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Green production and characterization of gold nanoparticles with *Capparis spinosa* extract and assessment of their efficacy against HCT-116 colon cancer cells

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Abstract

Capparis spinosa is native to the deserts of Najaf and Muthanna. Its bioactive compounds make it appropriate for different therapeutic uses in indigenous traditional medicine. The leaves of the plant were extracted by alcohol and the therapeutic efficacy and identification of active compounds were done by gas chromatography-mass spectrometry (GC-MS). The investigation revealed that the ethanolic extract was rich in bioactive compounds including phenols, coumarins, fatty acid esters and terpene derivatives. Then, the AuNPs were synthesised by green method. The synthesis was confirmed by UV-Vis spectroscopy. The surface plasmon resonance (SPR) peak was found at 528 nm and the particle size was found to be 68.3 ± 4.2 nm by dynamic light scattering (DLS). The particle size distribution was evaluated by using multi dispersion index (PDI). The PDI value obtained was 0.313 ± 0.02. The zeta potential was measured at −27.7 ± 1.5 mV, indicating an acceptable colloidal stability. Furthermore, the analysis of transmission electron microscopy confirmed the formation of spherical nanoparticles. ranging from 52 to 74 nm with minimal aggregation. The cytotoxicity assays showed that the extract was more efficacious to cause cell death, as compared to the crude extract with IC50 values of 47.8 ± 2.0 and 102.5 ± 3.7 µg/ml, respectively. The present results suggest the potential therapeutic efficacy of biosynthesised gold nanoparticles from *Capparis spinosa* as anticancer therapy.

Keywords: Gold Nanoparticles; *Capparis spinosa*; HCT-116 Cells; GC–MS; DLS

1. Introduction

Colorectal cancer is a type of malignant disease and ranks second in cancer deaths worldwide. Among the conventional treatments are chemotherapy, radiation and surgery, all of which are constantly improving. However, other issues such as increased toxicity, poor selectivity to cancer cells and drug resistance remain. Therefore, the search for a more secure and efficacious therapy strategy is ongoing (Sung *et al*., 2021).

The special physical and chemical properties of gold nanoparticles (AuNPs) such as their large surface area, biocompatibility, colloidal stability and ease of biosurface modification have increased the interest in nanotechnology in the pharmaceutical and medical fields. Gold nanoparticles have proved useful for cancer treatment, bioimaging and drug delivery (Dykman and Khlebtsov, 2012).

Recently, there has been an increasing interest in the synthesis of green of nanoparticles by ethanolic extracts of plant as electron donors (reducing substances) instead of toxic and hazardous materials employed in the traditional methods. The bio-reducing and capping agents play an important role in the production of stable nanoparticles which are acceptable in the environment. Active phytochemicals are terpenes, flavonoids and phenols. This method is simple, economical and safer than the conventional synthetic physical and chemical methods (Iravani, 2011).

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The wild caper (*Capparis spinosa* L.) is a plant of medicinal importance distributed in several Middle Eastern and Mediterranean countries including Iraq. It is a member of the Capparaceae family. It is used in traditional medicine for a number of diseases. Another study proved the presence of pharmacologically active constituents like flavonoids, phenols, alkaloids and glucosinolates with anti-inflammatory, antioxidant and anticancer activities (Tlili *et al*., 2011; Nabavi *et al*., 2016).

Conversely, plant extracts rich in phenolic compounds can effectively reduce gold ions to form stable nanoparticles with high bioactivity. The anticancer activity of these plants may be enhanced by the pharmacological properties of medicinal plants together with the nanoscale properties of gold nanoparticles compared to their non-encapsulated counterparts (Shankar *et al*., 2004; Ahmed *et al*., 2016).

The objective of this study was to evaluate the anti-cancer effects of *Capparis spinosa* alcoholic extract on cells line of HCT-116 colon carcinoma.

2. Methods and Materials

2.1. Preparation of Botanical Alcoholic Extract

Capparis spinosa leaves were collected from Najaf desert in the southwestern part of Najaf Governorate, Iraq, and then dried at ambient room temperature in the absence of sunlight. Then they were ground mechanically to a fine powder. Ethanol infusion technique by Harborne (1998) was used at a concentration of 70% for a period of 72 hours with continuous agitation. The crude alcoholic extract was obtained by filtering the extract through Whatman No. 1 filter paper and concentrating through rotary vacuum evaporation (low pressure). Until needed, it was stored at 4 °C in opaque, sealed containers.

2.2. GC-MS Analysis of the Active Compounds

The active chemicals contained in the extract were determined by GC-MS analysis of the alcoholic extract of *Capparis spinosa* as described by Adams (2007). Helium was used as carrier gas to inject the sample into the selected capillary column at a constant flow rate. Compound identification was carried out by retention time (RT) analysis and spectral comparison with the NIST database. The ratio of substances was calculated on the basis of chromatographic peaks area.

2.3. Gold Nanoparticles Biosynthesis

The alcoholic plant extract was used as reducing and bio-coating agent for the synthesis of gold nanoparticles. The plant extract was exposed to a solution of gold (III) chloride (HAuCl4) at room temperature with constant stirring (Shankar *et al*., 2004). The colour changed from light yellow to a dark ruby red indicating the formation of gold nanoparticles.

2.4. UV-Vis Spectroscopy Examination

The production of gold nanoparticles was confirmed by Ultraviolet-Visible spectroscopy. The spectra were recorded with a UV-Visible spectrophotometer in the wavelength range of 250–700 nm using deionised water as a reference (blank), following the method of Link and El-Sayed (1999).

Characterisation of alcoholic plant extract and suspension with biosynthesised gold nanoparticles and gold(III) chloride (HAuCl4) solution. The results showed that small and stable gold nanoparticles were formed with a strong surface plasmon resonance (SPR) peak at 528 nm.

2.5. Nanoparticle Size and Surface Distribution Analysis

The hydrodynamic diameter, polydispersity index (PDI) and zeta potential of the gold nanoparticles were analysed by dynamic light scattering (DLS). The mean size was found to be 68.3 ± 4.2 nm and the polydispersity index (PDI) was 0.313 ± 0.02, indicating a moderate size distribution. The surface potential was -27.7 ± 1.5 mV, showing strong colloidal stability of the nanoparticles.

2.6. Transmission Electron Microscopy (TEM) morphology

The morphology of the gold nanoparticles was examined by transmission electron microscopy (TEM). A drop of the nanoparticle solution was placed on a copper grid, dried and analysed. The microscopic pictures showed that the nanoparticles possessed a semi-spherical morphology with quite uniform dispersion and little aggregation. Size range 52-74 nanometres (Michen *et al*., 2015).

2.7. In vitro cytotoxicity evolution

The antitumor efficacy of unencapsulated plant extract and biosynthesised gold nanoparticles was evaluated against the human colon cancer cell line HCT-116 by MTT test, according to the standard protocol established by Mosmann in 1983. Cells were seeded in 96-well microplates at specified density and cultured in RPMI-1640 media supplemented with the (FBS) 10% and antibiotics (1%) at 37°C in a water-saturated atmosphere condition with 5% CO2.Once the desired cell density was reached, the cells were treated with different concentrations of free plant extract and gold nanoparticles (12.5–200 µg/mL) for 24 h. After 3-4 hours of incubation, an appropriate final concentration of MTT solution was added to each well and the plates were incubated for another 3-4 h.

The growing medium was removed and the resultant crystals solubilized in DMSO. Absorbance was determined by use a micro plate spectrophotometer (at 570 nm). The viability of cell was determined as a percentage of the untreated control group using the equation: (%) Relative viability= (Mean absorbance of treated cells/relative to control cells) × 100.

2.8. Calculation of IC50 values

IC50 values for the free plant extract, gold nanoparticles and doxorubicin were determined by dose-response curves with appropriate statistical analysis. All the results are expressed as mean ± SD of three different studies (Kedare and Singh, 2011).

2.9. Statistical Analysis

All the studies were carried out in triplicates and results were expressed as mean ± standard deviation (Mean ± SD). Statistical analysis was performed using appropriate software and significance was accepted at $p < 0.05$ (Motulsky, 2014).

3. Results and discussion

3.1. Chemical Analysis of *Capparis spinosa* Extract

GC-MS analysis of the ethanolic extract of the plant revealed the presence of many bioactive components belonging to different chemical families (Table 1 and Figure 1). It contained coumarin derivatives, fatty acid esters, phenolic compounds, sulphur compounds, diterpenes and hydrocarbons. The major component of the extract was scoparone (16.08% of the total area) followed by phenacyl thiocyanate (13.66%) and butane, 2-isothiocyanato- (5.44%). Different amounts of different useful compounds including phytol, methyl eugenol, ethyl hexadecanoate and 2,4-ditert-butylphenol were detected. Results showed that alcoholic extract of *Capparis spinosa* is an important source of active secondary metabolites which can act as reducing and stabilising agents in the green synthesis of gold nanoparticles. They may also be cytotoxic to HCT-116 colon carcinoma cells. The use of phytochemicals in synthesis of metal nanoparticles and pharmaceuticals was reported by Al-Burki et al. (2026) and Iravani (2011).

Table 1 Major phytochemicals identified by GC–MS in the ethanolic extract

No.	Compound	RT (min)	Area (%)	Match Quality	Chemical Class	Reported Biological Relevance
1	Phenacyl thiocyanate	5.08	13.66	50	Organosulfur compound	Potential cytotoxic activity
2	Butane, 2-isothiocyanato-	8.31	5.44	96	Isothiocyanate	Anticancer, chemopreventive
3	Hexadecanoic acid, ethyl ester	22.42	4.95	99	Fatty acid ester	Antioxidant, antiproliferative
4	Oxalic acid, cyclohexylmethyl tetradecyl ester	19.50	3.19	50	Ester derivative	Bioactive lipid derivative
5	Pentafluoropropionic acid, tetradecyl ester	18.21	3.16	90	Fatty acid ester	Membrane-active compound

6	Scoparone	22.50	16.08	90	Coumarin derivative	Anticancer, anti-inflammatory
7	Scoparone	22.76	2.84	92	Coumarin derivative	Anticancer, apoptosis inducer
8	Octadecanoic acid, ethyl ester	24.24	2.75	98	Fatty acid ester	Antioxidant activity
9	Phytol	23.55	1.94	90	Diterpene alcohol	Anticancer, pro-apoptotic
10	Heptadecane	19.41	1.38	98	Hydrocarbon	Reported bioactivity
11	Methyl eugenol	16.08	1.87	98	Phenylpropanoid	Antioxidant, cytotoxic
12	Octadecanoic acid, propyl ester	25.06	1.44	98	Fatty acid ester	Antiproliferative potential
13	Linoleic acid ethyl ester	23.99	0.28	95	Unsaturated fatty acid ester	Anti-inflammatory
14	2,4-Di-tert-butylphenol	17.36	0.74	96	Phenolic compound	Antioxidant, anticancer
15	Tetracosane	25.92	0.75	97	Hydrocarbon	Reported antimicrobial activity

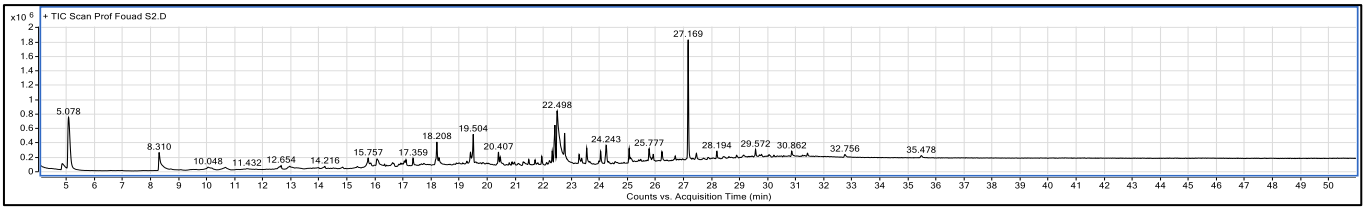

Figure 1 GC–MS chromatogram of the ethanolic plant extract showing the major identified phytochemicals

3.2. UV-Vis Absorption spectra of gold nanoparticles

UV-Vi's spectroscopy study showed that the colour of HAuCl4 solution turned from light yellow to deep ruby red after addition of plant extract (Table 2 and Figure 2), which indicates the formation of gold nanoparticles. The SPR peak of gold nanoparticles was observed as a distinct and symmetrical absorption band at around 528 nm which was quite atypical. The second extract of the plant showed a strong absorption band in the 280–330 nm region, due to phenolic compounds and coumarin derivatives.

The results confirmed the efficiency of the synthesis of gold nanoparticles using extract of *Capparis spinosa*. The symmetry of the SPR peak indicates the formation of small relatively stable nanoparticles confirming the results of Timoszyk and Grochowalska (2022) concerning the influence of plant extracts rich in active compounds on the reduction of gold ions and stabilisation of the obtained nanoparticles.

Table 2 UV–Visible spectroscopy analysis of biosynthesized gold nanoparticles

Sample	**Color observation**	**λmax (nm)**	**Peak characteristic**	**Interpretation**
$HAuCl_4$ solution	Pale yellow	No SPR peak	Flat baseline	Nanoparticles absence
Ethanolic plant extract	yellowish-brown	Broad absorption at 280–330 nm	broad band	Presence of phenolics and coumarins
Biosynthesized AuNPs	Dark ruby red	528 nm	Sharp symmetric SPR peak	Formation of small, stable, spherical AuNPs

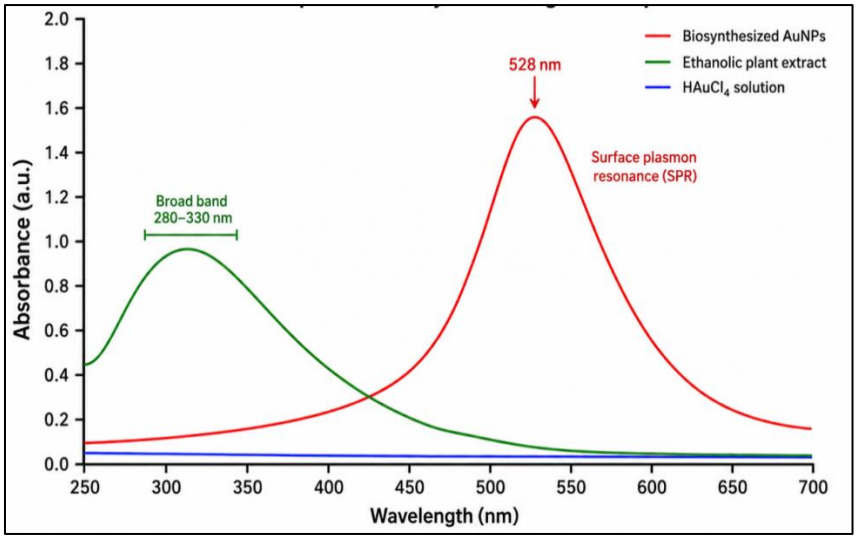

Figure 2 UV–Visible spectra of $HAuCl_4$ solution, ethanolic plant extract, and biosynthesized AuNPs showing the characteristic SPR peak at 528 nm

3.3. Zeta potential and Dynamic Light Scattering of Gold nanorods

DLS analysis showed that the average particle size of the bio-synthesized gold nanoparticles was 68.3 ± 4.2 nm and showed a good size distribution of the nanoparticles (Table 3). The measured Polydispersion Index (PDI) was 0.313 ± 0.02 which indicates a mean size distribution with a significant homogeneity between the particles (Figure 3). The zeta potential was −27.7 ± 1.5 mV, suggesting good colloidal stability from the electrostatic repulsion between the nanoparticles.

Capparis spinosa extract: A facile synthesis of stable gold nanoparticles. The measurements of dimensions, polydispersity index and zeta potential confirmed the efficiency of plant compounds in encapsulation and stabilisation of the synthesised nanoparticles, confirming the Dykman and Khlebtsov (2012) statements on the importance of surface properties and colloidal stability in medical and pharmaceutical applications of gold nanoparticles.

Table 3 DLS and zeta potential analysis of biosynthesized gold nanoparticles

Parameter	Result	Interpretation
Average particle size (nm)	68.3 ± 4.2	Nanosized particles with suitable dispersion
Polydispersity index (PDI)	0.313 ± 0.02	Moderate size distribution
Zeta potential (mV)	−27.7 ± 1.5	Good colloidal stability

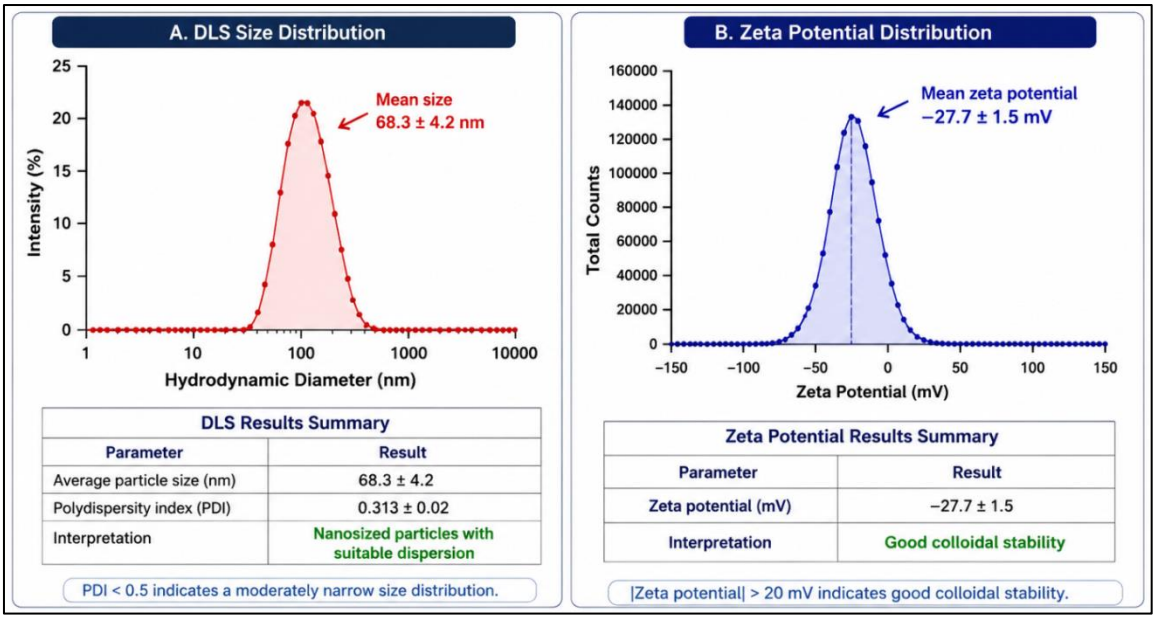

Figure 3 DLS size distribution and zeta potential analysis of biosynthesized gold nanoparticles indicating nanoscale particle size and colloidal stability

3.4. Morphology of Gold Nanoparticles by TEM

Table 4 and Figure 4 show successful synthesis of gold nanoparticles using *Capparis spinosa* extract. The particles were spherical in shape, narrow size range and low aggregation. The size of particle ranged between 52-74 nm which agrees with the results of DLS study. The high magnification images showed a homogeneous surface of the nanoparticles with a stable coating of the plant, which increased colloidal stability and decreased aggregation.

The results showed the effective regulation of phytochemicals on nanoparticles' production and stability. The results are in agreement with the ones published by Mehdi et al. (2025) and Shankar et al. (2004) on the efficacy of plant extracts for synthesizing stable and homogeneous gold nanoparticles for biomedical applications.

Table 4 Morphological characterization of biosynthesized gold nanoparticles by TEM

Parameter	Observation	Interpretation
Particle morphology	Nearly spherical	Successful nanoparticle formation
Particle distribution	Moderately dispersed	Fairly uniform distribution
Aggregation state	Minimal aggregation	Adequate colloidal stability
Range of particle size	52–74 nm	Similar to DLS data
Surface appearance	Regular surface texture	Stable phytochemical capping

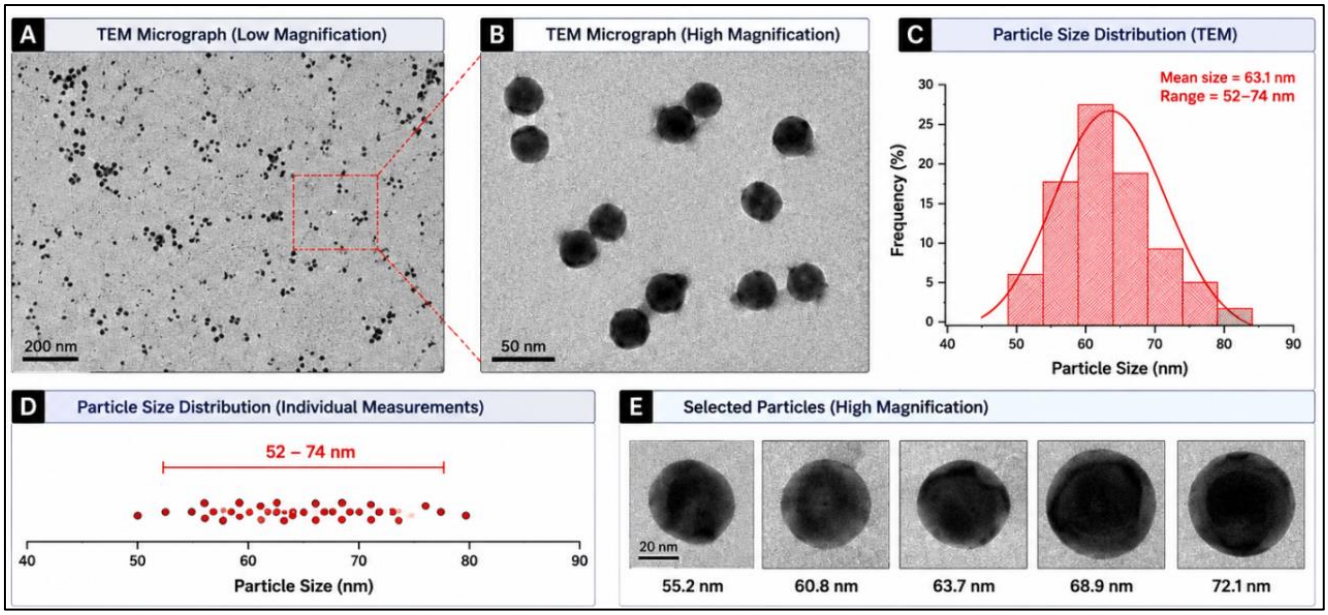

Figure 4 Biosynthesised Gold Nanoparticles: TEM based Morphological Characterisation. (A) Low-magnification TEM micrograph of the overall distribution of nanoparticles. (B) High magnification TEM image showing the almost spherical morphology of AuNPs. (C) Particle size distribution histogram from TEM analysis. (D) Individual particle size measurements in the range of 52–74 nm. (E) Representative images at high magnification of selected nanoparticles showing surface morphology and minimal aggregation

3.5. Cytotoxicity activity

As shown in Table 5 and Figure 5, the viability of HCT-116 cells decreased gradually with the increase of concentration of free plant extract, biosynthesised gold nanoparticles and doxorubicin. The cytotoxicity of the gold nanoparticles was higher than the free extract at all the concentrations. The free extract reduced the cell viability to 14.8 ± 1.2 % and 33.3 ± 1.9 % at 200 µg/mL.

The IC50 value of the gold nanoparticles (47.8 ± 2.0 µg/mL) was significantly lower than that of the free extract (102.5 ± 3.7 µg/mL) (Table 6) indicating that the anti-cancer efficacy was enhanced after conversion into nanoparticles. The most cytotoxic drug was doxorubicin (IC50=19.4±1.5 µg/mL).

The results showed that the active compounds were able to penetrate into the cancer cells and the bioavailabilities were improved by nanoencapsulation . The results are consistent with the findings of Dykman and Khlebtsov (2012) and Salman et al. (2026) that gold nanoparticles can augment the therapeutic efficacy of plant-derived anti-cancer drugs.

Table 5 Cytotoxic activity of free extract and biosynthesized AuNPs against HCT-116 cells after 24 h exposure

Concentration (µg/mL)	Free extract Cell viability (%)	AuNPs Cell viability (%)	Doxorubicin Cell viability (%)
12.5	91.5 ± 2.2	84.7 ± 1.9	71.3 ± 1.6
25	82.9 ± 2.6	69.4 ± 2.1	55.5 ± 1.9
50	68.8 ± 1.9	49.9 ± 2.4	37.2 ± 1.8
100	51.7 ± 2.5	31.6 ± 1.7	18.8 ± 1.3
200	33.3 ± 1.9	14.8 ± 1.2	7.9 ± 0.8

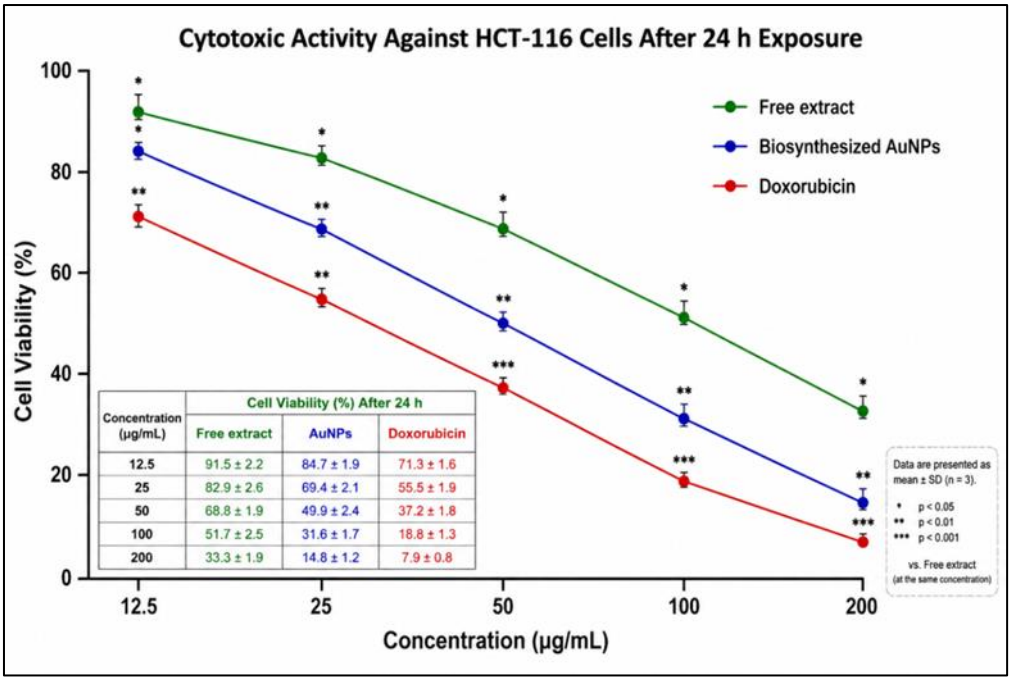

Figure 5 Cytotoxic activity of free extract, biosynthesized AuNPs, and doxorubicin against HCT-116 cells

Table 6 IC50 values of tested samples against HCT-116 cells

Sample	IC50 (µg/mL)
Free extract	102.5 ± 3.7
Biosynthesized AuNPs	47.8 ± 2.0
Doxorubicin	± 1.5

4. Conclusion

GC-MS study of alcoholic extract of *Capparis spinosa* revealed the presence of several active phytochemicals. UV-visible spectrophotometry, dynamic light scattering, analyiss of zeta potential and TEM imaging showed that the plant extract was a good agent for the environmentally friendly generation of solid gold nanoparticles. The biosynthesised AuNPs were of near-spherical morphology with good colloidal stability and nanosize. Furthermore, the AuNPs were found to be more cytotoxic to HCT-116 colon cancer cells than the unencapsulated plant extract, suggesting their potential as nanotherapeutic agents for future oncological applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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