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ORIGINAL RESEARCH

Green Synthesis of Gold Nanoparticles Using *Clerodendrum trichotomum* Thunberg for Antibacterial and Anticancer Applications

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Purpose: The potential use of gold nanoparticles (AuNPs) in healthcare research has increased dramatically in recent years, owing to advancements in their synthesis techniques, including green synthesis. Although *Clerodendrum trichotomum* is a popular medicinal plant that harbors many bioactive phytochemicals in various parts of the world, the green synthesis of AuNPs using this precious plant is still under investigation. Therefore, this study aimed to explore the green synthesis of AuNPs from *C. trichotomum* Thunberg leaves (CTT-AuNPs) and their antibacterial and cytotoxic properties.

Methods: AuNPs were synthesized from *Clerodendrum trichotomum* Thunberg and characterized using various microscopic and spectroscopic techniques. A serial dilution technique was used to determine the antibacterial activity of the synthesized CTT-AuNPs against two bacterial species, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The cytotoxicity assay was carried out in the breast cancer cell line (MCF-7), whereas the biocompatibility test was performed in the kidney cell line (293T).

Results: Multiple spectroscopic characterization techniques confirmed the successful synthesis of CTT-AuNPs. The average size of CTT-AuNPs was 19.1±2.2 nm. The results of this study revealed that CTT-AuNPs showed strong antibacterial activity against *S. aureus* and *K. pneumoniae*, which was further confirmed using a live/dead dual staining assay. CTT-AuNPs also significantly reduced the proliferation of MCF-7 breast cancer cells to 32.67% at 120 µg/mL after 24 hours of incubation. These green-synthesized CTT-AuNPs exhibited excellent cytobiocompatibility with 293T kidney cells. The dual staining method further confirmed the cytotoxicity and biocompatibility of CTT-AuNPs compared with chemically synthesized AuNPs.

Conclusion: This work will pave the way for the production of biocompatible AuNPs from *C. trichotomum* Thunberg that can be used in different disease treatments.

Keywords: antibacterial, biocompatibility, *Clerodendrum trichotomum* Thunberg, cytotoxicity, gold nanoparticles

Introduction

Since cancer and antimicrobial resistance (AMR) are the two biggest causes of death and their incidence is increasing every year, they represent a threat to global health. AMR was related to 4.71 million deaths in 2021, an increase every year, and it has been predicted that the AMR-associated death toll will increase to more than 8 million by 2050.[1] The improper use of antibiotics is the leading cause of AMR, prompting researchers to search for new anti-microbial drugs.[2] In contrast, more than 9 million new cancer-related deaths are recorded in 2022.[3] Resistance to chemotherapeutic drugs may cause more than 80% cancer-related death.[4] To combat growing health concerns related to drug resistance, researchers are now looking for alternative treatments. Since ancient times, plant-based medicines have been used to treat various diseases in humans. With advancements in the purification and characterization of plant-based phytochemicals, new approaches have been introduced for the discovery of cancer drugs from natural sources. More than 3500 plants

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exhibit promising cytotoxic activity against different cancer cells. More than 50% of all licensed anticancer medications have inextricable links with natural sources.[5]

In recent years, nanobiotechnology, which exploits nanoparticles to address various medical challenges, has received increasing attention in healthcare research. Nanoparticles considerably improve the safety and effectiveness of anticancer drugs in terms of safety and effectiveness.[6] Among the different metallic nanoparticles, AuNPs have several benefits for use in cancer therapy, including distinctive physiochemical characteristics such as stability, biocompatibility, high thermal activity, optical and electrical properties, high surface area-to-volume ratio, and surface chemistry, and they are easily fabricated into a variety of shapes and sizes.[7,8] Their small size offers more surface area relative to the total mass for interaction with biomolecules and allows easy penetration into cells, which is attributable to their strong cytotoxicity.[9] They can also be used to design antibacterial drugs because of their enhanced solubility, specificity, and reduced side effects.[10,11]

Green synthesis of AuNPs using medicinal plants is a sustainable process that maximizes therapeutic efficacy, specific binding, and targeted delivery. This may also reduce toxicity, which is a major concern in the discovery of novel anticancer drugs.[12] Several studies have reported minimal toxicity of AuNPs against non-cancerous cells. AuNP concentration, diverse sizes, shapes, and capping agents majorly affect their cytotoxic activity.[13] Apoptosis is induced in cancer cells via ROS-mediated mitochondrial dysfunction. In addition, AuNPs can inhibit cell cycle progression in the G_0/G_1 phase.[14] AuNPs also exhibit antibacterial activity by attacking the bacterial cell walls and DNA. Apart from their antibacterial activity, their antioxidant capacity also accounts for their faster wound-healing properties.[15]

In this study, the ornamental plant *Clerodendrum trichotomum* Thunberg was used for green synthesis of AuNPs. *C. trichotomum* Thunberg has long been used in Chinese folk medicine. This plant has also been found in Japan and South Korea. The biological activities of the different plant parts were explored. Several studies have reported the cytotoxic activity of this plant against human cancers, including gastric adenocarcinoma and nasopharyngeal, liver, blood, cervical, lung, and kidney cancer. This plant possesses many bioactive phytochemicals responsible for its cytotoxic properties. Phenylpropanoids, diterpenoids, and steroids are the major compounds found in this plant that show significant anticancer activity against different cancer cells. The antibacterial properties of various solvent extracts of this plant have been reported.[16]

The biosynthesis of nanoparticles was performed using different *Clerodendrum* species. AgNPs synthesized from *C. inerme, C. infortunatum, C. phlomidis, C. splendens, C. viscosum*, and *C. glandulosum* have been reported to exhibit antimicrobial, antioxidant, cytotoxic, and antiparasitic activity.[17–22] Aluminum and zinc oxide nanoparticles from *C. phlomidis, C. heterophyllum, and C. infortunatum* have antibacterial activities.[23–25] In the case of AuNPs, only *C. infortunatum and C. inerme* have been investigated, and their antibacterial, antioxidant, and antiproliferative capacities have been documented.[20,26] The phytochemicals in plant extracts reduce metal ions to nanoparticles, which may depend on their reduction potential and the functional groups in their structures. It has been reported that flavonoids and phenolic compounds in *Artemisia capillaris* extract synthesize AuNPs. The hydroxyl and amino groups in the compound structures play crucial roles in the reduction process. Au-ion reduction involves the oxidation of an aldehyde group to a carboxyl group.[27]

As mentioned earlier, *C. trichotomum* Thunberg is frequently used in Chinese traditional medicine. Dried leaves and stems of this plant are used to treat hypertension and inflammatory diseases.[28] However, there is a lack of data on nanoparticle synthesis from *C. trichotomum* Thunberg, despite its excellent bioactive properties. Therefore, this study aimed to investigate the synthesis of gold nanoparticles (CTT-AuNPs) from *C. trichotomum* and to evaluate their antibacterial and cytotoxic effects. To the best of our knowledge, this is the first report of synthesizing nanoparticles from the medicinal plant *C. trichotomum* Thunberg which may further improve their biomedical functions.

Methodology

Plant Material Collection

Clerodendrum trichotomum Thunberg was collected from a wild area native to tropical and subtropical regions. Its identification was performed by Dr. Zaheer (Department of Botany, Punjab University, Lahore, Pakistan). A voucher specimen of *Clerodendrum trichotomum* Thunberg was deposited at the herbarium of the Department of Botany, GC University, Lahore, Pakistan (CTT: GC. Herb. Bot. 240).

Plant Extraction

Plant extraction was performed using a previously described protocol[20] with slight modifications (Figure 1). Fresh leaves from *C. trichotomum* Thunberg (CTT) were harvested from *C. trichotomum*. After cleaning with deionized water (dH_2O), the leaves were oven-dried at 60 °C and finely crushed to obtain a powder. Fifteen grams of the powdered leaves were combined with 100 mL of dH_2O water and heated at 50–60 °C for 15 min. The mixture was filtered to obtain the plant extracts. The plant extract obtained from this process was stored at 4 °C in an airtight bottle until further use.

Green Synthesis of Gold Nanoparticles

Green synthesis of AuNPs using CTT was performed following a previously described protocol[20] with slight modifications (Figure 1). Briefly, 10.20 g $HAuCl_4$ was added to 100 mL of plant extract for the green synthesis of AuNPs (concentration of Au^{3+} in the mixture was 0.3001 M), and the mixture was agitated for 80 min at 65 ◦C. Following this, it was noticed that the color of the mixed solution changed to ruby red because of surface plasmon resonance, which showed that the necessary gold nanoparticles had formed. After centrifuging the nanoparticles twice at 3000 rpm, they were filtered and washed thrice with a solution of deionized water and ethanol. Subsequently, the gold nanoparticles were subjected to a 40 ◦C oven-drying process and a 3-hour muffle furnace calcined at 500 °C. The produced NPs were preserved in an airtight container for biological applications and characterization.

Characterization of CTT-AuNPs

Powder X-ray diffraction (XRD) with a wavelength (λ) of 0.154 nm over the 2θ range of 20°–80° was used to assess the crystallinity and purity of the CTT-AuNPs in powder form. Morphological characterization of the CTT-AuNP powder was performed using Transmission Electron Microscopy (TEM). Nano particle size analyzer was used to measure the size of the nanoparticles. A composition analysis was performed using Energy-Dispersive X-ray (EDX) spectroscopy.

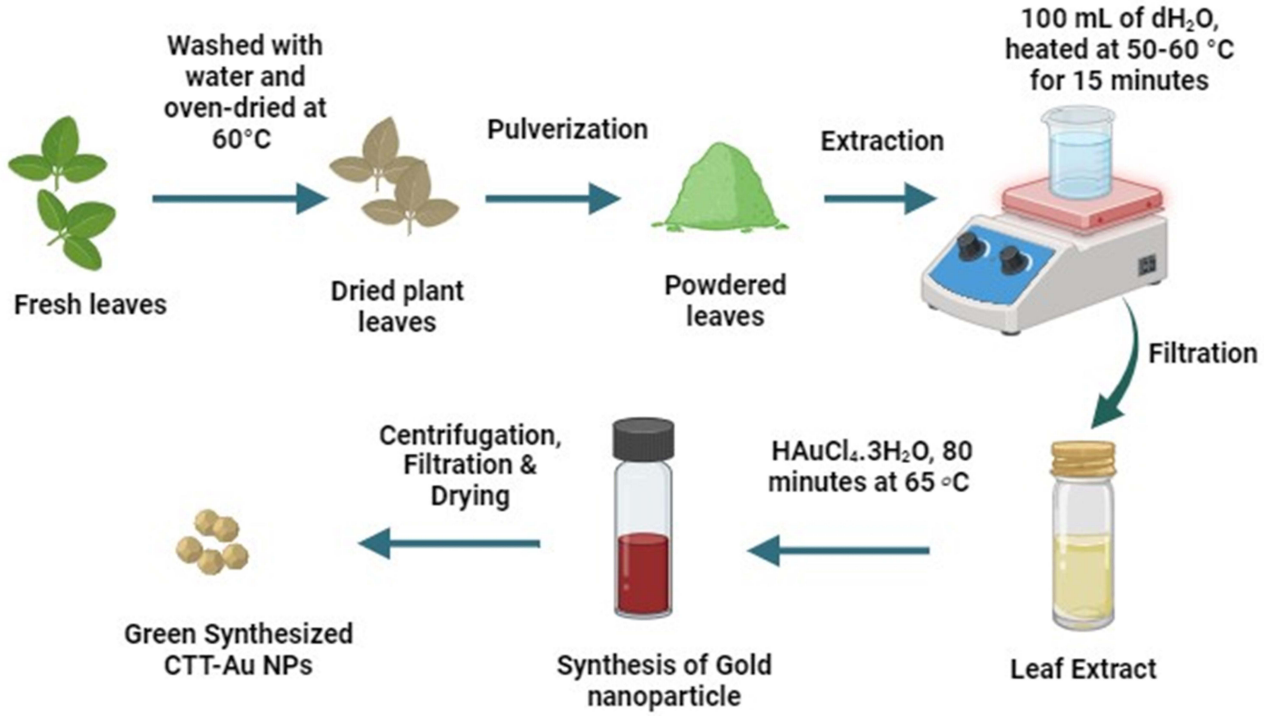

Figure 1 The leaf extract preparation and green synthesis of gold nanoparticles using *C. trichotomum* Thunberg (Created in BioRender.com).

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Antibacterial Assay

The antibacterial activity of CTT-AuNPs was assessed using a serial dilution method, as described previously.[29] The Gram-positive bacterium *Staphylococcus aureus* ATCC® 23235TM and gram-negative bacterium *Klebsiella pneumoniae* (ATCC® 13,883) were used in this experiment. Briefly, each bacterium (5×10^5 CFU/mL) was cultured overnight on agar plates at 37 °C. After incubation, bacterial colonies were diluted in PBS and adjusted to 1×10^7 CFU/mL. The bacterial culture (10 µL) was mixed with 1 mL of Muller-Hinton broth and added to each well of a 24-well plate at a final concentration of 1×10^5 CFU/mL. Next, 50 µL of CTT-AuNPs (250 µg/mL) were added to each well and incubated at 37 °C for 24 h. The serial dilution plate counting method was used to determine the bacterial count in each well, and the following formula was used to calculate the $\log_{10}$ reduction:[29]

$$Log_{10}\,Reduction = Log_{10}\,(CFU_b) - Log_{10}\,(CFU_a) \tag{1}$$

Where CFU_b and CFU_a are the number of bacterial colonies before and after incubation, respectively.

Live/Dead Bacteria Staining Assay

A confocal laser scanning microscope (CLSM, FV-1200, Olympus, Tokyo, Japan) was used to determine the bacterial cell viability using a previously described protocol. Each bacterium, at a confluency of 10^5–10^6 was inoculated into a 24-well microtiter plate and incubated for one hour. After incubation, the suspended cells were discarded and rinsed three times with saline water. Samples (250 µg/mL) were inoculated and incubated overnight. A live/dead bacterial viability kit was used, in which two fluorescent dyes, DAPI and propidium iodide (PI), were used to stain live (blue) and dead (red) bacteria, respectively.

Anticancer Activity

Cell Culture

Breast cancer cells (MCF-7 cells, commercially purchased from Sigma-Aldrich, Germany) were cultured in Dulbecco's Modified Eagle's medium (DMEM) in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C. The cells were cultured in DMEM for 24 h in a 96-well plate to obtain a cell confluence of 5×10^8 cells/well.

MTT Assay

The cytotoxicity of CTT-AuNPs was tested against MCF-7 cells using a previously described protocol.[29] CTT-AuNPs at a concentration of 120 µg/mL were applied to cancer cells and incubated for 24 h at 37 °C. Doxorubicin was used as a positive control. After incubation, the medium was discarded, and the cells were washed with phosphate-buffered saline. Each well was filled with 15 µL of MTT reagent (0.5 mg/mL), and the plate was incubated in the dark for 4 h at 37 °C. After 4 h, 150 µL of DMSO was added to each well and the absorbance was measured at 570 nm. The percentage of viable cells was calculated using the following equation:[29]

$$Cell\ viability = \frac{OD\ sample}{OD\ control} \times 100 \tag{2}$$

Live/Dead Cell Staining Assay

Fluorescent staining was used to further investigate the cytotoxicity of CTT-AuNPs using DAPI and PI staining. The same technique described for bacterial cell staining was used, except for the sample amount. CTT-AuNPs (10 µL, 120 µg/mL) were used in the assay. Each well was treated with 4 µg/mL of staining solution and incubated for 20 min. Images were taken at excitation wavelength of 488/545 nm using a confocal laser scanning microscope.

Biocompatibility Assay

Biocompatibility tests for CTT-AuNPs were performed using the same protocol as described in the previous section on the non-cancerous 293T kidney cell line. Chemically synthesized AuNPs (Chemi-AuNPs) were used to compare the biocompatibility of the CTT-AuNPs.

Results

Nanoparticle Characterization

The green-synthesized CTT-AuNPs were characterized by XRD, which revealed three diffraction peaks at 2θ= 37.78°, 44.02°, and 64.58° corresponding to the (111), (200), and (220) planes, respectively (Figure 2a). The nanoparticle size analysis revealed that the green-synthesized CTT-AuNPs had an average size of 19.1 ± 2.2 nm (Figure 2b). The EDX analysis further confirmed that the nanoparticles were mainly composed of gold (Au). The different peaks of C, N, and O in the spectra may be attributed to phytochemicals from the *C. trichotomum* Thunberg extracts coated on the nanoparticles (Figure 2c). TEM analysis revealed that the green-synthesized CTT-AuNPs were spherical and uniformly distributed (Figure 2d).

Antibacterial Assay

The antibacterial activity of CTT-AuNPs was tested against two bacteria, *S. aureus* and *K. pneumoniae*, along with the plant extract and chemically synthesized NPs. The data obtained from this study showed that green-synthesized CTT-AuNPs reduced bacterial growth better than the plant extract and chemically synthesized NPs (Chemi-AuNPs) (Figure 3). This study revealed that CTT-AuNPs exhibit bactericidal activity (log_{10} CFU/mL) against both *K. pneumoniae* (4.1 log_{10} CFU/mL) and *S. aureus*. (4.35 log_{10} CFU/mL) where CTT extract alone showed only bacteriostatic effect and (2.15 log_{10} CFU/mL) on *K. pneumoniae* and

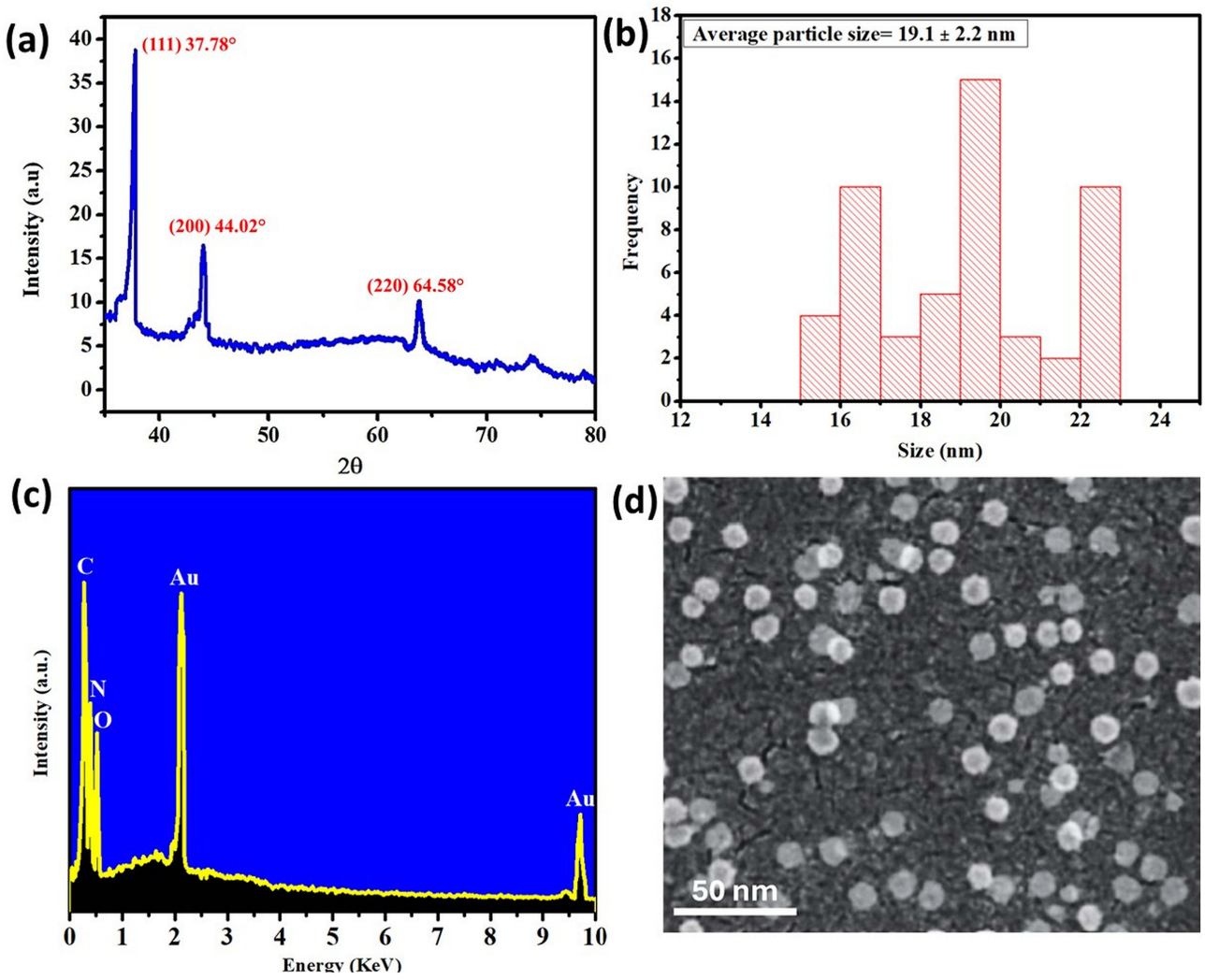

Figure 2 (**a**) XRD analysis; (**b**) size distribution; (**c**) EDX analysis and (**d**) TEM analysis of the green synthesized CTT-AuNPs.

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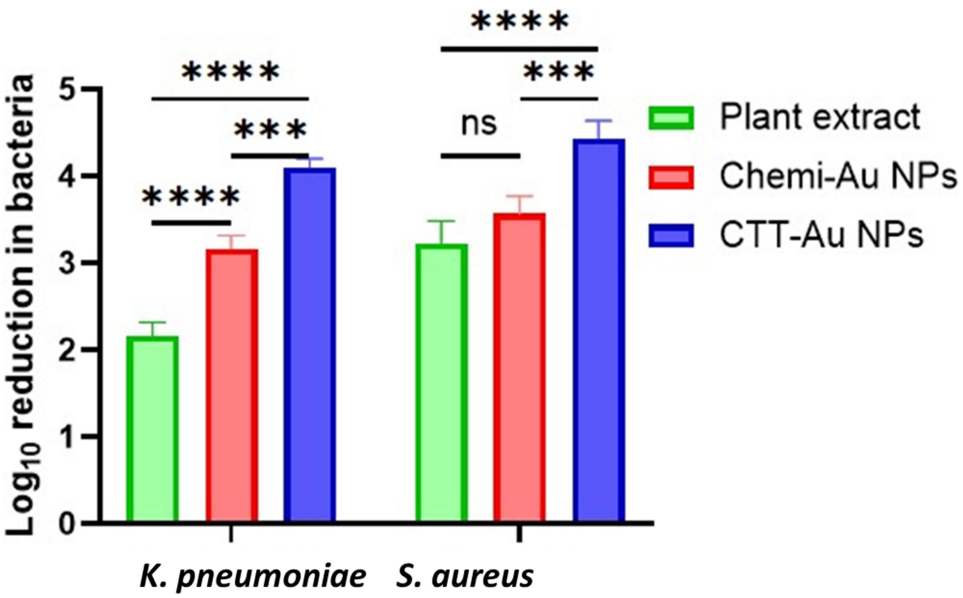

Figure 3 Log_{10} reduction of tested bacterial growth against the green-synthesized CTT-AuNPs compared to the plant extract and chemically synthesized Au-NPs (ns denotes $p > 0.05$, **** and *** denote $p < 0.0005$ and $p < 0.005$, respectively).

bactericidal effect (3.25 log_{10} CFU/mL) on *S. aureus*. In contrast, chemi-AuNPs exhibited better bactericidal effects than the plant extracts against both *K. pneumoniae* (3.1 log_{10} CFU/mL) and *S. aureus*. (3.45 log_{10} CFU/mL, respectively).

The DAPI/PI dual staining method was used to confirm the antibacterial activity of the green-synthesized CTT-AuNPs (Figure 4a–d). The results showed that most *S. aureus* and *K. pneumoniae* cells developed red fluorescence after

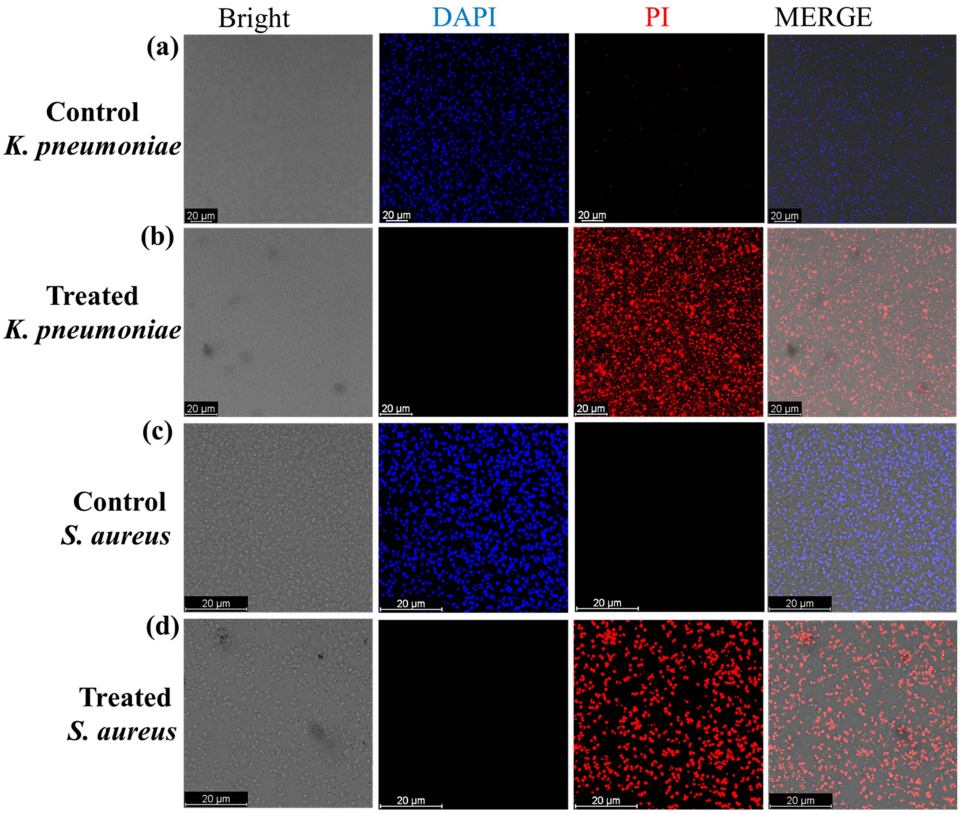

Figure 4 CLSM image of live/dead bacteria. (**a** and **c**) control and (**b** and **d**) Treated bacteria with green synthesized CTT-AuNPs.

treatment with green-synthesized CTT-AuNPs, confirming the presence of dead bacterial cells (Figure 4b and d). In the control, bacterial cells emitted blue fluorescence, confirming the presence of live cells (Figure 4a and c).

Cytotoxicity Assay

The cytotoxic effect of the green-synthesized CTT-AuNPs was evaluated against MCF-7 cells, along with that of the *C. trichotomum* Thunberg extract and chemically synthesized AuNPs. The results demonstrated that the green-synthesized CTT-AuNPs exhibited the strongest cytotoxicity against MCF-7 cells compared to the plant extract or chemi-AuNPs. CTT-AuNPs reduced MCF-7 cell viability to 32.67%, whereas the plant extract alone reduced cell viability to 68.33% at the same concentration after 24 h of incubation. Chemi-AuNPs reduced the cell viability to 50% after the same incubation period (Figure 5).

A dual-staining method using a fluorescent dye was used to confirm the cytotoxic effects (Figure 6a–d). The results showed the cytotoxic effect of the green-synthesized CTT-AuNPs, where most of the cells were red-stained, indicating dead cells (Figure 6d). In contrast, the plant extract and chemi-AuNPs showed less toxicity against MCF-7 cells (Figure 6b and c), which is represented in the image showing more blue-stained cells that represent live cells.

Biocompatibility Assay

The cytocompatibility of green-synthesized CTT-AuNPs was assessed in 293T kidney epithelial cells and compared with that of Chemi-AuNPs. Figure 7a–c show the cytocompatibility results. The data showed that CTT-AuNPs exhibited fewer toxic effects on 293T cells than Chemi-AuNPs. The results showed that Chemi-AuNP-treated cells were more red-stained (Figure 7b) than CTT-AuNP-treated cells (Figure 7c), indicating that CTT-AuNPs were more biocompatible.

Discussion

It has been reported that the leaf extract of *C. trichotomum* Thunberg contains a diverse array of biologically active phytochemicals, including flavonoids, phenolics, alkaloids, terpenoids, anthraquinones, carbohydrates, saponins, and tannins.[16] In the present synthesis process, these compounds play a crucial role in facilitating the reduction and stabilization of the nanoparticles. The proposed mechanism (Figure 8) for the green synthesis of CTT-AuNPs using plant extracts involves a synergistic reduction and stabilization process mediated by phytochemicals, such as

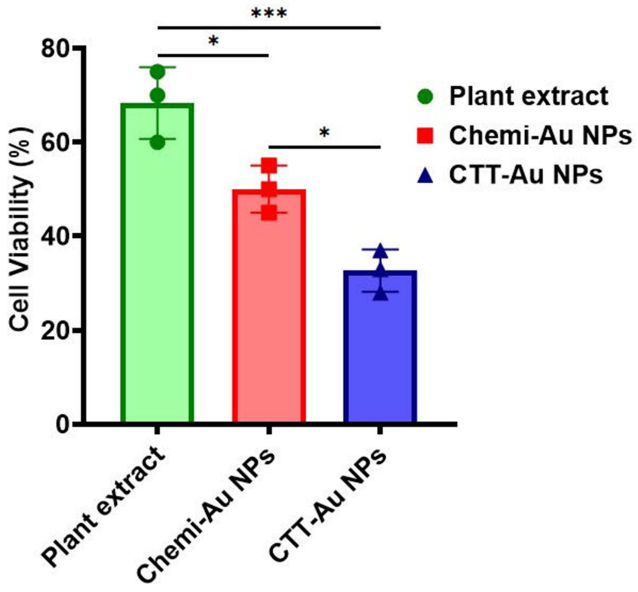

Figure 5 Cytotoxic activity of green synthesized CTT-AuNPs against MCF-7 cells after 24 h of incubation (*** and * denote $p < 0.005$ and $p < 0.05$, respectively).

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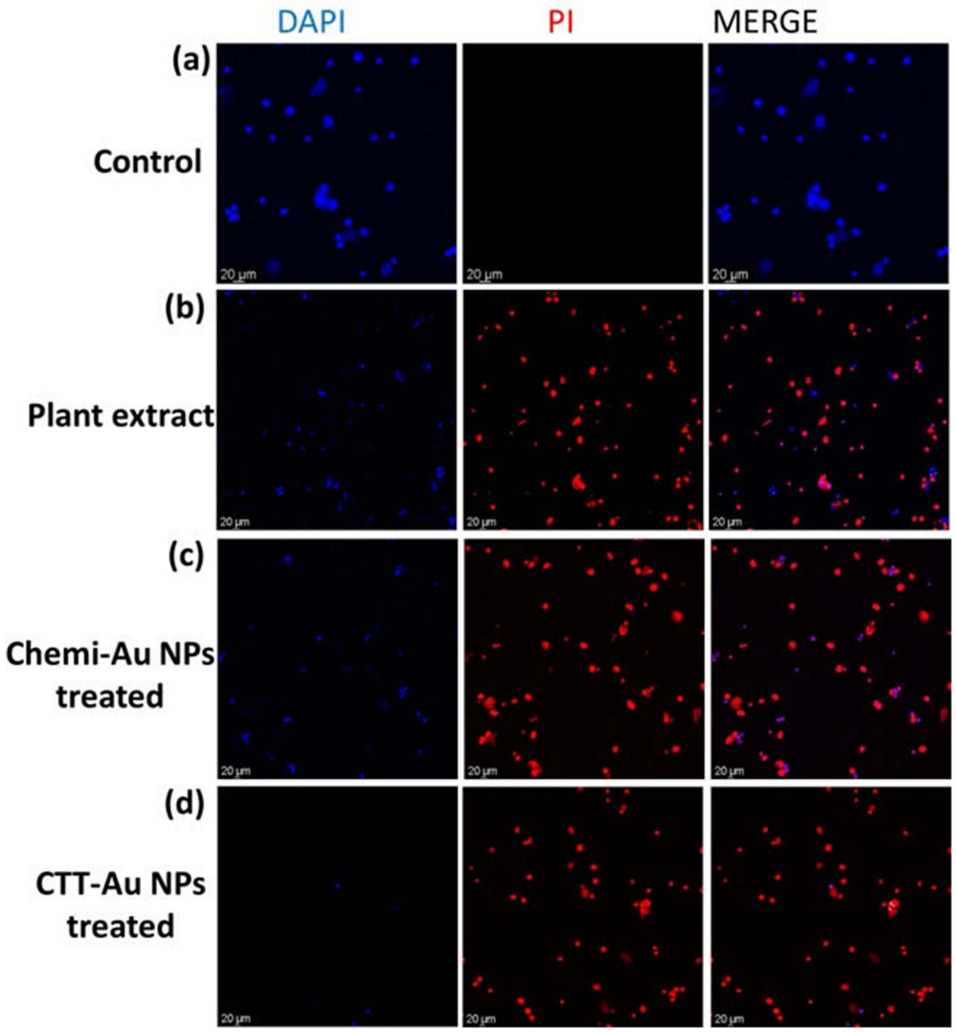

Figure 6 CLSM image of live/dead MCF-7 cells. (**a**) control; (**b**) plant extract, (**c**) cells treated with Chemi-AuNPs, and (**d**) cells treated with green synthesized CTT-AuNPs.

polyphenols, flavonoids, alkaloids, and saponins. The reduction of gold ions (Au^{3+}) to elemental gold (Au^0) occurs via a redox reaction facilitated by the electron-donating functional groups in phytochemicals, particularly the hydroxyl (-OH) groups in polyphenols and flavonoids. During this process, the reducing agents are oxidized, transitioning from their enol form to stable keto derivatives. Concurrently, the synthesized gold nanoparticles are capped and stabilized by various phytochemicals, including alkaloids, proteins, and saponins, which interact with the nanoparticle surface through functional groups such as hydroxyl, amine ($–NH_2$), and carbonyl (–C=O) groups. This capping process prevented nanoparticle aggregation and ensured stability and uniformity. The resulting nanoparticles are monodisperse and biologically compatible owing to the dual role of phytochemicals as reducing and capping agents. This eco-friendly, green synthesis method eliminates the need for hazardous chemical reductants or stabilizers and provides a sustainable approach for the production of AuNPs with potential applications in nanomedicine, catalysis, and environmental science.

This study aimed to synthesize AuNPs from a popular medicinal plant, *C. trichotomum* Thunberg, and to evaluate their antibacterial and anticancer properties. The green-synthesized CTT-AuNPs showed good bactericidal activity, with a $\log_{10}$ reduction of more than 3 $\log_{10}$ CFU/mL against both the bacterial species (Table 1). Generally, the bacteriostatic and bactericidal effects of an antibacterial agent are defined as <3 or ≥3 $\log_{10}$ CFU/mL, respectively.[30] In addition, CTT-AuNPs showed better antibacterial activity against the gram-positive bacterium *S. aureus* than against Gram-negative *K. pneumoniae*,

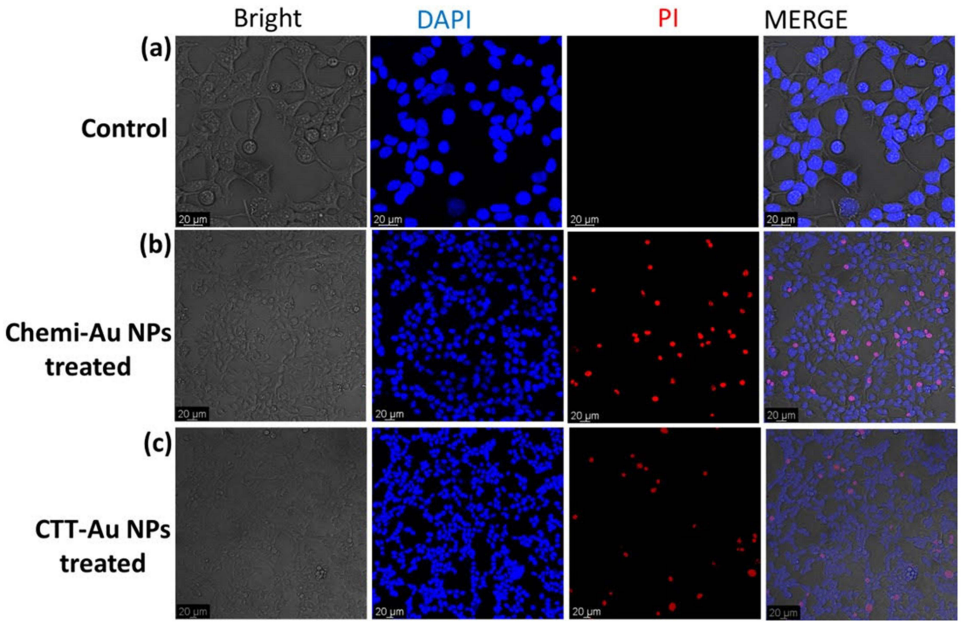

Figure 7 CLSM image of live/dead 293T cells. (**a**) control; (**b**) cells treated with chemically synthesized gold nanoparticles, and (**c**) cells treated with green synthesized CTT-AuNPs.

which is in line with the results of many previous studies.[18,20,29] This growth inhibition mechanism can be attributed to the cell wall structure, composition, and overall surface charge of the gram-positive bacteria. Gram-positive bacteria have a thicker cell wall layer, consisting of peptidoglycan and teichoic acid, than gram-negative bacteria. Both materials have a high negative charge on their surfaces, attracting positively charged metal nanoparticles. However, Gram-positive bacteria may have a higher negative charge than Gram-negative bacteria. A recent study showed that *Bacillus subtilis* (gram-positive) has a higher negative surface charge than *E. coli* (Gram-negative).[31] Although green-synthesized AuNPs exhibit increased bactericidal activity against gram-positive bacteria, some studies have shown that Gram-negative AuNPs are more sensitive to AuNPs.[32] In contrast, Ag NPs also showed greater sensitivity to gram-negative bacteria than to Gram-positive bacteria.[33] The generation of reactive oxygen species (ROS), which help to impair bacterial cells, may be one of the reasons for its antibacterial activity.

Based on previously reported studies, we proposed an antibacterial mechanism of green-synthesized CTT-AuNPs, as shown in Figure 9. They may exert antibacterial effects through a multifaceted mechanism. They penetrate the bacterial cell wall and disrupt membrane integrity via electrostatic interactions and oxidative stress, resulting in increased permeability.[38] Inside the cell, CTT-AuNPs may catalyze the formation of ROS, which induce DNA damage through strand breaks and nucleotide oxidation, inhibit protein synthesis by disrupting ribosomal function, and cause enzymatic inactivation by oxidizing functional groups or directly binding to active sites.[20] Furthermore, CTT-AuNPs impaired bacterial respiration by disrupting the electron transport chain, leading to energy depletion and metabolic collapse. Despite the upregulation of stress-related genes such as sodA, rpoS, and grxA,[40] cumulative oxidative stress and metabolic disruption overwhelm bacterial defense mechanisms. This cascade of damage culminates in cell death, highlighting the robust antibacterial mechanism of CTT-AuNPs and their potential against multidrug-resistant bacteria.

The DAPI/PI dual staining method confirmed the antibacterial activity of green-synthesized CTT-AuNPs. DAPI stains nucleic acids, whereas PI can only be detected in membrane-impaired bacterial cells, thus confirming cell death. Dead cells showed red fluorescence when the PI dye entered the damaged membrane. In contrast, live cells emit blue fluorescence under a microscope, confirming that the cells are intact and deter the PI dye from entering the cells.[41]

These CTT-AuNPs exhibited excellent cytotoxicity on MCF-7 cells but less compared to non-cancerous 293T cells, which was further confirmed by a dual staining assay. The physicochemical state of AuNPs, including their morphology (size and shape) and surface area, may play a crucial role in exerting high cytotoxicity toward cancer cells.[42] Small AuNPs can provide

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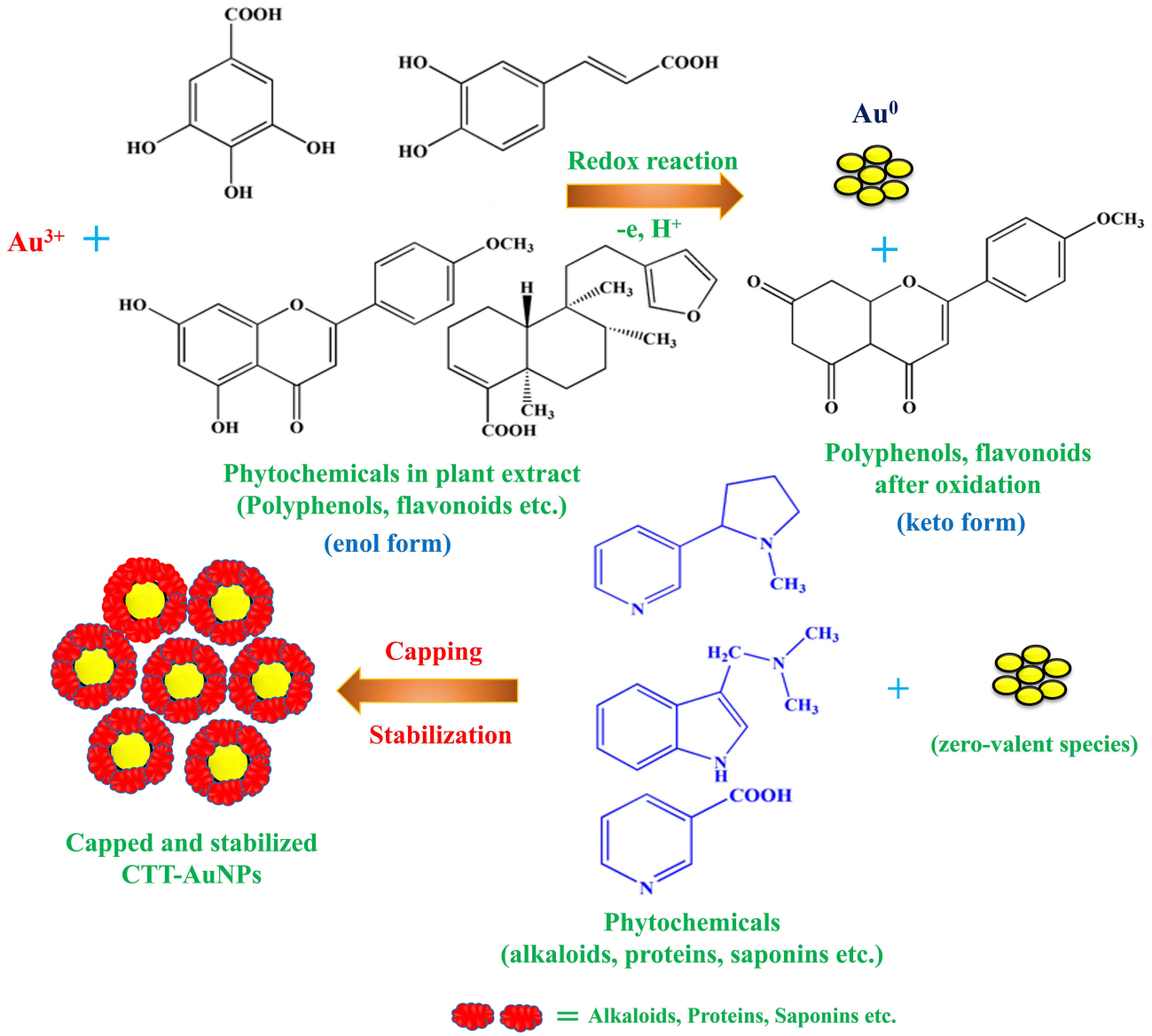

Figure 8 The schematic illustration depicts the proposed mechanism for the green synthesis of CTT-AuNPs utilizing the leaf extract of *C. trichotomum* Thunberg (Adapted from Khan et al[20] under Creative Commons Attribution (CC BY) license).

more surfaces to interact with bioactive molecules depending on their volume.[43] However, several reports have revealed that AuNPs synthesized from different Clerodendrum species have good cytotoxic effects on cancer cells (Table 2). The leaf extract of *C. infortunatum*, which is rich in clerodin, showed good cytotoxicity against leukemic cell lines.[26] *C. inerme* leaf extract significantly reduced the viability of MCF-7 cells after 24 hr.[20] Phytochemicals present in *Clerodendrum trichotomum* are also good sources of anticancer agents, as mentioned earlier.[16]

Moreover, the presence of CTT phytochemicals could be attributed to their enhanced biocompatibility. The increasing demand for AuNPs in the biomedical field has raised concerns regarding biosafety. Safe interactions between the host and AuNPs may confirm their safety and nullify the adverse effects on host immunity.[43] Therefore, it is crucial to test the cytobiocompatibility of green-synthesized AuNPs. Several previous studies have reported the biocompatibility of green-synthesized AuNPs.[20,47,48]

Table 1 Comparison of Antibacterial Activity of CTT-AuNPs With Other AuNPs Biosynthesized From Different Plants

Plant used	Size of NPs	Conc. of NPs	Bacteria used	Outcomes (Zone of inhibition)	References
Clerodendrum inerme	3–9 nm	250 µg/mL	*S. aureus*	13 mm	[20]
***Uncaria gambir* Roxb.**	11 nm	50 µg/mL	*S. aureus*	9 mm	[34]
Aloe vera	15 nm	40 µg/mL	*S. aureus*	17.7 mm	[35]
Peganum harmala	43.44 nm	200 µg/mL	*S. aureus*	25 mm	[36]
Garcinia kola	28 nm	75 µg/mL	*S. aureus*	10 mm	[37]
Jasminum auriculatum	8–37 nm	30 µg/mL	*K. pneumoniae* *S. aureus*	9 mm	[38]
Annona muricata	25.5 nm.	2 mg/L	*K. pneumoniae* *S. aureus*	49% and 40%, respectively	[37]
Areca catechu	13.7 nm	100 µg/mL	*K. pneumoniae* *S. aureus*	11 and 14 mm, respectively	[39]
***Clerodendrum trichotomum* Thunberg**	19.1±2.2 nm	250 µg/mL	*K. pneumoniae S. aureus*	Bacterial log reduction: 4.1 log_{10} CFU/mL and 4.35 log_{10} CFU/mL, respectively	This study

Conclusion

This study reports the successful synthesis of AuNPs from *C. trichotomum* Thunberg using an eco-friendly and green approach. The CTT-synthesized AuNPs were successfully characterized using TEM, SEM, XRD, and EDX. The synthesized AuNPs demonstrated a better log_{10} reduction in the growth of both Gram-positive and Gram-negative bacteria compared to the plant extract alone and commercial nanoparticles. Moreover, these green-synthesized particles showed excellent cytotoxic effects on breast cancer cells, while showing less detrimental effects on non-cancerous kidney cells (293T) than chemically synthesized gold nanoparticles, which proves their enhanced biocompatibility. These excellent antibacterial and cytotoxic properties of the biosynthesized AuNPs are due to the synergistic effect of bioactive phytochemicals present in the CTT extract. Therefore, this study demonstrated that using *the C. trichotomum* Thunberg leaf extract to produce CTT-AuNPs in an environmentally friendly manner with increased biological functions is a cost-effective and practical alternative to traditional chemical techniques.

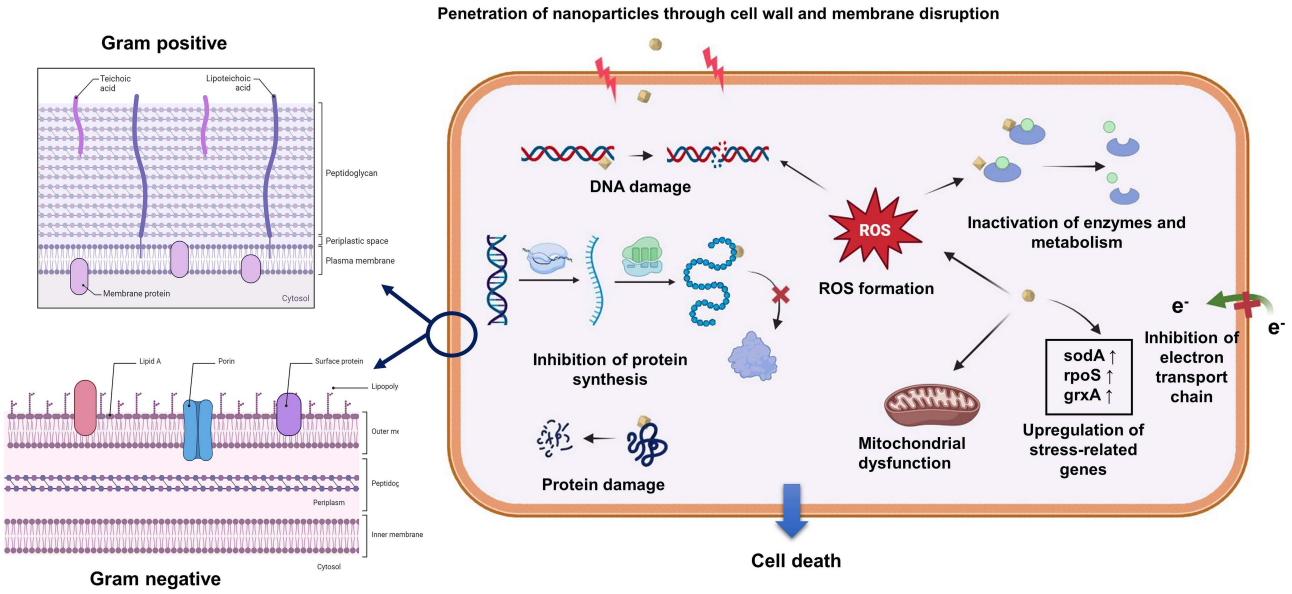

Figure 9 Effect of nanoparticles inside bacterial cells (Created in Biorender.com).

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Table 2 Comparison of the Cytotoxicity of CTT-AuNPs With Other Biosynthesized AuNPs From Different Plants

Plant used	Size of NPs	Cell lines	Outcomes	References
Clerodendrum infortunatum	33 ± 5 nm	Human monocyte leukemic cells (THP-I)	Cell viability decreased to 5% at a 5mM concentration of biosynthesized AuNPs	[26]
Crassocephalum rubens	20 ± 5 nm	Human breast cancer (MCF-7) and colorectal cancer (Caco-2) cells	IC_{50}: 125 and 250 µg/mL, respectively	[44]
Jasminum auriculatum	8–37 nm	Human cervical cancer cell lines (Hela)	IC_{50} value of 104 µg/mL	[38]
Dracocephalum kotschyi	7.9–22.63 nm	Human leukemia cells (K562) and HeLa cell lines	IC_{50}: 196.32 and 152.16 µg/mL, respectively	[45]
Backhousia citriodora	8.40 ± 0.084 nm	MCF-7 and the HepG2 liver cancer cells	IC_{50} values of 116.65 and 108.21 µg/mL, respectively	[46]
***Clerodendrum trichotomum* Thunberg**	19.1±2.2 nm	MCF-7 cells	Cell viability reduced to 32.67% at 120 µg/mL after 24 h of incubation.	This study

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

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