPs as safe, biocompatible agents with antioxidant, antibacterial, and anticancer potential. Future mechanistic and in vivo studies are needed to validate these findings and support their practical application.

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1. Introduction

The growing demand for novel therapeutic agents has accelerated the advancement of nanotechnology within biomedical sciences [1, 2]. Among the diverse range of nanomaterials, metal-based nanoparticles, particularly silver (Ag) and copper (Cu)- have attracted significant attention for their potent antimicrobial, antioxidant, and anticancer properties. Bimetallic nanoparticles, Ag-Cu composites, exhibit enhanced synergistic biological activities over their monometallic counterparts, attributable to improved surface reactivity, redox behavior, and physicochemical stability [3]. These nanomaterials can interact with biomolecules at the cellular level, making them promising candidates for pharmaceutical and biomedical applications including targeted drug delivery, cancer therapy, and antibacterial treatments [4].

Conventional chemical and physical methods for NPs synthesis frequently involve toxic reagents, high energy consumption, and the generation of hazardous by-products, raising environmental and safety concerns. These drawbacks have driven growing interest in sustainable alternatives. One such approach is plant-mediated green synthesis, which employs bioactive phytochemicals as inherent reducing and stabilizing agents, enabling the production of NPs through an environmentally benign and non-toxic process [5]. Bioactive constituents, including phenolics, alkaloids, flavonoids and terpenoids are crucial in facilitating the reduction of metal ions and stabilizing the resulting NPs. In recent years, numerous medicinal plants have been explored for their ability to mediate NPs synthesis, providing a safe and economical and efficient alternative to conventional fabrication methods [6].

Seidlitzia stocksii, a halophytic species with a long history of use in traditional medicine, contains abundant bioactive components such as polyphenols and flavonoids, known for their strong antioxidant and anti-inflammatory activities. Despite its recognized ethnopharmacological value, the application of *S. stocksii* in nanobiotechnology has received minimal scientific attention. The plant-based components offer a dual benefit by enabling NP synthesis while imparting intrinsic biological activities to the resulting nanomaterials. Employing *S. stocksii* extract for the green synthesis of Ag/Cu bimetallic NPs represents an innovative strategy for creating multifunctional nanotherapeutics with enhanced biocompatibility and reduced toxicity [7].

Biological assessment of green-synthesized Ag/Cu bimetallic NPs yielded encouraging results across multiple assays. Their antioxidant performance, evidenced by DPPH and FRAP analyses, indicates potent free radical scavenging capacity, which can be ascribed to synergistic effects of bimetallic composition and the phytochemical capping layer. Furthermore, their pronounced antibacterial capacity against both Gram-positive and Gram-negative bacterial strains highlights their potential in addressing microbial infections, a critical need in the context of rising antibiotic resistance [8]. Cytotoxicity assays on HepG2 liver cancer cells also demonstrate considerable anticancer activity, further highlighting the relevance of these NPs in oncological therapeutics [9].

An essential consideration in evaluating nanomaterials for biomedical use is their hemocompatibility and genotoxic potential. In this study, the hemolysis assay revealed negligible erythrocyte damage, remaining well within accepted safety limits and confirming the biocompatibility of synthesized NPs. Additionally, the Ames test verified the non-mutagenic nature of synthesized NPs, reinforcing their suitability for biomedical applications [10]. Overall, the combination of green synthesis and bimetallic NPs design offers a promising pathway towards creating multifunctional agents that combine therapeutic efficacy with environmental sustainability.

The primary aim of the study was to synthesize and characterize Ag/Cu bimetallic NPs using SSAE via a green synthesis method, and to investigate their multifunctional biological properties. The evaluation focused on their antioxidant, antibacterial, cytotoxic, and hemocompatibility profiles, as well as their genotoxic safety through the Ames mutagenicity assay. Ultimately, the research sought to demonstrate the biomedical potential and biocompatibility of these plant-mediated bimetallic NPs as promising candidates for therapeutic applications. The schematic of this investigation is presented in Fig. 1.

2. Methodology

2.1. Chemicals and reagents

Analytical-grade chemicals and reagents, including $AgNO_3$, $Cu(NO_3)_2 \cdot 3H_2O$, Folin–Ciocalteu reagent (FCR), Na_2CO_3, $AlCl_3$, quercetin 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeCl_3$, 2,4,6-Tripyridyl-S-triazine (TPTZ), CH_3COONa buffer, were nutrient agar media employed in this current project. Standard bacterial strains *Escherichia coli* (ATCC: 8739) and *Bacillus subtilis* (ATCC: 6633) were utilized for antibacterial analysis. Phosphate-buffered saline (PBS), Triton X-100, HepG2 cell line, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, trypsin, cisplatin and MTT reagent were also used. *Salmonella typhimurium* strains (TA98 and TA100), sodium azide (NaN_3), potassium dichromate ($K_2Cr_2O_7$), Vogel-Bonner minimal agar and deionized water were utilized.

2.2. Extract preparation

The *Seidlitzia stocksii* stem was collected from the District Karak, Khyber Pakhtunkhwa. After thorough washing with distilled water, it was subjected to drying under shade. The dried material was ground to get a fine powder. This fine powder (15 g) was mixed with deionized water (300 mL) and placed on an orbital shaker for continuous shaking for 8-12 h. The resulting mixture was allowed to be filtered to separate the insoluble residue. The crude aqueous extract was preserved at 4°C for further experimental investigations. The preparation of *Seidlitzia stocksii* mediated Ag/Cu bimetallic NPs has already been reported by our research group [5].

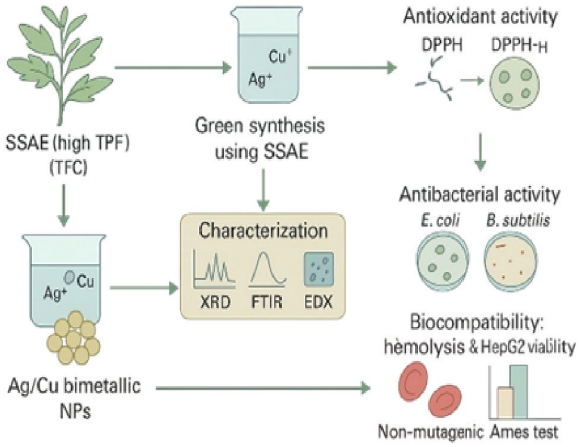

Fig. 1. Schematic for the green synthesis of Ag/Cu nanoparticles and applications.

2.3. Assessment of TPC and TFC

The TPC and TFC of SSAE were evaluated at various concentrations: 30, 60, 90, and 120 mg/mL. The TPC was quantified following the described method [11]. Briefly, FCR (500 µL) was allowed to interact with the sample stock solution (300 µL) and Na_2CO_3 (800 mM, 1 mL) to place it on an orbital shaker for 2 hours, followed by incubation to measure its absorbance at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800). Gallic acid was processed along with samples to express the results of TPC as mg of gallic acid equivalent (GAE/g of dry weight). The TFC was evaluated using the aluminum chloride assay following the method as described in the literature [12]. Samples and quercetin were processed under a similar set of experimental conditions and their absorbance was recorded at 510 nm to express the results of TFC as mg of quercetin equivalent (QE/gram of dry weight).

2.4. DPPH radical scavenging analysis

The antioxidant capacity of SSAE-mediated Ag/Cu NP was assessed by performing an in vitro assay of DPPH radical scavenging as described in literature [13]**.** The test sample (50 µL) from the stock solution was subjected to interact with 5 ml of 0.004% methanolic DPPH solution to incubate it for 30 min under dark conditions, followed by measuring the absorbance at 517 nm to represent DPPH scavenging potential using Eq. 1, where A_0 and D represented the absorbance of the control and sample.

$$\text{DPPH scavenging (\%)} = \frac{A_0 - \text{D}}{A_0} \times 100 \quad (1)$$

2.5. FRAP analysis

The FRAP analysis was carried out to assess the antioxidant capacity of SSAE-mediated Ag/Cu NPs following the protocol as described in the literature [14]. In this analysis, FRAP reagent (285 µL) was subjected to interact with each test sample (15 µL) at varying concentrations (30-120 mg/mL) to incubate for 20-30 min at 37 °C, followed by measuring absorbance at 593 nm to determine its reducing power by employing Eq 2. Ascorbic acid at 10 µg/ml was used as a control for this analysis. A_0 and F represented the absorbance of the control and test sample.

$$\text{FRP (\%)} = \frac{A_0 - \text{F}}{A_0} \times 100 \quad (2)$$

2.6. Antibacterial activity

The antibacterial activity of SSAE-mediated Ag/Cu NPs was evaluated against two bacterial strains including *Escherichia coli* (ATCC: 8739) and *Bacillus subtilis* (ATCC: 6633), following the method [15]. In this analysis, a freshly grown bacterial culture of 1.2×10^8 CFU/mL was used. For each sample, 30 mL of sterilized nutrient agar was poured into sterile Petri dishes, where spreading of the bacterial strain (0.1 mL) was carried out. Then, 100 µL of test sample from stock solution (30-120 mg/mL) was poured into 5 mm wells of nutrient agar media to incubate those 37°C for 24-36 hours. The zone of inhibition (ZOI) in mm was measured to express their antibacterial ability.

2.7. Membrane stabilization potential by hemolytic activity

The biocompatibility of SSAE-mediated Ag/Cu NPs was analyzed according to the methodology [16] with minor changes. In this analysis, heparinized containers were used to collect blood, and these were rinsed thoroughly with PBS of pH 7.4, following centrifugation at 4000 rpm for 5 min. The wash step was repeated 3-5 times using PBS to remove non-erythrocytic constituents. The resultant RBCs were suspended in the 20 mL of chilled PBS. The RBCs were counted under a microscope and maintained at a standardized concentration of 7.068 x 108 cells/mL. Then, RBC suspension (180 µL) was subjected to interaction with 20 µL of the sample (30-120 mg/mL), followed by incubation at 37°C with continuous agitation. After gentle mixing, centrifugation was performed at 1310xg to collect the supernatant (100 µL), followed by its dilution with PBS (900 µL), and measuring its absorbance at 576 nm. Negative and positive controls were also run along with the samples. Eq. 3 was employed for the percentage lysis of erythrocytes, where "A_s" denoted the absorbance of the sample.

$$\text{Lysis of erythrocyte (\%)} = \frac{A_S}{A_{\,triton\ X-100}} \times 100 \quad (3)$$

2.8. Cell viability analysis by MTT assay

The anticancer potential of SSAE-mediated Ag/Cu NPs was evaluated against hepatocarcinoma cell line HepG2 (RRID: CVCL_0027; ATCC, HB-8065, Manassas, VA, USA). These cell lines were maintained on DMEM media, where penicillin-streptomycin (1%) and FBS (10%) were supplemented, followed by incubation under CO_2 (5%) blanket at 37°C overnight. The morphology and proliferation were inspected using an inverted microscope. Trypsin was employed to harvest $HepG_2$ cells, followed by centrifugation at 360 rpm for 5 minutes, and then resuspended in fresh medium. Cell density was determined using a hemocytometer. Then, these cells (5×10^4)

were inserted into each well of the 96-well plate, followed by incubation. After 16 hours of seeding, the sample (50 µL) was supplemented from their stocks (30-120 mg/mL) and set to incubation for 48 hr, followed by the addition of MTT reagent (25 µL) to each well and further incubation to evaluate cytotoxicity. Formazan crystals were dissolved using 100 µL of solubilizing agent, and ELISA plate readers were used to record absorbance at 570 nm. Cisplatin (5 µL/well) served as the positive control, while wells containing only cells and growth medium were used as the negative control [17].

2.9. Mutagenicity analysis

The mutagenicity of SSAE-mediated Ag/Cu NPs was performed by using the reverse mutation test. In this analysis, fresh bacterial cultures were prepared according to standard pre-incubation assays for two mutant strains of *S. typhimurium*, strains TA98 and TA100. Firstly, 20 mL of Vogel-Bonner minimal agar medium (freshly prepared) was poured into petri plates and kept until it solidified. Subsequently, molten agar (2mL) was taken and mixed with the extract (100 µL) from the stock solution (30-120 mg/mL) in sterilized test tubes. Following this, 100 µL of the mutant *Salmonella* strains TA98 and TA100 were evenly spread onto the respective reaction mixtures. The mixtures were thoroughly mixed and immediately placed over agar plates. Blank control plates were prepared in parallel. For positive controls, 5 mg/plate of potassium dichromate ($K_2Cr_2O_7$) was used for the TA98 strain, and sodium azide (NaN_3) for TA100. The obtained findings were represented after revertant colony counts [18].

3. Results

3.1. Characterization and properties of the NPs

The synthesis and properties of Ag–Cu NPs prepared using SSAE have already been reported by our research group [5]. Briefly, XRD analysis confirmed the formation of bimetallic Ag–Cu nanoparticles, exhibiting distinct diffraction peaks corresponding to the crystalline phases of both silver and copper. These binary-phase signals align with face-centered cubic (FFC) crystal structures typical of Ag and Cu, indicating successful alloy formation or intimate coexistence of both metals at the nanoscale. FTIR spectroscopy detected characteristic absorption bands attributable to phytochemical moieties derived from SSAE. These included signals associated with hydroxyl, carbonyl, and aromatic functional groups typical of phenolics and flavonoids. The presence of these functional groups suggests reduced metal ions are capped and stabilized by bioactive compounds from the extract, aiding in both nanoparticle formation and colloidal stability. The EDX analysis confirmed the presence of both silver and copper within the NPs, validating their bimetallic composition. The spectra displayed strong signals for Ag and Cu, with minimal interference from impurities, demonstrating high elemental purity and efficient metal incorporation during green synthesis. The SEM analysis revealed predominantly spherical or slightly aggregated NPs morphology. The SEM images suggested relatively uniform particle shapes with moderate aggregation, likely due to interaction between metal cores and capping phytochemicals. Such clustering is commonly observed in plant-mediated syntheses, where organic coatings mediate interparticle interactions.

3.2. Total phenolic and flavonoid contents

Flavonoids are ubiquitous polyphenolic compounds distributed widely in the cellular and surface regions of plant tissues. These biologically active constituents have been reported to be present in abundance in plant matrices [19]. The antioxidant potential is correlated with the chemical structure of phenols, specifically the number and position of OH groups in them [20]. The results of TPC analysis of SSAE at a concentration range of 30-120 mg/mL revealed a dose-dependent trend. The highest TPC, 47.91 mg GAE/g DW, was recorded at 120 mg/mL. The TFC analysis revealed a similar trend, with 21.87 mg QE/g DW at 30 mg/mL, increasing gradually to 40.78 mg QE/g DW at 120 mg/mL (Figure 2). The presence of phenolic and flavonoid content underscores its efficacy in mitigating the stress induced by the oxidation triggered by free radicals. These reactive species largely influence biological macromolecules (DNA, protein, carbohydrates, and lipids), leading to

oxidative stress. Intake of natural antioxidants has been linked to lowering the risk of pathologies related to oxidative stress [21].

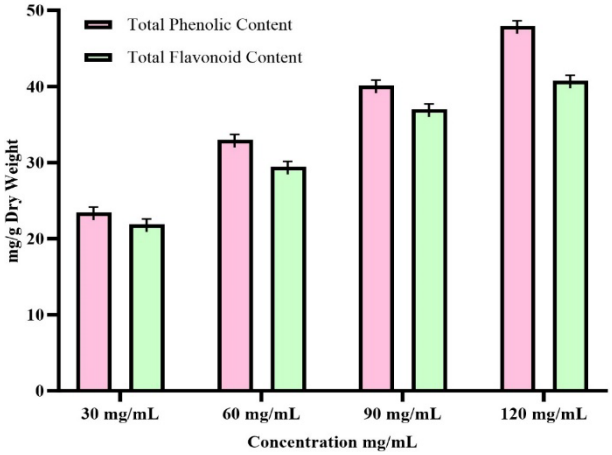

Fig. 2. Total phenolic and total flavonoid contents of Seidlitzia stocksii stem extract.

3.3. Antioxidant analysis

The antioxidant potential of SSAE-mediated Ag/Cu NPs was appraised by assessing the radical scavenging potential and reducing power of NPs. The antioxidant power analysis by DPPH revealed 20.87% inhibition at 30 mg/mL; it surges to 47.32% inhibition at 120 mg/mL. The reducing power analysis revealed 18.98% reducing power at the lowest concentration. While at the highest concentration of the test sample, 38.76% reducing power was recorded (Figure 3). Quercetin and ascorbic acid, which served as controls, exhibited 89.98% inhibition and 91.98% reducing power. The dose-dependent increase suggests potent antioxidant potential in counteracting free radicals. The presence of the Ag/Cu bimetallic compound largely influences its antioxidant potential. It is reported that metal NPs, including Au, Cu, Ag, and Fe, have enhanced antioxidant potential attributed to their high surface area and promising interaction with free radicals [22]. Our findings are supported by [3], citing Ag–Cu bimetallic NPs showed 59.18% radical scavenging potential. Research by [23] hypothesized that bimetallic NPs neutralize DPPH free radicals by donating electrons, confirming their antioxidant activity.

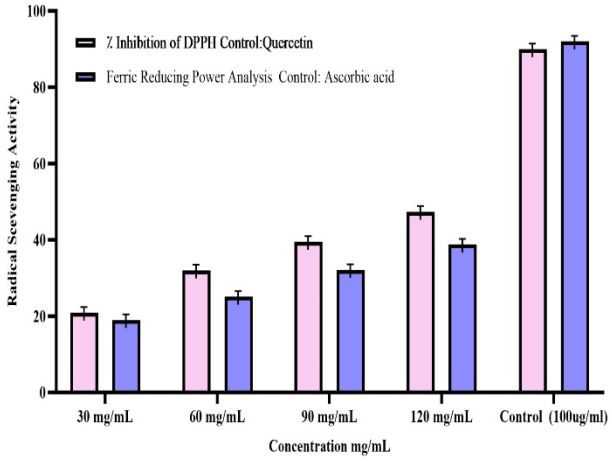

Fig. 3. Antioxidant activity of Seidlitzia stocksii-mediated Ag/Cu NPs.

3.4. Antibacterial activity

The rapid rise in antibacterial resistance against pathogenic bacteria necessitates a revolution in strategy for combating bacterial infection and chronic inflammation. Plant-derived NPs have shown great promise in this regard with enhanced antibacterial activity [8]. The antibacterial activity of SSAE-mediated Ag/Cu NPs was assessed against gram-positive bacterial strains, *Bacillus subtilis* and one gram-negative bacterial strain, *Escherichia coli*. The results revealed that NPs have more potent antibacterial activity against *E. coli* than *B. subtilis*. At a 30 mg/mL concentration, there are 10 mm and 12 mm ZOIs against *B. subtilis,* and *E. coli,* respectively. At 120 mg/mL, the antibacterial becomes more pronounced with 17 mm and 23 mm ZOI, respectively (Table 1). Our findings are in line with the results of the literature [24], where *Salvia officinalis*-mediated Ag-Cu NPs exhibited antibacterial capacity against various gram-positive and gram-negative bacteria, with the ZOI ranging from 9.6 mm to 35 mm. In the biomedical field, Ag NPs are extensively employeddue to their significant antibacterial potential [6]. Due to the biocidal potential of CuO-NPs, it is considered a promising candidate in wound healing. It also acts as a catalyst in various biomedical applications, such as biomedical imaging, cellular and drug delivery, and disease treatment [25].

Table 1. Antibacterial activity of SSAE-mediated Ag/Cu NPs.

SR#	Concentration	*Bacillus subtilis* (ATCC: 6633)	*Escherichia coli* (ATCC:8739)
1	30 mg/mL	10±0.43 mm	12±0.62 mm
2	60 mg/mL	13±0.76 mm	17±0.31 mm
3	90 mg/mL	15±0.57 mm	21±0.42 mm
4	120 mg/mL	17±0.46 mm	23±0.29 mm
5	Ciprofloxacin (1 mg/mL)	31±0.45 mm	33±0.32 mm

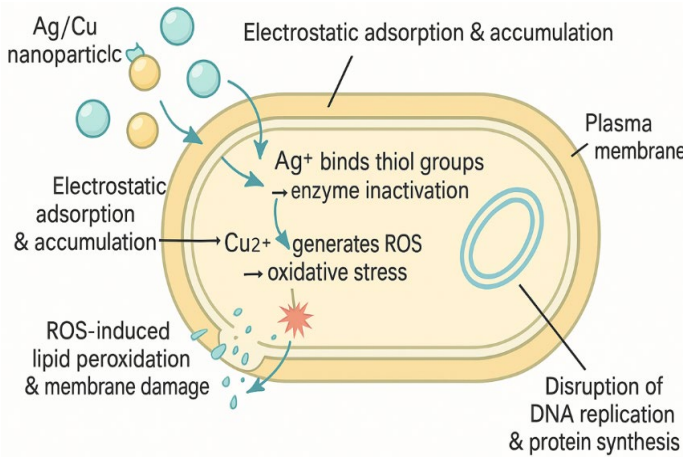

Fig. 2. Antibacterial activity mechanism of Seidlitzia stocksii-mediated Ag/Cu nanoparticles.

3.5. Hemolytic and cell viability analysis

Blood is the primary carrier of any foreign substance that gains entry into the body and carries it to organs, tissues, and erythrocytes. The biocompatibility analysis of the tested NPs is extremely crucial in creating a safety profile [7]. The hemolytic and cell viability analysis on the $HepG_2$ cell line was evaluated at different concentrations of 30, 60, 90, and 120 mg/mL (Table 2).

Results of hemolytic analysis exhibited 2.15% hemolysis at 30 mg/mL; the lysis increased with concentration to 3.67% and 4.76% at 90 and 120 mg/mL concentrations. The cell viability analysis on the $HepG_2$ cell line showed 96.76% cell viability at 30 mg/mL. The cell viability declined to 86.87% at 120 mg/mL. These results underscore the safety and biocompatibility of NPs, and the decrease in cell viability revealstheir anticancer activity. The findings by Babu et al. [4] reported the promising anticancer potential of *Linn*-mediated bimetallic NPs against the human cervical cancer (HeLa) and human lung cancer lines, with cell viability recorded as 90% and 92%, respectively. A similar research by Yang et al.[9] showed anticancer potential of Ag-Cu bimetallic NPs against the human alveolar cancer cell line.

Table 2. Hemolytic activity and cell viability analysis of Seidlitzia stocksii-mediated Ag/Cu NPs.

Sr#	Concentration	Percentage RBC lysis	$HepG_2$ cell viability (%)
1	30 mg/mL	2.15±0.67	96.76 ±1.22
2	60 mg/mL	2.98±0.73	93.01±0.78
3	90 mg/mL	3.67±1.21	88.98±1.32
4	120 mg/mL	4.76±1.13	86.87±1.31
5	Control	[a]100%	[b]16.89±1.42

[a] Triton X-100, [b] Cis-platin (5uL/mL)

3.6. Mutagenicity analysis

The Ames test is a widely employed assay for evaluating mutagenicity, utilizing histidine-dependent *Salmonella typhimurium* strains that carry a defective gene required for histidine biosynthesis [26]. This test is primarily mandated by regulatory agencies, including the FDA, U.S. and EPA, for assessing the mutagenicity of test samples before clinical or practical application [10]. Medicinal plants with potential carcinogenic or genotoxic activity would be detrimental, causing mutation leading to cancer [27]. The mutagenic potential of SSAE-mediated Ag/Cu NPs was evaluated against two *Salmonella* strains, *S. Typhimurium* TA98 & TA100, to notice frameshift mutation and base-pair substitutions, respectively. In all tested concentrations of NPs, the number of revertant colonies remains comparable to the negative control, underscoring no mutagenicity and genotoxicity. For the TA98 strain, the number of revertant colonies ranges from 522 to 543, while the negative control showed 529 revertant colonies, validating no significant increase in revertant colonies. For TA100, the revertant count was 619 to 659, while the negative control showed 773 revertant colonies (Table 3). There is a significant difference between the positive control and the test sample; no concentration-dependent enhancement in revertant colonies was observed, and there is no twofold increase in revertant colonies in the test sample. These results indicate clearly that the tested sample is non-mutagenic in nature. Our findings corroborate [28], citing the non-mutagenic potential of aqueous extract of *Ficus deltoidei* against TA98 & TA100.

Table 3. Mutagenicity evaluation of Seidlitzia stocksii-mediated Ag/Cu NPs.

Sr	Concentration	Mutant strains of *S. Typhimurium* (TA98)	Mutagenicity index (MI)	Mutant strains of *S. Typhimurium* (TA100)	Mutagenicity index (MI)
1	30 mg/mL	522±29	0.98	619±122	0.80
2	60 mg/mL	530±24	1.01	624±139	0.81
3	90 mg/mL	536±32	1.01	649±101	0.84
4	120 mg/mL	543±28	1.03	659±129	0.85
5	Blank (Negative control)	529±23	0.98	773±137	0.80
6	Potassium dichromate ($K_2Cr_2O_7$)	3446±250	6.51	-	
7	Sodium azide (NaN3)	-		4116±230	5.32

MI ≥ 2 is considered to be mutagenic. There is no progressive dose-dependent increase in revertant colonies, and the mutagenicity index shows no 2-fold increase in revertant colonies for the test sample.

4. Discussion

This study highlights the potent biological activities of SSAE-mediated Ag/Cu bimetallic NPs, suggesting their promise as multifunctional therapeutic candidates. Phytochemical profiling of SSAE confirmed a high TPC and TFC, which are believed to be key contributors in the reduction and stabilization process during NPs formation. These metabolites, owing to their intrinsic redox properties, not only drive the bio-reduction of metal ions but also form a phytochemical coating around NPs, therefore, improving their stability and enhancing their biological performance [20]. The antioxidant activity of synthesized NPs is likely due to the electron-donating capacity of the hydroxyl group in phenol combined with the high surface area of the bimetallic structure, which enhances their interaction with ROS [23].

Antioxidant assays validated the free radical scavenging and reducing potential of Ag/Cu NPs, aligning with the literature that highlights the role of transition metals, including Ag and Cu, in enhancing redox cycling and radical neutralization within bimetallic systems. The synergistic interplay between two metals enhances their ability to disrupt oxidative chain reactions through electron or hydrogen atom donation, thereby reducing cellular damage caused by oxidative stress [22]. Additionally, the phytochemical capping layer contributes to antioxidant defense by stabilizing free radicals through resonance, thereby offering a complementary mechanism of action.

The antibacterial activity observed against both Gram-positive and Gram-negative strains can be attributed to complementary bactericidal mechanisms of silver and copper ions. Ag^{+} ions interact with thiol groups in bacterial proteins and enzymes, causing structural denaturation and impairment of essential metabolic processes. In contrast, Cu^{2+} ions undergo Fenton-like reactions that generate OH radicals, which promote lipid peroxidation and compromise membrane integrity [24]. Moreover, the bimetallic configuration is believed to increase membrane permeability, facilitating greater NP penetration into microbial cells and consequently interfering with DNA replication and protein synthesis [6]. The observed dose-dependent enlargement of the ZOI further supports this cumulative antibacterial effect.

The biocompatibility of the synthesized NPs was confirmed through hemolytic and cytotoxicity assays. Hemolysis testing revealed minimal erythrocyte lysis, suggesting favorable membrane compatibility. This effect is likely attributed to the phytochemical coating derived from plant extract, which reduces surface reactivity and limits nonspecific interactions with cell membranes [7]. The MTT cytotoxicity assay demonstrated moderate antiproliferative effects against the HepG2 liver carcinoma cell line, indicating selective cytotoxicity potential. The underlying anticancer activity is likely mediated by the generation of ROS, which induces oxidative stress, resulting in mitochondrial dysfunction, DNA damage, and subsequently apoptosis in cancer cells [9]. The increased surface area of NPs enhances cellular uptake, leading to elevated intercellular ROS generation and activation of programmed cell death pathways.

The Ames test showed no evidence of mutagenicity at any of the tested concentrations. This indicates that phytogenic Ag/Cu NPs do not promote frame-shift mutations or base-pair substitutions in bacterial DNA, supporting their genetic safety. The lack of mutagenic effects may be associated with the natural phytochemical capping agents and the relatively stable interactions of NPs with DNA at non-toxic concentrations [10]. This attribute is particularly important for prospective clinical applications where long-term safety is essential. Overall, the findings demonstrate that SSAE-derived Ag/Cu bimetallic NPs are biologically active, non-toxic, and multifunctional, highlighting their potential in antimicrobial therapy, antioxidant supplementation, and cancer treatment.

4. Conclusions

This study demonstrated the green synthesis of Ag/Cu bimetallic NPs using SSAE, emphasizing their antioxidant, antibacterial and anticancer activities coupled with excellent biocompatibility and non-mutagenic properties. The phytochemicals present in SSAE were of prime importance to reduce and stabilize NPs, enhancing their biological efficacy. The synthesized NPs showed marked free radical scavenging activity, broad-spectrum antibacterial effects and selective cytotoxicity towards cancer cells, while exhibiting minimal toxicity to normal cells. These findings

highlight the potential of SSAE-mediated Ag/Cu NPs as safe and effective candidates for different biomedical applications.

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