Research Article

Sabah Ahmed Al-Zahrani, Ramesa Shafi Bhat*, Mona Awad Al-Onazi, Mona S. Alwhibi, Dina. A. Soliman, Nora Abdullah Aljebrin, Leenah Saleh Al-Suhaibani, and Sooad Al Daihan

Anticancer potential of biogenic silver nanoparticles using the stem extract of *Commiphora gileadensis* against human colon cancer cells

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Abstract: Plant-mediated silver nanoparticles are unique and are considered one of the best nanomaterials used in cancer research. We report a low-cost, eco-friendly process of green synthesis of AgNPs from *Commiphora gileadensis* stem extracts and evaluated their anticancer potential against colon cancer cell lines HCT-116, HT 29, and SW620. Anticancer activities were performed by an MTT assay and gene expression levels of four genes *CHEK1*, *CHEK2*, *ATR*, and *ATM* by the real-time polymerase chain reaction. Particles were initially confirmed by UV-visible spectroscopy. The morphology and stability of the particles were examined through TEM, zeta potential, and zeta sizer. GC-MS and FTIR were performed to examine the functional groups. The absorption peak was recorded at 430 nm; the average size recorded by TEM images was 13 nm, while the zeta potential and zeta sizer study showed aggregation in nanoparticles. Compared to *C. gileadensis* extracts, some of the FT-IR spectrum peaks were sight shifted with some new peaks in *C. gileadensis* AgNPs. *C. gileadensis* AgNPs were more toxic against HT29 followed by HTC116 and SW620. Expression levels of most of the genes in HCT116 and HT29 were increased by treatment whereas the gene expression level was least affected in SW620. *C. gileadensis* AgNPs have anticancer potential and need to be explored in cancer research.

*** Corresponding author: Ramesa Shafi Bhat,** Biochemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia, e-mail: rbhat@ksu.edu.sa
Sabah Ahmed Al-Zahrani, Mona Awad Al-Onazi, Nora Abdullah Aljebrin, Leenah Saleh Al-Suhaibani, Sooad Al Daihan: Biochemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia
Mona S. Alwhibi, Dina. A. Soliman: Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia

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1 Introduction

Green synthesis of metallic nanoparticles is a non-toxic, economical, and eco-friendly process [1]. Nanoparticles synthesized by biological methods need a biomolecule from plants, algae, microorganisms, etc. [2–4]. This kind of synthesis starts with a reaction between noble metal salts, and these biomolecules present in extracts get oxidized and reduce metal ions to metal nanoparticles [5]. The stability, size, and shape of the synthesized particles depend on the nature of biomolecules present in the extracts [5]. Metallic nanoparticles are in demand in many fields such as biomedical companies, chemical industries, cosmetics, and healthcare products [6]. Silver nanoparticles are considered one of the best nanomaterials among all metallic nanoparticles used in biomedical industries [7,8]. Silver nanoparticles can reduce the progressive development of cancer cells by hindering many signaling cascades responsible for the development and pathogenesis of tumors. Many research findings revealed that silver nanoparticles can kill human cancer cells with very little harm to normal cells [9,10]. However, silver nanoparticles prepared by physical, chemical, and biological methods are mostly preferred due to less toxicity and cost [11–15].

Green synthesis of silver nanoparticles by using plant extracts is simply a one-step reduction process with good yield [11,12]. Bioactive components present in the plant extracts act as reducing, capping, and stabilizing agents. Amino acids, alkaloids, proteins, polysaccharides, phenolics, terpenoids, and flavones present in plant extracts can act as reducing and capping agents [16]. Hydroxyl and carboxyl groups of these compounds have a unique property of

binding to metals to wrap around NPs to reduce aggregation thus making the particles stable once synthesized [17].

Commiphora gileadensis, an aromatic shrub, is used as a medicinal plant to treat many diseases in the N. Arabian population. Mainly, it is used as a pain reliever, a diuretic, and a laxative and to treat skin disorders. Its sap had an inhibitory effect against many pathogenic bacteria. Amiel et al. [17] reported (*E*)-caryophyllene as one of the main compounds of *C. gileadensis* with anticancer properties. Attributed to phytochemicals such as aliphatic alcohol glycosides, triterpenoids, sesquiterpenoids, β-caryophyllene, and flavonoids, its extracts are reported to show cytotoxicity against human prostate, liver, lymphocytic, and cervical cancer cell lines [18–20].

The use of nanoparticles in cancer therapy opened a new way of treatment. Various metal nanoparticles are widely being used in treating various cancer types. In comparison to chemical drugs, NP-based drugs are less toxic with low drug resistance and have shown promising results in anticancer therapies [21–24]. The present study investigated the synthesis of silver nanoparticles using the *C. gileadensis* stem extracts as a reducing agent. The anti-cancer potential of the aqueous extract of *C. gileadensis* and its silver nanoparticles was also assessed against three colon cancer cell lines HCT-116, HT 29, and SW620. We find it interesting to examine the anticancer effects of AgNPs synthesized from *C. gileadensis* which may be useful as a safe strategy in cancer therapy.

2 Material and methods

2.1 Collection of plant materials and extract preparation

The stem of *C. gileadensis* was collected from the Riyadh region of Saudi Arabia and washed with distilled water and then shade dried and powdered. The powdered stem was dissolved in the autoclave water with the ratio of 1:100 g·mL^{-1} and left on a magnetic stirrer for 48 h at 4°C. After filtration, the mixture was stored at −80°C for further experiments.

2.2 Gas chromatography-mass spectrometry

The hexane extract of *C. gileadensis* was analyzed phytochemically using a GC-MS [TRACE™ TR-35MS (Thermo Fisher Scientific)].

2.3 Biologically synthesized silver nanoparticles from *C. gileadensis* extracts

Silver nanoparticles were synthesized by dissolving silver nitrate ($AgNO_3$) solution in *C. gileadensis* extracts with a concentration of 2.5 mM $AgNO_3$ in the mixture.

2.4 Characterization of synthesized silver nanoparticles

The prepared nanoparticles were characterized by a UV-visible (UV-Vis) spectrophotometer (Thermo Scientific 1500, USA) ranging from 300 to 600 nm. The shape and size of the nanoparticles were determined by a transmission electron microscopy (TEM; JEOL JEM-1400 Plus). The average size of the nanoparticles was recorded by zeta sizer (Zetasizer Nano ZS90) using the technique of dynamic light scattering (DLS) and zeta potential by electrophoretic light scattering (ELS).

2.5 Fourier-transformed infrared (FTIR)

The functional groups present in the extract were monitored by FTIR using a Perkin Elmer FTIR-Spectrometer Spectrum (Spectrum BX) ranging from 4,000 to 400 cm^{-1}.

2.6 Analysis of cytotoxicity

2.6.1 Cell lines and cell treatment

Three different kinds of colon cancer cell lines HCT 116, SW620, and HT 29 were included in this study. Cell lines were treated with different concentrations of *C. gileadensis* extracts and *C. gileadensis* AgNPs (10, 20, 50, 100 µg) and compared with untreated cells after 48 h of the treatment. MTT assay was used to check the cell viability.

The expression level of four genes related to DNA damage response and cell cycle checkpoints (*CHEK1*, *CHEK2*, *ATR*, *ATM*) before and after the treatment with 50% cytotoxic concentration of *C. gileadensis* extracts and C. *gileadensis* AgNPs was measured using the RT-PCR (Applied Biosystems® Life Technologies, USA). Primer sequences and annealing temperature for RT-PCR are listed in Table 1.

Table 1: Primer sequences of ATM, ATR, ChK1, CHK2, and GAPDH

Primer	Sequence	Annealing temperature (°C)
ATM	Forward: 5′-GCA GAT GAC CAA GAA TGC AA-3′ Reverse :5′-GGC CTG CTG TAT GAG CAA AT-3′	60
ATR	Forward: 5′-GGGATGCCACTGCTTGTTATGAC-3′ Reverse: 5′-CTGTCCACTCGGACCTGTTAGC-3′	60
ChK1	Forward: 5′-CTTTGGCTTGGCAACAGT-3′ Reverse: 5′-CCAGTCAGAATACTCCTG-3′	60
ChK2	Forward: 5′-CTC GGG AGT CGG ATG TTG AG-3′ Reverse: 5′-CCAGTCAGAATACTCCTG-3′	57
GAPDH	Forward: 5′-GGTATCGTGGAAGGACTCATGAC-3′ Reverse: 5′-ATGCCAGTGAGCTTCCGTTCAGC-3′	60

2.7 Statistical analysis

GraphPad Prism® 9.0 statistical software (GraphPad Inc., USA) was used for the independent *T*-test. $p < 0.05$ was considered to be significant.

3 Results

3.1 Gas chromatography-mass spectrometry analysis of *C. gileadensis* extracts

The components detected by the GC-MS of *C. gileadensis* extracts are shown in Figure 1. GC-MS analysis of *C. gileadensis* extracts revealed the presence of some compounds with anticancer properties, which include thujone, d-carvone, propanoic acid, and eucalyptol.

3.2 Biosynthesis of silver nanoparticles

Green synthesis of silver nanoparticles started after mixing silver nitrate solution with *C. gileadensis* extracts. The reduction of silver ions to silver nanoparticles was observed by a color change from yellow to brown.

3.3 Characterization of synthesized silver nanoparticles

Initially, the process of formation of silver nanoparticles was monitored by UV-Vis spectral analysis where the

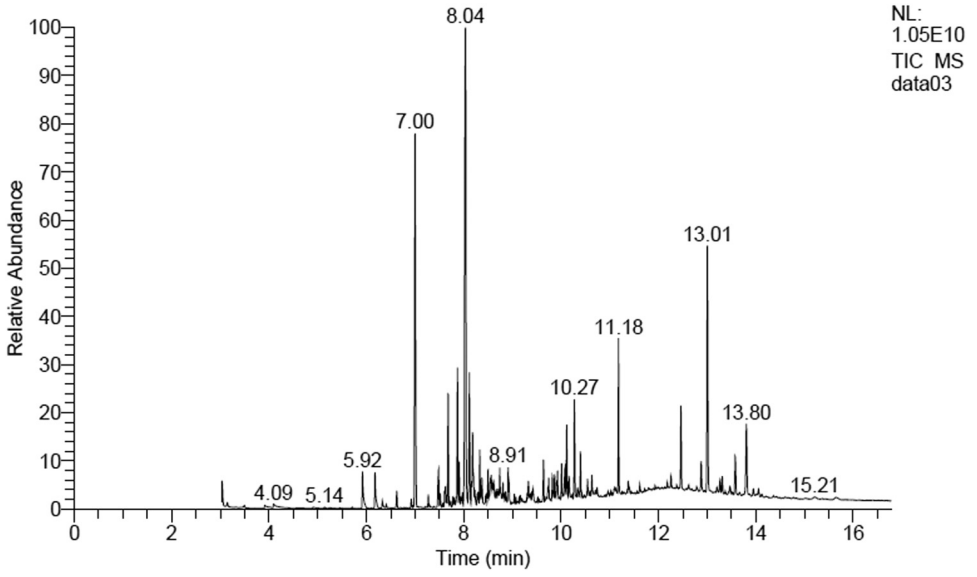


Figure 1: GC-MS analysis to detect the anti-cancer compound in *C. gileadensis* extracts.

surface plasmon resonance (SPR) absorption band was seen at 430 (Figure 1a). Figure 1b shows a TEM image of silver nanoparticles obtained from the *C. gileadensis* extracts confirming the formation of particles. Particles were spherical in shape with an average diameter of about 13 nm.The results of zeta sizer by dynamic light scattering (DLS) and zeta potential by electrophoretic light scattering (ELS) are shown in Figure 1c and d, respectively. The Z-average mean size of *C. gileadensis*-silver nanoparticles was 671.8 d.nm, with a polydispersity index (PDI) of 0.290, and the zeta potential was −22.5.

3.4 Fourier transformed infrared (FTIR) analysis

The IR spectrum of both *C. gileadensis* extracts and *C. gileadensis*-silver nanoparticles is shown in Figure 2. A slight shift of some IR spectrum peaks with the appearance of a few new peaks in *C. gileadensis* AgNPs was observed. The peaks at 3427.47, 1632.53, 1399.56, and 538 cm^{-1} seen in *C. gileadensis* extracts were shifted to 3406.91, 1625.27, 1376.80, and 534.96 cm^{-1} in *C. gileadensis* AgNPs, respectively. The appearance of new peaks at 3752.53 and 826.46 cm^{-1} was observed in *C. gileadensis* AgNPs (Figure 3).

3.5 Cytotoxic analysis

3.5.1 MTT assay

Cytotoxic analysis of *C. gileadensis* extracts and *C. gileadensis* AgNPs against human colon cancer cell lines HTC116, HT29, and SW620 through MTT assay is shown in Figure 4. *C. gileadensis* extracts were found more toxic

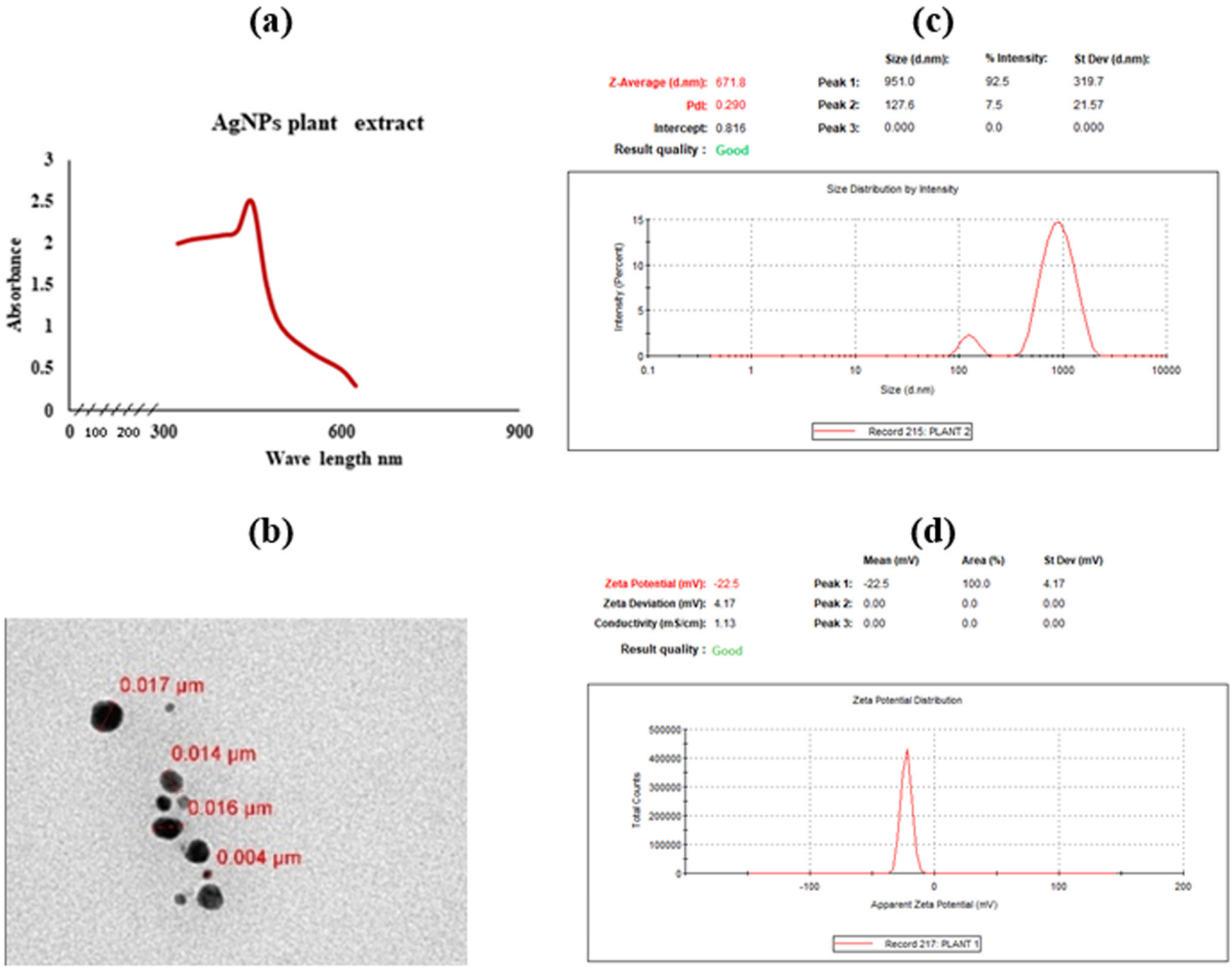


Figure 2: Characterization of biological synthesized *C. gileadensis* AgNPs: (a) UV-Vis spectra showing absorbance peak, (b) TEM micrograph, (c) zeta sizer–DLS, (d) zeta potential distribution–ELS.

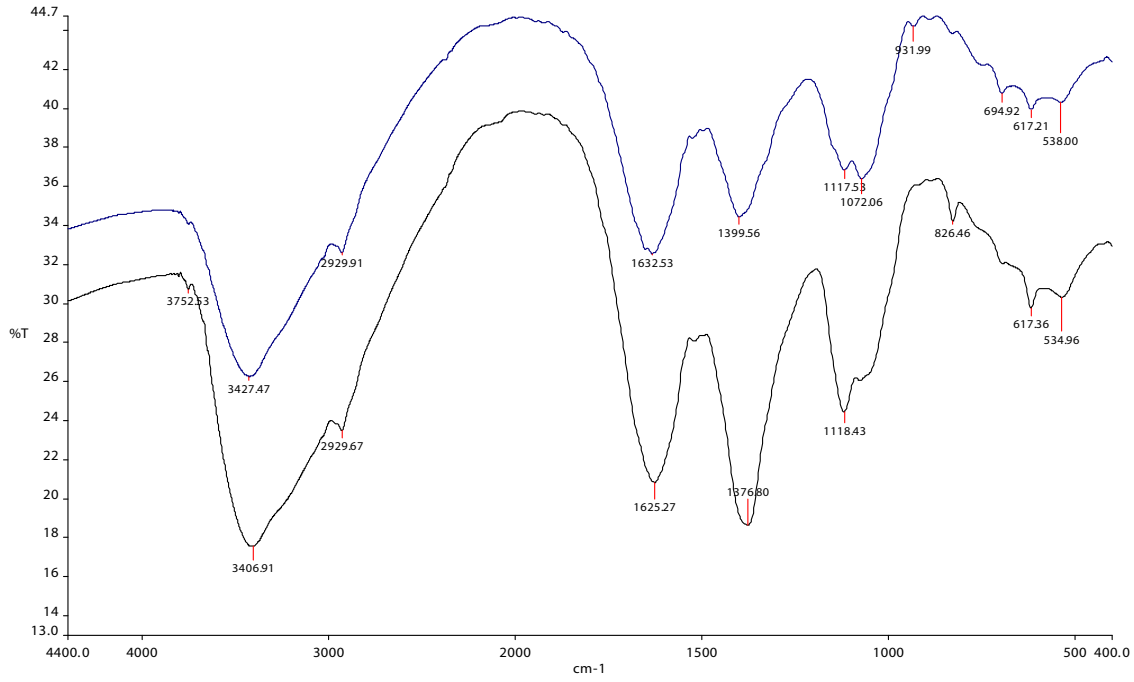


Figure 3: IR spectra of *C. gileadensis* extracts (blue color) and *C. gileadensis* AgNPs (black color).

at higher concentrations when compared to their biogenic AgNPs. Both extracts exhibited higher cytotoxicity against HT29 followed by HTC116 and SW620 in a dose-dependent manner.

3.5.2 RT-PCR

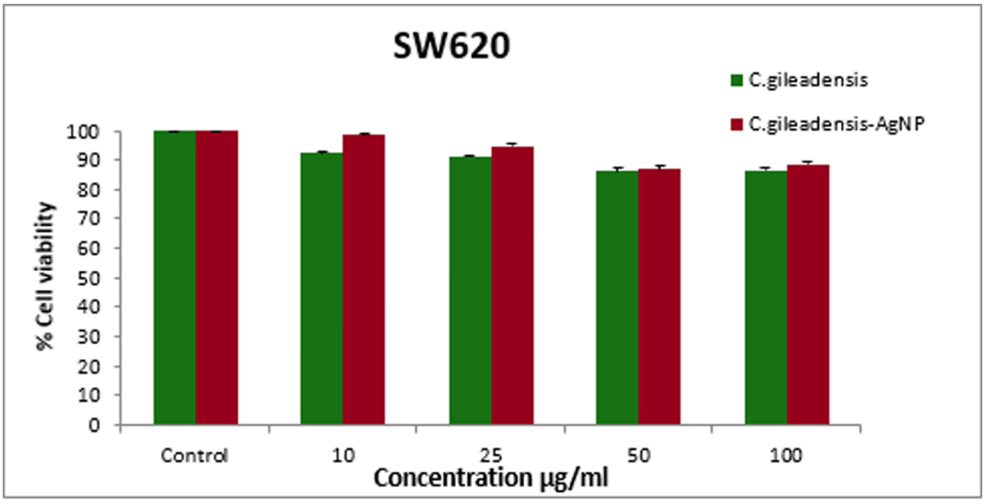
The expression level of *CHEK1*, *CHEK2*, *ATR*, and *ATM* genes before and after treatment with both extracts is shown in Figure 5. The expression level of most of the genes in HCT116 was increased by the *C. gileadensis* AgNPs treatment while *C. gileadensis* extract increases the expression level of most genes in HT29. Gene expression level was least affected in SW620 with both extracts.

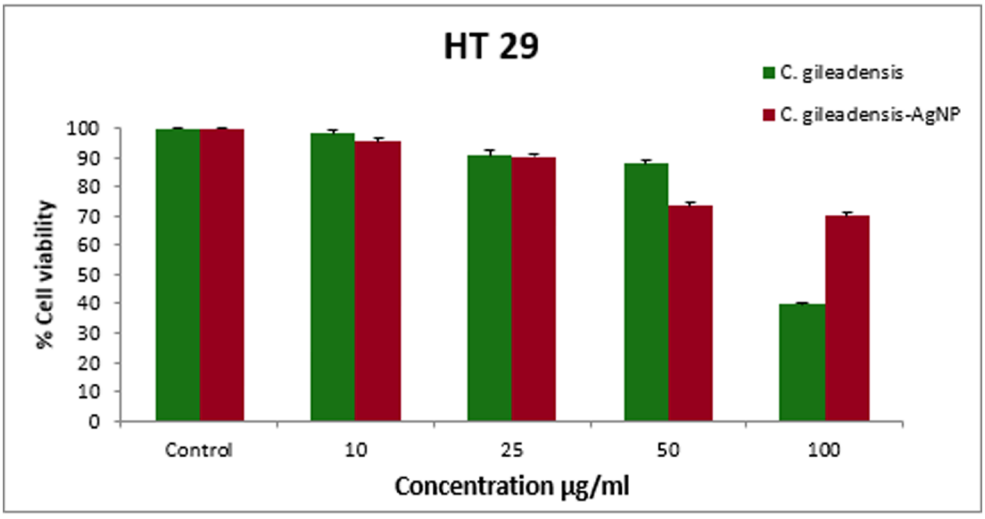
4 Discussion

Cancer treatment is the most challenging concern for scientists in the field of medicine as it is the major cause of death all over the world. Plants are being explored by many researchers all over the world to discover preventive therapies which can successfully reduce cancer incidence and mortality [25]. Several metallic nanoparticles, especially silver, are widely being tested for medicinal applications in cancer research. We prepared silver nanoparticles from *C. gileadensis extracts* and evaluated their antitumor activities.

C. gileadensis is rich in many bioactive compounds showing antitumor activity against cancer cell lines. Cytotoxicity of *C. gileadensis* extracts against human colon cancer cell line HTC116, HT29, and SW620 can be due to the presence of some active antitumor compounds such as thujone, D-carvone, propionic acid, and eucalyptol, which were detected in GC-MS results (Figure 1). Thujone and D-carvone are monoterpene ketones with promising anti-cancer potentials against many cancer cell lines. Thujone induces intracellular stress-mediated apoptosis, proteasome degradation, and mitochondrial disruption in different kinds of tumor cells [26]. D-carvone is reported to induce dose-dependent cytotoxic via ROS-mediated apoptotic cell death in human leukemic and cervix epithelioid carcinoma cells [27,28]. Eucalyptol is a monoterpenoid with active anticancer potential which can induce cancer cell death through ROS-mediated apoptosis. Propionic acid can increase microtubule-associated protein 1 A/1B-light chain 3 (LC3) in colon cancer cells to induce autophagy cell death [29].

Silver nanoparticles were successfully prepared from *C. gileadensis* extracts and were initially confirmed by surface plasmon resonance (SPR) with a peak at 430 nm (Figure 2a). The SPR peak range for silver nanoparticles is between 380 and 470 nm depending on the size and shape of the particle as small size particles absorb smaller wavelength [30]. TEM results showed that the average





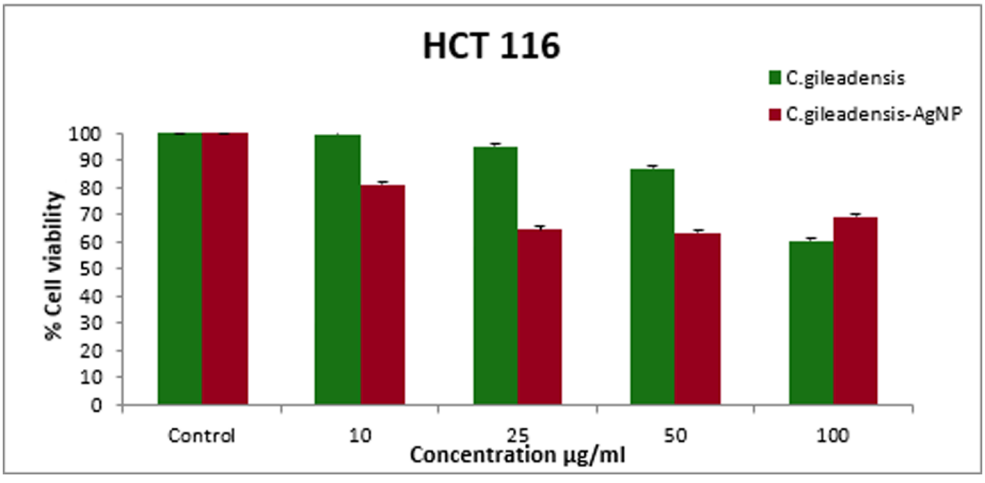


Figure 4: Cytotoxicity of *C. gileadensis* extracts and *C. gileadensis* AgNPs' extract against human colon cancer cell lines HTC116, HT29, and SW620.

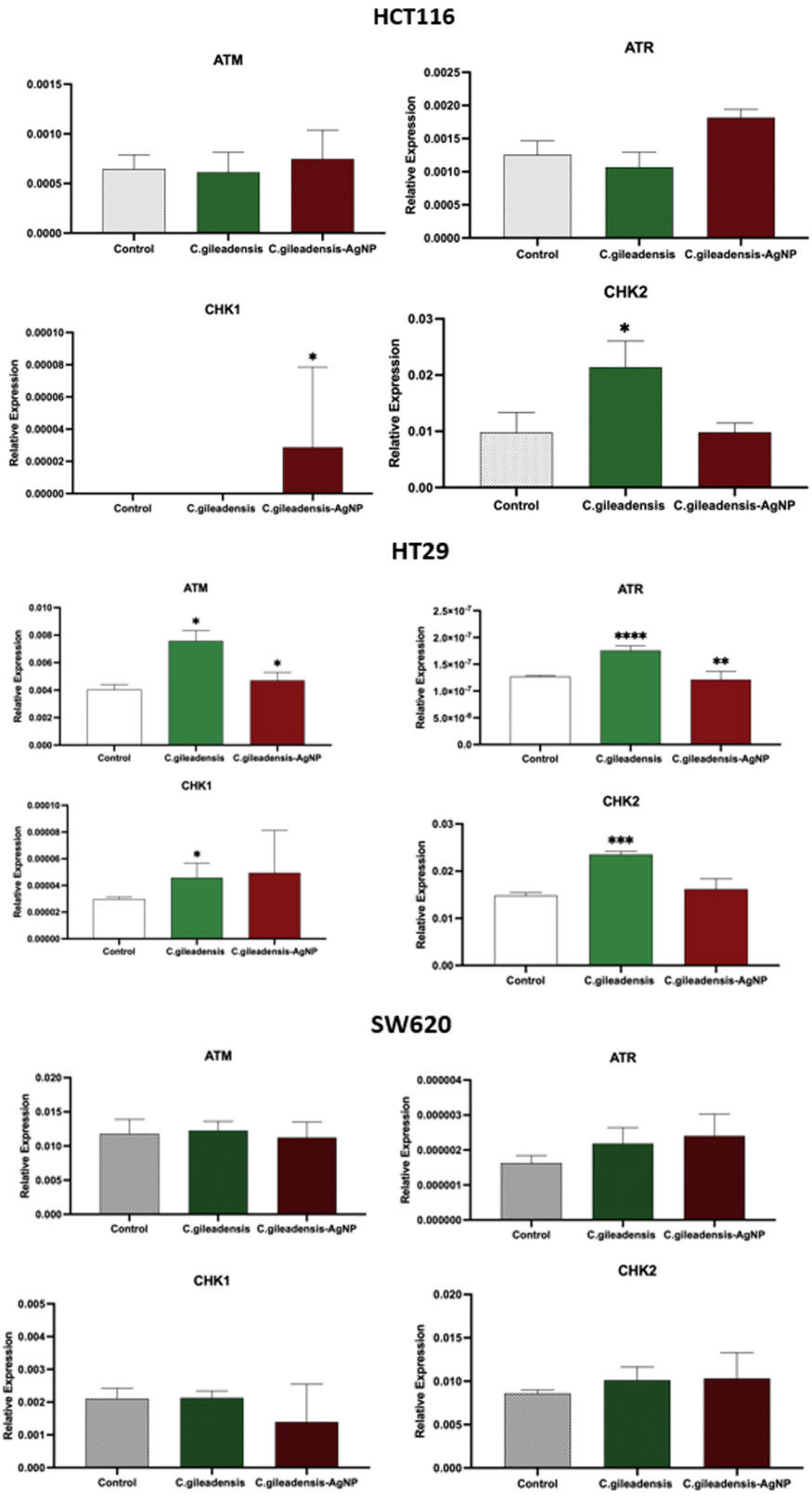


Figure 5: Gene expression fold change in cell lines treated with *C. gileadensis* extracts and *C. gileadensis* AgNPs.

particle size is 13 nm but the Z-average mean size of silver nanoparticles was 671.8 d.nm with a polydispersity index (PDI) of 0.290 which is acceptable for the pharmaceutical use as PDI less than 0.3 indicates monodispersity [31] (Figure 2). These results point toward little aggregation as DLS shows hydrodynamic diameter, but TEM shows the size of nanoparticles in the dried form [32].

The green process of synthesizing nanoparticles is mediated by active biomolecules present in plant extracts [33]; IR spectrum peaks (Figure 2) revealed the presence of compounds such as flavonoids, amide, alcohols, phenols, amines, and alkynes which are have capping and reducing properties and can transform metal ions to metal nanoparticles [34]. The peak shift in *C. gileadensis*-silver nanoparticles spectra at 3427.27–3,406 cm^{-1} is the specific hydroxyl functional groups in polyphenols and N–H stretching of amines. Additionally, the peak shift in the range from 1,500 to 1,200 cm^{-1} region assigned for proteins confirmed the interactions between the chemical functional groups present in proteins and phenols with silver nanoparticles [35]. Our results confirmed that proteins and phenols have the potential to act as reducing, stabilizing, and capping agents for silver nanoparticles [36].

Silver nanoparticles are found effective in killing different kinds of cancer cells *in vitro* and *in vivo* studies, and the cytotoxic efficiency of these particles depends on their size and shape [37]. The toxicity of AgNPs against cancer cells mainly depends upon the size of the particles as smaller particles can pass through the cell's membrane and subcellular organelles of cancer cells and the accumulated AgNPs in the cell causes cytotoxicity [38]. Size-dependent cytotoxicity of silver nanoparticles has been reported in human cancer cell lines [39]. AgNPs transport inside the cell starts with cell membrane receptor recognition and mostly occurs by endocytosis. AgNPs can induce many cell damages processed like oxidative stress, cell cycle rest, genotoxicity, chromosome aberration, and apoptosis in a cancer cell [40]. Silver nanoparticles can control cell proliferation and cell viability of cancer cells by regulating the expression level of many key genes related to DNA damage and cell cycle [41–43]. All three cell lines have a different origin. HT-29 belongs to female primary adenocarcinoma, HCT-116 to male primary carcinoma, and SW620 to male adenocarcinoma metastatic site. Also, SW620 is poorly differentiated among all three cell lines [44,45]. Both *C. gileadensis* and nanoparticle extracts showed cytotoxicity against all three cell lines in a dose-dependent manner, but the maximum toxicity was against HT29 and minimal against SW620. The expression level of ATM, ATR, CHK1, and CHK2 after treatment was also different in three cell lines. Cancer cell lines have unique genetic characteristics. HCT116 has mutated KRAS proto-oncogene while HT-29 and SW-620 have a mutation in the p53 gene and are used to study metastasis processes [46,47].

5 Conclusion

The extracts of *C. gileadensis* leaves are rich in phytochemicals with reducing and capping properties and have the capacity to transform silver ions into silver nanoparticles via a single-step reduction. *C. gileadensis*-mediated silver nanoparticles induced concentration-dependent cytotoxicity against colon cancer cell lines. *C. gileadensis*-based nanoparticles should be explored for some new therapeutic agents for the cancer treatment.

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