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Research paper

Biogenic silver nanoparticles synthesized via *Mimusops elengi* fruit extract, a study on antibiofilm, antibacterial, and anticancer activities

Nesrin Korkmaz [a,**], Yusuf Ceylan [b], Attia Hamid [c], Ahmet Karadağ [d], Ali Savaş Bülbül [e], Muhammad Nauman Aftab [c], Özge Çevik [f], Fatih Şen [g,*]

[a] Faculty of Science, Biotechnology Department, Bartın University, 74100, Bartın, Turkey
[b] Faculty of Science, Department of Molecular Biology and Genetics, Bartın University, 74100, Bartın, Turkey
[c] Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan
[d] Faculty of Arts and Sciences, Department of Chemistry, Yozgat Bozok University, 66200, Yozgat, Turkey
[e] Faculty of Science, Biology Department, Kahramanmaraş Sütçü İmam University, 46000, Kahramanmaraş, Turkey
[f] School of Medicine, Department of Biochemistry, Aydın Adnan Menderes University, 09100, Aydın, Turkey
[g] Sen Research Group, Department of Biochemistry, Dumlupınar University, 43000, Kütahya, Turkey

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ABSTRACT

Herein, a simple approach was used for synthesis of silver nanoparticles by *Mimusops elengi* liquid fruit extract. *Mimusops elengi* is an ornamental plant, well-known for its fragrant flowers. The fruit source acts as a reducing mediator. The reduction of silver ions of this work was explored by fruit extract in the solution. The characterization of prepared biogenic silver nanoparticles (AgNPs) was achieved by UV–vis spectroscopy, XRD analyses, and STEM. As a result of characterization, it showed a maximal absorbance in the UV–vis spectrum at a wavelength of ~431 nm. In the XRD analysis, five basic peaks attributed to Ag nanoparticles were observed. When STEM images were analysed, it was determined that Ag nanoparticles generally have a spherical shape and an average size of 43 nm AgNPs were tested antimicrobial activity with *Enterococcus durans*, *Listeria innocua*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae, Salmonella enteritidis, Salmonella kentucky, Staphylococcus epidermidis Staphylococus aureus*, *Xanthomonas*, and *Proteus vulgaris*. AgNPs demonstrated antibacterial activities against all tested Gram (+) and Gram (−) bacteria strains. AgNPs at a low concentration of 625 μg/mL exhibited bacteriocidal effects on some tested bacteria. Further, antibiofilm tests were also performed. In addition, AgNPs inhibited 86.36% of the biofilm layer formed by the *Escherichia coli* bacteria at a concentration of 1250 μg/mL. Last, but not least, anticancer activities were tested using human colon (HT-29) and breast (MCF7) cancer cell lines. AgNPs are one of the most effective IC50 values against HT-29 and MCF7 cancer cell lines, respectively, 155 μg/mL at 24 h and 179 μg/mL at 40 h. The rapid, eco-friendly and non-toxic synthesis of AgNPs obtained by green synthesis rises their potential to become an agent for biological activities such as antibacterial, antibiofilm, and anticancer.

1. Introduction

As a novel and multidisciplinary technology, nanotechnology has paved the way for an unlimited number of developments in several fields that get the aim to form atomic, molecular and supermolecular materials with enhanced features. To detect glucose, DNA and RNA detection, diagnosing diseases and control diseases and micro-electronics, nanoparticles are used in many medicinal fields [1–13]. Diverse approaches are proposed to meet the silver nanoparticle demands. Mostly, physical and chemical methods are very risky and costly [14]. Higher stability, solubility, and more yields have been provided by silver nanoparticles that are produced by biological methods. In addition to the simplicity of these methods, the products are non-hazardous and environment friendly. Microorganisms have been used to fabricate nanoparticles as well, but the degree of synthesis is slow due to the routes used in plant-facilitated synthesis [15]. To assess the synthesized nanoparticles, numerous analytical methods have been utilized, involving X-ray diffractometry (XRD), ultraviolet–visible spectroscopy (UV–vis

* Corresponding author.
** Corresponding author.
E-mail addresses: nkorkmaz@bartin.edu.tr (N. Korkmaz), fatih.sen@dpu.edu.tr (F. Şen).

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spectroscopy), and scanning electron microscopy (SEM) [16]. Silver nanoparticles are extensively used nanoparticles that reveal a broad scale of antibacterial activity. Hence, silver-based molecules have been extensively utilized in medicine to therapy of infections and burns. Their antimicrobial activities were examined by researchers [2–7,17–19]. The antimicrobial prospective of silver has rendered it an essential constituent of health-related products [20]. Related researches revealed the powerful antibacterial activities of silver nanoparticle compounds on both gram-positive and gram-negative bacteria, i.e., multidrug-resistant strains [3]. Its effect as an antifungal agent was also significantly observed [21]. As reported in related studies, silver nanoparticles are used in the treatment of wounds. Injuries such as abrasions, cuts, warts, burns, skin diseases and fungal diseases can also be treated with silver nanoparticles [22]. This biological action and cytotoxicity of silver nanoparticles are determined by considering a series of factors such as surface chemistry, size distribution, morphology of particles, particle structure and particle reaction in solution, efficacy of releasing of ion, cell type, and at least but not the last, the kind of reducing agents involved in some synthesis [23,24]. These physico-chemical properties of nanoparticles improve the biocapacity of therapeutic factors after being equally approved by systemic and local administration [25].

The green synthesis method, which is the favorite synthesis reaction in recent times, and the fact that silver get very essential biological activity compared to other metals inspired present study. In this research paper, we investigated for the first time the biosynthesis of silver nanoparticle compounds by reduction of silver ions using *Mimusops elengi* and analysed the antibacterial activities, biofilm inhibitory effects and anticancer activities. *Mimusops elengi* plant is an inexpensive ornamental plant and can be readily achieved compared to many endemic and rare plants studied in the literature. The rapid and renewable reaction of the AgNPs reaction is a major benefit for biological and medical applications.

2. Materials and methods

2.1. Plant selection and preparation of fruit extract

Mimusops elengi is an ornamental plant, most found in Pakistan. The genus *Mimusops elengi* belongs to the family Sapotaceae and consists of about eight hundred species that are dispersed in the tropical parts of the hemispheres. Among them, *Mimusops elengi*, generally known as Moulsari, is cultivated in country parks of Pakistan due to its fragrant flowers. Its fruit is known as Spanish cherry that has a reddish orange color when it is ripened.

To prepare the extract of *Mimusops elengi*, the fruits were collected from Government College University, Lahore, Pakistan, dried in an oven at 35 °C for 72 h, and crushed with pestle and mortar. 10 g of crushed fruit material was weighed and put into a flask. After the addition of boiled deionized water (300 mL), it was separated by filtration using Whatman filter paper-1 and stored at 4 °C for subsequent utilize.

2.2. Synthesis of silver nanoparticles

0.1 g of silver nitrate was weighed and transferred into 150 mL of deionized water. It was left for a while to dissolve completely. About 50 mL of crude aqueous fruit extracts were added into the solution of silver nitrate; then this beaker was placed on a stirring plate and heated at about 85 °C, 550 rpm. The synthesis method given in the literature was used in present study [5,6]. Silver concentration has been optimized because the reduction capacity of phytochemicals in each plant varies. The decrease of silver was verified by the change of the solution's color into brown. The production process was also followed by UV–visible spectroscopy.

2.3. Characterization of Ag nanoparticles

2.3.1. UV–visible spectroscopy

UV–vis spectroscopy method is an important method for the synthesized nanoparticles and chief characterization of these compounds. Silver nanoparticles have a unique optical property which occurs at specific wavelengths [26]. UV–Visible spectrophotometer analysis was performed by using UV–visible spectrophotometer (UV-Thermo scientific). One millilitre sample was added in a cuvette and consequently analysed at 25 °C. The stability of silver nanoparticles prepared by biochemical procedures was seen for more than 12 months. For UV–Visible spectroscopy, the nanoparticles which are of interest were re-suspended into spectrum scans, and sterile de-ionized water was performed using UV–Vis Spectrophotometer from Thermo scientific.

2.4. Scanning electron microscopy

The morphology, size, and shape of silver nanoparticles were evaluated by using a scanning electron microscope. SEM method is a surface imaging which has a potential of solving different particle sizes, the shape of nanoparticles, surface morphology and size of the synthesized particles at the nano and micro scales [27,28].

2.4.1. X-ray diffraction (XRD)

X-ray diffractometer with CuK radiation of 40 mA (Panalytical, Empyrean Advance) was employed for the analysis of both crystal and molecular structures of biogenic AgNPs. X-ray diffraction (XRD) is a prevalent analytical method that has been utilized, qualitative recognition of different molecules, examining the crystalline degree, particle sizes, isomorphous substitutions and quantitative resolution of chemical species.

2.5. Antimicrobial assay

The synthesized silver nanoparticle compounds were performed for their antibacterial activities against *Enterococcus durans, Listeria innocua, Bacillus subtilis* DSMZ 1971, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae, Salmonella enteritidis* ATCC 13075, *Salmonella kentucky, Staphylococcus epidermidis* DSMZ 20044. The strains were preserved on LB-Agar plates. The overnight culture was refreshed until 0.5 McFarland standard turbidity, and the suspension was then used for minimum inhibition concentration (MIC) assays. For this purpose, a 96-well plate was used. LB broth was added in the wells, and at the same volume, nanoparticles were put into the first well which followed by serial dilution. The MIC value was determined by UV–Visible spectrum. After analysing the values, the samples selected for minimum bacteriostatic/bacteriocidal concentration (MBC) were on LB-Agar plates and incubated at 37 °C for overnight. Next day, the growth pattern was checked to determine whether it's bactericidal or bacteriostatic in nature [29].

2.6. Antibiofilm test

Biofilm inhibition against bacteria *Enterococcus durans, Listeria innocua, Bacillus subtilis* DSMZ 1971, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae, Salmonella enteritidis* ATCC 13075, *Salmonella kentucky, Staphylococcus epidermidis* DSMZ 20044 was analysed by using Ag NPs from *M. elengi* fruit extract. Bacteria strains were grown in LB broth including a small amount of NaCl in order to induce biofilm layer until 0.5 McFarland standard turbidity was reached. Biogenic silver nanoparticles were primarily analysed at 5000 μg/mL and serially diluted twofold to 156.25 μg/mL, and the mixture was incubated at 37 °C for 48 h. All wells were washed and dried at room temperature. 95% methanol was added for 15 min of fixation, and all wells were stained with 0.1% crystal violet. Into the wells containing gram positive and negative bacteria, respectively 33% glacial acetic acid and 95% ethyl alcohol were added, and then all wells were examined with UV

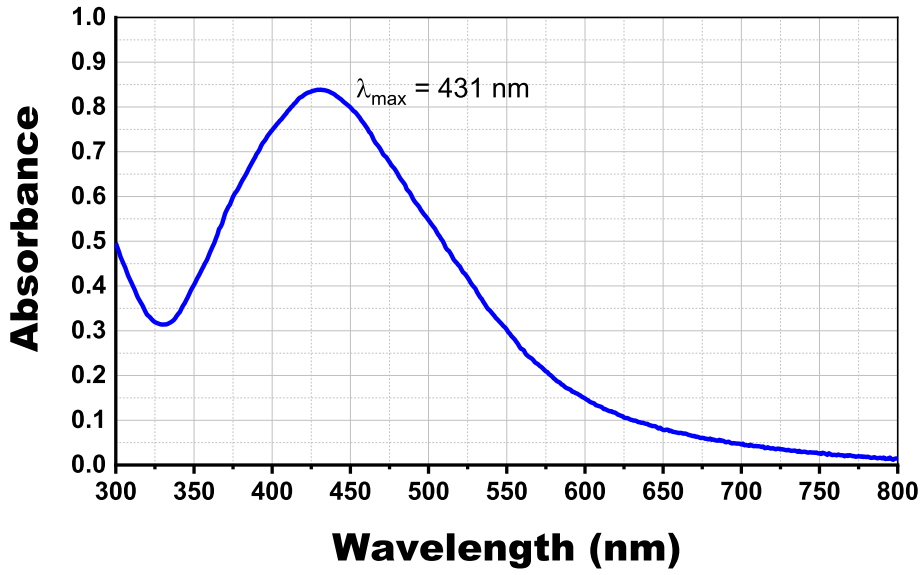

Fig. 1. The UV–Visible absorption spectrum of silver nanoparticles.

spectrophotometer. Results were used to calculate the biofilm inhibition percentage [30].

2.7. Anticancer activity

The anti-cancer activity of AgNPs was tested using human cancer cell lines HT-29 and MCF7. Human breast cancer cell line (MCF7) and colon cancer cell lines (HT-29) were obtained from the American Type Culture Collection (ATCC). Cells were cultured in DMEM containing 10% bovine fetal serum (FBS, F9665, Sigma, USA), 1% glutamine and 1% penicillin/streptomycin (P4333, Sigma) in a 75 cm^2 tissue culture flask at 5% CO_2 in incubator at 37 °C. The cytotoxicity of AgNPs was evaluated by MTT assay using HT-29 and MCF-7 cell lines. AgNPs (0.1, 1, 10, 100, 1000 μg/mL) including DMEM solution was added into 96-well plates and 2×10^4 cells were inoculated into wells. After incubation 24 h, MTT assay applied and OD values were tested using microplate reader (Multiscan, Thermo) at 570 nm.

3. Results and discussion

Advantages such as high efficiency, low cost and short reaction time in the production of nanoparticles have recently increased the interest on nanomaterials [31]. Like in many fields, the use of nanoparticles in modern medicine is increasing day by day. The shape, size, and monodisperity of nanoparticles make them essential for medical applications [22]. The green synthesis method, which is one of the methods used for the nanoparticles synthesis, is the present of nature for its rapid results, non-toxicity and eco-friendly. In this study, *Mimusops elengi* fruit extract was used for the synthesis of biogenic Ag nanoparticles. The change in the color of the biogenic AgNPs prepared indicates the reduction of the

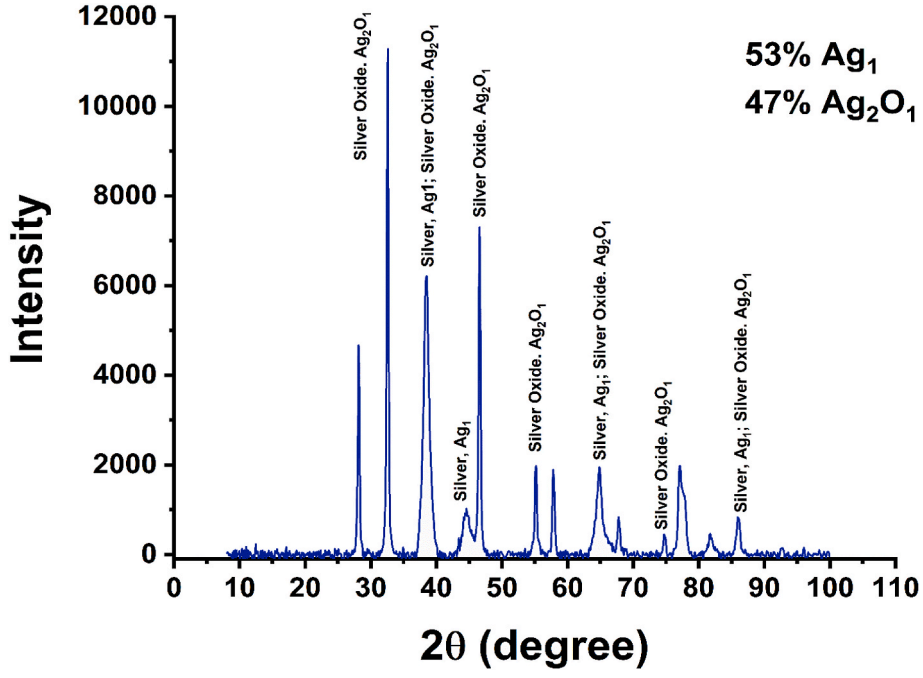

Fig. 2. X-ray diffraction pattern of silver nanoparticles.

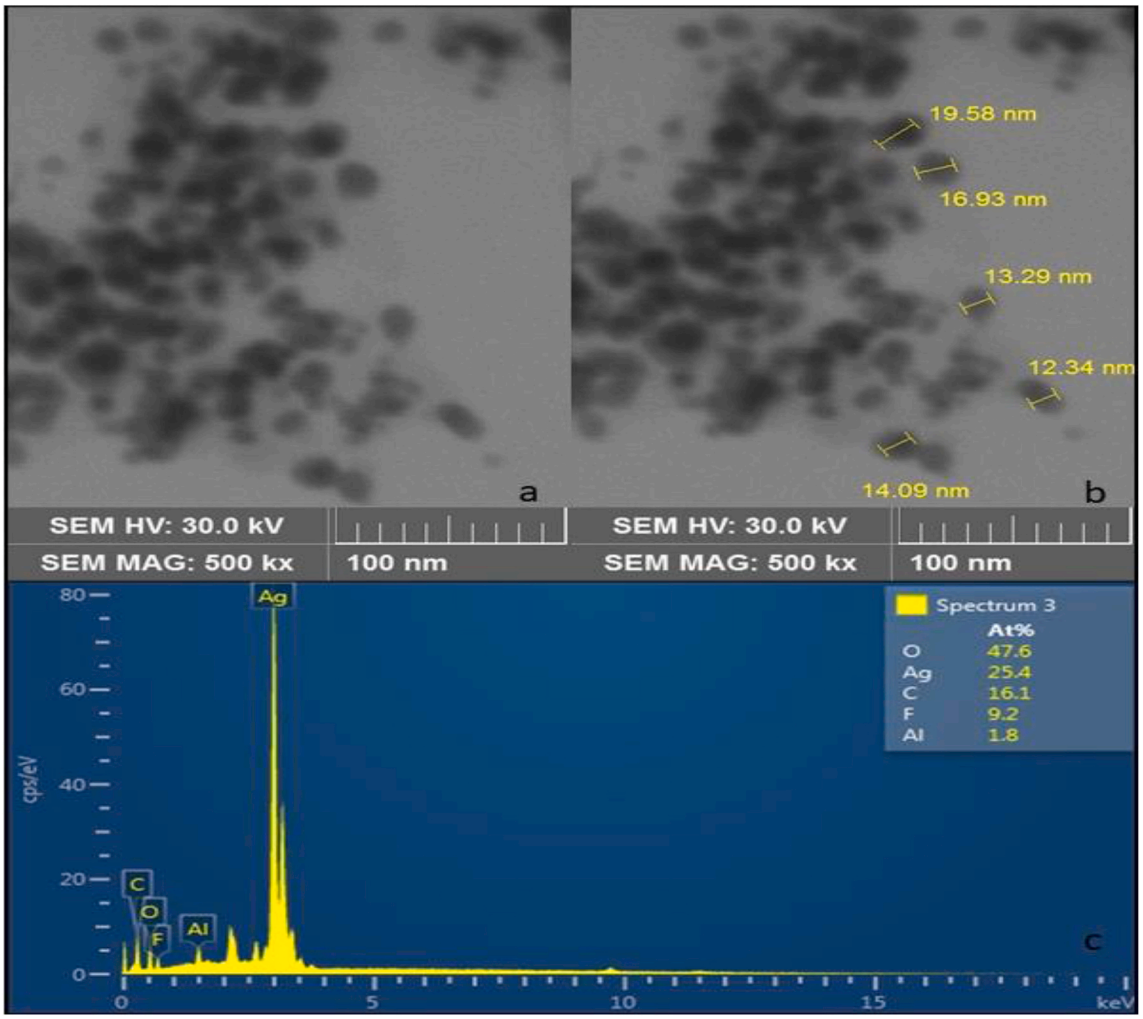

Fig. 3. (**a**,**b**) STEM images of the prepared nanomaterial and (**c**) EDX electron image showing the presence of AgNPs.

silver ion to the silver nanoparticle molecules through interaction with fruit extracts. The physical characterization of the nanoparticles was illuminated by UV–Vis, XRD and STEM analysis.

3.1. UV–visible spectroscopy

Biosynthesis of silver nanoparticle compounds was performed by using fruit extracts of *Mimusops elengi*. Light coloured fruit extracts were added into silver nitrate solution that changed into a dark brown color, as a result of the surface plasmon resonance property of silver. In the UV–visible spectroscopic observations of silver nanoparticles synthesized using *Mimusops elengi* fruit extract, a maximum absorbance was observed at 431 nm (Fig. 1). According to previous papers, it can be said that the spectrum values for Ag nanoparticles vary between 420 and 450 nm depending on the particle size, plant extraction concentration, chemical environment, and dielectric medium [29–35].

3.2. X-ray diffraction (XRD) analysis

XRD analysis was performed to confirm the UV–vis spectral analysis results and determine the crystal structure of silver nanoparticles synthesized by *Mimusops elengi*. The XRD pattern of AgNPs was shown in Fig. 2. Five main peaks at 32.56°, 38.45°, 44.69°, 64.86°, and 81.77° with respective interplanar spacing (d calculated) values of 2.74974, 2.34115, 2.0327772, 1.43748, and 1.17776 Å correspond to planes. The nanoparticles obtained according to X-Ray data are not pure Ag nanoparticles. The obtained nanoparticles consist of 53% pure Ag and 47% Ag_2O. While two peaks at 32.56° and 46.58° relate to Ag_2O, the peaks at 38.45° and 64.86° relate to a mixture of pure Ag and Ag_2O. The silver nanoparticles mean crystalline size calculated using the Scherrer equation. The particle size of the silver nanoparticles synthesized with *Mimusops elengi* extract obtained was approximately calculated as 43 nm. The results are compared to previous studies that are newly introduced to the literature; There are both smaller nanoparticles than the nanoparticles we synthesized [4,5,36] and results close to those obtained from our study [6,7].

Although the nanoparticles are easily synthesized by the green synthesis method, the experiment has been re-established three times for amount of product that can be used in characterization and biological studies since the amount of product obtained is small. The UV absorbance value and X-ray results of AgNPs obtained in each repetition were the same, which is very important for the renewability of the study.

3.3. Scanning transmission electron microscopy (STEM)

The morphology and particle size of the prepared nanoparticles were determined by using STEM. The high-resolution STEM can recognize the morphology of the nanoparticles smaller than 10 nm. The topography, structure and surface morphology of the silver nanoparticles were investigated by scanning electron microscopy that presented a well-defined spherical geometry of the nanoparticles without any agglomeration (Fig. 3). It exposed that the biogenic AgNPs are well spread and

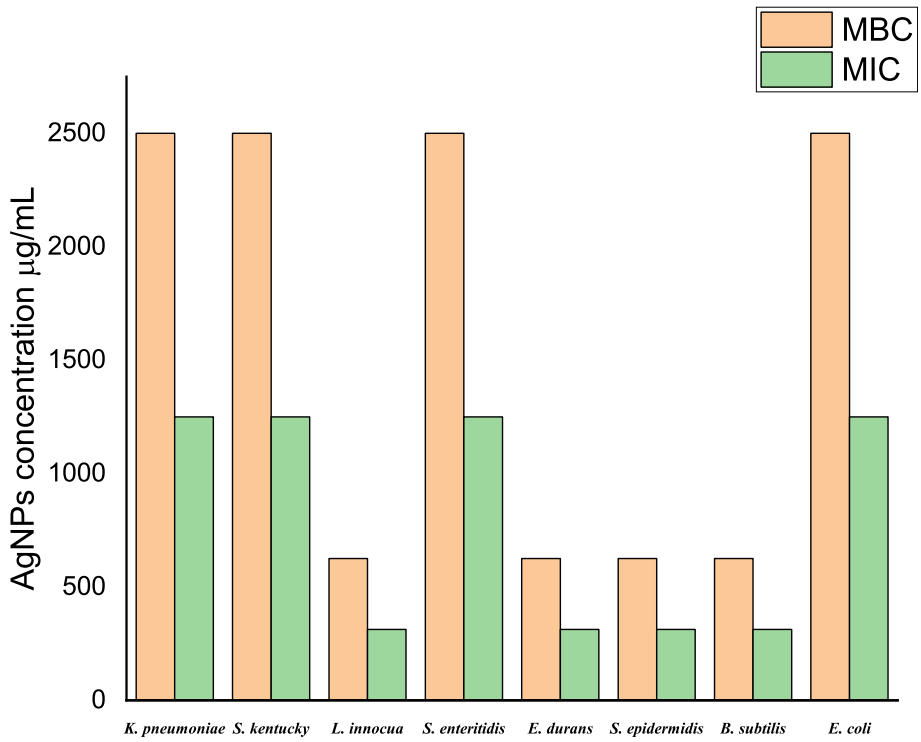

Fig. 4. The MIC and MBC concentration of AgNP derived from *Mimusops elengi* against bacteria.

principally spherical, although some of the nanoparticles were irregular in size and shape; showed the size in the range of 12.34, 13.29, 14.09, 16.93, and 19.58 nm (Fig. 3 a-b). Previously acquired reports are much related to these results [5,15,36]. This showed that plants were efficiently involved in the formation synthesis of silver nanoparticles. In addition to STEM images of AgNPs, EDX electron image showing the presence of Ag nanoparticles was also taken. The presence of Ag nanoparticles was reported with the EDX image (Fig. 3 c).

3.4. Antibacterial activity

The antibacterial activity of Ag NPs synthesized from fruit extract of *M. elengi* displayed maximum activity against gram-positive and gram-negative bacteria at the concentrations of 312.5 µg/mL and 1250 µg/mL, respectively, and also minimum bactericidal concentrations were recorded against gram-positive bacteria at 625 µg/mL concentration and against gram-negative bacteria at 250 µg/mL concentration (Fig. 4). The results show that the effective concentration against gram-negative bacteria is higher than gram-positive bacteria. The difference in effective concentrations against different bacteria groups might be due to the different cell membranes of bacteria. Owing to their antimicrobial properties, Ag NPs have been used extensively in textile coatings, medicine, dye reduction, food storage, antiseptic creams. The antibacterial activity of Ag NPs depends entirely on their applied concentration. Previous study reported that a higher level of Ag NPs showed more inhibitory activity on microbial growth [6]. Also, others examined the antimicrobial effect of nanoparticles towards some bacteria like *S. aureus, E. coli, K. pneumonia* [26]. Due to physiochemical configuration of Ag NPs, they mainly get influence against bacteria by damaging intracellular biomolecules, attaching cell wall and membrane. Compared to the Ag NPs concentration in previous study, the concentration of the Ag NPs used in present study had an impact against *E. durans, B. subtilis* and *S. kentucky* bacteria at lower concentration. Whereas the Ag NPs did not demonstrate any effect against *S. epidermis* that was affected at low concentration in current study [14]. Silver ions, considered Lewis acid, readily react and disrupt their structure with sulfur and phosphorus-containing biomolecules such as cell membrane, proteins and DNA [37].

Table 1
Antibiofilm percentage of AgNPs (µg/mL) obtained from *Mimusops elengi.*

	5000	2500	1250	625	312.5
L. innocua	29.7	57.92	0	0	0
E. durans	72.87	79.52	3.45	11,96	12.5
S. epidermidis	38.59	33.33	42.1	0	0
B. subtilis	52.51	58.65	8.93	0	0
K. pneumoniae	72.91	80.4	62,69	0	0
S. kentucky	79.52	81.21	71.57	68.86	24.02
S. enteriditis	34.22	46.3	0	0	0
E. coli	71.91	83.44	86.36	0	0

Antimicrobial effects of Ag NPs have been observed in different studies. These reports exhibit several values for the MIC of the particles on the tested bacteria. In most of the reports, the MIC values were lower or higher than the MIC values of our results.

3.5. Biofilm inhibition

Biofilms are layers that form by the accumulation of substances such as polysaccharides, nucleic acids, carbohydrates, proteins bound to living or non-living surfaces. Extreme problems caused by biofilm layer threaten human health and environment in many areas like food industry, wastewater treatment plant, water supply network [7]. According to our results, biosynthesized Ag NPs demolish all biofilm layers at low concentrations (Table 1). The highest biofilm inhibition percentage of 86.36% was achieved with a concentration of 1250 µg/mL against *E. coli* strain. Previous study reported that the inhibition of *E. coli* biofilm formation was obtained with a concentration of 10 mM at the highest percentage [17]. The biofilm formations of *E. durans* and *S. kentucky* were inhibited by biosynthesized Ag NPs at all concentrations. At high percentage, biofilm formation was inhibited by eco-friendly Ag NPs that were synthesized from *M. elengi* which are not harmful to human health and environment like chemicals.

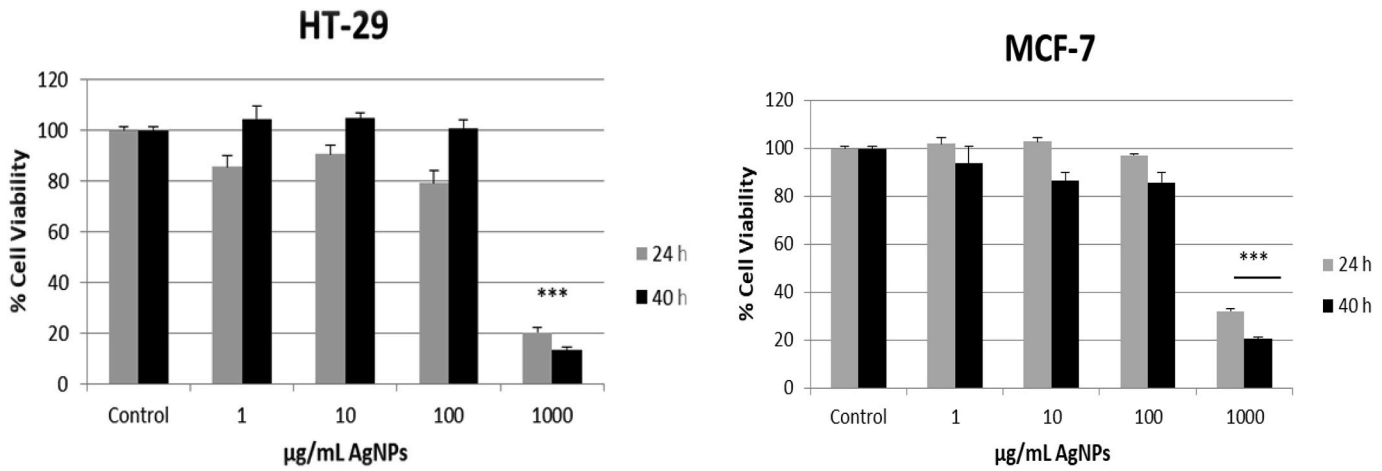

Fig. 5. HT-29 and MCF7 cell viability assay.

3.6. Anticancer activity

After administration of the AgNPs, cells were incubated for cytotoxic activity at 24 and 40 h by MTT assay. Both MCF-7 and HT-29 cells exhibited dose and time-dependent of AgNPs cytotoxicity. The value of inhibitory concentration IC50 of AgNPs was found to be 155 µg/mL at 24 h and 266 µg/mL at 40 h in HT-29 cells. However, the value to IC50 was found to be 206 µg/mL at 24 h and 179 µg/mL at 40 h in MCF-7 cells. As expected, increased nanoparticle concentrations reduced the viability of the cells (Fig. 5). Related to AgNPs similar results have been previously reported by other researchers through experimental studies [38–40]. Several studies reported that AgNPs were shown in different mechanism of cytotoxicity, internalization into cancer cells, a cascade of processes starts with loss of inner homeostasis and redox state destabilization. Ag^{+} produce by AgNPs oxidation to delivery into the cytoplasm and membranes of cells and resulting in cell destruction. Oxidative stress induces apoptosis, DNA damage and cell proliferation in cancer cells via exposure to nanoparticles [41]. A series of free radical waves damages mitochondrial and nuclear membranes and propagates oxidative stress. Additionally, in S-phase (DNA replication) of the cell cycle, damaged DNA is not repaired effectively because repair enzymes are blocked by Ag + ions and replication stops. Use of Ag NPs as good pro-apoptotic agents in cancer therapy seems to be reasonable. Toxicity of Ag NPs is shown through the intrinsic ROS-mediated mitochondrial apoptotic pathway. Any of these disruptions have been described as cytotoxic effects of Ag NPs of different origins; however, the most desirable one is the lethal apoptotic effect on cancer cells.

4. Conclusions

Nowadays chemicals and synthetic drugs have been subject to intense debate due to their effects on human health and environment. That's why scientists search for new products and new methods that do not have any adverse effects on human health and the environment. The physical characterization of the silver nanoparticles, at 431 nm UV-absorbance showed the maximum absorbance peak for Ag NPs. As a result of X-ray analysis, the average particle size was calculated 43 nm according to the Scherrer equation. It was found that the shape and size of nanoparticles demonstrate homogeneous distribution in STEM analysis. It was observed that metal nanoparticles produced from plants are more active and stable as compared to those produced from microorganisms. Ag NPs obtained by green synthesis inhibited the bacterial growth at a very low concentration, such as 0.3375 mg/mL. In addition, although there are few studies in the literature about biofilm inhibition of Ag NPs, in current study found that the biofilm inhibition of *E. coli* was 86.36% at a concentration of 0.125 mg/mL. According to our results, biosynthesized nanoparticles might be one of the best and most important means to fight with antibiotic-resistant microorganisms. Also, it has been observed that dose-dependent Ag NPs applications reduce the growth of MCF-7 and HT-29 cancer cells. It indicates that biogenic silver nanoparticles can be further analysed in another biomedical usage.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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