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Research article

In-vitro anticancer activity against Caco-2 cell line of colloidal nano silver synthesized using aqueous extract of *Eucalyptus Camaldulensis* leaves

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ABSTRACT

In the current study, we investigated the anticancer potential against human colon cells (Caco-2) of colloidal nanosilver (CN–Ag) produced in Syria using bioactive compounds in the aqueous extract of *Eucalyptus camaldulensis* leaves (AEECL). The formation of AgNPs was confirmed by UV-visible spectroscopy analysis with surface plasmon peak at 449 nm and their average size was found to be 12, 10, 23 nm by SEM, DLS and NTA respectively. This small size has confirmed the effective role of AEECL as capping agent. Further morphological characterization was done by EDS showed the presence of metallic silver. Zeta potential value (-23 mV) indicated the repulsion among the particles and stability of the formulation nanosilver. The anticancer effect of synthesized CN–Ag against Caco-2 has been tested. The cytotoxicity assay showed a dose-dependent and a time-dependent effect of CN–Ag. The high cytotoxicity of CN–Ag at low concentration (5μ/mL) open new prospects for the development of novel therapeutic approaches against human colon cancer Caco-2.

1. Introduction

Cancer is a dangerous class of disease involving abnormal tissue growth involving abnormal tissue growth that divides in uncontroll manner and spreads to almost anywhere in the body. The standard strategies used for the treatment of cancer diseases include surgical excision, chemotherapy, and radiotherapy either individually or in combination. However, these treatment approaches possess significant side effects. Recently scientists and researchers are working to discover new treatments for cancer-based on the use of new-featured materials. Nanotechnology has the potential to improve current approaches while reducing toxicity and negative effects associated with traditional treatments. Metal nanoparticles have a significant increase in usage in various fields, like electronics [1], catalyst [2], medicine [3] and biotechnology [4]. Silver nanoparticles are widely used in many fields especially at medical and health fields [5] because of their unique physicochemical, and biological therapeutic properties as antibacterial [6, 7, 8] antiviral [9], antifungal [10], anti-inflammatory [11, 12] and anticancer [13, 14]. Silver nanoparticles have been extensively carried out biomedical areas as treatment of wounds [15] and burns [16], water purification [17, 18], air filter [19], textile industry [20, 21] and healthcare products. In recent years, many eco-friendly methods [22, 23] have been employed in the synthesis of silver nanoparticles using biological materials like bacteria [24, 25], fungi [26, 27] and plant extracts. These methods are simple, nontoxic and acceptable for biomedical applications. Synthesis of AgNPs using plant extracts is simple, low-cost and does not require special conditions like in other chemical and biological methods. The present study deals with the synthesis of silver nanoparticles using an aqueous extract of *Eucalyptus Camaldulensis* leaves (AEECL). *Eucalyptus Camaldulensis* is the most common *Eucalyptus* genus of *Myrtaceae* family, widely planted in our region. Nevertheless, *Eucalyptus camaldulensis* leaves contain a wide range of bioactive compounds including saponins, tannins, flavonoids, carbohydrate and proteins [28], which play important roles as reducing and capping agent during the formation of

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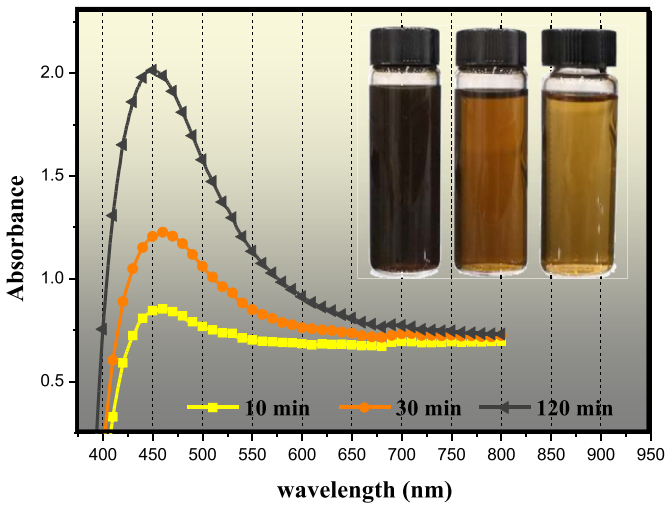

Figure 1. UV–Visible absorption spectrum of the synthesized silver nanoparticles at different times (10, 30, and 120 min). The inset is a photograph for changing colour within different time (10, 30, and 120 min).

nanoparticles [29, 30]. There are many articles published on the synthesis of AgNPs using different species of *Eucalyptus* such as *Eucalyptus corymbia* [31], *Eucalyptus globules* [32], *Eucalyptus critriodora* [33], and *Eucalyptus tereticornis* [34] etc… But, few studies have been reported in the using of aqueous extract of *Eucalyptus camaldulensis* leaves for synthesis silver nanoparticles [30].

To the best of our knowledge, this is the first report showing the potent anticancer of silver nanoparticles biosynthesized using AEECL extract against human colon cancer cells (Caco-2).

2. Materials and methods

2.1. Materials

Materials: silver nitrate ($AgNO_3$), MTT (3- (4,5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) and all other chemicals were purchased from sigma Aldrich. Freshly prepared distilled water was used throughout the experiments.

2.2. Preparation of the aqueous Eucalyptus camaldulensis leaves extract

In our previous study [30], we compared three different methods for preparing an aqueous extract of *Eucalyptus Camaldulensis* leaves (AEECL) and the chemical analysis showed that the extract prepared by boiling dried leaves for 10 min contains the highest amount of phenolic compounds and the better ability as free-radical scavenger compared to other prepared extracts. So it is the best candidate as a reducing agent for synthesis of silver nanoparticles. In this study, the same procedure has been used. Fresh and healthy *Eucalyptus Camaldulensis* leaves (ECL) were collected and cleaned with distilled water to remove all the dust and unwanted visible particles, then dried to constant weight [35] at 60 °C

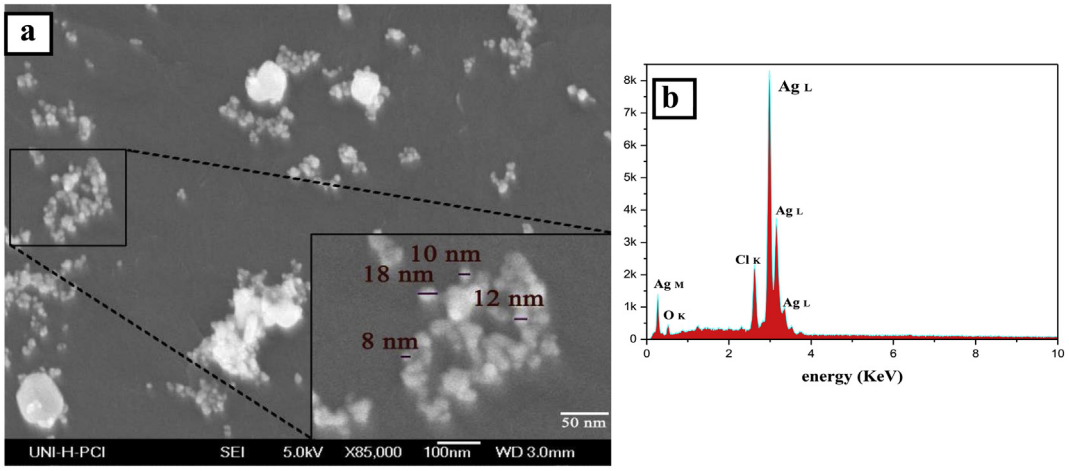

Figure 2. a) SEM image and b) EDX spectrum of silver nanoparticles prepared using aqueous extract of *Eucalyptus Camaldulensis* leaves.

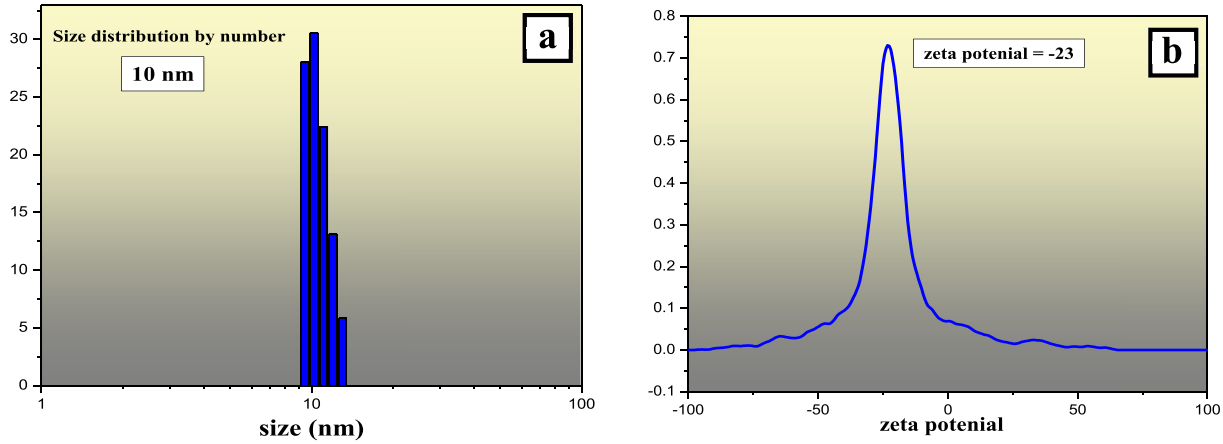

Figure 3. a) Particles size distribution by number and b) zeta potential of colloidal nanosilver synthesized using aqueous extract of *Eucalyptus Camaldulensis* leaves.

for 2 h to remove the residual moisture. Dried leaves were powdered and stored in a sealed bag until the extraction process. The aqueous extract was prepared by adding 3 g of dried ECL to 30 mL of distilled water and boiled for 10 min. After that, the macerate was centrifuged at 9500 rpm for 5 min to remove any particles. Then the resulting supernatant was filtered by Whatman No.1 filter paper and stored into a dark container at refrigerator temperature.

2.3. Synthesis of silver nanoparticles

An aqueous solution (0.02 M) of silver nitrate was prepared by dissolving 0.068 g of $AgNO_3$ in 20 mL of distilled water. Then 4 mL of the prepared aqueous *Eucalyptus Camaldulensis* leaves extract was added after it has been completed to 20 mL by adding distilled water. The mixture was kept under stirring at room temperature for almost half an hour to

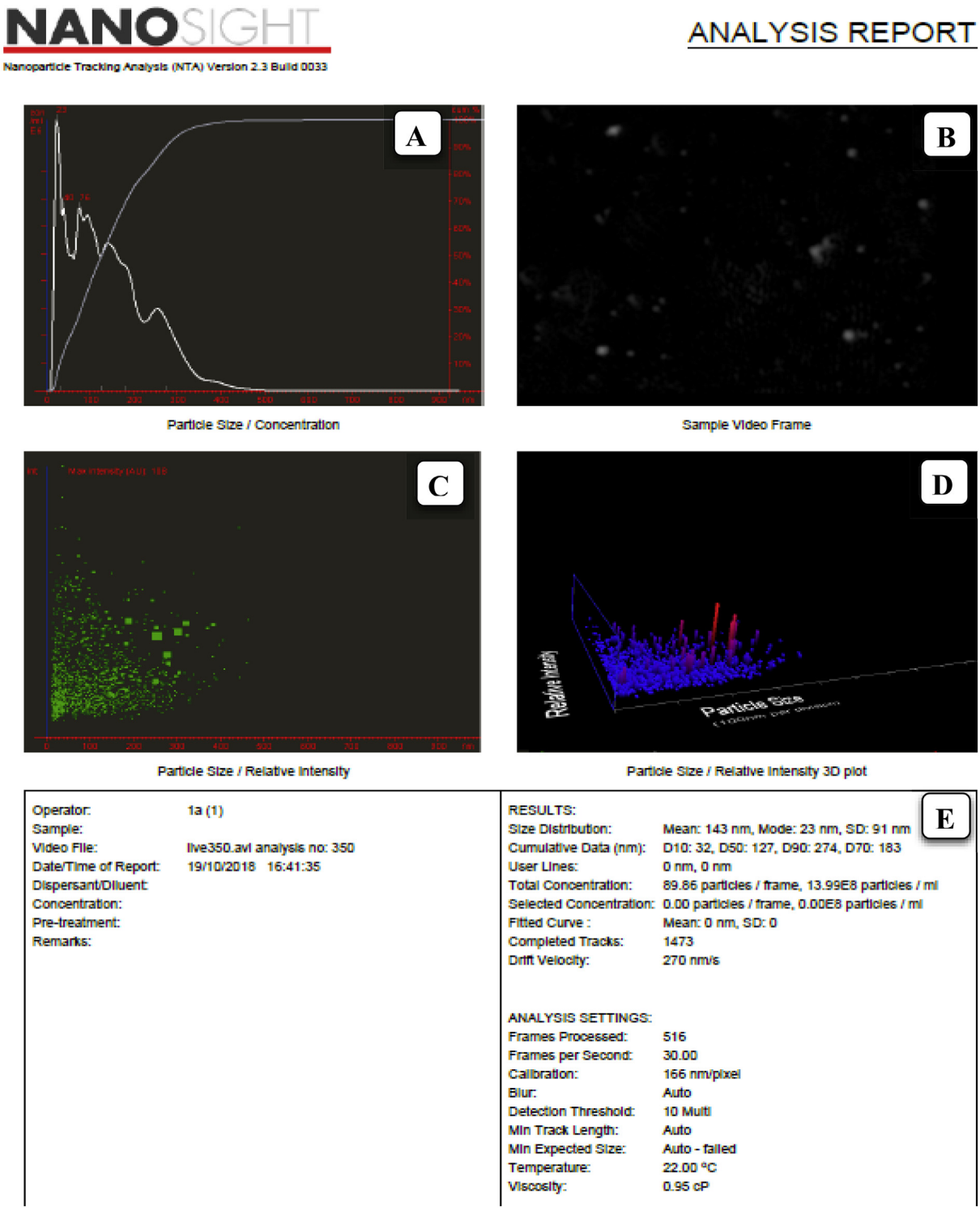

Figure 4. A) particles size distribution, B) A video frame, C) 2D scattergram, D) 3D plot and E) results from NTA for colloidal nanosilver synthesized using aqueous extract of *Eucalyptus Camaldulensis* leaves.

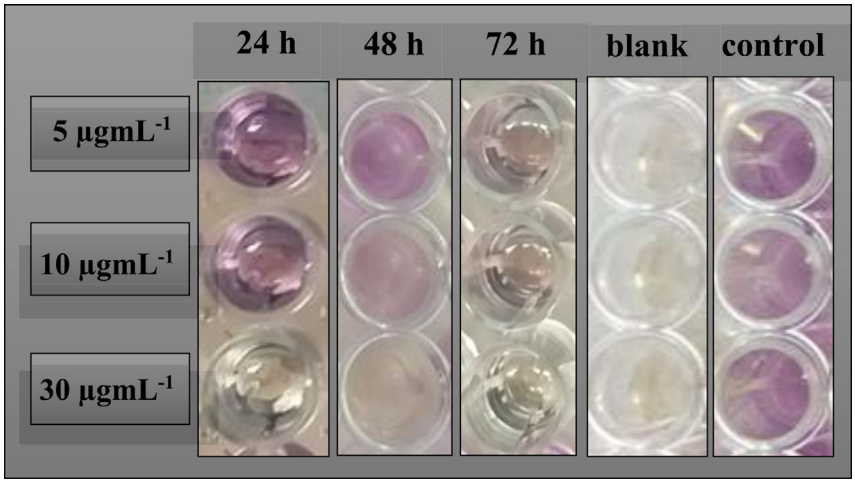

Figure 5. MTT assay for cytotoxicity investigation of colloidal nanosilver against Caco-2 cells at different concentrations 5, 10 and 30 μgmL^{-1} and various exposure times 24, 48 and 72 h.

ensure the homogeneous reduction process. The colourless solution of silver nitrate changed to brownish-yellow immediately and then changed to dark brown within a short period (10 min), indicating the formation of AgNPs. The colloidal nanosilver solution was kept at room temperature for 24 h to complete reduction.

2.4. Purification of biosynthesized silver nanoparticles

After 24 h of incubation, silver nanoparticles were separated by centrifugation at 9500 rpm for 10 min, the residue was collected and washed twice with distilled water and last with ethanol and dried at room temperature. This operation was applied to get rid of any uncoordinated biological materials and to remove the excess silver ions. The colloidal sample was prepared by adding a small amount of distilled water to the washed silver nanoparticles and dissolve it using the ultrasonic bath for 15 min

2.5. Characterization of silver nanoparticles

The preliminary characterization of AgNPs was carried out using UV-visible spectrophotometer (Cary 5000) and a cell with 1cm path length. The analysis was done after diluting a small aliquot of the suspension into distilled water (1:4). The baseline was corrected using the prepared AEECL extract. The surface morphology (size and shape) of silver nanoparticles was characterized by SEM (JSM-6700F JEOL GmbH). The sample was prepared by drop-casting, a small drop of CN–Ag was placed on a carbon-coated graphite grid and allowed to dry by using a hairdryer for 5 min. Then readings were taken at ×150K magnification with steady voltage (20 kV). EDS analysis was performed to confirm the presence of silver by detecting the rays being emitted from the NPs representing as peaks at particular electron volt. The particle size distribution and zeta potential of the prepared AgNPs were determined by the DLS technique using Zetasizer Nano ZS from Malvern. Zeta potential measurements were carried out by injecting 1mL of the sample into the zeta cell. For DLS analysis, 1mL of the sample was transferred into a plastic cuvette and automatically equilibrated in the instrument for a few minutes. An aliquot of CN-NPs was diluted with DI-water, then sonicated for 5 min before the measurements. All measurements were recorded in triplicate. The particle size distribution and concentration of Ag were also analyzed using Nanoparticle Tracking Analysis technology. To obtain optimum imaging concentrations, some samples were diluted in DI-water, A volume of 0.3 mL of each sample was measured 3 times with high sensitive camera. All data were analyzed using the instrument software (NanoSight™).

2.6. Cell line and culture

Human colon cancer (Caco-2) cell line was purchased from the Human cell culture laboratory in atomic energy commission of Syria. Caco-2 cells were cultured in RPMI 1640 medium containing 5% fetal bovine serum (FBS), 50 U/mL penicillin/streptomycin and 2mM L-glutamine. The cells were plated in 96-well plates at a density 2×10^4 cells/well and allowed to adhere overnight at 37 °C (95 % humidity, 5% CO2). Cultures were examined using an inverted microscope to evaluate the quality of confluence and confirming the absence of bacterial and fungal contaminants. The medium was replaced with fresh medium with varying concentrations of AgNPs suspensions (5, 10, and 30 µg/mL). These values were chosen according to the results of Kiran Jadhav *et al* [36] which demonstrated the absence of toxicity against normal human fibroblasts and blood erythrocytes at concentrations of silver nanoparticles less than 78 μgmL^{-1}. The cells were treated at three different time intervals (24, 48, and 72 h).

2.7. MTT assay

Cytotoxicity of the biosynthesized AgNPs was examined after 24, 48 and 72 h of treatment by MTT (Thiazolyl Blue Tetrazolium Bromide) assay. After incubation time, 20 µl of MTT (5 mg/mL) was added and the cells were incubated again for another 4 hrs at 37 °C in CO_2 incubator. The formazan crystals formed by mitochondrial reduction of MTT were solubilized in 150 µL of DMSO (dimethyl sulphoxide) which was added to each well and mixed, then the absorbance at 540 nm was measured using the microplate reader (biotek-epoch). Each experiment was performed in triplicate and the percentage of cell viability was calculated by the following formula:

$$\text{percentage of cell viability} = \frac{A_{treatment} - A_{blank}}{A_{control} - A_{blank}} \text{x}100$$

where the Blank is RPMI and AgNPs and the control is RPMI and cancer cells.

2.8. Statistical analysis

The experimental results were expressed as mean ± standard deviation of three independent experiments. The data were analyzed using one way ANOVA using origin program version 8.5.

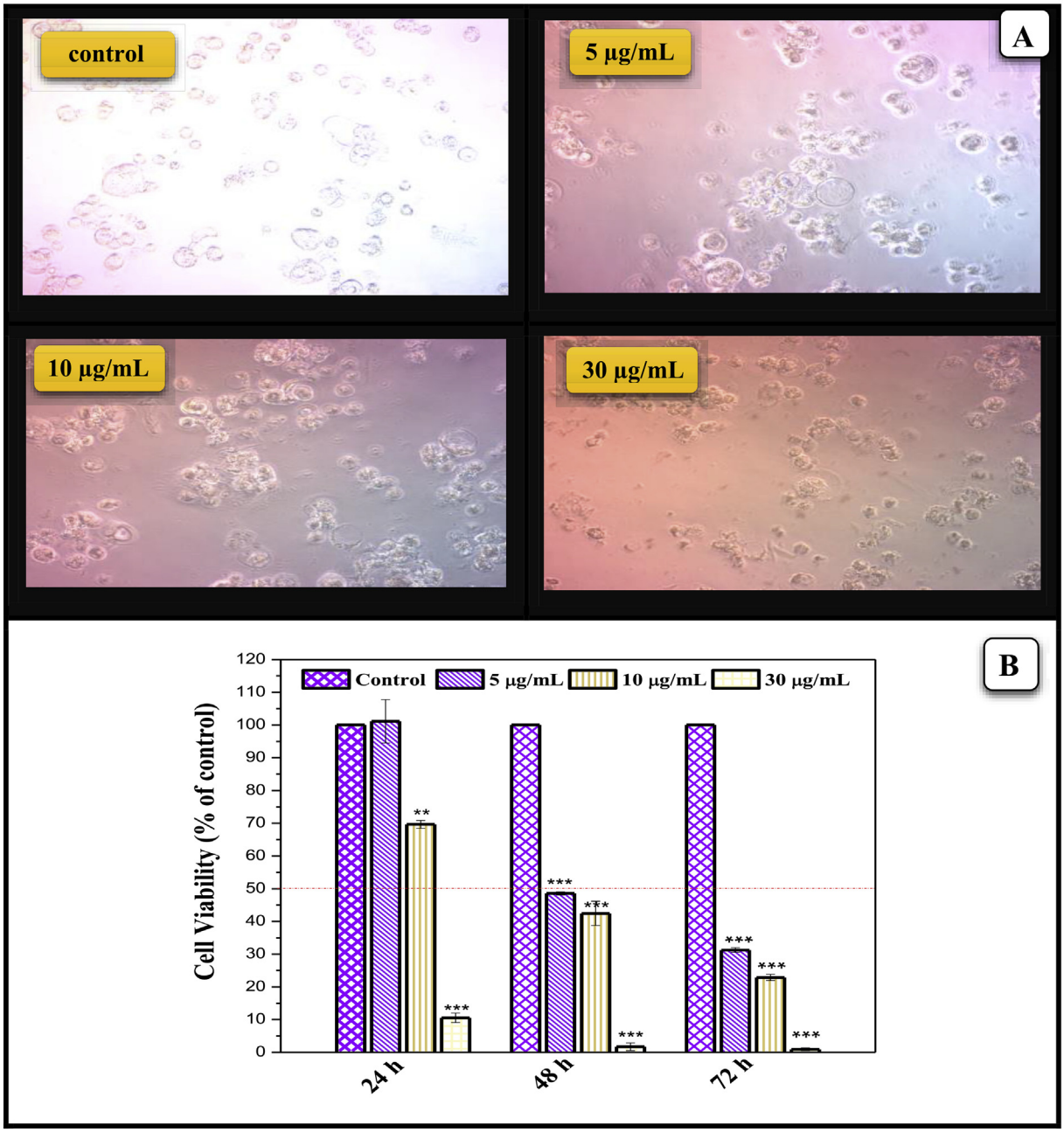

Figure 6. A) Morphological changes by inverted microscope (x40) in Caco-2 cells treated with colloidal nanosilver after 48h. B) Caco-2 cells viability at different concentrations: 5, 10, and 30 µg/mL and different exposure times: 24, 48, and 72 h. Results of MTT assay are represented as mean number ± SD of three independent experiments. Statistical significance was assessed by a one way ANOVA: $^{**}p < 0.05$ was considered significant; $^{***}p < 0.001$ very highly significant compared with the control.

3. Results and discusion

A rapid change of mixture colour from pale yellow to brownish-yellow within 10 min (see the inset Figure 1), indicated the high anti-oxidant activity of *Eucalyptus camaldulensis* leave aqueous extract which related to a wide range of bioactive compounds containing in the aqueous extract (AEECL) such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, amino acids and proteins [28]. These different biomolecules play an important role as reducing, chelating and capping agents [37]. Flavonoids contain many functional groups involved in the formation of silver. Some flavonoids can chelate silver ions at positions involving the carbonyl and hydroxyl (–OH) groups. It can also release a reactive hydrogen atom that can reduce metal ions to form silver nanoparticles [38]. Terpenoids also play an important role as a capping and reducing agent. It could be adsorbed on the surface of AgNPs enabling their reduction from Ag^{+} and preventing them from agglomeration [39, 40]. Amino acid can also reduce and bind silver ions through amino and carbonyl groups [41].

Figure 1 shows the UV–Visible absorption spectrum of the synthesized AgNPs at different incubation time (10, 30, and 120 min). The presence of single SPR peaks at 449 nm give first evidence of silver nanoparticles formation. Increasing the reaction time resulted in a gradual increasing of SPR peaks intensity. The level of absorption reached and 2.2 after 2 h of incubation, thus indicating that more Ag-NPs are formed as the period of reaction increases [42]. The SEM analysis was further employed to investigate the size and morphology of the

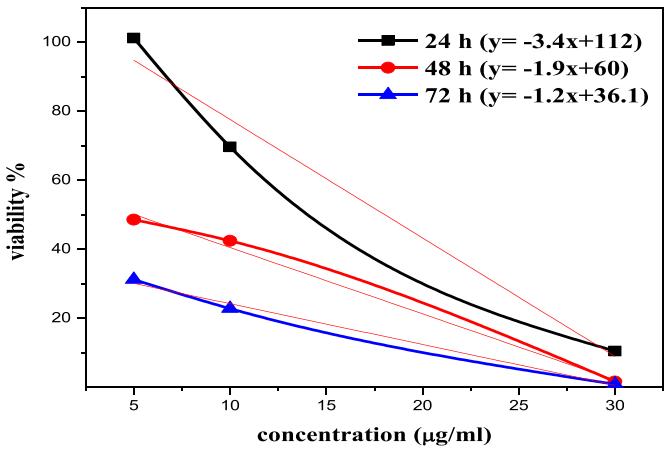

Figure 7. A linear regression for a dose-dependent curve of colloidal nanosilver on Caco-2 cell line at different concentrations 5, 10 and 30 µgmL^{-1} and various exposure times 24, 48 and 72 h.

synthesized EC-AgNPs as depicted in Figure 2(a). SEM image of AgNPs indicated that the nanoparticles were spherical and mostly dispersed with small size (12 nm). This result can be correlated with the single SPR spectrum obtained in the UV-visible spectrophotometric analysis. This indicates that *Eucalyptus camaldulensis* leaves served as a good reductant and a capping or a stabilizing agent in the synthesis of AgNPs. Additionally, the presence of metallic silver in the synthesized EC-AgNPs was confirmed by elemental composition (EDS) analysis (Figure 2(b)).

The EDS spectrum showed an intense sharp silver peak at 3 keV, which is the characteristic absorption of metallic silver. The other weak signals of Cl and O elements were also observed, which may be due to biomolecules and phytoconstituents of *Eucalyptus camaldulensis* attached to the surface of silver nanoparticles. Similar results have been included in the literature previously [34,43]. The particles size Vs number distribution graph obtained from the dynamic light scattering analysis (DLS) of colloidal nanosilver CN–Ag synthesized by the bioreduction process using AEECL were shown in Figure 3. It indicates that the average size of AgNPs was around 10 nm. For the confirmation of monodispersity, DLS results indicate 0.304 PDI, which depicts that the nanoparticles are well dispersed in the used solvent i.e. water. Comparing the average size of AgNPs from the two calculation technologies (SEM and DLS) reveals that the dimension values are highly correlated. The zeta potential describes the degree of stability of colloidal dispersion nanoparticles. The zeta potential of synthesized CN–Ag was -23 mV, which indicates the high stability of AgNPs. A negative value suggests that the surface of the nanoparticles is negatively charged as shown in Figure 3, which causes a strong electrostatic repulsion force between the particles, thus increases the stability of the formulation AgNPs without adding any capping agent. This is very important for using at a therapeutic propose. NanoTracking Analysis measurement revealed diameter distributions of the CN–Ag with a mode size of 23 nm (see Figure 4). The total concentration of EC-AgNPs measured with NTA was 13.99 E8 particles/mL.

3.1. Cytotoxicity and determination of half maximal inhibitory concentration of CN–Ag (IC_{50})

In the present investigation, Human colon cancer (Caco-2) cell line was employed to evaluate the anticancer potential activity of CN–Ag. The cytotoxicity of colloidal nanosilver was investigated using a colourimetric MTT assay. Caco-2 cells were treated with CN–Ag at different concentrations 5, 10 and 30 µgmL^{-1} and evaluated for cell viability after various exposure times 24, 48 and 72 hrs (see Figure 5).

The morphology of Caco-2 cells treated with CN–Ag was significantly changed in comparing with the untreated cells. The treated cells showed apoptotic-like symptoms such as cell shrinkage and irregularity in their shapes which observed in Figure 6(A). The bar diagram of the MTT cell viability assay results represented in Figure 6(B). Significant differences were showed statistically between the treated and control cells. This indicated the anticancer activity of CN–Ag on the tested cell line. However, a further increase in dose and time cause cell death which confirm the dose and the time-dependent response relationship. The higher concentration of CN–Ag (30 µgmL^{-1}) was found to be very toxic against Caco-2 cell line. It has a high capacity of reducing the cell viability percentage lower than 10%. In the other hand, the other concentrations (5 µgmL^{-1} and 10 µgmL^{-1}) significantly inhibit the cell growth by more than 50% and 70% after 48h and 72h exposure time respectively. The inhibition of cell growth by increasing the concentration of CN–Ag could be attributed to the apoptotic effect of silver nanoparticles. Effect of silver nanoparticles in the induction of apoptosis has been studied previously and different mechanisms of the potential cytotoxicity of silver nanoparticles have been investigated such as activation of apoptotic caspases, DNA fragmentation, nuclear fragmentation, ROS generation, leakage of LDH and also altering mitochondrial membrane potential [14, 44, 45, 46]. The IC_{50} of CN–Ag was calculated using linear regression for a dose-dependent curve that obtained the effect of different concentrations of silver nanoparticles on Caco-2 cell line as showed in Figure 7. The IC_{50} value was found to be 18 µgmL^{-1} for 24 h of incubation. This result showed that the CN–Ag could be more effective than phosphate-buffered AgNPs used by Meike van der Zande *et al* against Caco-2 cell line [47], where the tested doses in their work (5 and 25 µgmL^{-1}) displayed mild cytotoxicity against Caco-2 cells after 24 h of treatment. Also, another study by Bouwmeester *et al* showed the absence of toxicity for phosphate buffered AgNPs up to 50 *µgmL*$^{-1}$ to Caco-2 cells [48].

Notably, the concentration 5 µgmL^{-1} in our study reduced the cell viability to 50% subtracted to the control after 48 h and that confirmed the time-dependent of CN–Ag cytotoxic effect. So increasing exposure time reduces the effective concentration against Caco-2 cells. That result was obtained after 72 h of exposure; all the applied concentrations of CN–Ag demonstrated less than 30% of cell viability.

4. Conclusion

A simple, inexpensive, eco-friendly and green method using an aqueous extract of *Eucalyptus camaldulensis* leaves has reported for synthesis a small size silver nanoparticles 12 nm without any protecting or capping reagents. The cytotoxicity assay against Caco-2 cell line showed a dose-dependent and a time-dependent effect of CN–Ag. A small concentration (5 µgmL^{-1}) reduced the cell viability to 50% after 48h of exposure. The high cytotoxicity of CN–Ag at low concentrations open new prospects for the development of novel therapeutic approaches against human colon cancer Caco-2.

Declarations

Author contribution statement

Raghad Zein, Ibrahim Alghoraibi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chadi Soukkarieh: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Alaa Salman: Performed the experiments.

Abdalrahim Alahmad: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] S. Liu, M.C. Yuen, R. Kramer-Bottiglio, Reconfigurable electronic devices enabled by laser-sintered liquid metal nanoparticles, Flex. Print. Electron. 4 (2019), 015004.
[2] M.S.J. Khan, T. Kamal, F. Ali, A.M. Asiri, S.B. Khan, Chitosan-coated polyurethane sponge supported metal nanoparticles for catalytic reduction of organic pollutants, Int. J. Biol. Macromol. 132 (2019) 772–783.
[3] B.A. Aderibigbe, Metal-based nanoparticles for the treatment of infectious diseases, Molecules 22 (2017).
[4] D. Shcharbin, I. Halets-Bui, V. Abashkin, V. Dzmitruk, S. Loznikova, M. Odabaşı, Ö. Acet, B. Önal, N. Özdemir, N. Shcharbina, M. Bryszewska, Hybrid metal-organic nanoflowers and their application in biotechnology and medicine, Colloids Surf. B Biointerfaces 182 (2019) 110354.
[5] A.-C. Burduşel, O. Gherasim, A.M. Grumezescu, L. Mogoantă, A. Ficai, E. Andronescu, Biomedical applications of silver nanoparticles: an up-to-date overview, Nanomaterials 8 (2018) 681.
[6] R. Salomoni, P. Léo, A. Montemor, B. Rinaldi, M. Rodrigues, Antibacterial effect of silver nanoparticles in Pseudomonas aeruginosa, Nanotechnol. Sci. Appl. 10 (2017) 115–121.
[7] F.A. Qais, A. Shafiq, H.M. Khan, F.M. Husain, R.A. Khan, B. Alenazi, A. Alsalme, I. Ahmad, Antibacterial effect of silver nanoparticles synthesized using murraya koenigii (L.) against multidrug-resistant pathogens, Bioinorg. Chem. Appl. 2019 (2019) 1–11.
[8] M.M.R. Mollick, B. Bhowmick, D. Maity, D. Mondal, I. Roy, J. Sarkar, D. Rana, K. Acharya, S. Chattopadhyay, D. Chattopadhyay, Green synthesis of silver nanoparticles-based nanofluids and investigation of their antimicrobial activities, Microfluid. Nanofluidics 16 (2014) 541–551.
[9] S. Park, H.H. Park, S.Y. Kim, S.J. Kim, K. Woo, G. Ko, Antiviral properties of silver nanoparticles on a magnetic hybrid colloid, Appl. Environ. Microbiol. 80 (2014) 2343–2350.
[10] N.T. Khan, M. Mushtaq, Determination of antifungal activity of silver nanoparticles produced from Aspergillus Niger, Biol. Med. 9 (2017) 1–4.
[11] F.E. Meva, J.O.A. Mbeng, C.O. Ebongue, C. Schlüsener, Ü. Kökçam-Demir, A.A. Ntoumba, P.B.E. Kedi, E. Elanga, E.N. Loudang, M.H.J. Nko'o, E. Tchoumbi, V. Deli, C.C. Nanga, E.A.M. Mpondo, C. Janiak, Stachytarpheta cayennensis aqueous extract, a new bioreactor towards silver nanoparticles for biomedical applications, J. Biomater. Nanobiotechnol. 10 (2019) 102–119.
[12] G. Kannayiram, A. Sandhya, S. Sowmiya, S. Valarmathi, D. Joseph, Anti-inflammatory activity of nigella sativa silver nanoparticles: biochemical study, Asian J. Pharmaceut. Clin. Res. (2019) 346–349.
[13] Ş. Yeşilot, Ç. Aydın Acar, Silver nanoparticles; a new hope in cancer therapy? East. J. Med. 24 (2019) 111–116.
[14] K. Venugopal, H.A. Rather, K. Rajagopal, M.P. Shanthi, K. Sheriff, M. Illiyas, R.A. Rather, E. Manikandan, S. Uvarajan, M. Bhaskar, M. Maaza, Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum, J. Photochem. Photobiol. B Biol. 167 (2017) 282–289.
[15] F. Paladini, M. Pollini, Antimicrobial silver nanoparticles for wound healing application: progress and future trends, Materials (Basel) 12 (2019) 2540.
[16] L.G. Wasef, H.M. Shaheen, Y.S. El-Sayed, T.I.A. Shalaby, D.H. Samak, M.E. Abd El-Hack, A. Al-Owaimer, I.M. Saadeldin, A. El-mleeh, H. Ba-Awadh, A.A. Swelum, Effects of silver nanoparticles on burn wound healing in a mouse model, Biol. Trace Elem. Res. (2019).
[17] K.N. Jackson, J.A. Smith, J.N. Edokpayi, New method for the deposition of metallic silver and metallic copper on full-size porous ceramic water filters, Environ. Eng. Sci. 36 (2019) 2–11.
[18] S. Bharti, S. Mukherji, S. Mukherji, Water disinfection using fixed bed reactors packed with silver nanoparticle immobilized glass capillary tubes, Sci. Total Environ. 689 (2019) 991–1000.
[19] S.H. Mirjalili, M.R. Nateghi, F. Kalantari-Fotooh, Preparation of silver nanowire/expanded polytetrafluoroethylene and polypropylene nanocomposites via all solution process method for antibacterial applications, J. Text. Inst. (2019) 1–9.
[20] K. Hasan, M. Pervez, M. Talukder, M. Sultana, S. Mahmud, M. Meraz, V. Bansal, C. Genyang, A novel coloration of polyester fabric through green silver nanoparticles (G-AgNPs@PET), Nanomaterials 9 (2019) 569.
[21] P. Sivaranjana, E. Nagarajan, N. Rajini, N. Ayrilmis, A.V. Rajulu, S. Siengchin, Preparation and characterization studies of modified cellulosic textile fabric composite with in situ-generated AgNPs coating, J. Ind. Text. (2019), 152808371985531.
[22] T.M. Abdelghany, A.M.H. Al-Rajhi, M.A. Al Abboud, M.M. Alawlaqi, A. Ganash Magdah, E.A.M. Helmy, A.S. Mabrouk, Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A Review, Bionanoscience 8 (2018) 5–16.
[23] Z. Vaseghi, A. Nematollahzadeh, O. Tavakoli, Green methods for the synthesis of metal nanoparticles using biogenic reducing agents: a review, Rev. Chem. Eng. 34 (2018) 529–559.
[24] C. Saravanan, R. Rajesh, T. Kaviarasan, K. Muthukumar, D. Kavitake, P.H. Shetty, Synthesis of silver nanoparticles using bacterial exopolysaccharide and its application for degradation of azo-dyes, Biotechnol. Rep. 15 (2017) 33–40.
[25] A. Javaid, S.F. Oloketuyi, M.M. Khan, F. Khan, Diversity of bacterial synthesis of silver nanoparticles, Bionanoscience 8 (2018) 43–59.
[26] A. Shahzad, H. Saeed, M. Iqtedar, S.Z. Hussain, A. Kaleem, R. Abdullah, S. Sharif, S. Naz, F. Saleem, A. Aihetasham, A. Chaudhary, Size-controlled production of silver nanoparticles by Aspergillus fumigatus BTCB10: likely antibacterial and cytotoxic effects, J. Nanomater. 2019 (2019) 1–14.
[27] M. Guilger-Casagrande, R. de Lima, Synthesis of silver nanoparticles mediated by fungi: a review, Front. Bioeng. Biotechnol. 7 (2019) 1–16.
[28] A. Ibrahim Al, I. Mohammed A, Phytochemical screening and antibacterial activity of Eucalyptus camaldulensis's leaves and bark extracts, Asian J. Sci. Res. 12 (2019) 202–210.
[29] Y. Zhou, W. Lin, J. Huang, W. Wang, Y. Gao, L. Lin, Q. Li, L. Lin, M. Du, Biosynthesis of gold nanoparticles by foliar broths: roles of biocompounds and other attributes of the extracts, Nanoscale Res. Lett. 5 (2010) 1351–1359.
[30] I. Alghoraibi, C. Soukkarieh, R. Zein, A. Alahmad, J.-G. Walter, M. Daghestani, Aqueous extract of Eucalyptus camaldulensis leaves as reducing and capping agent in biosynthesis of silver nanoparticles, Inorg. Nano-Metal Chem. (2020) 1–8.
[31] M.J. Sila, M.I. Nyambura, D.A. Abong'o, F.B. Mwaura, E. Iwuoha, Biosynthesis of silver nanoparticles from *Eucalyptus corymbia* leaf extract at optimized conditions, Nano Hybrids Compos. 25 (2019) 32–45.
[32] K. Thakur, I. Bala, Evaluation of effectiveness of biologically synthesized silver nanoparticles of Eucalyptus globules leaf extract against pathogenic and acne-inducing bacteria, J. Nanomed. Nanotechnol. 8 (2017).
[33] S. Paosen, S. Jindapol, R. Soontarach, S.P. Voravuthikunchai, Eucalyptus citriodora leaf extract-mediated biosynthesis of silver nanoparticles: broad antimicrobial spectrum and mechanisms of action against hospital-acquired pathogens, APMIS 127 (2019) 764–778.
[34] M.S. Kiran, V.S. Betageri, C.R.R. Kumar, S.P. Vinay, M.S. Latha, In-Vitro antibacterial, antioxidant and cytotoxic potential of silver nanoparticles synthesized using novel Eucalyptus tereticornis leaves extract, J. Inorg. Organomet. Polym. Mater. (2020).
[35] O.R. Alara, N.H. Abdurahman, O.A. Olalere, Ethanolic extraction of flavonoids, phenolics and antioxidants from Vernonia amygdalina leaf using two-level factorial design, J. King Saud Univ. Sci. 32 (2017) 7–16.
[36] K. Jadhav, S. Deore, D. Dhamecha, R. H R, S. Jagwani, S. Jalalpure, R. Bohara, Phytosynthesis of silver nanoparticles: characterization, biocompatibility studies, and anticancer activity, ACS Biomater. Sci. Eng. 4 (2018) 892–899.
[37] N. Mayedwa, N. Mongwaketsi, S. Khamlich, K. Kaviyarasu, N. Matinise, M. Maaza, Green synthesis of nickel oxide, palladium and palladium oxide synthesized via Aspalathus linearis natural extracts: physical properties & mechanism of formation, Appl. Surf. Sci. 446 (2018) 266–272.
[38] G. Marslin, K. Siram, Q. Maqbool, R.K. Selvakesavan, D. Kruszka, P. Kachlicki, G. Franklin, Secondary metabolites in the green synthesis of metallic nanoparticles, Materials (Basel) 11 (2018) 940.
[39] Z.-R. Mashwani, M.A. Khan, T. Khan, A. Nadhman, Applications of plant terpenoids in the synthesis of colloidal silver nanoparticles, Adv. Colloid Interface Sci. 234 (2016) 132–141.
[40] M. Parlinska-Wojtan, M. Kus-Liskiewicz, J. Depciuch, O. Sadik, Green synthesis and antibacterial effects of aqueous colloidal solutions of silver nanoparticles using camomile terpenoids as a combined reducing and capping agent, Bioproc. Biosyst. Eng. 39 (2016) 1213–1223.
[41] V.V. Makarov, A.J. Love, O.V. Sinitsyna, S.S. Makarova, I.V. Yaminsky, M.E. Taliansky, N.O. Kalinina, "Green" nanotechnologies: synthesis of metal nanoparticles using plants, Acta Naturae 6 (2014) 35–44. http://www.ncbi.nlm.nih.gov/pubmed/24772325.
[42] S.K. Balavandy, K. Shameli, D.R.B.A. Biak, Z.Z. Abidin, Stirring time effect of silver nanoparticles prepared in glutathione mediated by green method, Chem. Cent. J. 8 (2014) 11.
[43] K. Ali, B. Ahmed, S. Dwivedi, Q. Saquib, A.A. Al-Khedhairy, J. Musarrat, Microwave accelerated green synthesis of stable silver nanoparticles with Eucalyptus globulus leaf extract and their antibacterial and antibiofilm activity on clinical isolates, PloS One 10 (2015), e0131178.
[44] M. Bin-Jumah, M. Al-Abdan, G. Albasher, S. Alarifi, Effects of green silver nanoparticles on apoptosis and oxidative stress in normal and cancerous human hepatic cells in vitro, Int. J. Nanomed. 15 (2020) 1537–1548.
[45] Y.-G. Yuan, S. Zhang, J.-Y. Hwang, I.-K. Kong, Silver nanoparticles potentiates cytotoxicity and apoptotic potential of camptothecin in human cervical cancer cells, Oxid. Med. Cell. Longev. 2018 (2018) 1–21.
[46] D. Maity, S. Pattanayak, M.M.R. Mollick, D. Rana, D. Mondal, B. Bhowmick, S.K. Dash, S. Chattopadhyay, B. Das, S. Roy, M. Chakraborty, D. Chattopadhyay, Green one step morphosynthesis of silver nanoparticles and their antibacterial and anticancerous activities, New J. Chem. 40 (2016) 2749–2762.
[47] M. van der Zande, A.K. Undas, E. Kramer, M.P. Monopoli, R.J. Peters, D. Garry, E.C. Antunes Fernandes, P.J. Hendriksen, H.J.P. Marvin, A.A. Peijnenburg, H. Bouwmeester, Different responses of Caco-2 and MCF-7 cells to silver nanoparticles are based on highly similar mechanisms of action, Nanotoxicology 10 (2016) 1431–1441.
[48] H. Bouwmeester, J. Poortman, R.J. Peters, E. Wijma, E. Kramer, S. Makama, K. Puspitaninganindita, H.J.P. Marvin, A.A.C.M. Peijnenburg, P.J.M. Hendriksen, Characterization of translocation of silver nanoparticles and effects on whole-genome gene expression using an in vitro intestinal epithelium coculture model, ACS Nano 5 (2011) 4091–4103.