Cytotoxicity of green-synthesized silver nanoparticles by *Adansonia digitata* fruit extract against HTC116 and SW480 human colon cancer cell lines

Research Article

Fatimah Basil Almukaynizi, Maha H. Daghestani, Manal A. Awad, Arwa Althomali, Nada M. Merghani, Wadha I. Bukhari, Norah M. Algahtani, Shatha S. Al-Zuhairy, Ahlam M. AlOthman, Eman A. Alsenani, Badrih O. Alojayan, Khulud S. Al-Saif, and Ramesa Shafi Bhat*

Abstract: Nanoparticles synthesized from plants are being explored for cancer treatment therapies all over the world. This study reported the eco-friendly and low-cost method for the green synthesis of silver nanoparticles (AgNPs) from *Adansonia digitata* fruit as a reducing and capping agent. The anti-cancer potential of synthesized particles was explored against HTC116 and SW480 colon cancer cell lines. Prepared AgNPs were characterized by ultraviolet-visible spectroscopy, zeta potential, transmission electronic microscopy, scanning electronic microscopy, Fourier transform infrared, and energy-dispersive spectrum. The cytotoxicity was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and expression levels of four genes (*CTNNB1*, *APC*, *LRP5*, and *LRP6*) were checked by reverse transcription polymerase chain reaction. The sharp peak of surface plasmon resonance at 400 nm confirms the formation of AgNPs. Dynamic light scattering showed average sizes of 16.34 nm with a polydispersity index of 0.193. *A. digitata* AgNPs were spherical with slight aggregated. AgNPs were more cytotoxic than *A. digitata* extract and decrease the expression of *CTNNB1* and *LRP6* genes while *LRP5* gene expression was increased in both cell lines. APC gene expression was decreased in SW480 but increased in HTC116 with treatment. Overall, this study suggested that AgNPs synthesized by *A. digitata* fruit extract can be an attractive candidate for anticancer applications.

Keywords: *Adansonia digitata*, silver nanoparticles, gene expression, human colon cancer cell line

1 Introduction

Silver nanoparticles (AgNPs) possess many attractive properties due to which many scientific communities are showing interest in their therapeutic applications [1]. AgNPs are being used in many commercial items, such as soap catheters and bandages to curb infection against pathogenic agents [2–5]. They show promising results in nanomedicine as drug delivery vectors, theranostics agents, and anti-cancer agents [1,6–8]. AgNPs can be synthesized by many chemical and physical methods, which may have some shortcomings as some of these methods need high energy or may produce toxic by-products [9,10]. Particularly, chemically produced nanoparticles give out many harmful chemicals, which are not encouraging for biomedical uses. These issues or disadvantages were solved by preparing AgNPs by green methods by using biological assets, such as plants [11,12], microbes [13], algae [14], and yeasts [15]. However, plants are mostly used due to their low cost and the...
presence of rich bio-reducing agents [11,12]. The oxidation process of various biomolecules present in plants is mainly responsible for the reduction of silver ions to AgNPs [11]. The synthesized AgNP stability mostly depends on the nature of biomolecules present in the extract. AgNPs’ size and shape depend on the concentration of plant extract as increases in plant extract lead to the formation of a large number of nanoparticles [16]. All plant parts, such as flowers, bark, leaves, fruits, and seed, have the capacity to synthesize nanoparticles in an eco-friendly manner [17]. Some of the most recent studies showing cytotoxicity of green-synthesized nanoparticles using various parts of plants against various cancer cell lines are listed in Table 1.

Due to promising cytotoxicity activity, AgNPs are attaining interest worldwide to achieve an effective cancer treatment. The unique optical properties, size, and conductivity of these particles play an essential role in cytotoxic capacity and drug delivery for treating cancer in addition to cancer diagnosis [3,8]. Nanoparticles synthesized by medicinal plants are being explored for cancer treatment therapies because these particles have shown a controlled effect on many cancer cell lines [11,31].

International Agency for Research on Cancer placed colorectal cancer (CRC) as the third most commonly diagnosed malignancy in men and the second place in women [32]. The advanced stage of CRC is treated by only one approved drug, that is 5-fluorouracil, and its treatment is still a major challenge for researchers [33]. It has been found that AgNPs can induce DNA damage and chromosomal aberrations in many cancer cell lines [34].

In the present study, we explored the anticancer potential of green-synthesized AgNPs from *Adansonia digitata* fruit extract on HT2116 and SW480 colon cancer cell lines. *A. digitata* is full of nutrients and is used to treat many diseases, such as diarrhea, malaria, and microbial infections [35,36]. Its excellent antioxidant content reveals its high antimicrobial, antiviral antioxidant,

| No. | The plant used as reducing agent | Part of plant used | AgNPs’ characteristic | Cell type used for cytotoxic assay | Ref | year |
|-----|---------------------------------|--------------------|------------------------|----------------------------------|-----|------|
| 1   | *Acanthospermum australe*       | Whole plant        | Spherical in shape     | Peripheral blood mononuclear cells | [18] | 2021 |
| 2   | *Chaetomorpha lignistica*       | Whole algae        | Spherical in shape     | Human colon cancer cell lines HT29 and HCT116 | [19] | 2021 |
| 3   | *Camellia sinensis*             | Leaf               | Spherical, polygonal, capsule | Human cervical epithelioid carcinoma – HeLa cells | [20] | 2021 |
| 4   | *Rosmarinus officinalis*        | Leaf               | Round-shaped capsule   | Human breast cancer cell lines – MB 231 | [11] | 2021 |
| 5   | *Jasminum officinale* L.        | Leaf               | Spherical in shape     | Human bladder cancer cell line – 5637 | [21] | 2021 |
| 6   | *Achillea millefolium*          | Flower             | Spherical in shape     | Human breast cancer cell line – MCF-7 | [22] | 2021 |
| 7   | *Abelmoschus esculentus*        | Flower             | Spherical in shape     | Lymphoblastic leukemia cell line – MOLT-4 | [23] | 2021 |
| 8   | *Diospyros malabarica*          | Fruit              | Spherical in shape     | Human lung carcinoma epithelial cell lines – A-549 | [24] | 2021 |
| 9   | *Aloe vera*                     | Gel                | Spherical in shape     | Human primary glioblastoma cell line – U87-MG | [25] | 2021 |
| 10  | *Ferula foetida*                | Gum                | Spherical in shape     | Human breast cancer cell line – MCF-7 | [26] | 2021 |
| 11  | *Juglans regia*                 | Green husk         | Spherical in shape     | Human breast cancer cell line – MCF-7 | [27] | 2021 |
| 12  | *Zea mays*                      | Corn silk          | Spherical in shape     | Rat cardio myoblasts – H9c2 | [28] | 2021 |
| 13  | *Mangifera indica*              | Seeds              | Round shape            | Normal fibroblast               | [29] | 2021 |
| 14  | *Rhodiola imbricata*            | Roots              | Spherical in shape     | Human breast cancer cell line – MCF-7 | [30] | 2022 |


and anti-inflammatory properties. Its fruit is rich in flavonoids, phytochemicals, amino acids, fatty acids, vitamins, and minerals [35,36]. *A. digitata* fruit pulp is very rich in vitamin C and antioxidants. *A. digitata* fruit is approved as a nutritional product by statutory bodies for use in certain nutritional products [37]. The European Commission has recognized it as a food supplement [38,39]. Demand for *A. digitata* fruit has increased in the cosmetic industry due to its fatty acid content.

Although green synthesis of AgNPs from *A. digitata* has been reported in the literature, the anticancer potential of the green-synthesized *A. digitata* AgNPs is not reported. This novel study describes the green synthesis of AgNPs by a simple and eco-friendly process by using *A. digitata* fruit extract and unveiled its anticancer activities against different colon cancer cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay followed by gene-expression profiling methods of four genes (CTNNB1, APC, LRP5, and LRP6).

### 2 Materials and methods

#### 2.1 Fruit extract

Fresh *A. digitata* cultivated in the Kordofan region of Sudan was included in this study. Twenty grams of fruit pulp were soaked in 200 mL of distilled water and boiled for 30 min. The extract was filtered after cooling and centrifuged at 10,000 rpm for 10 min. The collected supernatant was stored at 4°C.

#### 2.2 Biosynthesis of AgNPs

Silver nitrate (Fisher) solution was mixed with the above-mentioned fruit extract with a final concentration of 5 mM. The mixture was heated at 60°C for 10 min. The reduction of silver ions to AgNPs was observed by a color change from light yellow to brown and was confirmed by measuring absorbance from 300 to 600 nm with the help of a ultraviolet (UV)-visible spectrophotometer (Perkin Elmer, Lambda 25).

#### 2.3 Characterization of green-synthesized *A. digitata* AgNPs

The first step to examine the green synthesis of *A. digitata* AgNPs was by monitoring the color change followed by recording absorbance from 300 to 600 nm to get the absorbance peak. Then, the average size of *A. digitata* AgNPs was recorded by analyzing zeta potential (ZEN3600, Malvern) by electrophoretic light scattering (ELS) in a disposable folded capillary cell (DTS1060). The *A. digitata* AgNPs were loaded on a carbon-coated copper grid and analyzed on transmission electron microscopy (TEM) JEM-1400 plus JEOL and field emission scanning electron microscopy (SEM) JSM-7610F (Joel). Samples of *A. digitata* fruit extract and AgNPs were placed directly in potassium bromide cells and analyzed for bio-reducing functional groups recorded by infrared spectroscopy (Nicolet 6700; Thermo Scientific) the range from 4,000 to 400 cm⁻¹. Energy-dispersive X-ray spectroscopy was used to validate the presence of specific elements present in the sample.

### 2.4 Cell culture

Human colon cancer cell lines HTC116 and SW480 provided by Central Research Laboratory King Saud University were cultured in Eagle’s minimum essential medium (Stem Cell Technologies) under humidified incubator having with 5% CO₂. Trypsin (Sigma) was used to harvest the cells followed by washing in phosphate buffered saline (PBS) (Sigma) and then used for further experiments.

#### 2.5 Cytotoxic activity of green-synthesized *A. digitata* AgNPs

Cancer cells were seeded in a 96-well plate at a density of $2 \times 10^5$ cells/well in 100 µL of optimized medium grown to a density of $2 \times 10^5$ cells/well for 24 h and then exposed to different test concentrations of *A. digitata* extract and green-synthesized *A. digitata* AgNPs separately for 48 h. Finally, 100 µL of MTT (Sigma Aldrich, UK) was added at 37°C at a final concentration of 5 mg·mL⁻¹. The 96-well plate was kept in the dark for 2 h before the medium containing MTT was removed. One hundred microliters of dimethyl sulfoxide (Ajax Finechem Pty Ltd, Australia) were added to dissolve formazan crystals. The 96-well plate was also shaken for 15 min in the dark to help dissolve the formazan crystals. The optical density (OD) of each treatment was measured at absorbance 490 nm using a 96-well plate reader (Molecular Devices; SPECTRA max-PLUS384). Each experiment was performed in four replicates. Values of optical densities were normalized according to the control (untreated cells).
2.6 Gene expression analysis

Gene expression analysis was done after treating cells with IC\textsubscript{50} of green-synthesized \textit{A. digitata} AgNPs and \textit{A. digitata} fruit extract separately. Cells were incubated for 24 h and then were harvested for RNA extraction.

2.7 RNA isolation and RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) was performed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The cDNA was stored at −20°C until the RT-PCR experiment was carried out. The GAPDH gene was used as an internal control. The oligonucleotide sequences are listed in Table 2. The RT-PCR was done on a LightCycler Viia™ 7 Instrument (Viia™ 7; Thermo Fisher Scientific). The data were obtained using LightCycler Viia™ 7 software 1.0 (Viia™ 7; Thermo Fisher Scientific).

Relative mRNA expression levels were then normalized by using the mRNA level of the reference gene (GAPDH) as an endogenous control in each sample. mRNA data were analyzed using the comparative Ct method.

3 Results and discussion

3.1 Characterizations of green-synthesized \textit{A. digitata} AgNPs

The formation of \textit{A. digitata} AgNPs was detected by a color change from light yellow to brown, which happened due to the reduction of AgNO\textsubscript{3} to form AgNPs. We synthesize spherical AgNPs in 10 min by mixing silver nitrate solution at 60°C with fruit extract, which was...
Figure 3: Zeta potential distribution of green-synthesized A. digitata AgNPs.

Figure 4: (a) TEM micrograph and (b) SEM micrograph of the green-synthesized A. digitata AgNPs.
prepared by incubation fruit pulp in boiling water and left overnight, thus accompanying the green chemistry policy. High temperature decreases the diameter of AgNP due to the increasing intensity of the surface plasmon resonance (SPR) band as a result of the bathochromic shift [40]. The sharp peak of SPR at 400 nm in the UV region confirms the formation of AgNPs (Figure 1). This technique is considered one of the basic and quick methods to explore AgNPs [41]. The peak of absorbance is due to the vibration of electrons in synthesized AgNPs [42]. Normally, AgNPs show absorption peaks in the range of 380–470 nm [43].

Green synthesis of A. digitata-mediated AgNPs was confirmed by dynamic light scattering (DLS), which showed average sizes of 16.34 nm, as shown in Figure 2. The polydispersity index was 0.193, indicating small size distribution and homogeneous population of prepared AgNPs [41]. Polydispersity index less than 0.3 specifies monodispersed and can be used for pharmaceutical purposes [44]. Zeta potential was −0.39 as shown in Figure 3. Nanoparticles’ interface in deionized water is negatively charged. Generally, the surface charge appears after nanoparticle preparation, due to the ionizing groups present in the medium [45]. The charge gained by dispersed nanoparticles plays a major role in the stability preventing aggregation [46]. The surface morphology, size, and 3D structure of green-synthesized A. digitata AgNPs were observed by TEM and SEM (Figure 4). TEM results clearly show that AgNPs were spherical and with a size range from 9 to 20 nm, which agrees with the results shown by DLS. The most recent studies listed in Table 1 show almost the same size and shape of AgNPs. TEM is an ultrathin image displaying a 2D structure while SEM demonstrates the 3D structure of a material [47]. Although the spherical structure of A. digitata AgNPs was confirmed by SEM micrograph, the recorded size was in the range of 32.8–37.8 nm, indicating the cluster or aggregation of A. digitata AgNPs [41]. The energy-dispersive spectrum (EDX) of the A. digitata AgNPs exhibited strong signals for the sodium, potassium, and chlorine regions. The presence of other elements sulfur and magnesium was also observed in the spectrum (Figure 5).

3.2 Fourier transform infrared spectroscopy (FT-IR)

Fruit extract of A. digitata is very rich in flavonoid, tannin, saponin, alkaloid, and phenols [35,36]. These secondary metabolites help in the reduction process and also act as a capping agent due to which nanoparticles do not aggregate once formed [48]. FT-IR measurements were carried out on A. digitata extract and its reduced form to recognize the changes in the bonds followed by reduction of the metal precursors and capping of A. digitata AgNPs using an FT-IR spectrophotometer. As displayed in Figure 6a and b, the absorption peaks are 3,439 cm⁻¹ assigned for the OH bond of alcohols/phenols, 2,916 cm⁻¹ assigned for the C–H stretch of alkanes, 2,394 cm⁻¹ assigned for the N–H bond of ammonium ions, 1,629 cm⁻¹ assigned for the N–H bend of amines, 1,413 cm⁻¹ assigned for the O–H bend of carboxylic acids, 1,349 cm⁻¹ assigned for the C–H bend of alkenes, 1,068 cm⁻¹ assigned for the C–N bond of aliphatic amines, and 466 cm⁻¹ assigned for the C–H deformation of alkenes, reflecting its complex nature. The FT-IR spectrum of synthesized NPs shows 3,430 cm⁻¹ assigned for the O–H bond of alcohols/phenols, 2,344 cm⁻¹ assigned for the C–C bond of terminal alkynes, and 1,638 cm⁻¹ assigned for the N–H bond of primary amines and proteins.

Most of the peaks that appeared in the A. digitata extract disappeared after the synthesis of A. digitata AgNPs. Based on the FT-IR analysis, it is confirmed that the broad peaks of phenols and proteins act as reducing stabilizing and capping agents and for AgNPs. Our results are in agreement with previous studies showing that the
presence of biomolecules in plant extract mediates the process of biosynthesis of nanoparticles [47].

### 3.3 Cancer-cell cytotoxicity

We use the MTT assay to examine the impact of *A. digitata* AgNPs and *A. digitata* fruit extract on the cell viability of cancer cell lines. Both extracts decrease the cell viability in a dose-dependent manner in the range from 3.12 to 100 μg·mL⁻¹ against human colon cancer cell lines HTC116 and SW480. IC₅₀ values of test samples show higher cytotoxicity of *A. digitata* AgNPs against SW480 followed by HTC116. Remarkable differences in cell cytotoxicity were shown between *A. digitata* AgNPs and *A. digitata* fruit extract (Figure 7a and b). At all fixed concentrations, the cytotoxicity effect of the *A. digitata* AgNPs was higher than the *A. digitata* extract as shown in Figure 7a and b. The overall results showed that green-synthesized nanoparticles were more active than pure fruit extract on colon cell lines’ death and showed strong cytotoxicity. Many studies have highlighted the effective role of AgNPs against any kind of cancer *in vitro* and *in vivo* [49]. Generally, the zeta potential, which depends on the surface charge, is important for the stability of the

---

**Figure 6:** IR spectra of (a) *A. digitata* extract and (b) green-synthesized *A. digitata* AgNPs.
nanoparticle suspension and is also a major factor in the initial adsorption of nanoparticle on the cell membrane, and the zeta potential and size thus affect the nanoparticle toxicity. The size, shape, and charge of synthesized AgNPs in the current study were acceptable to reveal anticancer properties as small size particles are more active in showing the anticancer activity \[49\]. The size of an AgNP is the main factor to determine its toxicity toward biological systems as particles that are small in size can pass through cells or subcellular organelles of a cell line \[50\]. Gliga et al. \[51\] reported the size-dependent cytotoxicity against human cancer cell lines showing small AgNPs as more cytotoxic as compared to larger particles. AgNPs are proven safe for healthy cells while toxic against cancerous cells.

Several experiments in vitro and in vivo have shown that AgNPs can up- or downregulate the expression of many key genes and regulate important signaling pathways to control the cell proliferation and viability of cancer cells \[52–54\].

Abnormal stimulation of the WNT/\(\beta\)-catenin signaling pathway is observed in CRCs with activating mutations in the catenin beta 1 (\(\text{CTNNB1}\)) gene encoding \(\beta\)-catenin, inactivating adenomatous polyposis coli (\(\text{APC}\)) gene mutations \[55\]. Also, the low-density lipoprotein receptor-related protein 6 (\(\text{LRP6}\)), an indispensable co-receptor for WNT, is overexpressed in colorectal adenocarcinomas \[56\]. Currently, many clinical trials are being done to see the responses of therapies inhibiting Wnt/\(\beta\)-catenin signaling pathways in patients with CRC. However, chemoresistance is one of the major challenges in these trials. In this study, the expression level of these four genes \(\text{CTNNB1}, \text{APC}, \text{LRP5}\), and \(\text{LRP6}\) was measured after treating the cells with \(\text{A. digitata}\) AgNPs and \(\text{A. digitata}\) fruit extract to

---

Figure 7: Cytotoxicity of \(\text{A. digitata}\) extract and green-synthesized \(\text{A. digitata}\) AgNPs on human colon cancer cell lines SW480 and HTC116 following 24 h exposure.
explore its possible role in anticancer mechanisms. Figure 8 presents the results as a chart showing gene expression fold change in the control (without treated) cancerous colorectal cell line and treated cell line. Normally SW480 expresses only mutant APC and whereas HCT116 expresses wild-type APC but mutant CTNNB1 [57]. Mutation in APC and CTNNB1 occurs in more than 80% of colon tumors and is one of the initial events that contribute to colon cancer origin [58]. APC acts as a negative regulator of the canonical WNT signaling pathway through proteasomal degradation of β-catenin, which is expressed by the CTNNB1 gene [59].

A. digitata AgNPs and its aqueous extract show low expression of the APC gene in SW480 as compared to untreated cells, but it was slightly more expressed in HCT116 cells on treatment. Expression of the CTNNB1 gene was decreased in both types of cell lines on treatment. These observations clearly show the inhibitory effect of A. digitata AgNPs and its aqueous extract against chosen cell lines. Alteration in low-density lipoprotein-related receptors 5 and 6 (LRP5/6) genes are linked to the development of cancer in humans [60]. The expression of LRP5 can regulate the expression of serotonin by inhibiting the expression of tryptophan hydroxylase 1 (Tph1), which is the rate-limiting biosynthetic enzyme for serotonin [61]. Serotonin has been found to promote CRC by the modulation of DNA repair mechanisms and immune response [62]. LRP5 was highly expressed in both cell lines after treatment with A. digitata AgNPs and A. digitata extract. Anti-cancer potential of both extracts in our results can be revealed by an elevated level of low-density lipoprotein-related receptors 5 since LRP5 can downregulate tyrosine hydroxylase and decrease serotonin levels [63]. We find a remarkable decrease in

---

**Figure 8:** Gene expression fold change in SW480 and HTC116 cell lines treated with A. digitata extract and green-synthesized A. digitata AgNPs.
expression of the LRP6 gene in both cell lines with treatment. Reducing LRP6 expression has been reported to inhibit the cell proliferation and delay tumor growth [64]. Also, LRP6 is a co-receptor for WNT and is overexpressed in colorectal adenocarcinomas in association with increased WNT/β-catenin signaling [65].

4 Conclusions

Studies showing molecular mechanisms behind the anticancer potential of green-synthesized AgNPs are limited. AgNPs were successfully synthesized from A. digitata fruit extract, which acts as reducing, capping, and stabilizing agents in the process. The synthesis procedure was simple, low cost, and eco-friendly. SPR at 400 nm confirms the formation of AgNPs. Analytical characterization, such as TEM, SEM, and ELS, supports the overall morphology of synthesized AgNPs. A. digitata AgNPs and A. digitata fruit extract showed potent anti-cancer potential.

Funding information: This research project was supported by Researchers Supporting Project number (RSP2022R495) King Saud University, Riyadh, Saudi Arabia.

Author contributions: Fatimah Basil Almukaynizi: methodology; Maha H. Daghestani: project administration, resources; Manal A. Awad: methodology; Arwa Althomali: methodology; Nada M. Merghani: methodology; Wadha I. Bukhari: methodology; Norah M. Alqahtani: methodology; Shatha S. Al-Zuhairy: methodology; Aham M. AlOthman: methodology; Eman A. Alsenani: methodology; Badrih O. Alojayan: methodology; Khulud S. Al-Saif: methodology; Ramesa Shafi Bhat: writing – original draft.

Conflict of interest: The authors state no conflict of interest.

References

[1] Lee SH, Jun BH. Silver nanoparticles: synthesis and application for nanomedicine. Int J Mol Sci. 2019;20(4):865.
[2] Pascu B, Negrea A, Clopec M, Davidescu CM, Negrea P, Gherman V, et al. New generation of antibacterial products based on colloidal silver. Materials (Basel). 2020;13(7):1578.
[3] Ioan-Avram N, Anton F, Maria S, Denis F, Ovidiu O, Ecaterina A. Silver-based materials for biomedical applications. Curr Org Chem. 2014;18:173–84.
[4] Nakamura S, Sato M, Sato Y, Ando N, Takayama T, Fujita M, et al. Synthesis and application of silver nanoparticles (Ag NPs) for the prevention of infection in healthcare workers. Int J Mol Sci. 2019;20(15):3620.
[5] Paladini F, Pollini M. Antimicrobial silver nanoparticles for wound healing application: progress and future trends. Materials (Basel). 2019;12(16):2540.
[6] Burdușel AC, Gherasim O, Grumezescu AM, Mogoantă L, Fical A, Andronescu E. Biomedical applications of silver nanoparticles: an up-to-date overview. Nanomaterials (Basel). 2018;8(9):681.
[7] Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, et al. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol. 2018;16(1):71.
[8] De Matteis V, Cascione M, Toma CC, Leporatti S. Silver nanoparticles: synthetic routes, in vitro toxicity and theranostic applications for cancer disease. Nanomaterials (Basel). 2018;8(5):319.
[9] Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci. 2016;17(9):1534.
[10] Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385–406.
[11] Daghestani M, Al Rashed SA, Bukhari W, Al-Ojayan B, Ibrahim EM, Al-Qahtani AM, et al. Bactericidal and cytotoxic properties of green synthesized nanosilver using Rosmarinus officinalis leaves. Green Process Synth. 2020;9(1):230–6.
[12] Makarov V, Love A, Sinitsyna O, Makarova S, Yaminsky I, Taliansky M, et al. “Green” nanotechnologies: synthesis of metal nanoparticles using plants. Acta Nat. 2014;6:35–44.
[13] Fayaz AM, Balaji K, Kalaichelvan P, Venkatesan R. Fungal based synthesis of silver nanoparticles – an effect of temperature on the size of particles. Colloids Surf B Biointerfaces. 2009;74:123–6.
[14] Shiny P, Mukherjee A, Chandrasekaran N. Marine algae mediated synthesis of the silver nanoparticles and its antibacterial efficiency. Int J Pharm Pharm Sci. 2013;5:239–41.
[15] Niknejad F, Nabil M, Daie Ghazvini R, Moazeni M. Green synthesis of silver nanoparticles: advantages of the yeast Saccharomyces cerevisiae model. Curr Med Mycol. 2015;1(3):17–24.
[16] Skandalis N, Dimopoulou A, Georgopoulou A, Gallios N, Papadopoulos D, Tsipas D, et al. The effect of silver nanoparticles size, produced using plant extract from arbutus unedo, on their antibacterial efficacy. Nanomaterials (Basel). 2017;7(7):178.
[17] Hano C, Abbasi BH. Plant-based green synthesis of nanoparticles: production, characterization and applications. Biomolecules. 2022;12:31.
[18] Mussin J, Robles-Botero V, Casañas-Pimentel R, Rojas F, Angieollla L, Martín-Martínez ES, et al. Antimicrobial and cytotoxic activity of green synthesis silver nanoparticles targeting skin and soft tissue infectious agents. Sci Rep. 2021;11:14566.
[19] Al-Zahrali SA, Bhat RS, Al Rashed SA, Mahmood A, Al Fahad A, Alamro G, et al. Green-synthesized silver nanoparticles with aqueous extract of green algae Chaetomorpha lignostica and its anticancer potential. Green Process Synth. 2021;10:711–21.
Cytotoxicity of green-synthesized AgNPs by A. digitata fruit extract

[20] Riaz M, Mutreja V, Sareen S, Ahmad B, Faheem M, Zahid N, et al. Exceptional antibacterial and cytotoxic potency of monodisperse greener AgNPs prepared under optimized pH and temperature. Sci Rep. 2021;11:2866.

[21] Elhawary S, EL-Hefnawy H, Alzahraa FA, Sobeh M, Mostafa E, Osman S, et al. Green synthesis of silver nanoparticles using extract of jasminum officinal L. leaves and evaluation of cytotoxic activity towards bladder (5637) and breast cancer (MCF-7) cell lines. Int J Nanomed. 2020;15:9771–978.

[22] Karimi S, Mahdavi, Shahri M. Medical and cytotoxicity effects of green synthesized silver nanoparticles using Achillea millefolium extract on MOLT-4 lymphoblastic leukemia cell line. J Med Virol. 2021;93(6):3899–906.

[23] Devanesan S, Aisalhi MS. Green synthesis of silver nanoparticles using the flower extract of abelmoschus esculentus for cytotoxicity and antimicrobial studies. Int J Nanomed. 2021;14(16):3343–56.

[24] Bharadwaj KK, Rabha B, Pati S, Choudhury BK, Sarkar T, Gogoi SK, et al. Green synthesis of silver nanoparticles using diospyros malabarica fruit extract and assessments of their antimicrobial, anticancer and catalytic reduction of 4-nitrophenol (4-NP). Nanomaterials (Basel). 2021;11(8):1999.

[25] Alwhibi MS, Soliman DA, Awad MA, Alangery AB, Al Dehaish H, Alwasel YA. Green synthesis of silver nanoparticles: characterisation and its potential biomedical applications. Green Process Synth. 2021;10(1):412–20.

[26] Devanesan S, Ponnurungan K, Aisalhi MS, Al-Dhabi NA. Cytotoxic and antimicrobial efficacy of silver nanoparticles synthesized using a traditional phytoproduct, asafoetida gum. Int J Nanomed. 2020;15:4351–62.

[27] Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari MR. Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. Int J Nanomed. 2018;13:2017–24.

[28] Li R, Pan Y, Li N, Wang Q, Chen Y, Pan Y, Li N, Wang Q, Chen Y, Phisalaphong M, et al. Green synthesis of silver nanoparticles by green synthesis method using Mangifera indica seed aqueous extract and its potential catalytic, antioxidant and growth-inhibitory activities. Colloids Surf A: Physicochem Eng Asp. 2020;598:124827.

[29] Donga S, Chanda S. Facile green synthesis of silver nanoparticles using Mangifera indica seed aqueous extract and its antimicrobial, antioxidant and cytotoxic potential (3-in-1 system). Artif Cell Nanomed Biotechnol. 2021;49(1):292–302.

[30] Kapoor S, Sood H, Saxena S, Chaurasia OP. Green synthesis of silver nanoparticles using Rhodiola imbricata and Withania somnifera root extract and their potential catalytic, antioxidant, cytotoxic and growth-promoting activities. Bioprocess Biosyst Eng. 2022;45:365–80.

[31] White BD, Duan C, Townley HE. Nanoparticle activation methods in cancer treatment. Biomolecules. 2019;9(5):202.

[32] Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89–103.

[33] Kelly C, Bhuva N, Harrison M, Buckley A, Saunders M. Use of raltitrexed as an alternative to 5-fluorouracil and capecitabine in cancer patients with cardiac history. Eur J Cancer. 2013;49:2303–10.

[34] Nallanthighal S, Chan C, Murray TM, Mosier AP, Cady NC, Reliene R. Differential effects of silver nanoparticles on DNA damage and DNA repair gene expression in Ogg1-deficient and wild type mice. Nanotoxicology. 2017 Oct;11(8):996–1011.

[35] Muthai KU, Karori MS, Muchugi A, Indeika AS, Dembele C, Mng’omba S, et al. Nutritional variation in baobab (Adansonia digitata L.) fruit pulp and seeds based on Africa geographical regions. Food Sci Nutr. 2017;5(6):1116–29.

[36] De Caluwé W, Halamová K, Van Damme P. Adansonia digitata L.: a review of traditional uses, phytochemistry and pharmacology. Afr Focus. 2010;23(1):11–51.

[37] Buchmann C, Prehsler S, Hartl C, Vogl CR. The importance of baobab (Adansonia digitata L.) in rural west African subsistence-suggestion of a cautionary approach to international market export of baobab fruits. Ecol Food Nutr. 2010;49:145–72.

[38] FDA (U.S. Food and Drug Administration) agency response letter GRAS notice no GRN 000273.2009. http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm174945.htm.

[39] Li XN, Sun J, Shi H, Yu LL, Ridge CD, Mazzola EP, et al. Profiling hydroxycinnamic acid glycosides, iridoid glycosides, and phenylethanoid glycosides in baobab fruit pulp (Adansonia digitata). Food Res Int. 2017;99(Pt 1):755–61.

[40] Bindhu MR, Umadevi M. Silver and gold nanoparticles for sensor and antibacterial applications. Spectrochim Acta Part A: Mol Biomol Spectrosc. 2014;128:37–45.

[41] Bhat RS, Almusallam J, Al Daihan S, Al-Dbass A. Biosynthesis of silver nanoparticles using Azadirachta indica indica leaves: characterisation and impact on Staphylococcus aureus growth and glutathione-S-transferase activity. IET Nanobiotechnol. 2019;13:498–502.

[42] Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Appl Nanosci. 2016;6:399–408.

[43] Rahman A, Kumar S, Bafana A, Lin J, Dahoumane SA, Jeffreys CA. Mechanistic view of the light-induced synthesis of silver nanoparticles using extracellular polymeric substances of Chlamydomonas reinhardtii. Molecules. 2019;24(19):3506.

[44] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57.

[45] Wang T, Jin X, Chen Z, Megharaj M, Naidu R. Green synthesis of Fe nanoparticles using eucalyptus leaf extracts for treatment of eutrophic wastewater. Sci Total Env. 2014;446:210–3.

[46] Rauwel P, Küüsal J, Ferdov S, Rauwel E. A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. Adv Mater Sci Eng. 2015;2015:682749.

[47] El-Ansary A, Warsy A, Daghhestani M, Merghani NM, Al-Dbass A, Bukhari W, et al. Characterization, antibacterial and neurotoxic effect of green synthesized nanosilver using Ziziphus spinosa Christi aqueous leaf extract collected from Riyadh, Saudi Arabia’. Mater Res Express. 2018;5:025033.

[48] Marslin G, Siram K, Maqbool Q, Selvakesavan RK, Kruszka D, Kachlicki P, et al. Secondary metabolites in the green synthesis of metallic nanoparticles. Mater (Basel). 2018;11(6):940.

[49] Wang ZX, Chen CY, Wang Y, Li FXZ, Huang J, Luo ZW, et al. Ångström scale silver particles as a promising agent for low toxicity broad-spectrum potent anticancer therapy. Adv Funct Mater. 2019;29:1808556.
Rohde MM, Snyder CM, Sloop J, Solst SR, Donati GL, Spitz DR, et al. The mechanism of cell death induced by silver nanoparticles is distinct from silver cations. Part Fibre Toxicol. 2021;18:37.

Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol. 2014;11:11.

Farah MA, Ali MA, Chen SM, Li Y, Al-Hemaid FM, Abou-Tarboush FM, et al. Silver nanoparticles synthesized from Adenium obesum leaf extract induced DNA damage, apoptosis and autophagy via generation of reactive oxygen species. Colloids Surf B Biointerfaces. 2016;141:158–69.

Mytych J, Zebrowski J, Lewinska A, Wnuk M. Prolonged effects of silver nanoparticles on p53/p21 pathway-mediated proliferation, DNA damage response, and methylation parameters in HT22 hippocampal neuronal cells. Mol Neurobiol. 2017;54:1285–300.

Yang T, Yao Q, Cao F, Liu Q, Liu B, Wang XH. Silver nanoparticles inhibit the function of hypoxia-inducible factor-1 and target genes: insight into the cytotoxicity and antiangiogenesis. Int J Nanomed. 2016;11:6679–92.

Schatoff EM, Leach BI, Dow LE. Wnt signaling and colorectal cancer. Curr Colorectal Cancer Rep. 2017 Apr;13(2):101–10.

Raisch J, Côté-Biron A, Rivard N. A role for the WNT co-receptor LR6 in pathogenesis and therapy of epithelial cancers. Cancers (Basel). 2019;11(8):1162.

El-Bahrawy M, Poulsom R, Rowan AJ, Tomlinson IT, Alison MR. Characterization of the E-cadherin/catenin complex in colorectal carcinoma cell lines. Int J Exp Pathol. 2004;85(3):175.

Kwong LN, Dove WF. APC and its modifiers in colon cancer. Adv Exp Med Biol. 2009;656:85–106.

Parker TW, Neufeld KL. APC controls Wnt-induced β-catenin destruction complex recruitment in human colonocytes. Sci Rep. 2020;10:2957.