Research Article

Cymbopogon proximus Aqueous Extract for Green Synthesis of Silver Nanoparticles: Preparation, Characterization and Bioactivities Assessment

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Abstract | This study aimed to explore the biosynthesis of silver nanoparticles (AgNPs) using *Cymbopogon proximus* (CP) extract; a medicinal plant with a rich phytochemical profile. The AgNPs were produced by mixing water extract of CP with 1 mM silver nitrate solution. The synthesized AgNPs were characterized using UV-Vis spectroscopy, dynamic light scattering (DLS), zeta potential analysis (ZP), and transmission electron microscopy (TEM). The antioxidant activity was determined using several assays, including total antioxidant activity (TAA), total phenolic content (TP), and total flavonoid content (TF). Chemical composition of the aqueous extract was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The antimicrobial activity was evaluated using the disc diffusion method against several bacterial and fungal strains. Cytotoxicity was assessed on Wi38 human lung fibroblast cell line using the MTT assay. A color change and a surface plasmon resonance peak in the UV–Vis spectrum confirmed the production of AgNPs.. The nanoparticles (NP) were spherical, stable, and exhibited an average size of less than 100 nm. GC-MS analysis confirmed the existence of numerous bioactive chemicals, including piperitone, elemol, and β-eudesmol. AgNPs-CP and CP extract unveiled substantial antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*. The highest inhibition zone diameter was observed against *C. albicans* (32 mm). Cytotoxic analysis revealed a dose-dependent response with an IC_{50} value of 240.67 ± 4.2 μg/ mL, indicating moderate cytotoxic potential at higher concentrations. AgNPs synthesized using CP extract demonstrated promising antioxidant, antimicrobial, and cytotoxic properties. The findings of this study support the potential application of the AgNPs-CP as a preservative in food/ feed additives.

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Introduction

Nanotechnology is an interdisciplinary field that has revolutionized modern science and technology by enabling the manipulation of matter at the atomic, molecular, and supra-molecular levels. Among the diverse range of nanomaterials developed over recent decades, metallic nanoparticles

(NPs), particularly AgNPs, or silver NPs -have attracted much interest, because of their distinct physicochemical characteristics and wide range of biological activity. These NPs exhibit a high surface-to-volume ratio, distinctive optical properties, and potent antimicrobial, antioxidant, and anticancer effects, rendering them worthwhile in numerous applications, including drug delivery systems, wound dressings, water purification, and diagnostic tools (Fahim *et al*., 2024).

Conventional AgNPs synthesis methods are hazardous and energy-intensive, posing environmental and health risks. A shift towards greener, sustainable methods, such as using plant extracts, offers cost-effectiveness and environmental friendliness. Green extracts like *Cynara scolymus, Azadirachta indica,* and *Zingiber officinale* have been effectively used for NPs biosynthesis, resulting in various bioactivities (El-Chaghaby *et al*., 2022; Rathod *et al*. 2024; Shahzadi *et al*., 2025).

Green synthesis of NPs using biological agents, especially plant extracts, has emerged as a viable alternative to traditional techniques. Plants come in a range of bioactive compounds such as polyphenols, alkaloids, flavonoids, terpenoids, and proteins that can effectively reduce metal ions to NPs and stabilize them by capping their surfaces. The choice of plant species plays a crucial role in determining the size, shape, and stability of the synthesized NPs, as the nature and concentration of phytochemicals vary widely across different plant types (Awad *et al*., 2024).

Cymbopogon proximus, commonly known as camel grass, is an aromatic perennial grass native to arid and semi-arid regions of Africa and Asia. It is a member of the *Poaceae* family and is well-adapted to extreme environmental conditions, thriving in sandy, rocky, and nutrient-poor soils. In Egypt, *C. proximus* is found in several regions, including the Sinai Peninsula, the eastern desert, and the western desert, where it plays a role in both natural ecosystems and traditional practices (Abdel-Moatamed, 2024). Traditionally, *C. proximus* has been used in Egyptian and North African herbal medicine for its diuretic, antispasmodic, analgesic, and antimicrobial activities (Essa *et al*., 2024). The plant's essential oil, rich in bioactive constituents, is utilized in aromatherapy, herbal infusions, and perfumery. It's reported that its pharmacological effects have been ascribed to the existence of several phytochemicals like flavonoids, tannins, phenolics, and other secondary metabolites, which also make it a promising candidate for NPs biosynthesis (Wahyuni *et al*., 2024).

The utilization of *C. proximus* extract in the biosynthesis of AgNPs presents a main benefit: as it promotes the use of an underutilized, naturally abundant plant while advancing the green nanotechnology. Phytochemicals in *C. proximus* have the ability to function as stabilizing agents, which stop NPs from sticking together, and reducing agents, which change Ag^+ ions into Ag^0. This method improves the biocompatibility and functioning of the final AgNPs while reducing the requirement for hazardous chemicals (Amr *et al*., 2023).

Herbal nanomaterials have emerged as promising candidates in therapeutic applications due to their biocompatibility, biodegradability, and potent biological activity. However, despite the growing body of literatures on green synthesis and biological evaluation, significant gaps remain in understanding the mechanisms underlying their therapeutic action, stability, and targeted delivery. Furthermore, there is a need for standardized methods to assess their safety and efficacy (Yadav *et al*., 2025). Therefore, the objectives of the present study were to synthesize AgNPs using an aqueous extract of *C. proximus* and characterize the resulting NPs using various analytical techniques. Additionally, the antioxidant capacity, antimicrobial potential against pathogenic microbial strains, and cytotoxicity are evaluated to assess their suitability for biomedical applications. This integrative approach not only contributes to the field of green nanotechnology but also highlights the value of a native Egyptian plant in developing multifunctional nanomaterials for therapeutic and antimicrobial use.

Materials and Methods

Preparation of Cymbopogon proximus extract

Cymbopogon proximus samples were collected from a local herbal farm in Qalyubia, Egypt, washed, and air-dried at room temperature in the shade. After drying, an electric grinder was used to finely crush the leaves. For 3 h, 50 g of the powdered plant material were extracted with 500ml dist. water while being continuously stirred at 60 °C. Afterward, the mixture was filtered using Whatman No. 1 filter paper and the filtrate was kept at 4 °C for use in the production of NPs.

Green synthesis of silver nanoparticles (AgNPs-CP)
Silver nanoparticles were synthesized using an eco-friendly approach by adding 1 ml of *C. proximus* aqueous extract to 25 ml of silver nitrate ($AgNO_3$) (1 mM) solution. The reaction mixture was continuously stirred at 40 °C for 30 min. in darkness. A visible color change from pale greenish-yellow to dark brown signified the successful formation of AgNPs. After centrifugation of the NPs for 15 min. at 6,000 g, they were washed three (3) times with dist. water, dried overnight at 50 °C in a hot air oven and kept at 4 °C for later processing.

Antioxidant activity assays
The antioxidant capacity of *C. proximus* extract was assessed using several standard analytical methods as reported in details in the previous study conducted by Rawash *et al.* (2025), and the absorbancies for the different assays were determined using a UV-Vis spectrophotometer (SpecorD250 plus, Analytik Jena, Germany).

Total antioxidant activity (TAA)
The phosphomolybdenum assay outlined by Prieto *et al.* (1999) was used to measure total antioxidant capacity. For this assay, 300 µL of the extract was combined with 3 mL of reagent solution that contained 0–6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. For 90 min. the reaction mixture was incubated at 95 °C in a water bath. Following cooling at room temperature, absorbance at 695 nm was measured. Results were reported as mg of ascorbic acid equivalents per kg dry weight (mg AAE/ kg DW) of the sample with ascorbic acid being used to construct a calibration curve.

Total phenolic content (TP)
With minor modifications, Singleton and Rossis (1965) colorimetric Folin–Ciocalteu method was used to measure the extracts total phenolic contents. Briefly 500 µL of 10 % (v/v) Folin–Ciocalteu reagent were combined with 100 µL of the extract. 400 µL of 7.5 % (w/v) sodium carbonate solution were added after the reaction had been run for 5 min. The mixture was vortexed and let to stand for 30 min. at room temperature in darkness. At 765 nm, the absorbance was measured. Using a standard calibration curve with gallic acid as a reference standard, the results were reported as mg gallic acid equivalents per kg of extract (mg GAE/ kg).

Total flavonoid content (TF)
According to Chang *et al.* (2002), the total flavonoid content was determined using the aluminum chloride colorimetric technique. 100 µL of 10 % (w/v) aluminum chloride solution and 100 µL of 1 M potassium acetate were combined with 500 µL of the extract in an aliquot. Dist. water was used to bring the volume up to 5 mL. The absorbance was measured at 415 nm following 30 min. of incubation at room temperature. The results were reported as mg of quercetin equivalents per kg of dry weight (mg QE/ kg DW) of the sample with quercetin serving as a reference standard.

Gas chromatography–mass spectrometry (GC-MS) analysis
Gas chromatography–mass spectrometry (GC-MS) was used to characterize the *C. proximus* extract's chemical profile. An Agilent 7890A gas chromatograph and an Agilent 7000A mass spectrometer equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thicknesses) were used for the analysis. At the rate of 15 °C/ min., the oven temperature was raised from 50 °C to 310 °C, with a final hold of 10 min. A helium carrier gas flow rate of 1.5 mL/ min. was employed and the injector temperature was set at 280 °C. A mass range of 50–550 m/z was scanned by the mass spectrometer, which was run at 70 eV and at an ion source temperature of 270 °C. Compound identification was based on comparisons with mass spectral libraries, including NIST 17, Wiley 275, Palisade 600, and ADAMS 2001 as previously reported by Rashad *et al.* (2025).

Characterization assays of AgNPs-CP
UV–visible spectroscopy: Silver nanoparticles (AgNP) formation was assessed using UV-visible spectrophotometry (SpecorD250 plus, Analytik Jena, Germany) and performing a spectral scan across the 300–500 nm range. A characteristic surface plasmon resonance (SPR) peak that typically appears between 400 and 450 nm was used to identify AgNPs production.

Dynamic light scattering (DLS) and zeta potential (ZP)
A Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) was used to assess the zeta potential (surface charge) and particle size distribution of the produced plant extract-loaded silver nanoparticles (AgNPs-CP). The hydrodynamic diameter of the nanoparticles was ascertained using

Dynamic Light Scattering (DLS), providing details on their size distribution in colloidal suspension based on the Brownian motion of particles and scattered light intensity fluctuations. Measurements remained taken at 25 °C at a detection angle of 173° with a He-Ne laser (λ = 633 nm). Data were examined using the cumulant method to derive the Z-average diameter and polydispersity index (PDI).

Zeta potential (ZP) was measured using electrophoretic light scattering to establish the nanoparticles surface charge, an indicator of colloidal stability. Samples were suspended in dist. water at an appropriate concentration to avoid multiple scattering effects and then filled into a folded capillary cell to analyze. A zeta potential value of more than ±30 mV typically indicates good stability *via* electrostatic repulsion between particles.

Transmission electron microscopy (TEM)
The size, morphology, and distribution of the synthesized AgNPs-CP were characterized using Transmission Electron Microscopy (TEM) (A JEOL JEM-2100, JEOL Ltd., Tokyo, Japan). Samples were prepared by placing a drop of the nanoparticle suspension onto a carbon-coated copper grid (400 mesh) and allowing it to air-dry at room temperature. Excess solution was wicked off with a filter paper to ensure a thin film suitable for imaging (Williams and Carter, 2009). A TEM running at a 200 kV accelerating voltage was used for TEM imaging.

Antimicrobial activity assessment
The antimicrobial efficacy y of *C. proximus* extract and AgNPs-CP was tested using the disc diffusion method against selected microbial strains of *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *Salmonella typhi* (ATCC 6539), *Aspergillus Niger* (ATCC 16888) and *Candida albicans* (ATCC 10221). The control antibiotic for the bacteria was Gentamycin and for the fungi was fluconazole. All tests were done in three replicate.

Sterile discs (6 mm diameter) were loaded with 50 µL of each sample and aseptically placed on the surface of Mueller-Hinton agar (MHA) seeded individually with 100 µl (2×10^6 cells/ ml) of the bacterial strains, and Sabouraud dextrose agar (SDA) individually seeded with 100 µl (10^6 cfu/ ml) of the fungal strains. All plates were incubated at 37 °C for 24 h and 72 h for the bacterial and fungal plates, respectively. After incubation, the inhibition zones were measured (mm) using a calibrated ruler to assess the antimicrobial activity of the extract and the AgNPs-CP (Crisan *et al*., 2024).

Cytotoxicity evaluation
Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) on Wistar Institute-38 (Wi38) normal human lung fibroblast cells, the cytotoxic potential of both AgNPs-CP and Doxorubicin (positive control) was assessed. The cells were kept at 37 °C in an incubator with 5 % CO_2 in Dulbecco's Modified Eagle Medium (DMEM media) supplemented with 10 % fetal bovine serum. Due to differences in chemical nature and mode of action, doxorubicin (a conventional chemotherapeutic agent) was tested at lower concentrations (up to 100 µg/ mL) compared to AgNPs-CP. Cells were treated with varying concentrations of AgNPs-CP (ranging from 125 to 1000 µg /mL) for 24 h. Three wells served as the controls, getting only DMEM media, while 0.1 ml of each dilution was examined in separate wells. Afterward, the MTT reagent was added. After incubation for 4 h, the absorbance was recorded using 96-well plate-reader at 560 nm. The IC_{50} value was calculated to determine the concentration required to inhibit 50 % of cell viability (Halkai *et al*., 2019). All tests were done in three replicate.

Statistical analysis
Three separate tests were used to gather the data, and SPSS software for Windows was used for analysis. The mean ± standard deviation (SD) of the triple experiments was used to express the data.

Results and Discussion

Antioxidant assays
The analysis of CP extract revealed consistent trends in total antioxidant activity (TAA), total phenolic content (TP), and total flavonoid content (TF). These parameters are critical for assessing the antioxidant potential of plant-based extracts and presented in Table 1. Recently *Cymbopogon proximus* (CP) as natural sources of antioxidants, antimicrobials, and green sources of nano silver materials have received much attention in literatures. Results of the present work showed that the antioxidant assays had high levels of antioxidant activity, flavonoids, and phenolic content observed in CP, which underscore CP extract potential as a powerful antioxidant agent. A TAA of

9274.87 mg AAE/kg was notably high, contributing to the extract's ability to mitigate oxidative stress in biological systems. Flavonoids and phenolics are critical secondary metabolites that play essential roles in plant defense and widely recognized for their health-promoting effects, including reducing oxidative damage and inflammation (Roy *et al.*, 2022).

Table 1: *Total antioxidant capacity, total flavonoid content and total phenolic content of Cymbopogon proximus extract (CP).*

	***Cymbopogon proximus* extract**
Total antioxidant activity (mg AAE/ kg)	9274.867 ± 564.7
Total flavonoid content (mg QE/ kg)	1112.733±12.17
Total phenolic content (mg GAE/ kg)	2245.733 ±10.7

[*] Results are reported as means of 3 replicate ± Standard deviation (SD).

The substantial levels of TF (1112.73 mg QE/kg) and TP (2245.733 mg GAE/kg) suggest that CP extract could be effective in managing conditions related to biotic or abiotic oxidative stresses. The correlation between these bioactive compounds and antioxidant potential is well-established, supporting their use in pharmaceutical and nutraceutical applications (Bereksi-Reguig *et al.*, 2024).

Identification of bioactive compounds of CP using GC/MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the CP extract revealed a diverse range of phytochemicals, including terpenes, flavonoids, fatty acids, and sterol compounds. The retention time (RT), compound name, and area sum percentage % of each identified component are summarized in Table 2. GC-MS chromatogram is presented in Figure 1.

The GC-MS analysis of CP extract revealed a complex profile rich in bioactive constituents, predominantly terpenoids, flavonoids, fatty acids, and phytosterols. These compounds are closely associated with the plant's traditional use and its scientifically validated antioxidant and antimicrobial properties. Furthermore, the biosynthesis of AgNPs using CP extract likely benefited from the presence of these phytochemicals, which act not only as reducing and stabilizing agents but also as synergistic enhancers of the NP's biological activity.

Piperitone (26.8 %) was identified as the major component, consistent with previous studies on *C. proximus* essential oil (Apu *et al.*, 2022). As a monoterpene ketone, piperitone exhibited strong antimicrobial activity, particularly against Gram-(-) and Gram-(+) bacteria. When used in the synthesis of AgNPs, it may contribute both to the reduction of silver ions and the enhancement of the NP's antimicrobial efficacy. A previous study conducted by Moglad *et al.* (2022) assessed that piperitone reached 45 % (Wahyuni *et al.*, 2024). The difference in types and percentages of the essential oils may be attributed to the season of cultivation, geographical region, and storage and extraction methods (Aly *et al.*, 2025).

Table 2: *Active compounds in Cymbopogon proximus (CP) extract detected by Gas chromatography/ mass spectrometry (GC/MS).*

Retention time (min.)	Compound name	Area sum %
3.5	trans-Z-α-Bisabolene epoxide	0.79
3.65	eudesmol derivative	0.81
3.75	4(15)-Selinene-11,12-diol	1.08
3.97	2',5'-Dimethoxyflavone	2.69
4.3	α-terpineol	1.17
4.7	D-α-Tocopherol succinate	0.53
5.99	Camphene	0.89
6.18	8-Carboxy-3-methylflavone	3.16
7.5	Undecane	3.78
7.66	Geranyl isovalerate	2.01
8.27	Phytol	1.09
8.51	9-cis-Retinoic acid	0.48
9.15	Kampferol-3,4'-dimethyl ether	0.36
9.97	Thymol	2.66
10.32	3,2'-Dimethoxyflavone	1.66
10.36	6-Methyl-γ-ionone	6.66
11.16	Luteolin 6,8-c-diglucoside	0.68
11.29	3',4',7-Trimethylquercetin	1.18
12.13	Isovitexin	0.91
14.27	Isohumulone	1.54
14.61	Palmitic Acid	3.75
15.18	linoleic acid	2.11
15.59	Octadecanal	1.89
16.09	Stearic acid	2.58
16.3	Piperitone	26.8
16.8	Salicylic acid β-D-O-glucuronide	1.16
17.1	Caryophyllene oxide	1.73
17.7	Elemol	9.06
17.89	α-eudesmol	8.64
18.68	β-Sitosterol	2.07
19.9	Arachidic acid methyl ester	2.9
22.69	Tetracosanoic acid, methyl ester	2.97

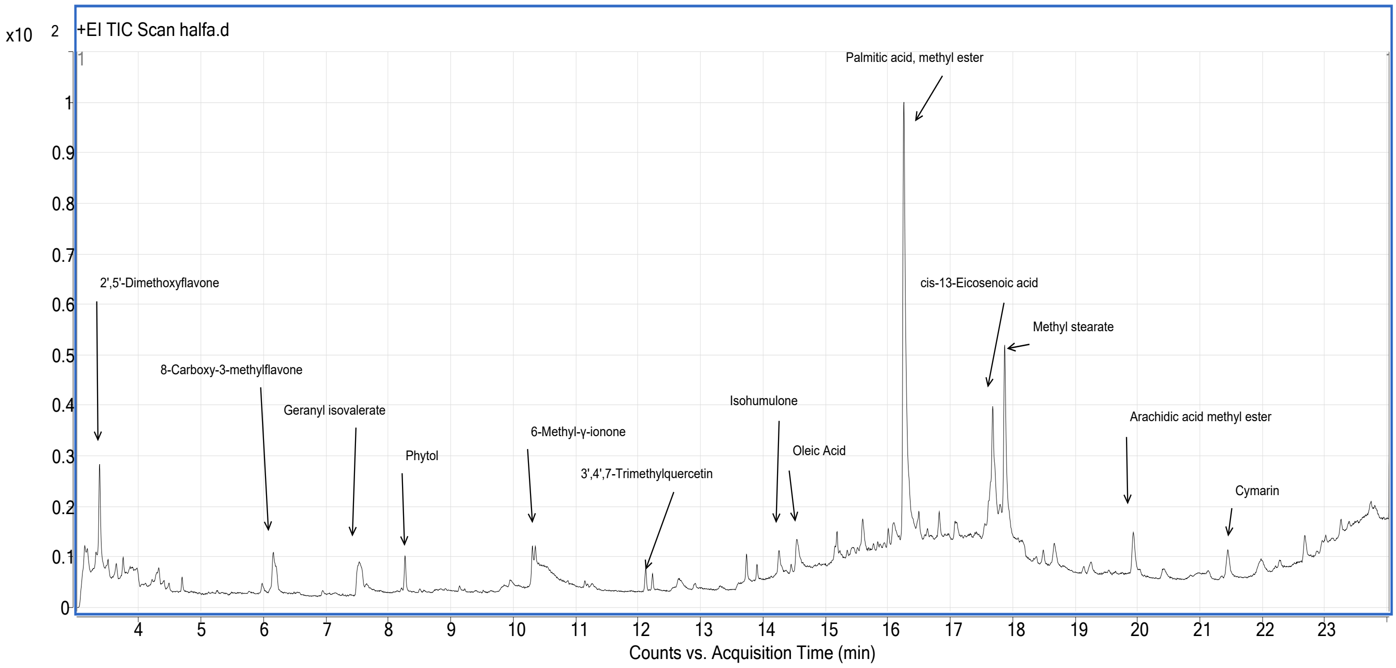

Figure 1: *Gas chromatography- mass spectrometry chromatogram of Cymbopogon proximus (CP) extract.*

Sesquiterpenes such as Elemol (9.06%) and α-eudesmol (8.64 %) were present in high concentrations (Yagi *et al.*, 2020). These oxygenated sesquiterpenes are known for their potent antimicrobial, anti-inflammatory, and antifungal properties. Their presence in the CP extract likely supported the formation of a stable capping layer around AgNPs, improving biocompatibility, and boosting antimicrobial effects through synergistic mechanisms.

Flavonoids, including 8-carboxy-3-methylflavone (3.16 %), 2',5'-dimethoxyflavone (2.69 %), and thymol (2.66 %) are strong antioxidants that played a vital role in the reduction of silver ions to elemental silver. Their antioxidant activity contributed to the stabilization of NPs, preventing agglomeration, and increasing their surface reactivity. These phytochemicals may also impart additional therapeutic effects to the AgNPs, particularly in combating oxidative stress and microbial infections.

Fatty acids such as palmitic acid (3.75 %), stearic acid (2.58 %), and linoleic acid (2.11 %) may further assist in the NP's formation by modulating surface characteristics and enhancing membrane permeability in microbial cells, contributing to the AgNPs' antimicrobial potential.

The detection of β-sitosterol (2.07 %) and geranyl isovalerate (2.01 %) added further bioactivity, as these compounds are often associated with anti-inflammatory, antioxidant, and antimicrobial avtivities (Drug and Eda, 2024).

Overall, the phytochemical composition of CP extract not only facilitated the green synthesis of AgNPs but also significantly enhanced their antioxidant and antimicrobial performance. The synergistic interaction between the bioactive compounds and AgNPs resulted in a multifunctional nanomaterial with promising applications in medicine, particularly for combating microbial infections and oxidative stress-related disorders (Awad *et al.*, 2024).

Nanoparticle s morphology and dispersion of AgNPs-CP

The CP extract's color changed from light yellow to brownish black upon the addition of $AgNO_3$, indicating the formation of AgNPs (Hemlata *et al.*, 2020). A single peak at λmax (448 nm) was observed in the AgNPs-CP's UV–vis spectra at various wavelengths scanning from 300 to 600 nm (Figure 2).

Transmission electron microscopy (TEM)

The TEM image analysis is presented in Figure 3. Regular shapes were observed, with well dispersed NPs and others clustered. The images revealed the presence of predominantly spherical NPs with diameters ranging from 15 to 45 nm, which aligns with previously reported values for AgNPs synthesized using CP extract (Al-Rubaye *et al.*, 2020).

Zeta potential analysis of AgNPs-CP

Zeta potential (ZP) is a measure of the surface charge of NPs or particles suspended in a liquid.

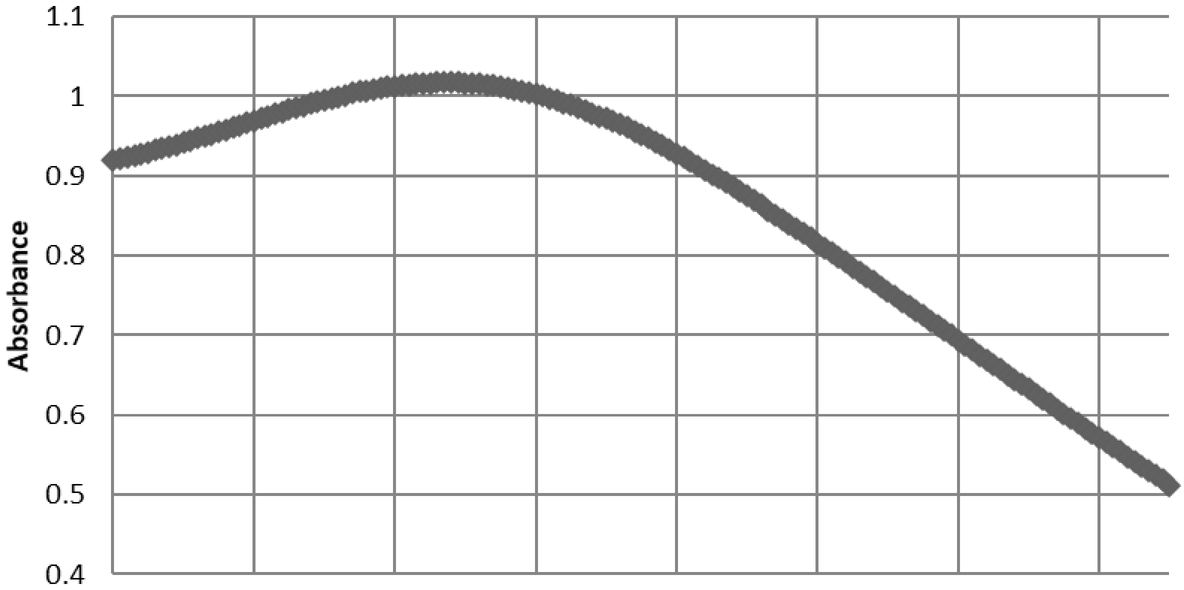

Figure 2: *UV–Vis spectrum of the AgNPs-CP.*

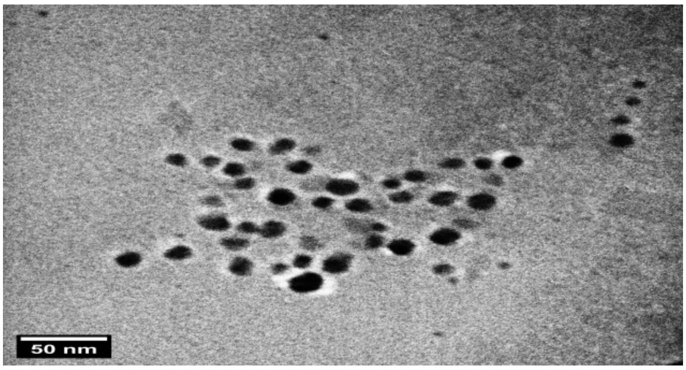

Figure 3: *Transmission electron microscope (TEM) image of AgNPs-CP showing spherical NPs with diameters ranging from 15 to 45 nm.*

Referring to Figure 4, it was observed that ZP of -35.6 mV indicated that the particles in suspension had a significant negative surface charge. This charge typically provided electrostatic stability, helping to prevent agglomeration of the particles and their stablity in a suspension for an extended period (Awad *et al*., 2024).

DLS analysis of AgNPs-CP

The DLS measurement suggested that the average size of the particles was 42 nm (Figure 5). This value provides insight into the size distribution of the particles.

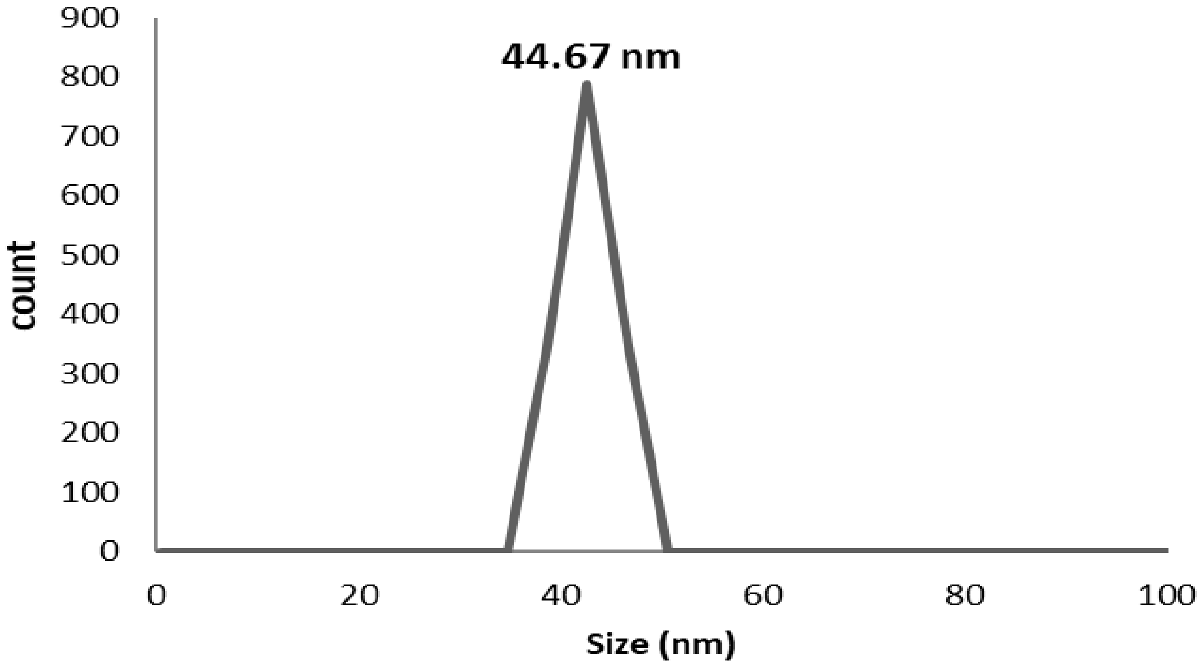

Figure 4: *Particle size distribution spectrum of AgNPs-CP as determined by dynamic light scattering (DLS) analysis.*

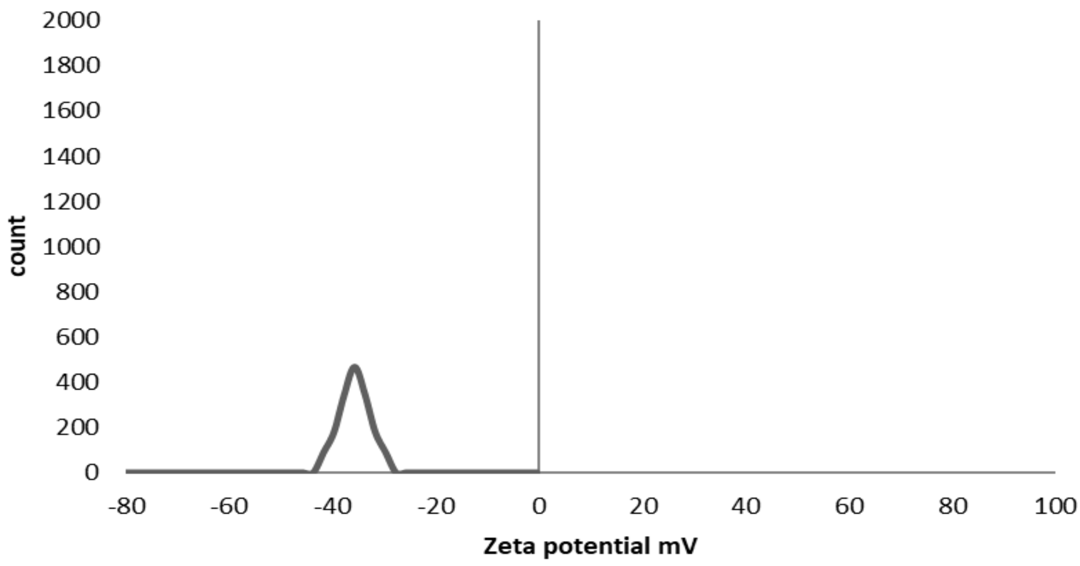

Figure 5: *Zeta potential (ZP) distribution spectrum of CP-AgNPs.*

A smaller size, like 42 nm, typically means the particles could be in the nanoparticle range and had high surface area-to-volume ratios. Notably, the particle sizes measured *via* TEM were smaller than those obtained through DLS (42 nm). This discrepancy arose because DLS measurements account for contributions from capping agents and solvation effects, leading to an apparent increase in particle size (Mohamed *et al*., 2022). These small sizes are favorable for several applications that require fine control over particle behavior, such as in nanomedicine, and environmental applications, including sensors and/ or catalysis. A previous study proved that AgNPs-CP had a size of 47 nm (Apu *et al*., 2022). Meanwhile, another similar study conducted by Alobaid *et al*. (2022) reported that the assessed AgNPs-CP size was 139.9 nm.

Antimicrobial activity of CP extract and AgNPs-CP

The present study showed that CP extract can produce AgNPs-CP using $AgNO_3$ through a green synthesis method. The antimicrobial efficacy of CP extract and its synthesized AgNPs-CP was evaluated against a panel of bacterial and fungal strains, and the results were compared to those of standard antibiotics (Table 3). The obtained findings demonstrated that both CP extract and AgNPs-CP possess considerable antimicrobial efficacies, with variable effectiveness across different microbial strains.

Among Gram-(+) bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis* exhibited notable susceptibility to both CP extract and AgNPs-CP. The inhibition zones for *B. subtilis* were 28 ± 2 mm (CP extract) and 27 ± 2 mm (AgNPs-CP), which were comparable to the standard antibiotic (27 ± 1 mm). Similarly, *S. aureus* showed consistent inhibition across all treatments, with zones measuring 26 ± 1 mm (CP and AgNPs-CP) and 27 ± 1 mm (antibiotic). These results suggest that both the plant extract and its NPs are effective against Gram-(+) bacterial pathogens, attributed to the disruption of bacterial cell wall synthesis or membrane integrity facilitated by bioactive components and NPs action (Victor *et al*., 2022).

Table 3: *Antimicrobial activity of Cymbopogon proximus (CP) extract and AgNPs-CP.*

Sample/ Name of microrganism	CP	Ag-NPs-CP	Control antibiotic
Bacillus subtilis (ATCC 6633)	28±2	27±2	27±1
Staph aureus (ATCC 6538)	26±1	26±1	27±1
Escherichia coli (ATCC 8739)	18±1	20±0.1	22±1
Salmonella typhi (ATCC 6539)	28±0.1	25±0.1	28±1
Candida albicans (ATCC 10221)	32±1	30±1	29±0.2
Aspergillus Niger ATCC 16888	NA	NA	40±1

*Where; *NA: No activity. The control antibiotic for the bacteria was Gentamycin and for the fungi was fluconazole at a tested concentration of 1.0 mg/ ml. The diameter (mm) of the zone of inhibition is expressed as mean of 3 replicate ± Standard deviation (SD).*

In the case of Gram-(-) bacterial strains, *Salmonella typhi* and *Escherichia coli* showed varied responses. *E. coli* demonstrated slightly higher sensitivity to AgNPs-CP (20 ± 0.1 mm) compared to the crude extract (18 ± 1 mm), though both were less effective than the antibiotic control (22 ± 1 mm). This indicates that the NPs may enhance the permeability or binding efficacy to the bacterial membrane, improving the antibacterial activity (Apu *et al*., 2022). Conversely, *S. typhi* showed a marginally reduced zone of inhibition with AgNPs-CP (25 ± 0.1 mm) compared to the extract (28 ± 0.1 mm) and the control antibiotic (28 ± 1 mm). This could be attributed to differences in cell wall structure, efflux mechanisms, or NPs interaction kinetics in this particular strain. Similarly, a prior study demonstrated that AgNPs had a potent antibacterial effect against both Gram (+) and Gram (-) bacteria (Apu *et al*., 2022).

For fungal strains, *Candida albicans* was highly sensitive to both treatments, with the CP extract showing the highest inhibition zone (32 ± 1 mm), followed by AgNPs-CP (30 ± 1 mm) and the standard antifungal (29 ± 0.2 mm). This superior performance of the crude extract suggests the presence of potent antifungal compounds in *C. proximus* that may act synergistically or independently of silver ions (Wahyuni *et al*., 2024).

Interestingly, neither the CP extract nor the AgNPs-CP expressed antifungal activity against *Aspergillus niger*, while the standard antifungal agent showed a strong inhibitory effect (40 ± 1 mm). The resistance of *A. niger* to both treatments may be attributed to its thick cell wall, robust spore structure, and/ or its intrinsic resistance to the bioactive phytochemicals and the used NP's formulations. In several similar previous studies, CP essential oil exhibited stronger antibacterial activity against the Gram-(+) bacteria (*i.e., B. subtilis and S. aureus*) than against the Gram-(-) bacteria (*i.e., E. coli*) (Apu *et al*., 2022; Abdel Moatamed, 2024).

Overall, the study highlights the broad-spectrum antimicrobial potential of *Cymbopogon proximus*, particularly against Gram-(+) bacteria and yeast-like fungi such as *C. albicans*. Meanwhile, AgNPs-CP generally enhanced or maintained the antimicrobial efficacy of the extract, although their activity varied depending on the strain of the microorganism (Victor *et al*., 2022). These findings support the potential use of *C. proximus*-based NPs as natural and effective antimicrobial agents, though further studies on mechanisms of their action and optimizations are warranted.

The cytotoxic effects of AgNPs-CP

The cytotoxic effects of the tested compounds on WI38 cells (human lung fibroblast normal cells) were evaluated using the MTT assay and are shown in Figure 6. The optical density (OD) values were used to calculate cell viability, toxicity, and the half-maximal inhibitory concentration (IC_{50}) values, which are presented in Table 4. The cytotoxic potential of AgNPs synthesized using *Cymbopogon proximus* extract (AgNPs-CP) was evaluated against the WI38 cells using the MTT assay across a concentration range of 31.25 to 1000 µg/ mL. The observed cytotoxicity was dose-dependent, with higher concentrations showing greater inhibitory effects on cell viability.

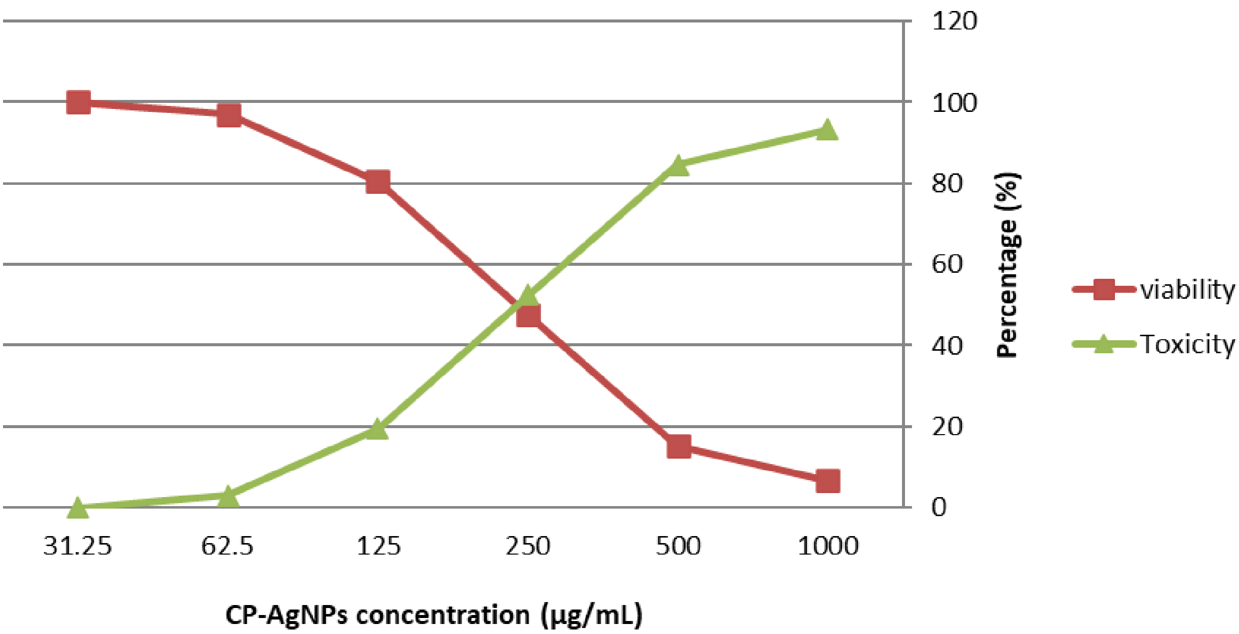

Figure 6: *Effects of different concentrations of CP-AgNPs on the cytotoxicity and viability of WI-38 normal human fibroblast cells.*

Table 4: *Cytotoxicity and cell viability of AgNPs-CP and doxorubicin against WI-38 normal human fibroblast cells as determined by the MTT assay.*

ID	µg/ mL	Mean OD	±SD	Viability %	Toxicity %	Half-maximal inhibitory concentration (IC_{50}) ± SD
WI38	--	0.701	0.0063	100	0	µg
Ag-NPs-CP	1000	0.047	0.0030	6.704	93.295	240.67 ± 4.2
	500	0.107	0.0067	15.311	84.688	
	250	0.333	0.0085	47.551	52.448	
	125	0.565	0.0070	80.551	19.448	
	62.5	0.680	0.0109	97.052	2.948	
	31.25	0.701	0.0017	100	0	
Dox-oru-bicin	100	0.020	0.0006	2.773	97.226	10.23 ± 0.13
	50	0.023	0.0098	3.183	96.816	
	25	0.063	0.0098	8.549	91.450	
	12.5	0.242	0.0120	32.969	67.030	
	6.25	0.592	0.0051	80.809	19.190	
	3.125	0.732	0.0026	99.863	0.1364	

*Where; *OD refers to optical density. Results are reported as means of 3 replicate ±Standard deviation (SD).*

At the highest tested concentration (1000 µg/ mL), AgNPs-CP exhibited strong cytotoxicity and reduced cell viability by approximately 93.3 %, indicating significant anti-proliferative effects at this dose. The obtained IC_{50} value was 240.67 ± 4.2 µg/ mL, signifying the concentration at which 50 % of cell viability was inhibited. This moderate IC_{50} value reflects a balanced profile that is potent enough to demonstrate cytotoxicity, and at the same time not being highly toxic to normal cells at the tested lower concentrations.

Cytotoxicity recorded 84.7 % at 500 µg/ mL, 52.4 % at 250 µg/ mL, and 19.4 % at 125 µg/ mL, indicating increasing cytotoxicity with higher concentrations. At the lowest concentrations (62.5 and 31.25 µg/mL), cell viability reached 97.05 % and 100 %, respectively, suggesting negligible cytotoxicity and supporting the biocompatibility of AgNPs-CP at lower doses.

Doxorubicin; used as a positive control, exhibited potent cytotoxicity against WI38 normal cells with an IC_{50} value of 10.23 ± 0.13 µg/ mL (Figure 7). At the highest tested concentration (100 µg/mL), it reduced cell viability to 2.77%, confirming its strong anti-proliferative effect. Compared to AgNPs-CP (IC_{50} = 240.67 ± 4.2 µg/mL), doxorubicin displayed significantly higher toxicity, underscoring the relatively safer profile of the biosynthesized AgNPs at lower concentrations.

These results suggest that AgNPs-CP are relatively safe for normal cells at concentrations below the IC_{50} threshold and demonstrate selective cytotoxic effects at higher doses. The cytotoxicity observed at elevated concentrations may be attributed to oxidative stress induced by silver ions, disruption of mitochondrial function, and/ or NP-mediated interference with cellular processes.

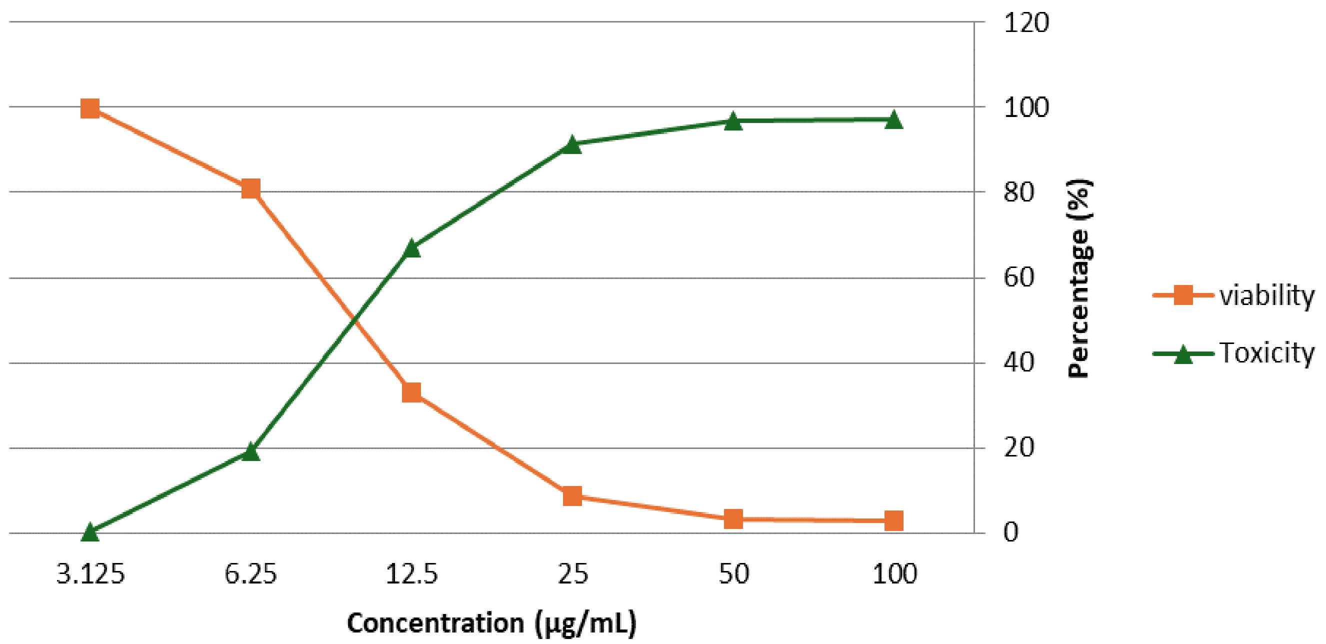

Figure 7: *Cytotoxic and viability effects of doxorubicin (positive control) on WI-38 normal human fibroblast cells.*

The moderate cytotoxic profile, combined with the previously demonstrated antimicrobial and antioxidant activities of AgNPs-CP, positions them as promising candidates for biomedical applications such as antimicrobial coatings or cancer-targeted therapies, pending further investigations for selectivity and *in vivo* safety. Similarly, another study demonstrated that AgNPs-CP showed 20 % viability in HCT116 colon cancer cells (Alobaid *et al*., 2022). Furthermore, a previous study conducted by Elkholy *et al.* (2024) displayed the inhibitory cytotoxic concentration 50 % (CC 50) of green synthetized AgNPs against WI38 cells to be 5.87 µg/ mL.

Conclusions and Recommendations

Cymbopogon proximus (CP) has shown significant bioactive potential in the form of green-synthesized AgNPs-CP. The CP extract showed strong antioxidant activity and high contents of flavonoid and phenolic, indicating its ability to counteract oxidative stress. The bioactive phytochemicals piperitone, elemol, α-eudesmol, and flavonoid derivatives were crucial for the biosynthesis and stabilization of AgNPs. The synthesized AgNPs-CP showed strong colloidal stability and enhanced antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*. The cytotoxicity assessment on WI38 normal human fibroblast cells showed a dose-dependent response, with higher concentrations exhibiting cytotoxic effects; however, lower doses maintained high cell viability. This study suggests further exploration of CP-based nanocomposites for application in food and feed additives, and for biomedical applications, particularly in antimicrobial therapy and oxidative stress-related conditions.

From the obtained results of this study, it is recommended that future investigations should be directed towards the scale-up of green synthesis of AgNPs with C. proximus extract and the assessment of their use as natural preservatives in food and feed systems. Moreover, investigations of the mechanisms of antimicrobial and cytotoxic activity and extensive toxicological studies are also required to validate safety and efficacy of the AgNPs. Additionally, *in vivo* testing of the AgNPs on fresh food/feed matrices and analyzing their synergistic effects with the other antimicrobial compounds can enhance their practical utility.

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Novelty Statement

This study presents a novel and eco-friendly approach to synthesizing AgNPs using *C. proximus* aqueous extract and offers new insights by evaluating novel biological activities, especially cytotoxicity against WI-38 cells, which was not previously reported. The resulting AgNPs displayed significant antioxidant, antimicrobial, and moderate cytotoxic activities, highlighting their potential as natural preservatives for food and feed applications.

Author's Contribution

El-Shaimaa A. Rawash: Investigation, validation of results, writing original draft, writing review and editing
Sayed Rashad: Investigation, writing original draft, writing review and editing
Dina A. Anwar: Validation of results, writing original draft
Heba R. Eid: Writing review and editing
Ghadir A. El-Chaghaby: Supervision and revision for important intellectual content.

Conflict of interests
The authors have declared no conflicts of interest.

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