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Preparation and physical characterization of *Adonis vernalis* aqueous leaf extract-mediated green synthesized silver nanoparticles and its toxicity effect on breast cancer cells

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ABSTRACT

The specific characteristics of silver nanoparticles (AgNPs), especially anticancer and antimicrobial properties, have led to their widespread usage in biology. The study looked at the anti-cancer activity of AgNPs, which were produced by a one-step green synthesis based on the use of *Adonis vernalis* leaf extract. Characterization of reducing agents in plant extracts was performed using (FT-IR) infrared spectroscopy. The presence of adsorption peak at 442 nm in UV-visible absorbance of bio-fabricated AgNPs indicates the effectiveness of proposed method. Transmission electron microscope (TEM) and field emission electron microscope (FESEM) were used to investigate the morphology and confirmed the spherical shape of products. Phase analysis and determination of the synthesized product crystal lattice was performed using X-ray diffraction (XRD) method. The stability of particles was determined using zeta potential analyze. Energy-dispersive X-ray (EDX) analysis confirmed the formation of high purity AgNPs. The MTT assay was used to determine the anti-toxic properties of produced products and the results demonstrated that silver nanoparticles biosynthesized by *Adonis vernalis* extract can be used to treat breast cancer.

Keywords: *Green synthesis; Silver nanoparticles; Adonis vernalis extract; Breast cancer; MTT assay.*

1. Introduction

Breast cancer is one of the most common types of diseases in women, and many of them are affected by it every year [1]. Blood flow can transport cancer cells from damaged tissue to other parts of the body. Under these conditions, complications related to cancer are observed in the patient. Despite significant advances in medicine, studies have shown a relatively high mortality rate for this cancer, especially in women. Common methods in the treatment of cancer are radiotherapy and chemotherapy. The most important limitation of these methods has their relatively high side effects, which sometimes occur as serious complications such as damage to healthy body organs [2, 3] . Also, most of the drugs used in classical therapies cause the destruction of healthy tissue along with cancer cells. Therefore, research to find a treatment with

less side effects is the subject of many researches [2]. Today, nanotechnology is widely used in medicine and has high hopes for medicine and treatment for a variety of diseases [4]. Studies have shown the unique biological properties of AgNPs including antiplatelet, antiviral, antifungal, antibacterial, anticancer, antioxidant and antispasmodic at low concentrations, lead to its widespread uses applications. Other usages of AgNPs mentioned its direct use as a drug carrier and active ingredient in medical imaging [5-8]. AgNPs are generally produced in three different ways. These methods include chemical synthesis, physical synthesis, and biological synthesis. Meanwhile, the use of the first two methods suffered from main drawbacks including the usage of high production costs and/or hazardous chemicals [9, 10]. Therefore, the biological synthesis can be selected as a suitable alternative for the production of AgNPs [11]. These methods have advantages such as economic efficiency and low toxicity [12]. There are several sources for the production of AgNPs by biological synthesis, including plant parts, bacteria, fungi, algae, yeasts, and viruses [13, 14]. Meanwhile, green synthesis is preferred by plants as a reducing agent due to advantages such as low cost, lack of need for maintenance and cell culture in a particular environment and the possibility of using it on a large scale compared to other materials [15, 16].

Plant extracts due to their biologically active metabolites, including ascorbic acid, flavonoids, citric acid, phenols, terpenes, polyphenols, reductase and alkaloids, can be used as reducing and stabilizing agents to prepare AgNPs [11, 17]. To the best of our knowledge, no synthesis of AgNPs has been reported by *Adonis vernalis* (AV) extract. Accordingly, the most important innovation of the current study includes: Preparation and analysis of AgNPs with selected plant extract (AV-AgNPs) by UV-Visible spectroscopy, FTIR, EDX, FESEM, TEM, XRD, potential analysis of Zeta and the assessment of AV-AgNPs cell toxicity in breast cancer (MDA-MB-468) cell lines.

2. Experimental

2.1. Materials

The Av leaves used in the present study was procured from market (Kerman, Iran). Silver nitrate ($AgNO_3$) (Merck), Ethyl alcohol (96wt.%) (Atlas Shimi Co.) and double-distilled water (Zakaria Jahrom Co.) were purchase. Human breast cancer cell line, i.e., MDA-MB-468, was provided from Iranian biological resource cancer (IBRC). MTT prepared from Sigma-Aldrich (St. Louis, MO). Fetal Bovine Serum (FBS), RPMI 1640, Trypsin and Penicillin-Streptomycin solution were obtained from Gibco (Invitrogen, NY, USA).

2.2 Leaves extract preparation

To prepare *AV* plant extract, it was first cleaned with water to remove impurities and then dried at room temperature and outdoor air for 7 days. Then, 0.5 g of leaf powder was added to 50 mL of boiled twice-distilled water for 2 min. After this step, the output solution was centrifuged at 1500 rpm for 15 min and finally filtered using Whatman No. 40.

2.3. Preparation of AV-AgNPs

To produce AV-AgNPs, 40 mL of $AgNO_3$ (10 mM) and 15 mL of *Adonis vernalis* extract were mixed each other. It was then stirred for 2 hr at 37 °C at 1200 rpm by a magnetic stirrer. Conversion of Ag^+ to Ag° was occurred by changing the color of the solution from pale yellow to brown. To collect AV-AgNPs, a centrifuge with 3500 rpm was used for 15 min. The products were rinsed twice with distilled water that followed by ethyl alcohol to remove all of possible biomolecules and proteins in prepared samples. The pure AV-AgNPs, was dried at room temperature and used for further characterization.

2.4. Characterization of AV-AgNPs

In order to confirm the formation of AgNPs, the spectroscopic spectroscopy of ultraviolet absorption (droplet scanning, Ghana Analytical Company, Germany) was used with a resolution of 1 nm between 300 and 800 nm. Intermediate infrared analysis 400-4000 cm^{-1} was used using the Fourier transform infrared spectroscopy (FT-IR, Tensor 27, Bruker Co, Germany) to determine the effective groups in *Adonis vernalis* extract. Phase analysis of dried powder (AV-AgNPs) were done using X-ray diffraction (XRD) technique (X'Pert MPD, Philips Co) using Cu-K_α radiation (λ=1.54 nm) at 2θ=30-80 degrees with scan rate 0.02 °/sec at 30 mA and 40 kV. Energy Dispersive X-ray analysis (EDX) analysis (Pentafet model, United Kingdom) was employed for determination of chemical point analysis of products. Zeta potential (Horiba sz-100 zeta potential analyzer at 25 °C) was employed for determination of surface charges on synthesized AgNPs.

Transmission electron microscope (TEM; 208S

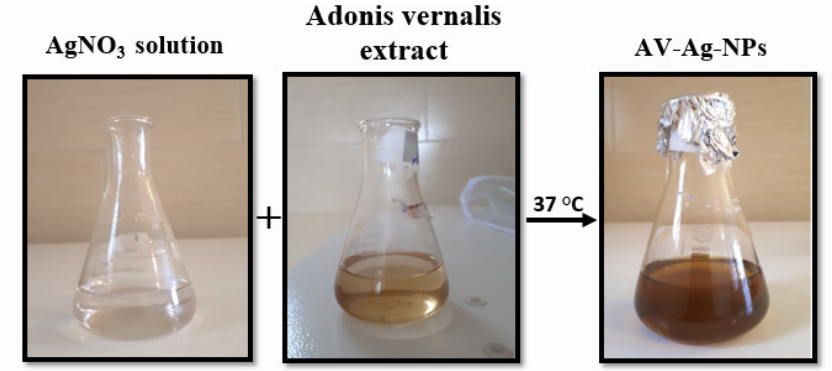

Fig. 1- The observed changes in color during the synthesis of AV-AgNPs using *Adonis vernalis extract*.

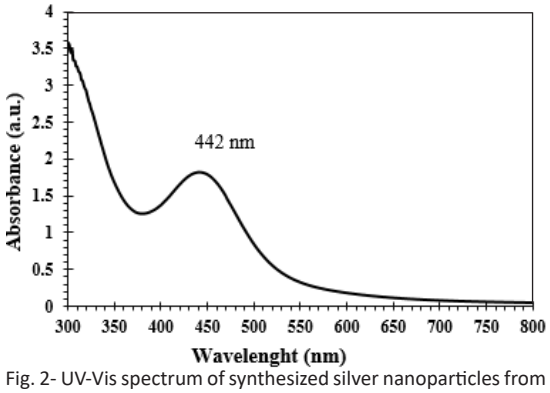

Fig. 2- UV-Vis spectrum of synthesized silver nanoparticles from aqueous leaf extract of *Adonis vernalis*.

Model) and field emission electron microscope (FESEM; MIRA3 Model) were used to investigate the morphological changes as well as the size of synthesized AgNPs. In this regards, the preparation of TEM sample was done by placement of sample drop on 300 mesh holey carbon film supported on a copper grid and drying it at ambient temperature. To provide a conductive sample for FESEM, powdered of AgNPs placed on double sided tape and coated with a thin film of gold. The histogram of size distribution was extracted from the FESEM images by measuring the diameters of at least 91 particles.

2.5. Anticancer assay

The RPMI 1640 medium consists of 10 % FBS serum, 100 units/mL penicillin and 100 mg/mL streptomycin in the atmosphere containing of 5 % CO_2 at 37 °C, was used for cell culture of the human breast cancer cell line (MDA-MB-468). IC_{50} defined as the at least concentration of inhibitor that decreased 50 % of the activity of cancer cells and used to compare the effect of *Adonis vernalis* leaf extract and AV-AgNPs on the inhibition of cancer cells biological activity by employment of 3-(4,-5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) in colorimetric technique. 2×10^4 cells/well were seeded into the 96 well culture plates. The analysis was followed by the addition of 0, 20, 40, 60, 80 and 100 µg/mL of *Adonis vernalis* extract and AV-AgNPs to wells after incubation time equal to 48 h. At the end of each time point, 200 µL MTT dye solution (5 mg/mL) was added to each well and the culture plates further incubated for 4 h at 37 °C. Then, the medium was removed and solubilization of the formazan crystals was done for 15 min by adding of 100 µL of dimethyl sulfoxide to each of the wells and incubated. The optical density was determined at 570 nm in an ELISA microplate reader (Biochrom Anthos, USA).

3. Results and discussion

3.1. Characterization of AV-AgNPs

The presence of surface plasmon resonance (SPR) properties in noble metals has created unique optical properties and their emergence as a suitable candidate in industrial applications. SPR can be attributed to the interaction between the resonant oscillations of the electron conductors at the nanoparticle surface due to the interaction with electromagnetic waves [16, 18, 19]. As shown in Fig. 1, the evolution of brown color instead of pale yellow of initial solution indicated the reduction of silver ions with *Adonis vernalis* extract due to the stimulation of plasma surface vibration in AgNPs. The adsorption of AV-AgNPs has formed a sharp peak at approximately 442 nm (Fig. 2) [16, 20].

Formation of nanoparticles in the range of 1 to

5 nm with a clear spherical and relatively uniform geometry, were confirmed in TEM (Fig. 3). The average size of spherical particles are about 36 nm based on FESEM image (Fig. 4a and c). These analyses indicated the formation of homogeneous AgNPs obtained with the use of *Adonis vernalis* extract as reducing and stabilizing agents. Particle agglomeration, especially during the preparation of FESEM samples, can be the reason for their larger size than the TEM image [21]. EDX analysis confirms the presence Carbon (C) and Oxygen (O) elements along with high-intensity silver peaks (Fig. 4b). The presence C and O signals can be attributed to molecules of organic compounds attached to nanoparticles or present in the sample coating [22]. Another reason for the presence of C as sharp peak can be related to the usage of C grid as substrate through EDX analysis [23].

Zeta potential was used to determine the stability of synthesized nanoparticles. The result of this analyze is shown in Fig. 5. The zeta potential of particles equal to -24.3 mV confirmed the acceptable stability of the prepared nanoparticles. According to studies, this potential is within the range of recommended for the distribution of nanoparticles and prevents them from sticking together [24, 25]. This observation is consistent with the reported that use of plant biomolecules as stabilizers. Such a features are effective in improving the properties of AV-AgNPs [21, 26].

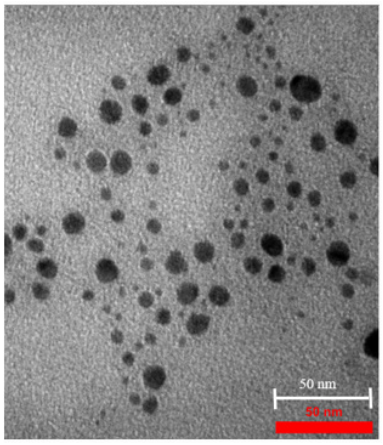

Fig. 3- TEM image of biosynthesized AV-AgNPs.

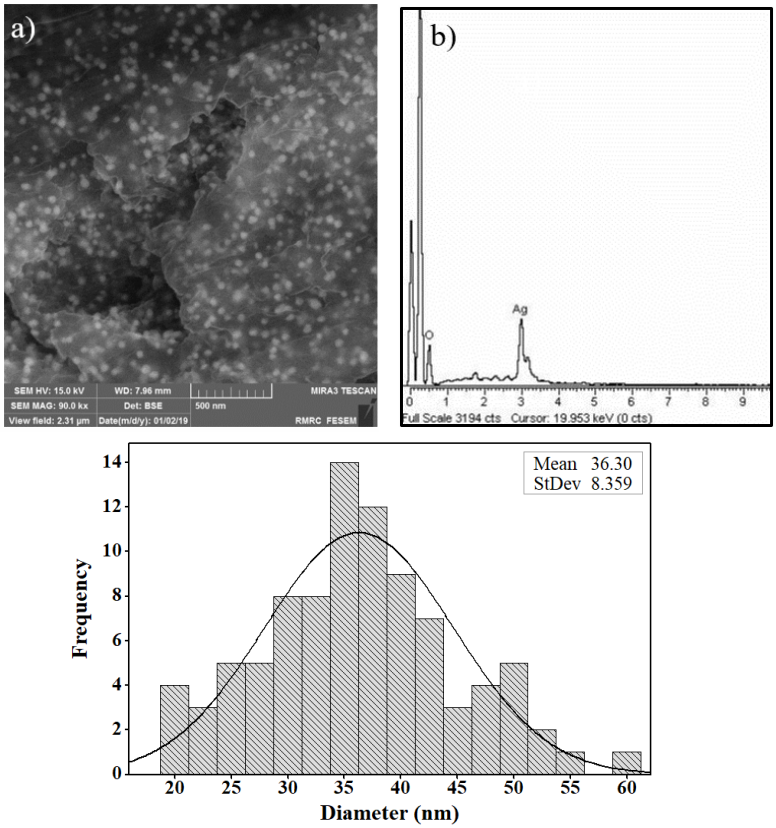

Fig. 4- (a) FESEM image; (b) EDX spectrum; and (c) particle size distribution histogram of AV-AgNPs based on FESEM image.

XRD spectra employed to investigate the crystalline nature of synthesized AV-AgNPs. Fig. 6 demonstrated by four main peaks at 2θ values of 38.25, 44.67, 64.51 and 77.58 were related to the (111), (200), (220) and (311), respectively as the same as JCPDS card number 04-0783 [27]. The XRD pattern is consistent with the results of other studies and indicates the face centered cubic (FCC) crystalline nature of the AV-AgNPs [26, 27]. Also, the crystallization of organic compounds of extract at the nanoparticle surface is responsible for the formation of noise in the XRD spectrum [28, 29]. The estimation of average crystal size of AgNPs were determined using Debyee Scherrer equation [16] was about 21 nm.

FTIR spectra were used to find the effective chemical groups in bioreduction of the Ag^{+} ions and capping agent of AV-AgNPs. A FTIR spectra of *Adonis vernalis* leaf extract and biosynthesized AV-AgNPs have been showed in Fig. 7. The peaks of AV-AgNPs and *Adonis vernalis* extract at (3437 and 3436), (1638.38 and 1638.33), (1385 and 1384), (1107 and 1112), (617 and 609) cm^{-1} corresponds to O-H stretching vibration of phenol and alcohols, C=O group of carbonyl, CH_3 groups of carbohydrate, C-O groups of alcohols, ethers and esters, C-H groups of aromatic compounds, respectively [8, 28]. These groups indicate that the AV-AgNPs synthesized from the extract are surrounded by some metabolites such as glycosides, flavonoids and phenolic compounds [29]. The major changes in the regions (1107 and 1112) and (617 and 609) cm^{-1} indicate the presence of more C-O and C-H groups in reducing or stabilizing agents of AV-AgNPs. The comparisons of the other spectra demonstrated the slight changes in their band positions. Therefore, they have a negligible role in reducing and stabilizing of AV-AgNPs.

3.2. Anticancer activity

AgNPs prepared silver ions that used directly to react with nucleic acids and DNA damage by destroying the mitochondrial respiratory chain, inhibiting the synthesis of DNA, inhibition of cell division and DNA replication, inducing apoptosis, inducing reactive oxygen species [30-32]. The cytotoxicity activity of AV-AgNPs at different

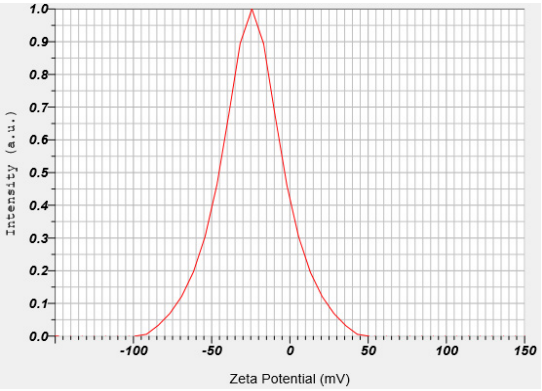

Fig. 5- The zeta potential of biosynthesis of AV-AgNPs from the *Adonis vernalis* extract.

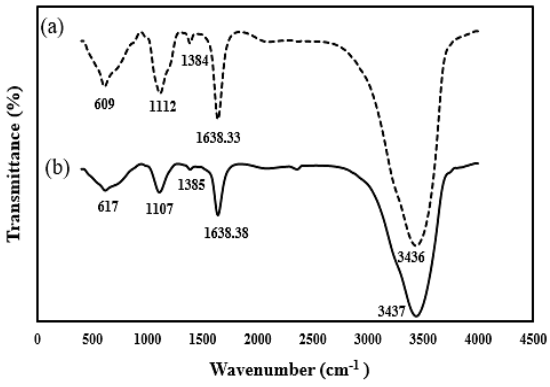

Fig. 7- FTIR spectra of (a) *Adonis vernalis* leaf extract and (b) biosynthesized AV-AgNPs using *Adonis vernalis* leaf extract.

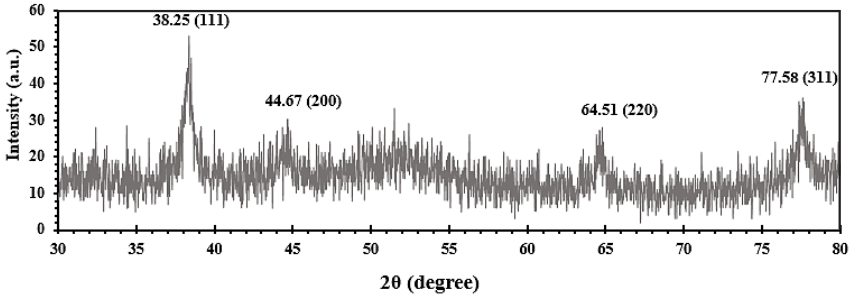

Fig. 6- XRD patterns of biosynthesized AV-AgNPs using aqueous leaf extract of *Adonis vernalis*.

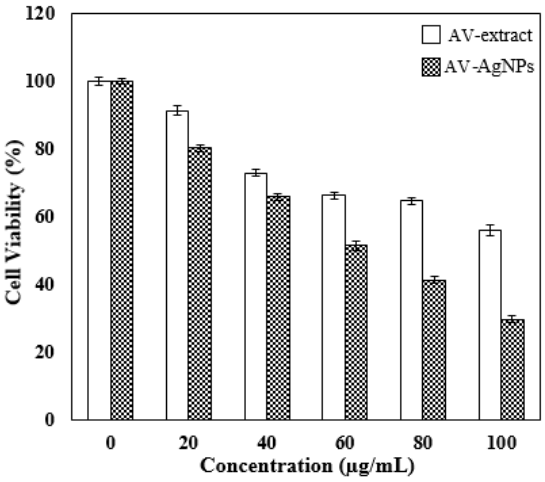

Fig. 8- Dose-dependent cytotoxicity effect of biosynthesized AV-AgNPs on the proliferation of MDA-MB-468 cell lines by MTT test.

concentrations against the MDA-MB-468 using MTT assay was evaluated (Fig. 8). The results revealed that any increase in AV-AgNPs content is reciprocal to the cell viability percentage and clearly shows a dose dependent cytotoxicity activity mediated biosynthesized AV-AgNPs. The IC50 value of AV-AgNPs and *Adonis vernalis* extract was detected to be 60 µg/ml and more than 100 µg/ml after 48 hr of treatment, respectively. Also, the cell viability of MDA-MB-468 cell lines with increasing the concentration from 0 to 100 µg/ml for the AV-AgNPs and Adonis vernalis extract decreased 70 % and 44% after 48h, respectively. The higher toxicity effect of AgNPs in the study could be attributed to their size, shape, particle surface as well as intriguing physicochemical properties [33].

4. Conclusion

The chemical compounds found in many medicinal plants in traditional medicine are suitable candidates for possible treatment of various diseases, including cancer. Accordingly, the use of medicinal plants to synthesize silver nanoparticles (green synthesis) can significantly enhance the unique properties of silver nanoparticles and is the subject of much research. In current work, the green synthesis of AgNPs was performed using *Adonis vernalis* extract of plant and its anticancer behavior on human cancer cells (MDA-MB-468) was investigated. The results showed that the extract has the ability to regenerate silver ions. Reduction and stability of nanoparticles synthesized by UV-Vis spectroscopy were investigated. The *Adonis vernalis* extract is useful in allowing robust control over morphology, size distribution and surface charge that were confirmed with zeta potential, TEM and FESEM techniques. Based on FTIR spectra, biomolecules of natural aqueous extract can effectively functionalized AgNPs and performed as capping and stabilizing agents. EDX analysis confirmed the existence of elemental silver. XRD spectra showed the crystalline nature of AV-AgNPs. By consideration of MTT assay results (IC_{50}), it was revealed inhibiting of 50 % breast cell line was occurred at 60 µgml^{-1}. In summary, after the comprehensive investigation of molecular mechanism, the prepared AV-AgNPs by proposed approach can effectively act as drug delivery and therapeutic agents for cancer.

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