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Eco-friendly synthesis of gold nanoparticles using *Gracilaria gracilis* with antioxidant potential and biocompatibility

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Gold nanoparticles have emerged as promising materials for drug delivery systems due to their unique biological and physicochemical properties. This study presents the synthesis and biological application of gold nanoparticles using red algae (*Gracilaria gracilis*) for the first time, offering a cost-effective and eco-friendly method. The biosynthesized nanoparticles (NPs) were characterized using various analytical techniques, including Transmission electron microscopy (TEM), Field emission scanning electron microscopy (FESEM), Energy dispersive X-ray analysis (EDX), UV-Vis-spectroscopy, Powder X-ray diffraction (PXRD), Fourier-transform infrared spectroscopy (FTIR), and Zeta-potential analysis. The UV-Vis spectrum confirmed the successful green synthesis of gold NPs. FESEM and TEM images revealed the spherical morphology of these NPs with an average size of 10.49 nm, and they were uniformly dispersed. The high gold content was further confirmed by EDX analysis. The crystalline size of the nanoparticles, as calculated from XRD data, was about 39.5 nm. The presence of bioactive compounds on the nanoparticles' surface was verified by FTIR analysis, which also correlated with a decrease in Zeta-potential values. The antioxidant activity was evaluated using the DPPH assay, which revealed dose-dependent radical scavenging effects. Cytotoxicity analysis demonstrated that the biosynthesized gold nanoparticles exhibited no significant toxicity toward human fibroblast cells. Furthermore, these nanoparticles showed excellent biocompatibility, indicating their potential applicability as effective alternatives in biomedical, pharmaceutical, and food industries.

Keywords Gold nanoparticle, Red algae, Green synthesis, Biocompatibility, Biomedical applications

Abbreviations

NPs	Nanoparticles
TEM	Transmission electron microscopy
FESEM	Field emission scanning electron microscopy
EDX	Energy dispersive X-ray analysis
PXRD	Powder X-ray diffraction
FTIR	Fourier-transform infrared spectroscopy
Bio-active	Biologically active
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
ABTS	2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid
FRAP	Ferric ion reducing antioxidant power
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
PG	Propyl gallate
AuNPs	Gold nanoparticles

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GPX	Glutathione peroxidase
SOD	Superoxide dismutase
MAAs	Mycosporin-like amino acids
WoRMS	World register of marine species
GG-AuNPs	Gracilaria gracilis synthesized gold nanoparticles
DMEM-HG	Dulbecco's Modified Eagle Medium-High Glucose
Pen-Strep	Penicillin-streptomycin
FBS	Fetal bovine serum
PBS	Phosphate buffer saline
MTT powder	3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
DMSO	Dimethyl sulfoxide
HAuCl ₄ ·3H ₂ O	Chloroauric acid
XRD	X-ray diffraction
HFF-3	Human foreskin fibroblast cell line

Antioxidants are natural molecules, either synthesized endogenously by cells or obtained exogenously from dietary phytonutrients that inhibit the formation of or neutralize biologically harmful agents such as reactive oxygen and nitrogen species (ROS/RNS)^{1–6}. They protect cells and biomolecules from oxidative stress induced by various environmental stimuli^{7–10}. Antioxidant capacity is commonly evaluated using DPPH, ABTS, and FRAP assays. For example, in the DPPH assay, the interaction between the sample and DPPH (a stable free radical) is monitored by spectrophotometric analysis. Natural antioxidants such as epicatechin, kaempferol, and naringenin exhibit potent anti-cancer, anti-inflammatory, and antioxidant activities. These compounds exert their effects through multiple mechanisms, including the induction of apoptosis in cancer cells, modulation of key signaling pathways such as MAPK and PI3K/AKT/mTOR, and scavenging of free radicals to protect cells from oxidative damage.

To meet some industrial demands, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been widely used. Although these compounds have been reported to exhibit anti-tumor effects due to their antioxidant activities, excessive consumption has been associated with various health concerns in human populations¹¹. Recent studies have highlighted the high toxicity and adverse side effects of synthetic antioxidants, including liver damage and carcinogenicity^{12–14}. These concerns have prompted researchers to focus on extracting natural antioxidants from plants and algae by optimizing culture conditions and employing various extraction techniques^{15–18}.

Gold nanoparticles (AuNPs) exhibit remarkable physicochemical properties that enable their use across various fields, including pharmacological and biomedical research^{19,20}. In recent years, AuNPs have emerged as promising candidates for biomedical applications and are being investigated as a novel class of antioxidant agents^{21,22}. AuNPs can interact with various biochemical groups due to their large surface-to-volume ratio²². They exhibit excellent biocompatibility, stability, and ease of functionalization, making them suitable for various diagnostic and therapeutic applications^{23,24}.

Unlike conventional antioxidants such as vitamins C and E, gold nanoparticles (AuNPs) are resistant to oxidation and do not produce toxic byproducts. Their size and structural characteristics can be precisely tuned to enhance their antioxidant performance. Studies have demonstrated that AuNPs promote the activity of key antioxidant enzymes, including glutathione peroxidase (GPX) and superoxide dismutase (SOD), thereby reducing oxidative damage. Additionally, studies have shown that AuNPs can inhibit lipid peroxidation, a harmful process that compromises cellular integrity^{25–27}. AuNPs exhibit superior antioxidant potential, making them promising candidates for various biological and biomedical applications.

Among various types of algae, red algae exhibit remarkable potential for the synthesis of antioxidant compounds. This capability is attributed to their high content of secondary metabolites such as phenolic compounds, polyphenols, flavonoids, and pigments like carotenoids, mycosporin-like amino acids (MAAs), as well as tannins, vitamin C, and certain antioxidant proteins^{28,29}. *Gracilaria gracilis* is one of the most extensively studied red macroalgae for nanoparticle synthesis. It belongs to the phylum Rhodophyta, and is registered under the taxonomic ID: urn:lsid:marinespecies.org:taxname:AphiaID:145,700 in the World Register of Marine Species (WoRMS). This alga is easy to cultivate and can tolerate fluctuations in salinity and temperature³⁰. Its natural habitat ranges from rocky shores to sandy seabeds.

Unlike previous studies that mainly employed plant extracts for gold nanoparticle biosynthesis, this study is the first to employ *Gracilaria gracilis* extract for this purpose, highlighting its unique phytochemical composition as a dual reducing and stabilizing agent, and demonstrating the production of well-crystallized nanoparticles with distinct structural features and promising biomedical applications.

Although several studies have investigated the green synthesis of gold nanoparticles using plant-derived extracts, there remains a lack of comprehensive research on algal-based synthesis, particularly using *Gracilaria gracilis*. Furthermore, the cytotoxic impacts of these biosynthesized nanoparticles on normal human fibroblast cells have not been thoroughly explored. Addressing this research gap, the present study reports a one-pot, eco-friendly synthesis of gold nanoparticles using *G. gracilis* (GG-AuNPs), followed by detailed physicochemical characterization. The antioxidant and cytotoxic properties of GG-AuNPs were further evaluated to assess their potential as safe and biocompatible nanomaterials with strong antioxidant capacity for future biomedical applications.

Materials and methods

Materials

A 24-karat gold bar was purchased from Parsis Gold Shop (Mashhad, Iran). Chemical reagents, including HNO_3 , HCl , NaOH , PVP, and NaBH_4 , were obtained from Merck (Germany). DMEM-High Glucose (DMEM-HG) medium was supplied by Dacell (Iran), while Pen-Strep, FBS, PBS, and trypsin were procured from Denazist (Iran). Culture flasks and Falcon tubes were purchased from SPL (Korea). Finally, MTT powder and dimethyl sulfoxide (DMSO) were obtained from Betacell (Iran).

Collecting algae and preparing the extract

G. gracilis (NCBI taxonomy ID: 2777; AphialID: 145700, World Register of Marine Species- WoRMS) samples were collected from the southern coast of Bushehr, Iran. The fresh samples were sun-dried and transported to Mashhad for further processing. For extract preparation, 10 g of the dried algal biomass was ground into a semi-powdered form and mixed with 100 ml of deionized water in a flat-bottomed flask, which was then covered with aluminum foil to prevent photo-degradation. The mixture was stirred continuously at 40 °C for 24 h using a magnetic stirrer (Medpip, Iran). The resulting solution was filtered.

Synthesis and neutralizing process of Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$)

To synthesize chloroauric acid, 0.3 g of a 24-karat gold bar was dissolved in a mixture of 5 ml of HNO_3 and 15 ml of concentrated HCl (37%) under continuous stirring at 50 °C using a magnetic stirrer. Once the gold had fully dissolved, 5 ml portions of concentrated HCl were added sequentially five times while gradually increasing the stirring speed and raising the temperature to 70 °C to remove residual nitric acid. The resulting solution was then diluted with deionized water, and the mixture was centrifuged at 10,000 RPM for 10-minute cycles (Universal-320R, Germany)³¹.

Synthesizing and characterizing gold nanoparticles

A 0.1 M chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solution was prepared by dissolving 240 mg $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in 6 mL of deionized water under magnetic stirring (Medpip, Iran) at room temperature. Three different concentrations of algal extract and 0.1 M gold chloride solution were prepared in covered beakers under continuous stirring at room temperature. The volume ratios of algal extract to $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ were as follows: 7:3 (7 ml extract + 3 ml gold solution), 8:2 (8 ml of extract + 2 ml gold solution), and 9:1 (9 ml of extract + 1 ml gold solution). The gold chloride solution was added dropwise to the alga extract to ensure uniform mixing and controlled reduction.

Characterization of the synthesized nanoparticles was performed using various analytical techniques. UV-visible Spectrophotometry (SHIMADZU Bio-spec-1601, Japan) was conducted in the wavelength range of 350–750 nm, with absorbance measurements recorded every 15 min for 4 h. Control samples included three concentrations of algal extract mixed with deionized water in the same ratios (9:1, 8:2, and 7:3). For further analyses, the nanoparticle-containing solutions were oven-dried at 40 °C overnight (Mettler UN55, Germany). The crystalline structure and phases of the synthesized gold nanoparticles were determined by X-ray diffraction (XRD) using a GNR Explorer diffractometer (Italy) operating over a 2θ range of 20° to 80°. Fourier-transform infrared spectroscopy (FTIR) (Thermo Nicolet AVATAR 370 FT-IR, USA) was employed to identify functional groups and molecular bonds involved in nanoparticle formation. Zeta potential analysis (Horiba SZ100, Japan) was used to determine the surface charge and colloidal stability of the nanoparticles. The morphology, size, and purity of the gold nanoparticles were examined using field emission scanning electron microscopy (FESEM, MIRA 3 TSCAN, Czech Republic) and transmission electron microscopy (TEM, LEO 915AB, Germany).

Antioxidant activity

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay for GG-AuNPs was performed according to previously reported methods³². In this assay, each test sample was diluted in distilled water to obtain the desired final concentration of GG-AuNPs. Subsequently, 1 mL of DPPH solution was added to 2.5 mL of the sample solution, and the mixture was incubated under dark conditions. Absorbance was measured at 518 nm, and the antioxidant activity was expressed as a percentage of radical scavenging. The negative control consisted of DPPH solution alone, while butylated hydroxyanisole (BHA) served as the positive control. The scavenging activity (%) was calculated using the following equation:

$$\text{Scavenging activity (\%)} = (\text{Absorption of control} - \text{Absorption of sample}) / \text{Absorption of control} \times 100$$

Cell culture

In this study, the human foreskin fibroblast cell line HFF-3 was used. The cell line was obtained from the Ferdowsi University of Mashhad (Iran). For cell culture, the cryopreserved cells were thawed and maintained in a complete culture medium consisting of Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG; 84%), supplemented with 15% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin (Pen-Strep). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 . The culture medium was replaced whenever a color change indicated nutrient depletion or pH variation. Cell morphology and confluence were monitored daily using an inverted microscope (Nikon Eclipse TS100, Japan). To avoid over-confluence (above 80–85%), subculturing was performed regularly to maintain optimal growth.

MTT assay

The MTT assay was performed to evaluate the in-vitro cytotoxicity of the green-synthesized gold nanoparticles (AuNPs). The assay was initiated when the cell culture reached approximately 85% confluence. Depending on the treatment design, the experiment was conducted over a period of 3 to 5 days. On the second day, cells were

exposed to the test compounds at various concentrations. The treatment solutions were freshly prepared by diluting the AuNPs in complete culture medium and were added to the wells after removing the spent medium. Each concentration was tested in triplicate, and fresh medium was added to the control wells. The plate was then incubated for 24, 48, or 72 h, depending on the experimental design. On the final day, the MTT assay was performed. A stock MTT solution was prepared by dissolving 5 mg of MTT powder in 1 mL of PBS. In a dark environment, 10 μ L of this solution was added to each well, followed by incubation for 3 h. Subsequently, 100 μ L of DMSO was added to dissolve the formazan crystals. The plate was covered with aluminum foil and placed on a shaker for 20 min. Absorbance was then measured at 570 nm using a microplate reader (EPOCH 2, BioTek Instruments, USA).

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). The Shapiro–Wilk test was used to assess the normality of data distribution. For normally distributed data, one-way ANOVA followed by Tukey's post hoc was applied, whereas the Mann–Whitney test was used for non-normally distributed data. A p -value < 0.05 was considered statistically significant. Statistical analyses were performed using Graphpad Prism version 8.4.3, and particle size distribution was analyzed using ImageJ and SPSS software.

Results and discussion

UV-Vis spectroscopy

Gold nanoparticles (AuNPs) were synthesized using different volume ratios of algal extract to chloroauric acid, and their formation was confirmed by UV–Vis spectrophotometry. The control sample exhibited no characteristic absorption peak, indicating the absence of AuNPs. As shown in Fig. 1, the 7:3 ratio displayed a broad absorption band, suggesting possible nanoparticle aggregation and non-uniform size distribution. In contrast, the 8:2 ratio exhibited a sharp and intense surface plasmon resonance (SPR) peak, indicating the formation of stable, well-dispersed, and uniformly sized AuNPs. This sample also demonstrated the strongest linear correlation with the Beer–Lambert law, confirming a high nanoparticle concentration and minimal aggregation. Therefore, an 8:2 ratio was identified as the optimal synthesis condition. Conversely, the 9:1 ratio displayed a weak and irregular peak, suggesting poor nanoparticle formation, most likely due to an insufficient amount of gold precursor.

Sung et al.³³ reported a positive linear correlation between nanoparticle formation and absorbance intensity over time, indicating that as the nanoparticle concentration increased, the absorbance peak height also rose before reaching a plateau. In gold nanoparticle synthesis, a strong and symmetric maximum absorbance peak corresponds to the highest nanoparticle concentration. Moreover, increasing the extract volume is directly associated with a faster reduction rate and accelerated reaction kinetics.

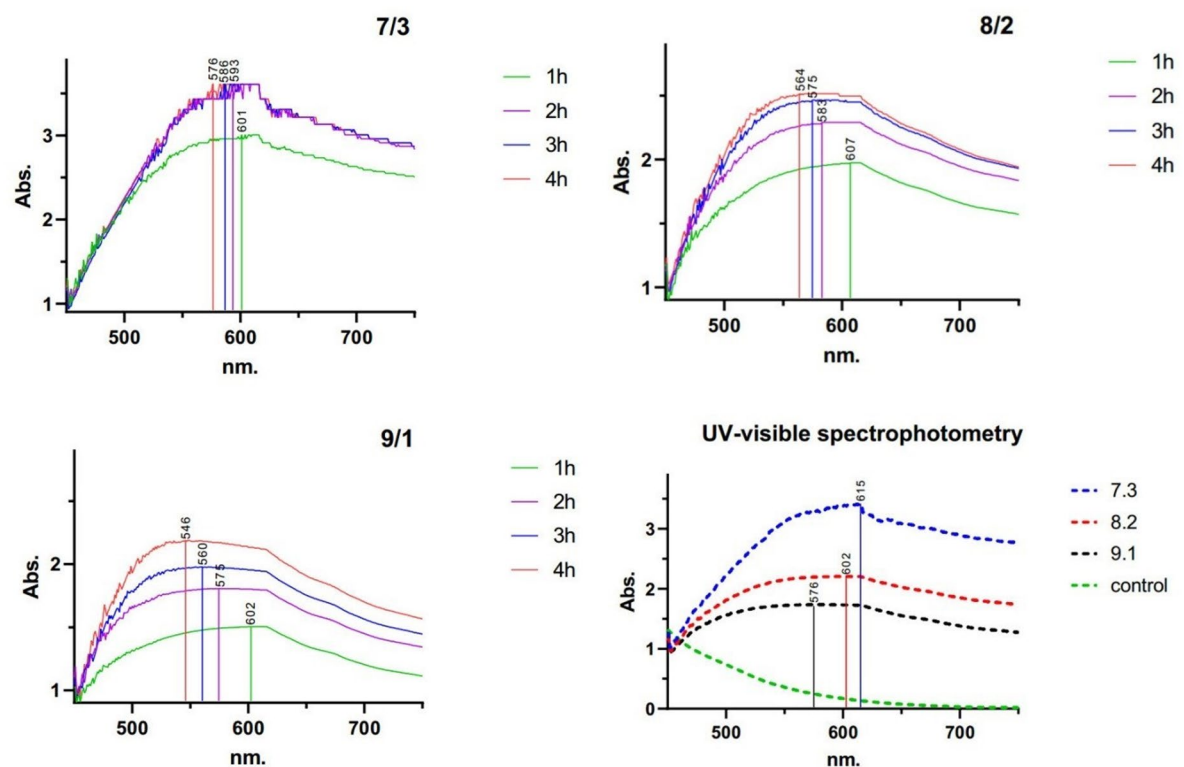


Fig. 1. UV-Vis spectrum of the biosynthesized GG-AuNPs.

Fourier transform infrared spectroscopy (FTIR)

The possible functional groups present in algae extract and GG-AuNPs were analyzed using FTIR spectroscopy. The FTIR spectrum of GG-AuNPs showed distinct absorption peaks corresponding to various biochemical groups associated with the algal coating. As shown in Fig. 2, a broad absorption band at 3423 cm^{-1} was attributed to the hydroxyl ($-\text{OH}$) stretching vibrations. The band observed at 2933 cm^{-1} corresponded to C-H stretching³⁴, while the carboxyl group exhibited characteristic stretching vibrations at 1624 cm^{-1} ³⁵. A peak at 1384 cm^{-1} was assigned to $-\text{OH}$ deformation, and the absorption band at 1052 cm^{-1} was related to polysaccharides³⁶. Additionally, the band at 596 cm^{-1} corresponded to N-H stretching vibration.

Field emission scanning electron microscopy (FESEM), transmission electron microscope (TEM), and Zeta-potential analysis

TEM images (Fig. 3A) reveal that green synthesized AuNPs are polycrystalline with rod-like, square, spherical, and semi-spherical structure. FESEM analysis (Fig. 3B) was conducted to examine the morphology and average particle size of the red algae-coated AuNPs. The micrographs revealed that the nanoparticles were predominantly rod-like and spherical in shape, with an apparent coating of biomolecules derived from the red algae. Gold nanoparticles (AuNPs) can be synthesized in a variety of shapes and sizes using green methods that employ plant and algal extracts as reducing and stabilizing agents. The biomolecules present in these extracts, such as polyphenols and polysaccharides, facilitate the reduction of gold ions and influence nucleation and growth processes, ultimately determining the morphology of the nanoparticles. By selecting different types of plant or algal extracts, researchers can obtain spherical, rod-shaped, triangular, or other anisotropic AuNPs^{20,37}. EDX analysis (Fig. 3C) confirmed the elemental purity of the synthesized GG-AuNPs, indicating the successful formation of gold nanoparticles. The mean particle size (Fig. 3D) was approximately 10.49 nm, demonstrating a uniform size distribution. The GG-AuNPs had a negative zeta-potential values in the mean of -18.6 mV (Fig. 3E). This range is considered as an incipient stability for colloids³⁸.

Powder X-ray diffraction results

The PXRD pattern of the biosynthesized GG-AuNPs is presented in Fig. 4. The characteristic diffraction peaks observed at 2θ values of 38.5° , 44.5° , 64.6° , and 77.5° correspond to the (111), (200), (220), and (311) crystallographic planes of the face-centered cubic (FCC) gold, confirming the crystalline nature of the synthesized nanoparticles. The presence of intense and well-defined peaks indicates a high degree of crystallinity³⁹. These findings are consistent with the data from the Joint Committee on Powder Diffraction Standards (JCPDS NO. 040784) and align well with previously reported PXRD patterns of green-synthesized gold nanoparticles^{35,40,41}. Crystallite size calculation of AuNPs was done using Scherrer's equation. It is found that the average size is $\sim 39.5\text{ nm}$ which closed with the particle size obtained from FESEM image of NPs (10.49 nm).

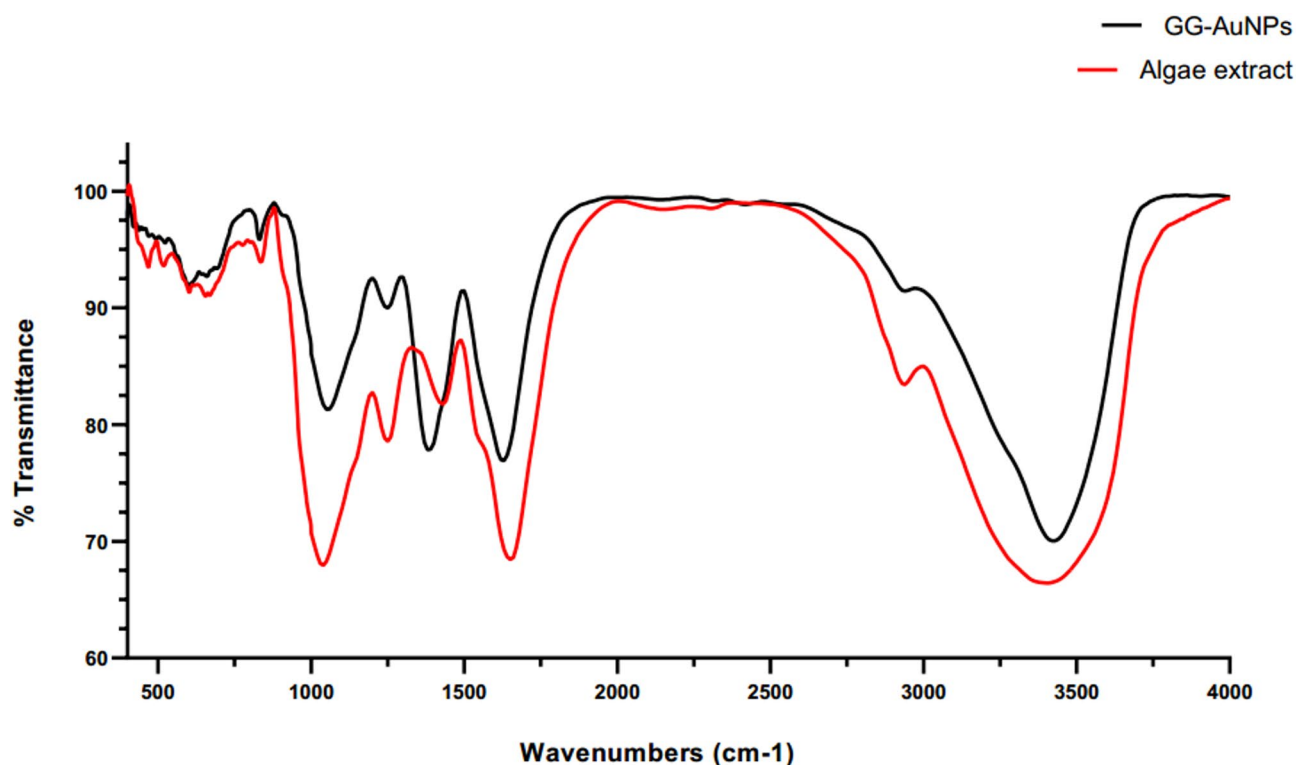


Fig. 2. FTIR spectrum of the algal extract and GG-AuNPs.

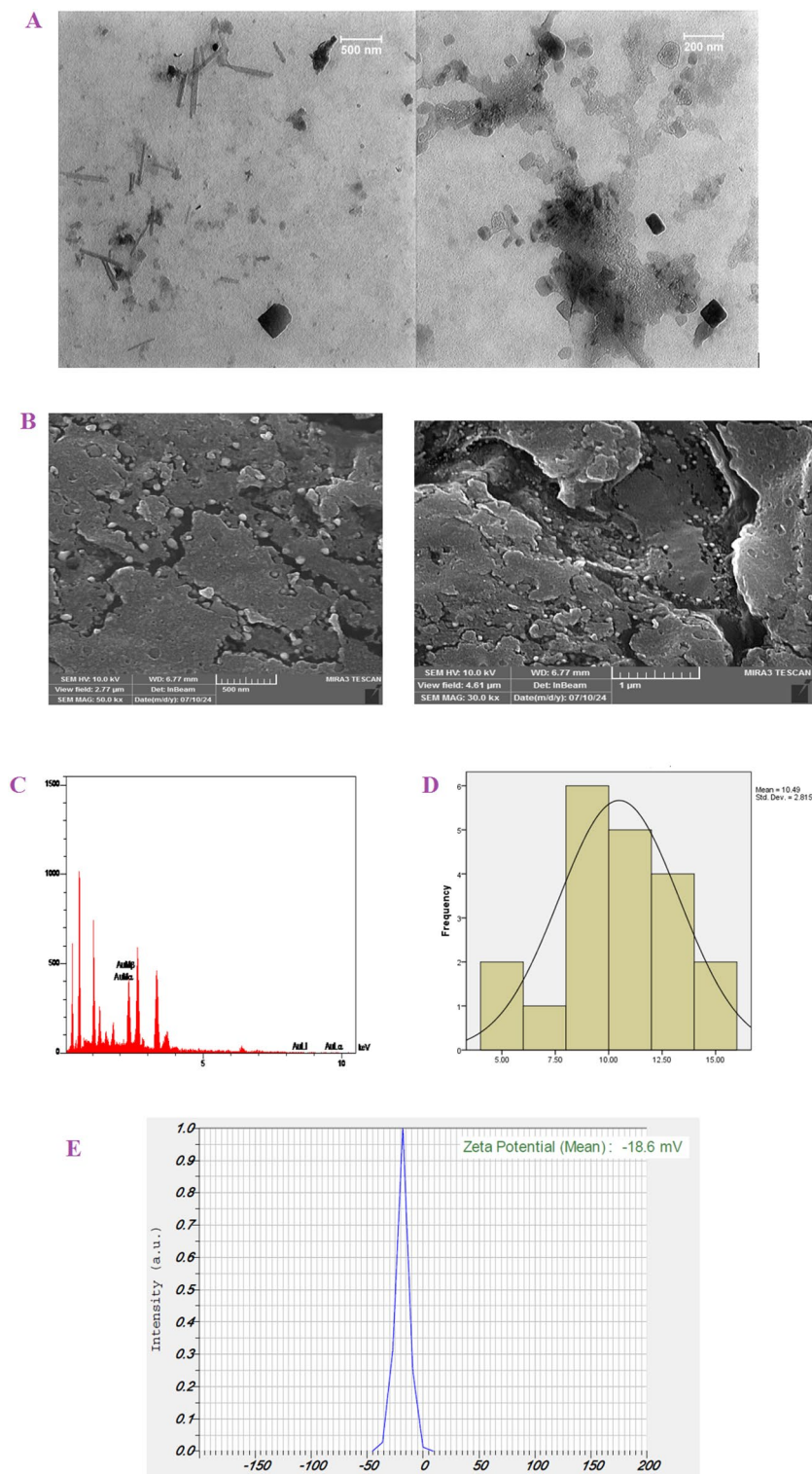


Fig. 3. (A): TEM images, (B): FESEM images, (C): EDAX examination, (D): particle size distribution, and (E): zeta potential of the biosynthesized GG-AuNPs.

Antioxidant results

Antioxidants are naturally occurring molecules produced by cells or plants that can inhibit the formation of toxic reactive species, such as reactive oxygen (ROS) and nitrogen (RNS) species^{42,43}. They protect tissues and cells from oxidative stress and environmental insults. Recent studies have demonstrated that gold nanoparticles (AuNPs) possess notable free-radical scavenging properties, providing cellular protection across several cell

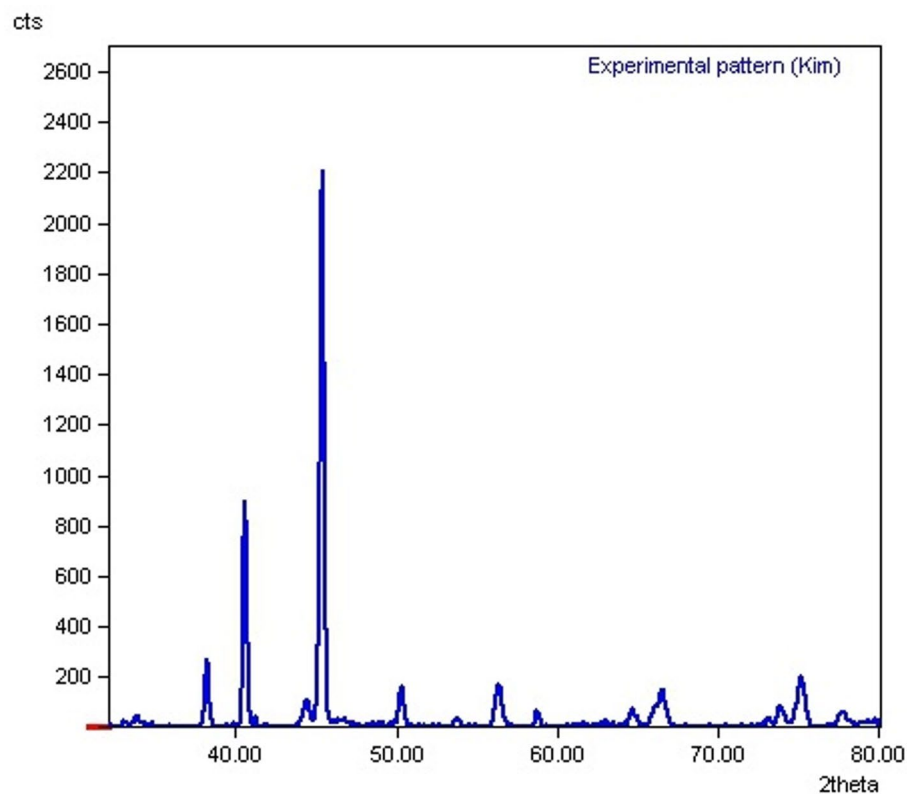


Fig. 4. Powder X-ray Diffraction results of bio-fabricated gold nanoparticles using red algae extract of *Gracilaria gracilis*.

lines^{44–46}. Among the standard methods for evaluating antioxidant activity, the DPPH radical assay is widely used because of its simplicity and reliability⁴⁷.

As shown in Fig. 5, the biosynthesized AuNPs derived from red algae exhibited strong DPPH radical-scavenging activity, comparable to that of butylated hydroxyanisole (BHA), a well-known industrial antioxidant. The scavenging efficiency of AuNPs increased in a dose-dependent manner, indicating potent antioxidant capacity. These findings suggest that AuNPs could serve as effective antioxidant agents for various industrial and biomedical applications.

In general, the antioxidant behavior of AuNPs can be explained by two principal mechanisms: (1) Chain-breaking and (2) Inhibitory (Fig. 6). The chain-breaking mechanism involves electron or proton transfer from the antioxidant to a free radical, forming a stable hydroperoxide (ROOH). The inhibitory mechanism comprises two pathways: (i) Superoxide-mimic activity, in which AuNPs trap superoxide anions and release hydrogen peroxide and oxygen; and (ii) Catalase-mimic activity, in which hydrogen peroxide decomposes into water and oxygen under neutral pH conditions⁴⁴.

The remarkable antioxidant performance of GG-AuNPs can be understood from their nanoscale features and surface chemistry. Because of their small size (~10 nm), these particles expose a very high surface area that provides abundant reactive sites for direct electron donation to neutralize free radicals. The phytochemicals from *Gracilaria gracilis* such as phenolic compounds and flavonoids, act as natural capping agents that not only stabilize the nanoparticles but also endow them with hydrogen- and electron-donating abilities. This biological coating works in concert with the intrinsic redox nature of gold atoms, which can shift between Au⁰ and Au⁺ states, enabling rapid electron transfer reactions that detoxify reactive oxygen species. Altogether, these size-dependent and surface-mediated effects explain the strong radical-scavenging potential observed for the biosynthesized GG-AuNPs and highlight their promise as safe and effective antioxidant nanomaterials.

Cell toxicity activity of biosynthesized gold nanoparticles

Gold nanoparticles (AuNPs) possess a wide range of applications across various fields, particularly in biomedicine and pharmaceuticals. However, their potential cytotoxicity remains a major concern for human health. Nanoparticles ranging from 1 to 100 nm in size are small enough to enter cells via endocytosis. Studies have demonstrated that nanoparticles can utilize multiple cellular uptake pathways, allowing their translocation to diverse tissues where they may disrupt normal biological functions. Furthermore, the physicochemical properties of nanoparticles play a crucial role in determining their toxicity^{48–50}.

The green synthesis of nanoparticles should be prioritized because it minimizes the use of toxic chemicals during fabrication. Numerous studies have examined the mechanisms underlying nanomaterial toxicity, emphasizing size- and structure-dependent effects^{51–54}. The size of gold nanoparticles plays a critical role in

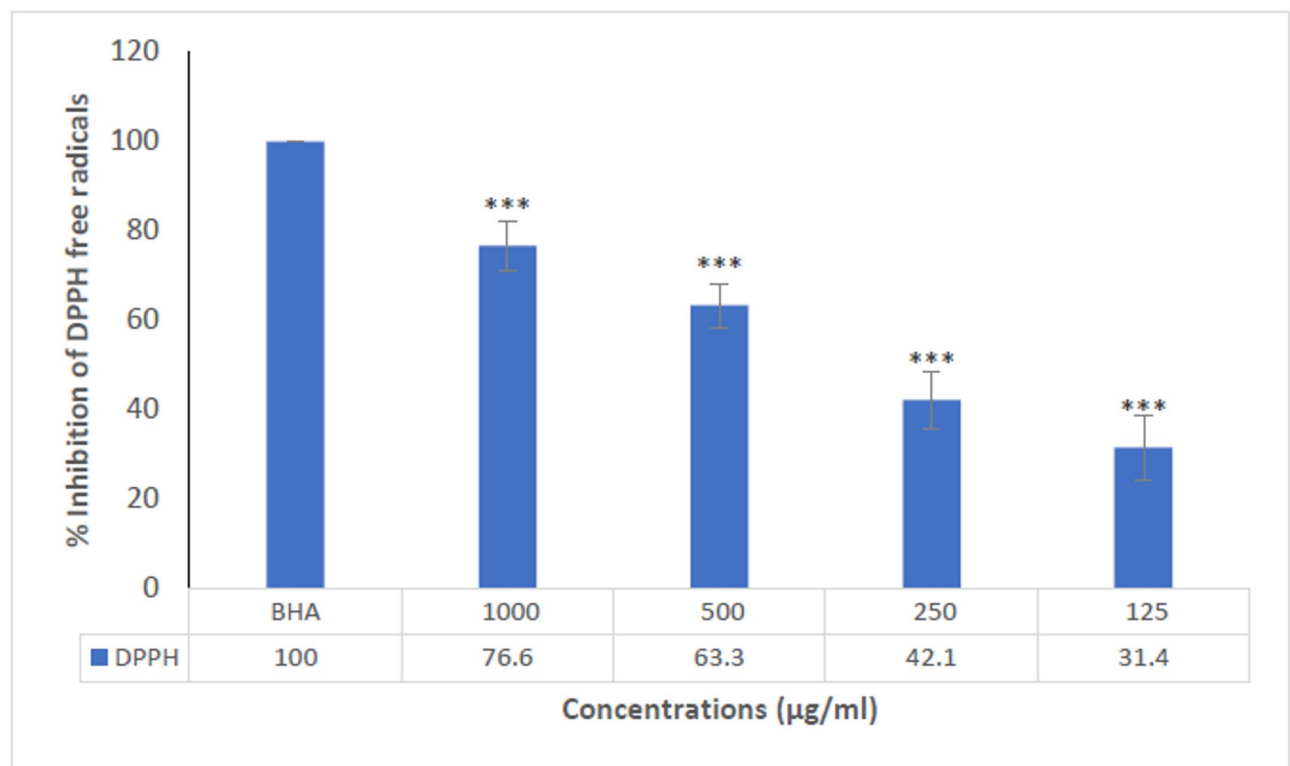


Fig. 5. The inhibitory effect of gold nanoparticles synthesized with algal extract on DPPH free radicals compared to BHA. Significant changes are shown by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. ns = not significance.

determining their biocompatibility, as smaller particles may reduce nonspecific organ accumulation and thereby minimize potential toxic effects⁵⁵. Consequently, evaluating the cytotoxicity of synthesized nanoparticles is essential for assessing their biosafety⁵⁶. As illustrated in Fig. 7, the biosynthesized AuNPs derived from red algae demonstrated negligible toxicity toward human fibroblast cells, indicating their potential suitability for diverse industrial applications. One of the primary mechanisms responsible for AuNP-induced cytotoxicity is the generation of reactive oxygen species (ROS)⁵⁷. The key regulatory factors involved in this process are summarized in Fig. 8.

Challenges and future perspectives

Gold nanoparticles (AuNPs) have recently garnered significant attention due to their unique physicochemical properties. A growing area of research is the “green synthesis” of these nanoparticles, which utilizes biological sources to create them in an eco-friendly manner^{58,59}. The synthesis of AuNPs using different algae species results in particles with varied characteristics. The size and shape of these nanoparticles are crucial as they significantly influence their biological activity. For instance, AuNPs from *Chondrus crispus* and *Porphyra linearis* are spherical with a size of approximately 16.9 nm, whereas those from *Gelidium corneum* are polyhedral and larger, at 44.2 nm⁶⁰. In contrast, nanoparticles synthesized from the green alga *Ulva rigida* are smaller, around 9 nm⁶¹, while the red seaweed *Halymenia venusta* produces larger spherical particles of 81 nm⁶². Some syntheses produce a mix of shapes; for example, *Caulerpa prolifera* yields particles that are spherical, hexagonal, and triangular⁶³. This diversity in size and shape allows for a broad spectrum of interactions with biological systems, making algae-derived AuNPs versatile tools for various medical applications.

Antioxidants exert their protective effects through multiple mechanisms that mitigate oxidative stress^{64–66}. One primary mode is the inhibitory mechanism, in which antioxidants suppress the initiation or propagation of free radical formation, often by chelating metal ions or quenching reactive species before they can trigger chain reactions. Another important mode is the chain-breaking mechanism, where antioxidants donate hydrogen atoms or electrons to existing free radicals, terminating the radical chain reactions and stabilizing reactive intermediates. These complementary mechanisms enable antioxidants to prevent cellular damage and lipid peroxidation, thereby maintaining redox homeostasis and protecting biomolecules from oxidative injury.

One of the most promising applications of biotechnology and nanosciences is in cancer therapy^{67–69}. Several studies have demonstrated the significant antitumoral and cytotoxic effects of algae-derived AuNPs, often mediated by the induction of apoptosis. Nanoparticles from red algae such as *Chondrus crispus*, *Gelidium corneum*, and *Porphyra linearis* have been shown to induce apoptosis in monocytic cancer cell lines⁶⁰. Specifically, AuNPs from *Gracilaria foliifera* exhibited a remarkable 92.13% anticancer activity against MCF-7 breast cancer cells at a concentration of 188 µg/ml⁶¹. Furthermore, nanoparticles from *Halymenia pseudofloresii* showed potential cytotoxic activity against A549 lung cancer cells with an IC₅₀ value of 19.02 µg/mL⁷⁰, while

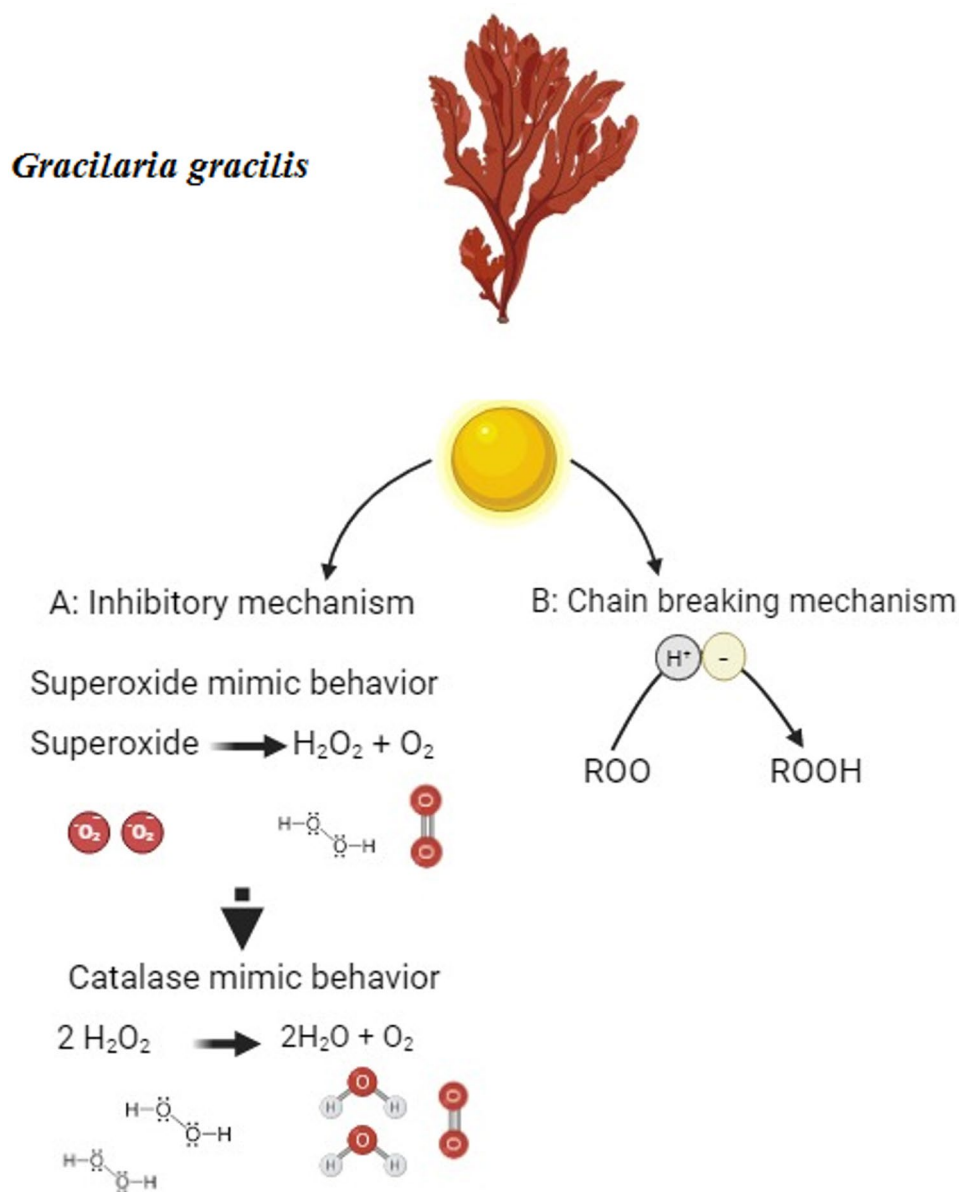


Fig. 6. Gold nanoparticles: antioxidant mechanism of action.

those from *H. venusta* also displayed antiproliferative properties validated by reactive oxygen species (ROS) assays⁶². Adding to the evidence, AuNPs from *Halodule uninervis* have also shown the ability to inhibit cancer cell viability and induce apoptosis⁴¹. A crucial aspect of cancer therapy is minimizing harm to healthy cells. In this regard, AuNPs from *Gracilaria verrucosa* are particularly noteworthy, as they showed no cytotoxic effect against healthy human embryonic kidney (HEK-293) cells but displayed effective cellular uptake, suggesting a favorable safety profile for targeted therapies⁷¹.

Beyond cancer, these nanoparticles possess potent antimicrobial and antifungal properties. AuNPs synthesized from *Ulva rigida*, *Cystoseira myrica*, and *Gracilaria foliifera* have exhibited significant activity against dermatophytic fungi such as *Trichosporon cataneum* and *Trichophyton mantigrophytes*⁶⁰. Similarly, AuNPs from *C. trinodis* showed particularly high antifungal activity against pathogens like *Aspergillus niger* and *Alternaria alternate*, respectively⁶³. In terms of antibacterial action, AuNPs have proven effective against both Gram-positive and Gram-negative bacteria. Nanoparticles from *Gelidiella acerosa* were found to effectively kill *S. aureus*⁷², and those from the green microalga *Dunaliella salina* displayed a significant bactericidal power against Gram-positive bacteria⁷³.

The therapeutic potential of algae-derived AuNPs extends to anti-inflammatory, antioxidant, and antidiabetic applications. AuNPs from *Gelidium corneum* have shown the ability to inhibit pro-inflammatory cytokines IL-6 and TNF α , suggesting a role in treating chronic inflammatory diseases like rheumatoid arthritis⁶⁰. Similarly, those from *Cystoseira myrica* demonstrated a 64.2% inhibitory effect on protein denaturation, a marker of inflammation⁶¹. The antioxidant capabilities are also notable, with AuNPs from *Porphyra linearis*⁶⁰ and *Halymenia*

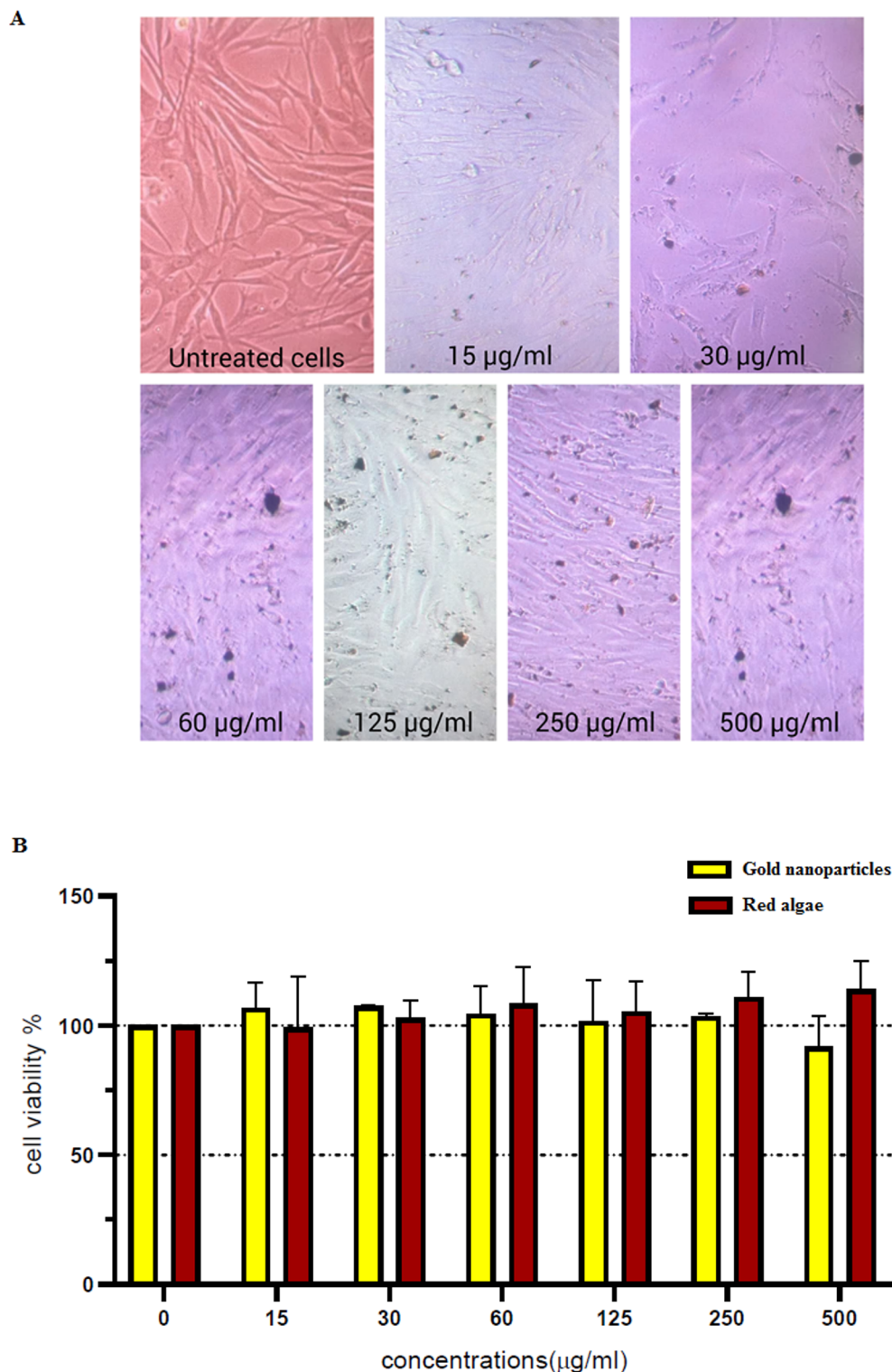


Fig. 7. (A) Human fibroblast cell (HFF-3) morphology treated with different concentrations of GG-AuNPs; (B) Data analysis of cell viability percentage against human fibroblast cells and red algae.

species^{62,70} showing excellent free radical scavenging activity; nanoparticles from *Halymenia pseudofloresii*⁷⁰, for instance, effectively inhibited 71.87% of DPPH radicals at a 50 µg/mL concentration. Furthermore, researchers have identified other unique applications, such as the antidiabetic potential of nanoparticles from *Corallina officinalis*⁷⁴. This is further supported by findings that AuNPs from *Gelidiella acerosa* exhibit strong inhibitory activity against alpha-amylase and alpha-glucosidase enzymes, key targets in managing diabetes⁷⁵. Lastly, the diverse bioactivity is highlighted by the schistolarvicidal (larva-killing) activity of *C. myrica* AuNPs against

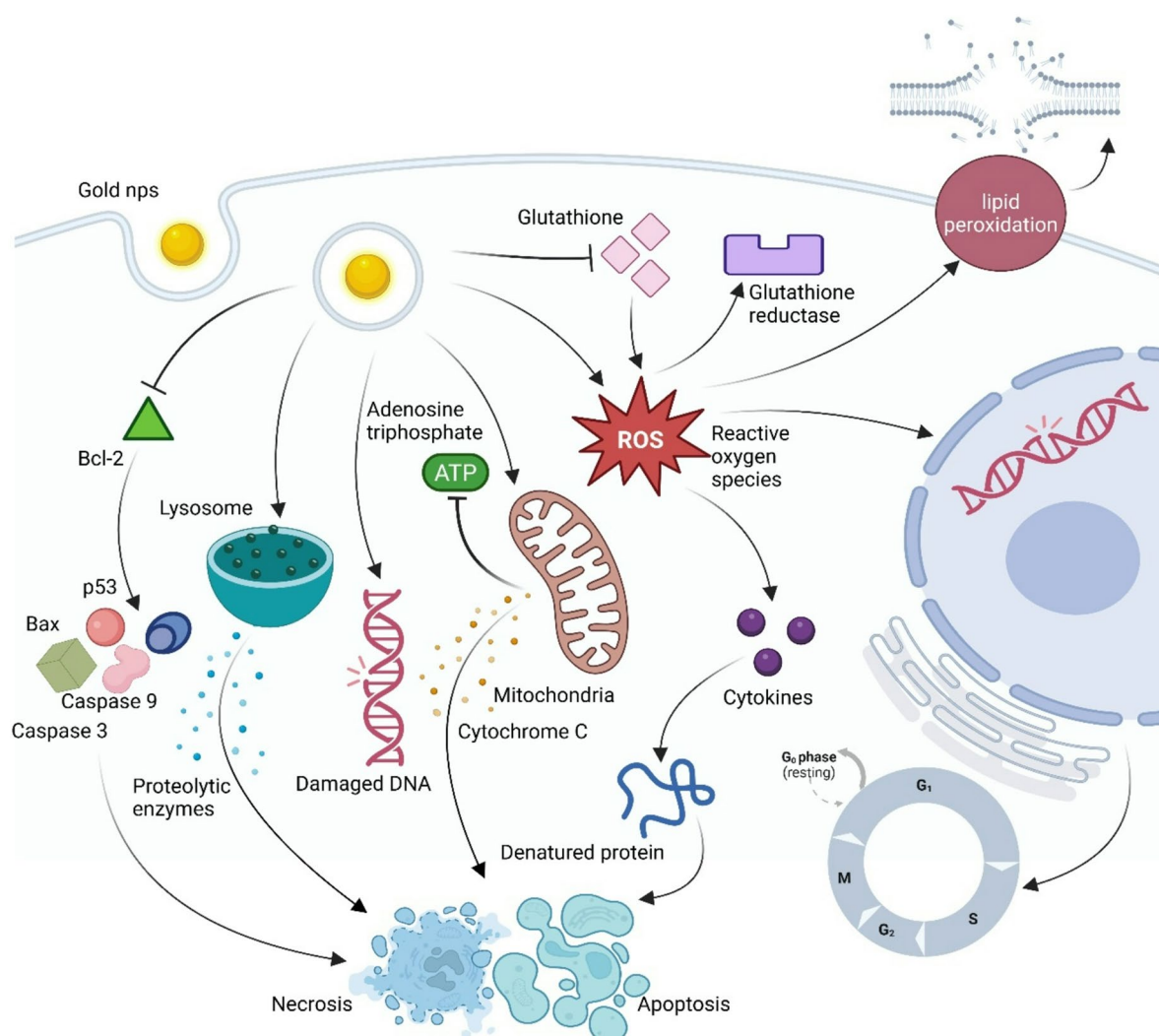


Fig. 8. Some of the major cytotoxic activities of gold nanoparticles.

Schistosoma mansoni cercariae⁶³. In conclusion, the green synthesis of gold nanoparticles using marine algae is a highly promising and sustainable approach for developing novel biomedical tools. The resulting nanoparticles exhibit a wide range of sizes and shapes, which contribute to their diverse and specific biological activities. From inducing apoptosis in cancer cells while showing safety towards healthy cells, to combating pathogenic microbes with measurable efficacy and targeting specific enzymes in metabolic diseases, algae-derived AuNPs have demonstrated significant and multifaceted potential. This research paves the way for new, effective, and environmentally friendly treatments for some of the most challenging diseases facing modern medicine.

Conclusion

In this study, gold nanoparticles were successfully synthesized using *Gracilaria gracilis* extract through a cost-effective and eco-friendly green approach. Comprehensive characterization by UV-Vis-spectroscopy, PXRD, FESEM, TEM, Zeta-potential, EDX, and FTIR confirmed the formation of uniformly dispersed, spherical nanoparticles with an average size of 10.49 nm, a crystalline size of about 39.5 nm, high gold content, and the presence of bioactive surface compounds. The nanoparticles exhibited excellent antioxidant activity in a dose-dependent manner and showed no significant cytotoxicity toward human fibroblast cells, highlighting their strong biocompatibility. The novelty of this work lies in the first-time use of *Gracilaria gracilis* as a dual reducing and stabilizing agent, which enabled the production of highly monodisperse and structurally stable gold nanoparticles with promising biomedical and pharmaceutical potential. Nevertheless, this study is limited to in-vitro assay, and further investigations, including in-vivo evaluations, long-term stability tests, and mechanistic studies, are required to fully validate their safety and efficacy. Overall, these findings demonstrate that biosynthesized gold nanoparticles from *Gracilaria gracilis* represent a sustainable and innovative platform for future applications in medicine, food technology, and nano-pharmaceuticals.

Data availability

The authors declare that the data supporting the findings of this work are available within the manuscript.

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Author contributions

KR, SG, AHM, and METY designed the work. KR and METY synthesized the nanoparticle. KR, SG, AHM, and METY performed the study and analyzed data. SG, AHM, and METY approve the data. All authors read and approve the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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