Green synthesis of silver nanoparticles from aqueous leaf extract of Pomegranate (Punica granatum) and their anticancer activity on human cervical cancer cells

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Received 13 November 2017
Accepted for publication 8 March 2018
Published 6 June 2018

Abstract
Plants contain different important phytochemicals that can be used as a potential treatment for various ailments including cancer. The green synthesis of silver nanoparticles from the extract of different plant parts has gained a wide range of engrossment among the researchers due to its unique optical and structural property. The aim of this study is green synthesis of silver nanoparticles from the aqueous leaf extract of pomegranate (Punica granatum) and to investigate its anticancer activity on human cervical cancer cells (HeLa). The synthesis of silver nanoparticle was witnessed by the colour change from golden yellowish to dark brownish, UV-visible spectral analysis gave a characteristic surface plasmon absorption peak at 420 nm. Further morphological characterization was done by Zeta potential where the size analysis was depicted to be 46.1 nm and zeta potential as $-67.2\text{mV}$. Fourier transform infrared spectroscopy (FTIR) inferred 3 intense sharp peaks at 3393 cm$^{-1}$, 1638 cm$^{-1}$, 667 cm$^{-1}$, confirmed the presence of flavonoids and polyphenols. The scanning electron microscopy (SEM) analysis with energy diffraction spectroscopy (EDS) confirmed the presence of silver nanoparticles with size ranged from 41.69 nm to 69.61 nm. X-ray diffraction (XRD) confirmed the crystallographic nature of silver. The cell proliferation activity of nanoparticles was tested by 3, 4, 5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay where the $50\%$ inhibitory concentration ($IC_{50}$) was found at $100\mu\text{g ml}^{-1}$ inhibiting $50\%$ of HeLa cell line. The anticancer activity of nanoparticles was determined by lactate dehydrogenase (LDH) assay where $100\mu\text{g ml}^{-1}$ showed $50\%$ of cytotoxicity. Furthermore, the anticancer property of nanoparticles was confirmed by the DNA fragmentation assay.

Keywords: silver nanoparticles, aqueous leaf extract pomegranate, human cervical cancer cells

Classification numbers: 2.00, 2.05, 4.02, 5.00
Mechanism and also it acts as a medium to detect and diagnose body disorders [5].

Green synthesis of nanoparticle is an important methodology that has been used in the synthesis of metallic nanoparticles, being an eco-friendly method (less toxic to human and environment), using different parts of any selected plants (having medicinal effect) [6]. The pomegranate plant (Punica granatum) is considered an important traditional source to treat perilous ailments due to its high content of various important phytochemicals [7]. These important phytochemicals make pomegranate as a rich source of antioxidant. The phytochemical analysis of pomegranate leaves has interpreted the presence of flavones, luteolin, glycosides, alkaloids, organic acids and tannins. There are strong evidences from several research articles and papers regarding the anticancer properties of tannins, flavones, alkaloids. The pomegranate leaf extract contains important polyphenols like gallotannins, ellagitannins, punicalagin and punicalin which is considered as important phytochemicals for the treatment of cancer [8]. So, we hypothesize that aqueous leaf extract of pomegranate contains rich amount of phytochemicals can aid the rate of inhibiting the abnormal cell proliferation.

The phytochemicals being released in the body might not have a good efficacy towards the targeting of the cancer cells due to its low diffusion characteristics and failure of overcoming the anatomical barriers [9]. Since the structure and nanosize of the silver particle makes it successful in easy diffusion and overcoming the barriers [10], it can be assumed that the green synthesis silver of nanoparticle from the pomegranate leaf can target the cancer cells and have successful anticancer property. Researches have been done on the synthesis of silver nanoparticles from pomegranate peel, seed, flower, husk, juice [11]. However, little or no research is performed in synthesis of nanoparticles from pomegranate leaf extract. Therefore, the present research focuses on the green synthesis of silver nanoparticles from pomegranate aqueous leaf extract. Cancer is one of the deadly diseases, which resulted in the death of millions of people worldwide. Cervical cancer generally arises from the cervix and is caused by Human Papilloma Virus. Cervical cancer is the fourth common cause of cancer and eventually death in women. This cancer results in the formation of warts around the genital areas [12]. However it is preventable and curable if the proper treatment is given and detected at an early stage. In this study the green synthesized nanoparticles were tested on cell cytotoxicity and apoptosis (inducing nuclear fragmentation) on HeLa cell lines. The unique morphological characteristics of silver nanoparticles may overcome barrier and reach the target cells.

2. Materials and methods

2.1. Materials

Silver nitrate procured from Sisco Research Laboratories Chemicals of Analytical Grade. All other chemicals and reagents required for the experimental assay were of Analytical Grade. DMEM medium, MTT, DMSO, Tris base, EDTA, NADH, Sodium pyruvate, RNase, Ethanol, Agarose, EiBr, DNA ladder were purchased from Himedia. The human cervical cancer cells (HeLa cells) were purchased from National Centre for Cell Science (Pune, India).

2.2. Sample preparation

Pomegranate leaves were collected from SRM University campus, Kattankulathur, Chennai. The leaves were dried under shade for 5 d and were subjected to grinding in mixer grinder. Fine powder was obtained and stored in container for future use.

2.3. Preparation of silver nanoparticles

Briefly 2 g of pomegranate leaf powder was treated with 30 ml of distilled water in 100 ml beaker and boiled at 60°C for 10min. Subsequently, leaf extract was cooled and filtered through whatman filter paper and stored at 4°C. The silver nitrate concentration was maintained at 1 mM. 5 ml of leaf extract was added to 95 ml of silver nitrate to make up the volume to 100 ml and kept for incubation at different time intervals in the dark at room temperature.

2.4. Characterization of silver nanoparticles

2.4.1. UV-visible spectral analysis. The UV-Visible spectral analysis for silver nanoparticle was performed according to [13]. The spectral analysis was tested using UV3600—Shimadzu UV-vis-NIR Spectrophotometer. Briefly 3 ml of the reaction mixture was taken in the quartz cuvette for spectral analysis. The spectroscopic readings were taken at different time intervals of incubation (1, 2, 3, 4, 5 and 6h). Spectral analysis was done in the range of wavelengths from 300 nm to 700 nm.

2.4.2. Zeta potential analysis. To study the particle size based on the refractive index and to check the stability of the silver nanoparticles that has been synthesized from the aqueous leaf extract of pomegranate. The Zeta potential analysis for silver nanoparticle was tested according to the method of [14]. The reaction mixture was collected in 15 ml centrifuge tube and centrifuged at 10,000rpm for 20 min. Washing step was repeated for 3 times with deionized water after vortexing and sonicating every time. Finally, the supernatant was discarded and the pellet was stored in 4°C for future use. The pellet was re-suspended in deionized water and subjected for zeta potential analysis.

2.4.3. Fourier transforms infrared spectroscopy (FTIR). The FTIR analysis is done in order to capture the obtained infrared spectrum. The analysis is done mainly to record the high resolution spectral data. The FTIR analysis was performed according to the method of [14] using IRTracer-100 Fourier Transform Infrared Spectrophotometer. The reduced silver solution was centrifuged at 10,000 rpm for 20 min and the pellet was washed three times with deionized water. The obtained pellet was finally re-suspended with 1 ml of deionized water,
ground with KBr pellets and was subjected to FTIR analysis. A drop of the reaction mixture was used for the FTIR measurement.

2.4.4. Scanning electron microscopy (SEM). The SEM analysis was performed according to the method of [15]. SEM analysis was carried out further with Fusing Ultra 55 Model-II Carl Zeiss SEM to study the morphology, size, composition and the distribution of nanoparticles. A small drop of silver nanoparticles was placed on carbon coated copper grid and allowed to dry by using the mercury lamp for 5 min. Then readings were taken at a magnification of 10000×, 25000×, 50000× and 75000× with steady voltage.

2.4.5. Energy diffraction spectroscopy (EDS). Energy Diffraction Spectroscopy was carried out with INCA energy EDS system where the emission of x-rays from the nanoparticle was collected. This analysis is mainly done according to [15] for the elemental analysis and characterization of the chemicals in the sample. The x-ray detector detects the rays being emitted from the nanoparticles representing as peaks at particular electron volt hence confirming presence of element silver.

2.4.6. X-ray diffraction. X-ray diffraction study is a rapid diagnostic system which is utilized as an evidence for the presence of crystalline structure and can give a data on unit cell measurements. The material to be analyzed is finely ground, homogenized, and normal mass piece along with bulk composition was retrieved according to the method of [15]. The synthesized silver nanoparticles were dried in the form of the pellet and a tiny nanoparticle film was placed on the XRD grid for the analysis.

2.5. Anticancer activity of silver nanoparticles on human cervical cancer cells (HeLa cells)

2.5.1. MTT cell viability assay. The MTT assay was performed according to the method of [16]. Briefly, 5000 HeLa cells per well were seeded in 96 well plate. After seeding the cells, the sample (silver nanoparticles) was treated with different concentrations (50, 100, 150, 200 and 250 µg ml⁻¹) and the plate was incubated for 24 h at 37 °C in CO₂ incubator. After incubation, the reaction mixture was discarded and 200 µl of fresh media was added to each well and OD was measured at 570 nm.

2.5.2. Lactate dehydrogenase (LDH) cell cytotoxicity assay. The LDH cell cytotoxicity assay was carried according to the method of [17] with little modification. Briefly, 2 × 10⁵ HeLa cells per well were seeded in 6 well plate and incubated in CO₂ incubator until the cells reach confluent growth. Then, the cells were treated with quercetin (positive control) and different concentrations (50, 100, 150, 200 and 250 µg ml⁻¹) of silver nanoparticles and plate was incubated for 24 h at 37 °C in CO₂ incubator. After incubation 50µl of supernatant was taken from each well and treated with 2 ml of Tris EDTA-NADH buffer and incubated at 37 °C for 30 min. Then, the reaction mixture was treated with 200 µl of freshly prepared sodium pyruvate and OD was measured at 340 nm for every 15 s for 3 min.

2.5.3. DNA fragmentation assay. The DNA fragmentation assay was carried out based on the method of [18] with slight modification. After silver nanoparticles treatment, cells were collected and washed with PBS at 4 °C. Subsequently, cells were centrifuged at 4500 rpm for 3 min. The obtained pellet was suspended with DNA lysis buffer and incubated on ice for 60 min. After incubation 20 µl of RNase (20 mg ml⁻¹) was added and incubated for 1 h at 37 °C. Then, 20 µl of Proteinase K (20 mg ml⁻¹) was added. Follows, the cell suspension were centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatant was aspirated and transferred to sterile micro centrifuge tube. Subsequently, the DNA was precipitated with addition of 0.15 M NaCl and ice cold absolute ethanol. Further, the precipitated DNA was washed with 70% ethanol and air dried for 20 min and eventually dissolved with sterile distilled water. The quantity of isolated DNA was measured by spectrophotometer. The DNA of the sample (100 µg ml⁻¹), control and DNA ladder were electrophoresed on an ethidium bromide containing agarose gel (1.5%). The gel was visualized and photographed in a gel documentation system.

2.5.4. Statistical analysis. The statistical analysis was performed using one-way analysis of variance (ANOVA) with Graph Pad Prism7 software. The obtained data were represented as mean ± standard error of the mean (SEM). In all graphs, P values less than 0.05 was statistically significant between control and sample.

3. Results

3.1. Synthesis of silver nanoparticles

The aqueous leaf extract of pomegranate was added with silver nitrate. After incubation, the reaction mixture changed its colour from golden yellowish to dark brownish as depicted in figure 1. The colour change occurs due to the presence of the reducing agent which reduces the silver salts into silver ions.

3.2. Characterization of silver nanoparticles

3.2.1. UV-spectrum analysis. The silver nanoparticles (Ag NPs) were incubated at different time interval (1, 2, 3, 4, 5, 6 h) before testing with UV-vis spectroscopy. The spectral analysis exhibited strong absorbance at 420 nm after 1 h incubation. This property is specific for presence of silver nanoparticles. The spectral band range between 400 nm and 450 nm is the evidence for the presence of surface plasmon resonance (SPR) of Ag NPs. However, the intensity of the absorbance was increased with time dependent manner as depicted in figure 2.
3.2.2. Dynamic light scattering assay. The size of the Ag NPs synthesized from aqueous leaf extract of pomegranate was determined by dynamic light scattering measurement technique. The size of the Ag NPs is 46.1 nm as depicted in figure 3.

3.2.3. Zeta potential. The zeta potential of Ag NPs synthesized from aqueous leaf extract of pomegranate is $-67.2$ mV as depicted in figure 4. This range of value being high provides the confirmation regarding the repulsion among the nanoparticles and increases the stability of the formulation.

3.2.4. Fourier transform infrared spectroscopy analysis. FTIR analysis determines the presence of important chemical groups in the silver nanoparticles. The obtained spectroscopic data indicated the bonding of Ag NPs with a functional group of leaf extract through abridging linkage. The IR spectrum of Ag NPs synthesized from aqueous leaf extract of pomegranate is depicted in figure 5. The IR spectrum exhibited the strong peaks at 667 cm$^{-1}$, 1638 cm$^{-1}$ and 3339 cm$^{-1}$ which correspond to presence of alkenes, carbonyl groups and phenolic compounds, respectively. Hence, the obtained results shows that presence of phenolic compounds in the pomegranate leaf extract may involved in the reduction and stabilization of nanoparticles.

3.2.5. Scanning electron microscopy (SEM). The SEM analysis was performed at magnification of 10,000×, 25,000×, 50,000× and 70,000× respectively as depicted in figure 6. The size of the Ag NPs synthesized from the aqueous leaf extract of pomegranate was within the range of 40 – 70 nm. The obtained results showing that capping of the bioactive compounds from the aqueous leaf extract of pomegranate on the Ag NPs, which is indicated by the formation of clusters at several points.

3.2.6. Energy diffraction spectroscopy (EDS). The EDS analysis of the synthesized Ag NPs from aqueous leaf extract of pomegranate confirmed the presence of silver element in the sample. The peaks around 3.2 keV correspond to the binding energies of AgL with atomic weight percentage as 0.55. The result showing the peaks as depicted in figure 7 for the presence of silver element.
3.2.7 X-ray diffraction (XRD). The XRD pattern of Ag NPs synthesized from aqueous leaf extract of pomegranate was determined by reducing AgNO₃ to Ag. The XRD investigation indicated six diverse diffraction tops at 2θ estimations of 27.48°, 32.02°, 38.26°, 46.04°, 57.38° and 59.61° which recorded the planes (111), (333), (200), (331), (222) and (220) respectively as depicted in figure 8 which is carried out with the calculation of particle size with the help of Debye–Scherer equation [19]. Hence the Ag NPs were found to be monodispersed and crystalline in nature.

3.3. Anticancer property

3.3.1 MTT cell viability assay. The effect of Ag NPs synthesized from aqueous leaf extract of pomegranate on viability of human cervical cancer cells were determined using MTT
assay. The result showed as depicted in figure 9, the viability of human cervical cancer cells were significantly decreased by Ag NPs in a dose-dependent manner. Since, the Ag NPs effectively inhibits the growth potential of HeLa cells, 50, 100, 150, 200 and 250 µg ml⁻¹ concentration was taken for further analysis.

3.3.2. Lactate dehydrogenase (LDH) cell cytotoxicity assay. To investigate the cytotoxic effect of silver nanoparticles synthesized from aqueous leaf extract of pomegranate on human cervical cancer cells was determined by LDH cytotoxicity assay. The LDH is present in all normal mammalian cells but, the LDH is released from the damaged or necrotic cells. Hence, the estimation of amount of LDH release can facilitate to investigate the toxic effect of the chemicals or nanoparticles. The morphological evidence of the silver nanoparticles treated HeLa cells were depicted in figure 10 and indicates that Ag NPs treatment inhibited cell proliferation and induced cell senescence in human cervical cancer cells in a concentration dependent manner.

3.3.3. DNA fragmentation assay. The DNA fragmentation assay was performed to investigate the effect of Ag NPs synthesized from aqueous leaf extract of pomegranate on the induction of apoptosis in human cervical cancer cells. To determine the influence of Ag NPs on DNA damage, the Human cervical cancer cells were treated with effective concentration of sample 100 µg ml⁻¹ (This effective concentration was selected based on the MTT cell viability assay) for 24 h. Follows, DNA was isolated from treated and control cells and electrophoresed on agarose gel (1.5%).

The result showed that Ag NPs induced apoptosis by cleaving nuclear DNA of human cervical cancer cells by forming ladder pattern as depicted in the figure 11. The control cells (untreated) have not shown any fragmented DNA.

4. Discussion

4.1. Characterization of Ag NPs

The aqueous extract of pomegranate leaf was added to the silver nitrate solution for the synthesis of Ag NPs. The colour change from golden yellow to dark brown took place within 10 min depicting the silver nitrate salt got reduced to silver as represented in figure 1. The pomegranate leaf extract is depicted to contain important phytochemicals such as flavones, tannins, and other polyphenols which act as a reducing agent to give the reduced silver ions from silver nitrate. The obtained colour change confirming the synthesis of Ag NPs was compared to the similar type of colour inference was observed during the synthesis of Ag NPs from Ocimum sanctum, Moringa oleifera and Carica papaya leaf extract [20–22]. There was a colour change from light brown or yellowish to dark brown which is depicted that the silver salt got reduced to silver. A similar colour change was also reported when silver nitrate got reduced by aqueous neem leaf extract and walnut leaf extract [23]. The process of oxidation and the reduction reaction is responsible for the colour change, which is the indicator for Ag NPs synthesis.

The UV-vis spectral analysis represented in figure 2 determines the surface plasmon resonance, where sharp broad peaks were obtained at 420 nm. This particular analysis was confirmed and justified when similar type of absorption spectra was observed within the range of 400 – 430 nm, which is at higher energy as that obtained by with aqueous extract of neem leaf [24]. The similar type of spectral analysis was done during synthesis of Ag NPs using aqueous olive leaf extract [25]. The spectral analysis done for the synthesized nanoparticles with aqueous leaf extract of Saccharina japonica was in the same range that is within the range of 400 – 450 nm. The peak occurring in this range confirms the synthesis and the presence of Ag NPs [26].

For the determination of the particle size and the stability of Ag NPs, the zeta potential analysis was done as shown in figures 3 and 4 the structural characteristic of nanoparticles get altered due to a number of factors including incubation time, temperature, pH and the method used for nanoparticle preparation. Water was used as a dispersant for the zeta potential analysis of synthesized silver nanoparticles. The zeta potential value can either be negative or positive. The negative potential value depicted from the synthesized Ag NPs due to the entrapment of the bioactive compound present in the extract. The similar type of analysis was made during the synthesis of Ag NPs using aqueous extract of Urtica dioica [27]. The aqueous Pedaliun murex leaf extract used for the synthesis of Ag NPs had shown the similar type analysis [28].

The obtained result of FTIR confirmed that the aqueous leaf extract of pomegranate is potential in performing the role of reducing and stabilizing the silver nanoparticles. Three peaks were obtained as shown in figure 5. The broad peak appearing at 3339 cm⁻¹ is indicated for O – H functional group determining the presence of phenolic groups. The stalwart sharp broad peak at 1638 cm⁻¹ corresponds to C = O stretching vibration. The band at 667 cm⁻¹ indicates C – Cl alkyl halide type of vibration stretch. A similar type of FTIR peaks were
observed during the analysis of chemical composition of Ag NPs synthesized using *Saccharina japonica* leaf extract [26]. The *Memecylon edule* aqueous leaf extract used for the Ag NPs synthesis had shown similar type of peaks [29]. A similar output of FTIR peaks were observed during the synthesis of Ag NPs using aqueous neem leaf extract [24]. These bands infers the presence of polyphenols and flavonoids. Similar types of peaks were observed during the presence of flavonoid and polyphenol compounds in *Viscum album* and *Allium sativum* leaf extracts [30].

The hydrogen bonding along with the electrostatic interactions between synthesized Ag NPs and bio-organic topping atom as depicted in figures 6–9 showing the free scattering of Ag NPs. The Ag NPs being in the range of 40 – 66 nm confirms the characteristic size of the nanoparticles. The similar type of analysis was made during synthesis and characterization of Ag NPs using *Cannonball* leaves and their cytotoxic activity against MCF-7 cell line where the size of the synthesized nanoparticles where in the similar range which is justified to the obtained SEM analysis result of Ag NPs synthesized from pomegranate leaf extract [31]. The same type of SEM analysis is done during the green synthesis of Ag NPs from pomegranate peel extract. Upon SEM analysis the particle size obtained for the synthesized Ag NPs were in the range of 40 – 80 nm [32]. So the nanoparticle which comes in the range of 0 – 100 nm is considered to be the silver nanoparticle.

The EDS is used to analyze the presence of the elemental silver in the given sample, which is a confirmatory study. The obtained peak at certain electron volt showing the chemical and elemental characterization as shown in figure 10. The similar type of elemental analysis was made during the synthesis of Ag NPs from neem leaf (*Azadirachta indica*) extract [33]. The aqueous extract of neem leaves was used for the synthesis of Ag NPs, and upon synthesizing the Ag NPs the dried pellet was subjected to EDS study, where the presence of elemental silver in the range of 0 – 100 nm is considered to be the silver nanoparticle.
silver was shown. The presence of silver element was justified in the Ag NPs synthesis from pomegranate peel and pomegranate juice extract [34, 35]. Hence, the confirmation for the presence of silver was determined on performing this diffraction analysis, the peaks were observed at 3 – 3.5 keV.

XRD study is performed to determine the nature and structure of particles. The crystalline shape and nature of Ag NPs is depicted in figure 11. Similar study was done in characterization of Ag NPs by green synthesis method using pomegranate peel extract [34]. The same type of study was confirmed the crystallographic nature of Ag NPs synthesized from pomegranate juice extract [35]. Similar type of result was obtained in the XRD study of Ag NPs synthesized from the leaf extract of *Catharanthus roseus* which confirms that the Ag NPs which have been synthesized are crystalline in structure. These results report that green synthesis Ag NPs exhibits crystallographic nature [36].

### 4.2. Anticancer property

The silver nanoparticle has the capacity of reducing the cell viability percentage of HeLa cell line which is represented in figure 12. With increase in Ag NPs concentration the cell viability gradually decreased. Similar types of results were reported using different types of mammalian cell lines with Ag NPs [37]. The similar type of dose response curve was observed during the treatment of the Hep-2 cells with the Ag NPs. The concentration of Ag NPs was treated in the range of 15 – 1000 μg ml⁻¹. Hence, cell viability decrease for Ag NPs in dose dependent manner [38]. In another study, reported that cell viability of Hep-G2, PC3 and Vero cells were decreased with increasing the concentration of four biosynthesized nanoparticles [42] the obtained result is similar to our investigation. The treatment of Hep-G2 cells with 4.7 μg ml⁻¹ Ag NPs for 24 h and 48 h decreased cell viability to 61% and 37% respectively [43]. The reported result is dissimilar with our result, the reason may be the source and cell line used.

The dose response curve as depicted in figure 13 shows that the amount of LDH is increased upon increasing the dose concentration of silver nanoparticles between 50 μg ml⁻¹ and 250 μg ml⁻¹. This result confirms that Ag NPs have a cytotoxic effect on this HeLa cell line releasing lactate dehydrogenase in the media. The similar type of response curve and cytotoxic effects of Ag NPs was reported on rainbow trout cell lines and primary hepatocytes [39]. A significant validation of LDH assay was made during the assessment of the toxic effect of Ag NPs on rat lung epithelial Type-I cell line R3/1 which is similar to the obtained response curve [40].

The apoptosis assay (DNA fragmentation assay) the pattern of DNA bands are shown in figure 14. The fragmented DNA was observed in the third lane which is the signature of apoptosis. This result reports that Ag NPs synthesized from pomegranate leaf extract induce apoptosis in human cervical cancer cells. The similar type of banding pattern is observed in the investigation of the toxic effect of Ag NPs synthesized from *Ganoderma neoijaponicum* against the human breast cell lines [41].

### 5. Conclusion

The Ag NPs were synthesized using the aqueous leaf extract of pomegranate. The synthesized Ag NPs have the potential to reduce the silver nitrate into silver which is shown by the colour change from golden yellow to dark brown. Furthermore, the morphological characterization was performed by UV-vis spectral analysis showing surface plasmon absorption’s shift towards higher wavelength with increase in reaction time and the peak was obtained at the wavelength of 420 nm. The size of the Ag NPs was 46.1 nm, which is within the characteristic standard size of the nanoparticle (0 – 100 nm). The zeta potential of the Ag NPs –67.2 mV confirming the repulsion among the particles and the stability of the formulation. The FTIR analysis confirmed the presence of phenolic compounds and flavonoids. In addition, presence of the silver in the synthesized nanoparticles from aqueous leaf extract of pomegranate was confirmed with EDS and SEM analysis showed that bioactive compounds present in the aqueous leaf extract of pomegranate was capping with Ag NPs size ranging from 41.69 nm to 69.61 nm. The XRD studies have shown that Ag NPs were in crystallographic structure. The MTT cell viability assay result showed that Ag NPs inhibited the human cervical cancer cells growth in dose dependent manner and the LDH cell cytotoxicity assay showed that Ag NPs increased the human cervical cancer cells toxicity percentage in dose dependent manner. Follows, DNA fragmentation assay result showed that Ag NPs (100 μg ml⁻¹) has induced apoptosis by fragmenting the DNA. Based on this study, we suggest that Ag NPs synthesized from aqueous leaf extract of pomegranate could be used for drug delivery and further this can be used as a therapeutic agent for cancer after thorough study of molecular mechanism.
Acknowledgments
The authors would like to acknowledge deep regards and
grateful to Dean and Head of the department, Department
of biotechnology, school of bioengineering, SRM Institute
of Science and Technology. We are thankful to the Direc-
tor of Engineering & Technology and Management of SRM
Institute of Science and Technology for funding chemicals
required for the project.

References

[1] Nair L S and Laurenccin C T 2007 Silver nanoparticles:
synthesis and therapeutic applications J. Biomed.
Nanotechnol. 3 301
[2] Salata O V 2004 Applications of nanoparticles in biology and
medicine J. Nanobiotechnol. 2 1
[3] Khandelwal N, Kaur G, Kumar N and Tiwari A 2014
Application of silver nanoparticles in viral inhibition: a new
hope for antivirals Dig. J. Nanomater. Biostrocr. 9 175
[4] Vardhana J and Kathiravan G 2015 Biosynthesis of silver
nanoparticles by endophytic fungus Pestalotiopsis psacista
isolated from the leaves of Psidium guajava linn Int. J.
Pharm. Sci. Rev. Res. 31 29
[5] Mohammadian A, Shojaosadati S A and Habibi R M 2007
Fusarium oxysporum mediates photogeneration of silver
nanoparticles Sci. Iran 14 323
[6] Zarfeshany A, Asgary S and Javannard S H 2014 Potent
health effects of pomegranate Adv. Biomed. Res. 3 100
[7] Chauhan S, Upadhyay M K, Rishi N and Rishi S 2011
Phytofabrication of silver nanoparticles using pomegranate
fruit seeds Int. J. Nanomater. Biostrocr. 1 17
[8] Nisha H M, Tamilselwari R, Jesurani S, Kanagesan S,
Hashim M, Catherine S and Alexander P 2015 Green
synthesis of silver nanoparticles from pomegranate (Punica
granatum) and analysis of anti-bacterial activity Int. J. Adv.
Technol. Eng. Sci. 3 297
[9] Ong C, Lim J Z Z, Ng C T, Li J J, Yung L Y L and Bay B H
2013 Silver nanoparticles in cancer: therapeutic efficacy
and toxicity Curr. Med. Chem. 20 772
[10] Acetinoua V C, Ahn S, Simu S Y, Singh P, Mathiyalagan R,
Lee H A and Yang D C 2016 Anticancer activity of silver
nanoparticles from Panax ginseng fresh leaves in human
cancer cells Biomed. Pharmacother. 84 158
[11] Fischer U A, Carle R and Kammerer D R 2011 Identification
and quantification of phenolic compounds from
pomegranate (punica granatum) peel, mesocarp, aril and
differently produced juices by HPLC-DAD–ESI/MS
Food Chem. 127 807
[12] Berrington D and Lall N 2012 Anticancer activity of certain
herbs and spices on the cervical epithelial carcinoma
(Hela) cell line Evid. Based Complement. Alternat. Med.
2012 564927
[13] Brahmachari G, Sarkar S and Ghosh R 2014 Sunlight-induced
rapid and efficient biogenic synthesis of silver nanoparticles
using aqueous leaf extract of Ocimum sanctum Linn with
enhanced antibacterial activity Org. Med. Chem. Lett. 4 18
[14] Amaladas T P, Savagami S, Devi T A, Ananthi N and
Velamal S P 2012 Biogenic synthesis of silver
nanoparticles by leaf extract of Cassia angustifolia Adv.
Nanosci. Nanotechnol. 3 045006
[15] Dimitrijevic R, Cvetkovic O, Miodragovic Z, Simic M,
Manojlovic D and Jovic V 2013 SEM/EDX and XRD
characterization of silver nanocrystalline thin film prepared
from organometallic solution precursor J. Min. Metall. B
49 91
[16] Satyavani K, Gurudeeban S, Ramanathan T and
Balasubramanian T 2011 Biomedical potential of silver
nanoparticles synthesized from calli cells of Citrullus
colocynthis (L.) Schrad J. Nanobiotechnol. 9 43
[17] Holder A L and Marr L C 2013 Toxicity of silver nanoparticles
at the air-liquid interface Biomed. Res. Int. 2013 328934
[18] Baharara J, Namvar F, Ramezani T, Mousavi M and
Mohamad R 2015 Molecules 20 2693
[19] Klug H P and Alexander L E 1954 X-ray Diffraction
Procedures (New York: Wiley)
[20] Singha G, Bhavesh R, Kasariya K, Sharma A S and Singh R P
2011 Biosynthesis of silver nanoparticles using Ocimum
santon (Tulsi) leaf extract and screening its antimicrobial
activity J. Nanopart. Res. 13 2981
[21] Prasad T N V K V and Elumalai E K 2011 Biofabrication of
Ag nanoparticles using Moringa oleifera leaf extract and their
antimicrobial activity Asian Pac. J. Trop. Biomed. 1 439
[22] Banala R R, Nagati V B and Karnati P R 2015 Green synthesis
and characterization of Carica papaya leaf extract coated
silver nanoparticles through x-ray diffraction, electron
microscopy and evaluation of bactericidal properties Saudi
J. Biol. Sci. 22 657
[23] Korbekandi H, Asghari G, Jalayer S S, Jalayer M S and
Bandegani M 2013 Nanosilver particle production using
Juglans regia L. (Walnut) leaf extract J. Nat. Pharm. Prod.
8 20
[24] Verma A and Mehata M S 2015 Controllable synthesis
of silver nanoparticles using neem leaves and their
antimicrobial activity J. Radiat. Res. Appl. Sci. 9 109
[25] Khalil M M H, Ismail E H, El-Baghdady K Z and Mohamed D
2014 Green synthesis of silver nanoparticles using olive leaf
extract and its antibacterial activity Arab. J. Chem. 7 1131
[26] Seecanthan T V M, Pandurangan M and Kim D H 2016 Green
synthesis: in vitro anticancer activity of silver nanoparticles
on human cervical cancer cells J. Clust. Sci. 27 671
[27] Jyoti K, Baunthiyal M and Singh A 2016 Characterization of
silver nanoparticles synthesized using Urtica dioica leaves
and their synergistic effects with antibiotics J. Radiat.
Res. Appl. Sci. 9 217
[28] Anandalakshmi K, Venagobal J and Ramasamy V 2016
Characterization of silver nanoparticles by green synthesis
method using pedalium murex leaf extract and their
antibacterial activity Appl. Nanosci. 6 399
[29] Tamizhamud E and Kantha D A 2011 Memecylon edule
leaf extract mediated green synthesis of silver and gold
nanoparticles Int. J. Nanomed. 6 1265
[30] Trifunsci S, Munteanu M F, Agotici V, Pintea S and Gligor R
2015 Determination of flavonoid and polyphenolic
compounds in Viscum album and Allium sativum extracts
Int. Curr. Pharm. J. 4 382
[31] Devaraj P, Kumar P, Aarti C and Renganathan A 2013
Synthesis and characterization of silver nanoparticles
using cannonball leaves and their cytotoxic activity against
MCF-7 cell line J. Nanotechnol. 2013 598328
[32] Phongtongpasuk S and Poadang S 2016 Green synthesis
of silver nanoparticles using pomegranate peel extracts Adv.
Mat. Res. 1131 227
[33] Ahmed S, Saifullah S, Ahmad M, Swami B L and Ikram S 2016
Green synthesis of silver nanoparticles using Azadirachta
indica aqueous leaf extract J. Radiat. Res. Appl. Sci. 9 1
[34] Thilagavathi T, Renuka R and Priya R S 2016 Bio-synthesis of
silver nanoparticles using Punica granatum (pomegranate)
peel extract: a novel approach towards waste utilization Int.
J. Adv. Sci. Eng. 3 234
[35] Salem N M, Albanna I S and Awwad A 2016 Green synthesis of sulfur nanoparticles using punica granatum peels and the effect on the growth of tomato by foliar spray applications J. Environ. Nanotechnol. 6 83

[36] Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K and Thangamani S 2012 Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities Asian Pac. J. Trop. Biomed. 2 574

[37] Sambale F, Wagner S, Stahl F, Khaydarov R R, Schepere T and Bahnemann D 2015 Investigations of the toxic effect of silver nanoparticles on mammalian cell lines J. Nanomater. 2015 136765

[38] Satyavani K, Gurudeeban S, Ramanathan T and Balasubramanian T 2012 Toxicity study of silver nanoparticles synthesized from Suaeda monoica on Hep-2 cell line. Avicenna J. Med. Biotechnol. 4 35

[39] Connolly M, Fernandez-Cruz M L, Quesada-Garcia A, Alte L, Segner H and Navas J M 2015 Comparative cytotoxicity study of silver nanoparticles (AgNPs) in a variety of rainbow trout cell lines (RTL-W1, RTH-149, RTG-2) and primary hepatocytes Int. J. Environ. Res. Public Health 12 5386

[40] Han X, Gelein R, Corson N, Wade-Mercer P, Jiang J, Biswas P and Oberdörster G 2011 Validation of an LDH assay for assessing nanoparticle toxicity Toxicology 287 99

[41] Gurunathan S, Raman J, Abd M S N, John P A and Vikineswary S 2013 Green synthesis of silver nanoparticles using Ganoderma neo-japonicum imazeki: a potential cytotoxic agent against breast cancer cells Int. J. Nanomed. 8 4399

[42] Prasannaraj G and Venkatachalam P 2017 Green engineering of biomolecule-coated metallic silver nanoparticles and their potential cytotoxic activity against cancer cell lines Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 025001

[43] Ebrahiminezhad A, Bagheri M, Taghizadeh S M, Berenjian A and Ghasemi Y 2016 Biomimetic synthesis of silver nanoparticles using microalgal secretory carbohydrates as a novel anticancer and antimicrobial Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 015018