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Mohammad Mousavi-Khattat

University of Isfahan

Hamid Nourbakhshan

Nourdanesh Institute of Higher Education

Mehrnaz Roumi

University of Isfahan

Mahshid Ebrahiminejad

Islamic Azad University

Yasaman Fazeli

University of Isfahan

Zahra Shakeran (✉ shakeran123@gmail.com)

University of Isfahan <https://orcid.org/0000-0002-6829-870X>

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Anticancer activity of silver nanoparticles synthesized using extract of animal waste as a traditional medicine on MCF-7 human breast cancer cell line

Mohammad Mousavi-Khattat¹, Hamid Nourbakhshan², Mehrnaz Roumi¹, Mahshid Ebrahiminejad³, Yasaman Fazeli⁴, Zahra Shakeran^{1*}

1. Department of biotechnology, Faculty of Biological science and technology, university of Isfahan, Isfahan, Iran
2. Department of Biology, Nourdanesh Institute of Higher Education, Meymeh, Isfahan, Iran
3. Department of Mechanical Engineering, Najafabad Branch, Islamic Azad University, Najafabad, Iran.
4. Department of Physics, Faculty of Physics, University of Isfahan, Isfahan, Iran

*Corresponding author: Shakeran123@gmail.com

Abstract

Green synthesis methods are environmentally friendly, cost effective and nonhazardous for biomedical applications in comparison with other methods. The aim of the study was green synthesis of silver nanoparticles using medicinal animal dung extract as a reducing, capping and stabilizing agent for the first time among other synthesis methods of silver nanoparticles. Female donkey's dung was capable to reduce silver ions to nanoparticles and stabilize them. Silver nanoparticles with average sizes of 36 nm were synthesized and characterized by UV-Vis, FT-IR, XRD and TEM. Moreover, synthesized nanoparticles were analyzed in terms of anticancer activity by MTT assay on MCF-7 cell line. UV-Visible spectrophotometer showed an absorbance peak in the range of 414-433 nm. To identify the phytochemical coating of particles, FTIR analysis was used. Transmission electron microscope (TEM) images and X-ray diffraction (XRD) confirmed the formation of small spherical silver nanoparticles. The MTT assay revealed potent anticancer effects of the aqueous extract synthesized nanoparticles on MCF-7 cells, incubated for 24 hours. Based on the current findings, it is strongly believing that the use of donkey's dung offers large scale production of biocompatible silver nanoparticles that can be suggested to possess valuable anticancer agents against breast cancer cell lines.

Keywords: Silver nanoparticles, green synthesis, chemical synthesis, animal waste extract, breast cancer cells, anticancer drugs.

Introduction

Nanotechnology provides fields ranging from conventional chemical approaches to medical and environmental innovations with practical applications. Nanoparticles have emerged with leading contributions in a range of applications, including drug delivery ointments, nanomedicine, chemical sensing, data storage, cell biology, agriculture, cosmetics, textiles, food processing, photocatalytic organic coloring, antioxidants, and antimicrobial agents. (1-10). Nanoparticles can

be synthesized through various physical, chemical and biological approaches, and they possess sizes in the range of 1–100 nm with large surface area to volume ratio which makes them extraordinarily significant (11, 12). Typical chemical and physical synthesis methods have many disadvantages such as high cost, environmental and human hazards and the use of toxic chemical materials. Especially for biomedical purposes, nanoparticles that are chosen should be non-toxic and it's exploitable by use of biological macromolecules as the nanoparticle building blocks or its coating with biocompatible substances (13, 14). Such problems caught the attention of the new and alternate routes. Green synthesis of nanoparticles using plants and micro-organisms has been proven as a new source for nanoparticles synthesis and development (15, 16).

Green synthesis is a relatively novel and simple process which is more environmentally friendly and cheaper than the rest (17, 18). There are a variety of chemically diverse and bioactive molecules in the green synthesis precursors that include proteins /enzymes, amino acids , polysaccharides, polyphenols, aldehydes and ketones, which can reduce metal ions and stabilize nanoparticles to the desired shapes and sizes (19-21). Although green synthesis of nanoparticles, using plant extracts, has reported enormously, there are a few studies on removing materials from the animal bodies include reducing and stabilizing agents for the synthesis of nanoparticles. For example cow urine, honey, chitosan and silk as animal waste materials reported recently for biosynthesis of nanoparticles (22-25). One of these waste material is female donkey dung called Anbarnesa.

Anbarnesa is applied in Persian traditional and folk medicine for the treatment of bacterial and viral infectious diseases such as sinusitis, ear infections and cold, allergic diseases such as asthma and cough, tumors and vaginal cysts. Moreover, the findings have shown Anbarnesa was nontoxic to normal cells. According to the initial results of Anbarnesa, it may be used as a reducing and stabilizing agent for silver nanoparticle synthesis, due to have some phenolic groups in the extract(26, 27).

Cancer is a life-threatening illness and causes deaths worldwide (28). The annual cancer cases are expected to increase from 14 million in 2012 to 22 million in the next two decades, according to the WHO (29). However, most of the chemical methods are expensive and involve the use of toxic chemicals that pose various biological risks (30). Thus, the development of potent and effective antineoplastic drugs is one of the most persuaded goals. Recent advancements in nanotechnology spread the area of research based on metal based silver nanoparticles having bio-medical applications. Silver nanoparticles, in particular, have been the focus of increasing interest due to their excellent contribution for therapeutic purposes (31). Although there is a common traditional usage of Anbarnesa for antitumor proposes, experimental studies on its cytotoxicity has reported rarely (32). The anticancer effect of Anbarnesa may be attributed to the presences of many polar bioactive compounds in the plants which are eaten by animals such as alkaloids, flavonoids, tannins, terpenoids, and glycosides. These compounds are potent anti-tumor agents (33).

Therefore, in this study the aqueous extract of Anbarnesa was used to biosynthesize of the silver nanoparticles. Small size, high aspect ratio and penetrating ability of the nanoparticles and their own anticancer activity as well as anticancer activity of Anbarnesa, would be a promising combination for cancer treatment proposes (34). To confirm the increasing of nanoparticles anticancer effect due to the presence of extract materials, chemical nanoparticles were synthesized simultaneously and their anticancer activities were analyzed separately (35).

Materials and methods

Collection and extraction methods

Female donkey dungs were collected from Isfahan, Iran in May 2019 and washed several times with tap water, followed by distilled water. The matter was dried in the shade for a week. The dry matter was passed through a 50 mm mesh, to make a uniform powder. The extract was prepared by mixing 5 g of dry powder with 100 mL of deionized water, and then exposing the mixture to water bath at 80 °C for 15 min. The aqueous extract was prepared using Whatman filter paper No. 1 by filtration, and was held at 4 °C for further use.

Synthesis of silver NPs

about 50 ml of Silver nitrate solution (1 mM) was added drop wise to 1 ml of female donkey dung extract under continuous stirring (500 rpm) on a magnetic stirrer and the whole mixture was incubated overnight at 60°C. The color change (light yellow to dark red) was visible after the incubation period. The solid product was separated by centrifuge (14,000 rpm, 15 min, 25 °C), washed with deionized water twice, and freeze-dried for 24 h. The final AgNPs powder was stored in a room temperature for characterization and bioactivity studies. Also, chemically synthesized nanoparticles was provided through the similar method with the difference that is replacement of sodium citrate 1% with female donkey dung extract as a reducing and stabilizing agent at the same amounts.

Characterization of synthesized nanoparticles

UV–Vis spectral analysis was performed using UV-vis model Biowave II, UK for both green and chemical synthesis methods from the start of the reaction over 60 min with a time interval of 10 min between 300 and 600 nm. Two milliliter of each sample was pipetted into a cuvette and subsequently analyzed at room temperature. Dynamic light scattering (VASCOTM, Cordouan-tech, France) was used to analyze the average size of synthesized silver nanoparticles. In addition, to determine the surface charges and stability of the nanoparticles in the suspension, zeta potential (ζ -potential) of the nanoparticles were measured in water via Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) using material refractive index (RI) value of 1.54 and absorption of 0.00, controlled at 25°C with water dispersant refractive index of 1.330. The FT–IR (Fourier-transform infrared spectroscopy) spectra were recorded on FT-IR Spectrometer

6300, Jasco, Japan. The synthesized AgNPs sample was dried and ground and various modes of vibrations were recognized to confirm the functional groups present in AgNPs. For XRD analysis, the powdered nanoparticles were located in X-ray diffractometer (D8 Advanced X-ray diffractometer, Bruker, Germany) using a wavelength at 1.540598 Å (Cu K α) was used to confirm the silver content of synthesized nanoparticles. To verify the shape and confirm the diameter of the nanoparticles non-diluted samples of AgNPs were placed on grid for transmission electron microscopy (TEM).

Anticancer activity test:

In this study, we tested the anticancer effect of green and chemically synthesized AgNPs on MCF-7 cancer cell line as a model. MCF-7 kindly provided by the Royan Institute, Iran. The cells were cultivated under a humidified atmosphere containing 5 percent carbon dioxide in RPMI supplemented with 10 percent FBS at 37 ° C. After the confluence cells were trypsinised and died. The breast, ovarian, multidrug-resistant cell line was transferred to 96-well tissue cultivation plates at a density of 80%, 24 hours before diagnosis. After the confluence cells were trypsinised and died. Breast, ovarian, multi-drug - resistant cell line was transferred at a density of 80 percent to 96-well tissue cultivation plates, 24 hours before diagnosis. The medium was then replaced with fresh medium containing green/chemically synthesized AgNPs, the extract and silver nitrate at different concentrations (3.2–204.8 µg/ml), followed by incubation for 24 h at 37°C in the presence of 5% CO₂ in an incubator. 20µl / well of (5mg / ml) 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl — tetrazolium bromide (MTT) buffered saline solution was added after removal of the sample solution and washing with phosphate buffered saline (pH 7.4). After 4h incubation 100 mL DMSO was added to each well and the absorbance was measured immediately at 570 nm by using a microplate reader (Awareness Technology Stat Fax 3200 Microplate Reader, USA).

RESULTS

Characterizations and sample analysis

The extract of Anbarnesa was mixed with silver nitrate to synthesize AgNPs. The change in color of the mixture was an indication of AgNPs synthesis where the color of the solution changed from pale yellow to dark brown because of the process of reduction of Ag⁺ to Ag⁰ nanoparticles. Also during a chemical reaction for silver nanoparticle synthesis, the color of the solution was changed from transparent to dark gray after addition of sodium citrate as a reducing/stabilizing agent. These results indicated the biosynthesis of AgNPs. UV–vis spectra for both biosynthesized and chemically synthesized AgNPs gave the peaks at 425 nm after 1 h incubation (Figure 1B). The presence of an absorbance peak at about 420-430 nm clearly indicates the formation of AgNPs in the solution due to surface plasmon resonance (SPR) electrons present on the nanoparticle surface.

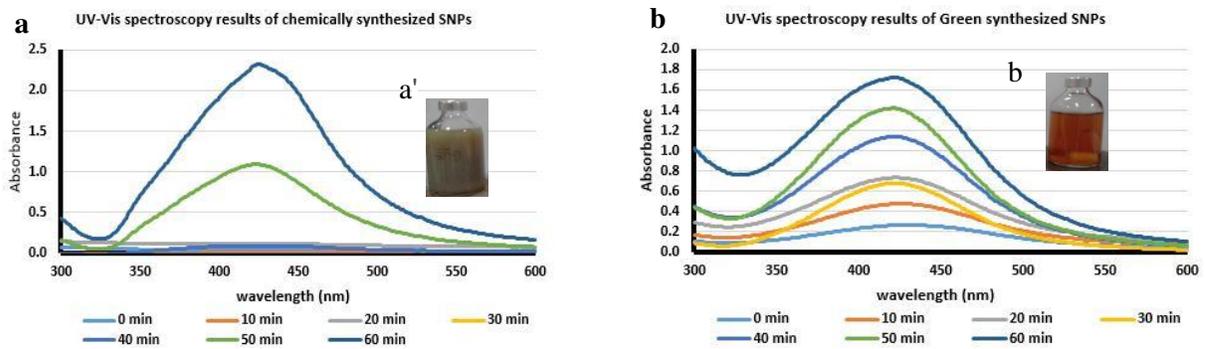


Figure 1. UV-Vis spectra of the silver nanoparticles synthesized through (a) chemical and (b) green methods. Color changes in chemical and green methods was demonstrated in a' and b' figures respectively.

Bio-molecules involved in the capping and reducing of green-AgNPs were analyzed by FTIR. The FTIR results of green-AgNPs showed main absorption peaks located at 616, 1077, 1355, 1629, 2923 and 3351 which characterizes several functional groups like C-Cl, CO, CH (stretch), C=C, CH (bending) and OH receptivity (Figure 7). The hydrodynamic diameter and size distribution of spherical silver nanoparticles were analyzed by DLS and the results showed the narrow size distribution for the green synthesized nanoparticles. The average nanoparticle size synthesized by green approach was around 35 nm (Figure 3a). The surface charges and stability of the nanoparticles in the suspension were measured through zeta potential and the values was about -47 meV for green synthesized nanoparticles (Figure 3b).

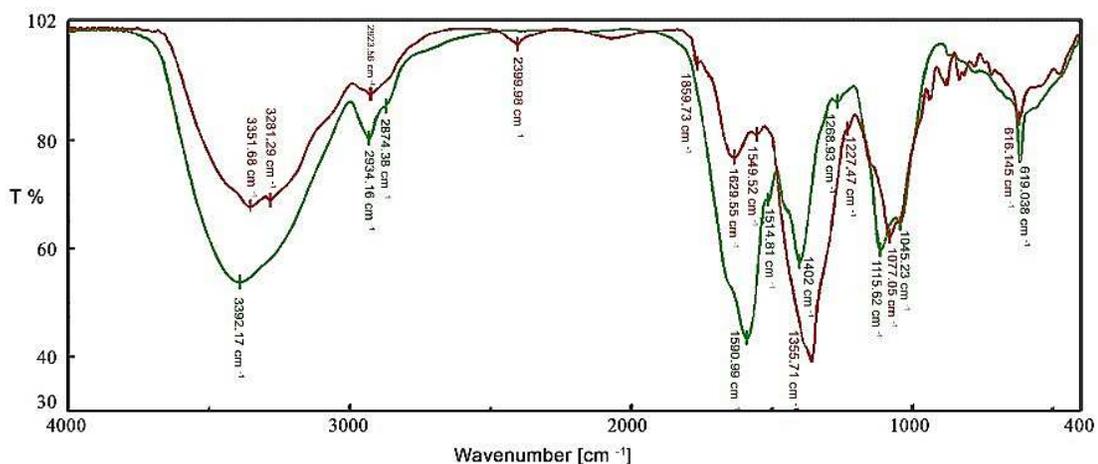


Figure 2. FTIR spectra of Anbarnesa extract (green curve) and silver nanoparticles synthesized by Anbarnesa extract (red curve).

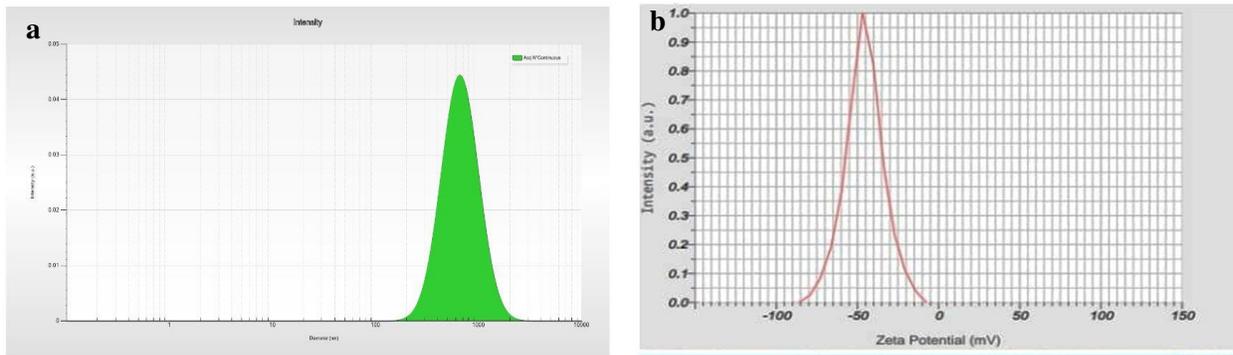


Figure 3. (a) DLS histograms and (b) Zeta potential histograms of green synthesized AgNPs

The typical XRD pattern of silver nanoparticles synthesized using Anbarnesa, is shown in Fig. 2a, b. Bragg reflections with 2θ values and its corresponding lattice planes, shown as 38.15° , 44.14° , 64.50° , 77.25° and 81.52° , indicates presence of silver and the amounts of 28° , 32.50° , 46.51° , 55.21° , 57.93° , 67.86° , 74.93° , 77.23° , 86.16° was an evidence of AgCl composition. TEM micrograph demonstrated that the majority of the particles were spherical in shape and the nanoscale range of nanoparticles obtained from DLS analysis was verified in the micrograph.

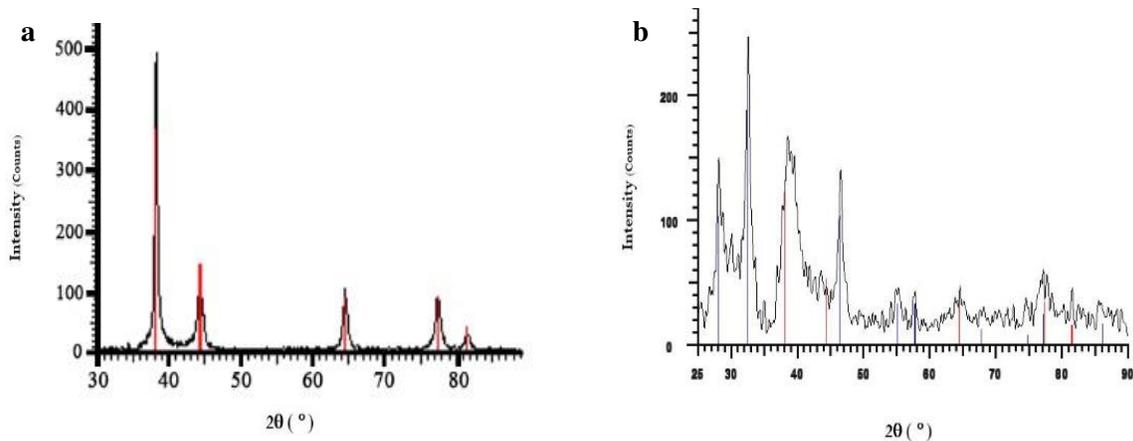


Figure 4. X-Ray diffraction spectra of (a) chemically and (b) green synthesized AgNPs

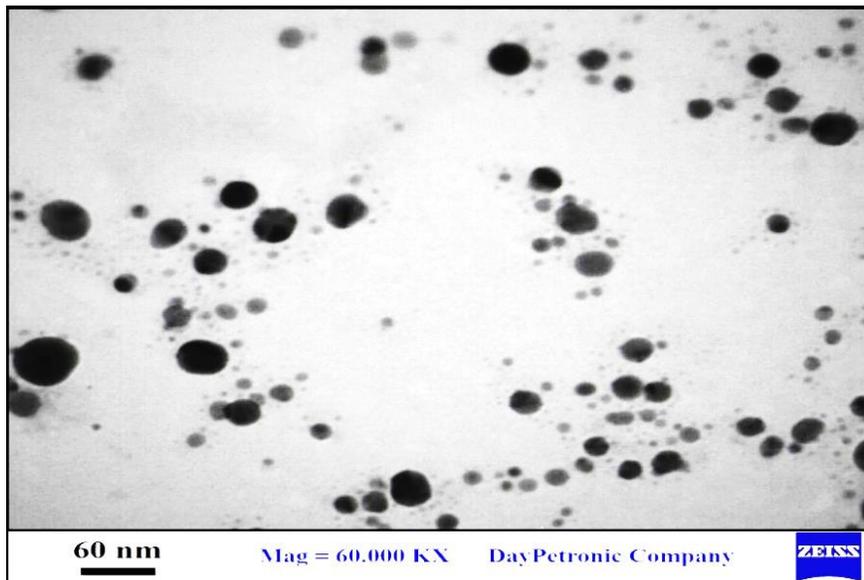


Figure 5. TEM micrographs and particle size distribution histogram of AgNPs synthesized using Tulsi extract

Anticancer assay

The cytotoxic effect of green synthesized silver nanoparticles, chemically synthesized ones, the extract of Anbarnesa and silver nitrate solution were determined separately on MCF-7 cells (Figure 6). The interaction of AgNPs with MCF-7 was studied because these cells are a well-established model for the identification of adverse effects of NPs and have an epithelial and non-invasive phenotype, as reported elsewhere (36). The anticancer potential of AgNPs was studied by MTT based cytotoxicity assay. In this cytotoxicity assay, increasing concentration of AgNPs significantly inhibits the growth of the MCF-7 cells for 24 h incubation. It was found that silver nitrate was the most effective material to inhibit the proliferation of cancer cells, and green synthesized silver nanoparticles, chemically synthesized ones, the extract of Anbarnesa was standing in the next rankings respectively. An IC₅₀ of 43.6 $\mu\text{g}.\text{ml}^{-1}$ was obtained eventually for green-AgNPs and 77.3 $\mu\text{g}.\text{ml}^{-1}$ for chemical-AgNPs. When MCF-7 cells were treated with a different dose of green synthesized silver nanoparticles, chemically synthesized ones, the extract of Anbarnesa and silver nitrate solution, morphological changes in cell morphology and structure were observed 24 h post-treatment under 40 \times magnification (Figure 7).

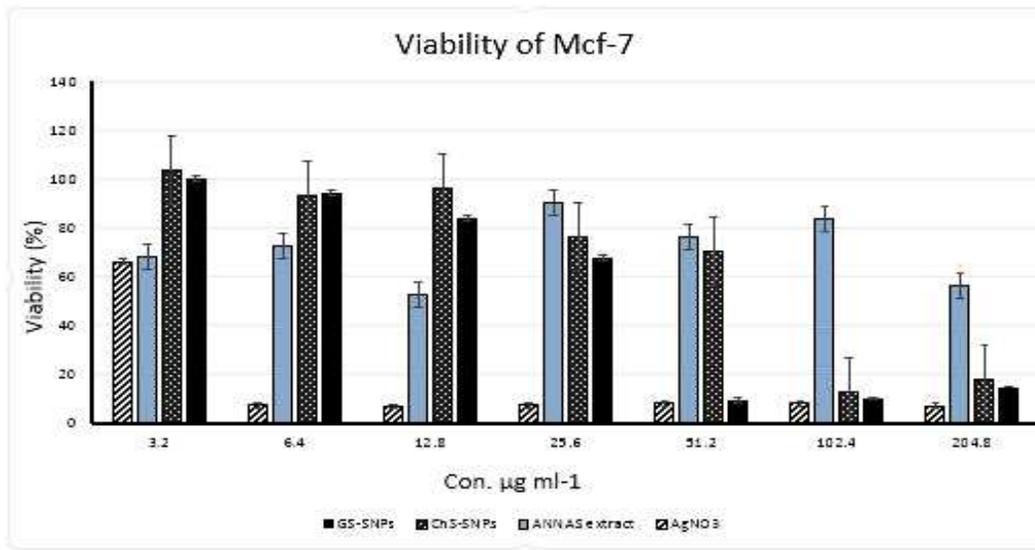


Figure 6. the viability percentage of the MCF-7 cells after incubation for 24 hours with different concentrations (3.2 µg.ml-1 to 204.8 µg.ml-1) of the green/chemically synthesized silver nanoparticles, Anbarnesa extract and silver nitrate solution.

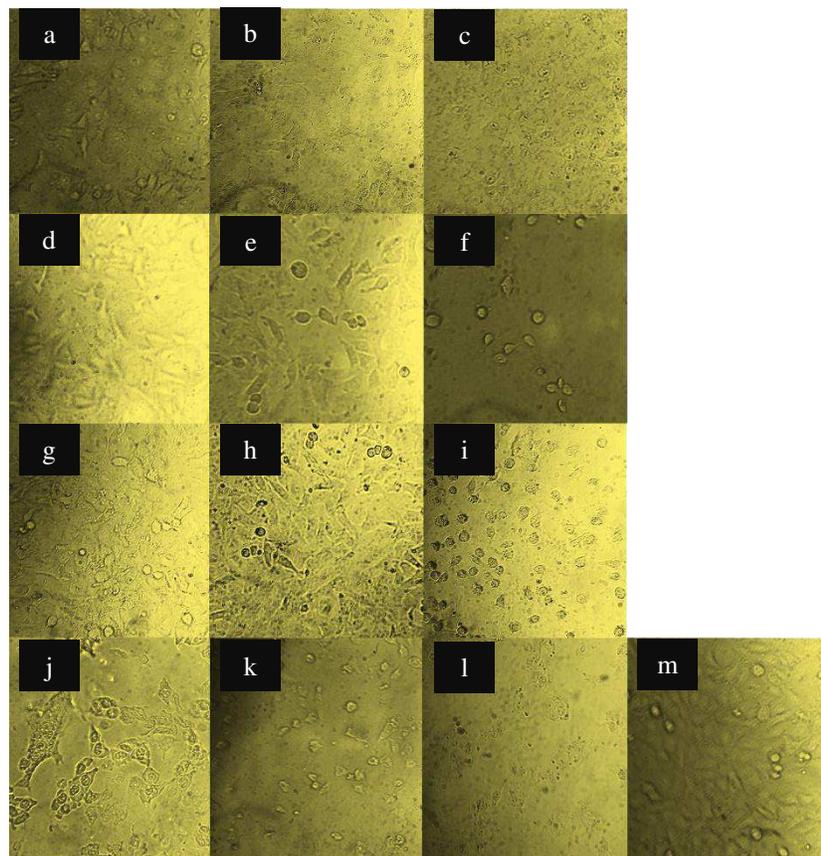


Figure 7. Morphological changes of MCF-7 cells after incubation with silver nanoparticles at the concentrations of 3.2, 25.6 and 204.8 $\mu\text{g}\cdot\text{ml}^{-1}$ for (a-c) silver nitrate solution, (d-f) Anbarnesa extract, (g-i) chemically synthesized nanoparticles and (j-l) green synthesized nanoparticles. The standard medium was prepared with no more material than culture media and the cells (m).

Discussion

One of the serious challenges for cancer therapy is related to the use of toxic agent as the anticancer factors with various sides effects. To solve the problem, novel anticancer nano-systems including metal nanoparticles have been developed (37). Production of nanoparticles due to have unusual chemical and electrical properties is the interesting area for research, especially in the field of clinical trials. Metals such as silver, which have a strong surface plasmon resonance are very important in the synthesis of nanoparticles (38, 39). Delivery of such anticancer nanoparticles to tumor interstitial spaces is based on either passive or active targeting and the passive targeting of the nanoparticles to tumor spaces can be succeeded by enhanced permeability and retention (EPR) effects (40). The size of nanoparticles plays an important role and particle diameters with average size of <200 nm have long circulation time in the bloodstream and show enhanced EPR effects (41). Therefore, particle sizes below 200 nm are offered to be ideal for anticancer systems. Concerns regarding the synthesis of these nano-systems, such as the use of chemicals precursor and toxic solvents, and the production of toxic byproducts have led to a new alternative, green synthesis approach. This environmentally friendly approach includes the use of biological agents, plants or microbes as reducing and capping agents. Green-chemistry synthesized silver nanoparticles gives a novel and possible alternative to chemically synthesized nanoparticles. This research focused on the preparation of AgNPs using an environmentally friendly, synthetic method mediated by animal waste. Several studies have shown the green synthesis of metal NPs, using aqueous plant extracts for various applications. Anbarnesa as a processed herbal material is cheaply and easily available, and there is no need to buy capping and reducing agents. Anbarnesa extracts have not been used to date in AgNP phytosynthesis. We successfully synthesized AgNPs using Anbarnesa aqueous extract, which acts as a reducing agent. As the first sign of nanoparticle formation, the light yellow AgNO_3 solution containing Anbarnesa extract changed to a dark brown color within 1 hour, whereas clear AgNO_3 solutions containing sodium citrate was changed to dark gray after the same time. The Anbarnesa mediated synthesis of AgNPs was completed within a very short time period, compared to other plant extracts (42, 43). This rapid synthesis was due to the excitation of the surface plasmon resonance effect and the reduction of metal ions into nanoscale particles (42). The absorbance of the UV-Vis spectrum gradually increased over time. however, in chemical synthesis method for nanoparticle synthesis the intensity of absorption increases suddenly showing the nucleation proceeds slow but the growing process is fast. In the XRD results, there

were few unassigned peaks which might be raised due to the crystallization of bio-organic phase or phytochemicals on the surface of the Green-AgNPs from Anbarnesa extract. Similar results were reported on the synthesis of AgNPs by leaf extracts of *Rosa rugosa* and *Hibiscus cannabinus* (44, 45). TEM images of the Green-AgNP samples reveal nanoparticles with an ellipsoidal and typical spherical morphology. The image also shows loosely bound particles created due to the effect of being treated with sonication. The approximate particle diameter was confirmed the results obtained from DLS analysis. The anti-proliferative effects of silver NPs were investigated in MCF-7 cell lines (human breast cancer cells). The most significant cancer therapeutic criteria are their effectiveness and selective toxicity (46). A drug's ability to cause cancer cell apoptosis while retaining normal cells is a crucial prerequisite for successful cancer treatment. In addition, since such agents work with the immune system to combat cancer, candidate drugs should not adversely affect the cells of the immune system. Green-AgNPs were synthesized in this study, showed good anticancer activity against breast cancer cell line, in while silver nitrate has the most anti proliferative effect on cancer cells, but due to its high toxicity against normal cells, its usage in the body would be hazardous and it's not appropriate for biomedical uses.

Conclusion

In the study aqueous extract of Anbarnesa applied to green synthesis of silver nanoparticles and its anticancer activity analyzed. The results indicated anticancer activities of dispersed green-AgNPs synthesized by extract of Anbarnesa were higher than chemically synthesized AgNPs on MCF-7 breast cancer cell line. The Anbarnesa extract containing silver nanoparticles may therefore be a possible alternative agent for the treatment of human breast cancer with decreased cytotoxicity against normal cells.

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Data openly available in a public repository that issues datasets with DOIs

References

1. Chen T, Wu W, Xiao H, Chen Y, Chen M, Li J. Intelligent Drug Delivery System Based on Mesoporous Silica Nanoparticles Coated with an Ultra-pH-Sensitive Gatekeeper and Poly(ethylene glycol). *ACS Macro Letters*. 2016;5(1):55-8.
2. Carabineiro SAC. Applications of Gold Nanoparticles in Nanomedicine: Recent Advances in Vaccines. *Molecules*. 2017;22(5).
3. Roy K, Biswas S, Banerjee P. 'Green'synthesis of silver nanoparticles by using grape (*Vitis vinifera*) fruit extract: characterization of the particles and study of antibacterial activity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2013;4(1):1271-8.

4. Kaur R, Singh J, Tripathi S. Incorporation of inorganic nanoparticles into an organic polymer matrix for data storage application. *Current Applied Physics*. 2017;17(5):756-62.
5. Abdal Dayem A, Hossain MK, Lee SB, Kim K, Saha SK, Yang G-M, et al. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. *International journal of molecular sciences*. 2017;18(1):120.
6. Fukui H. Development of new cosmetics based on nanoparticles. *Nanoparticle Technology Handbook*: Elsevier; 2018. p. 399-405.
7. Bharathi D, Josebin MD, Vasantharaj S, Bhuvaneshwari V. Biosynthesis of silver nanoparticles using stem bark extracts of *Diospyros montana* and their antioxidant and antibacterial activities. *Journal of Nanostructure in Chemistry*. 2018;8(1):83-92.
8. Fathima JB, Pugazhendhi A, Oves M, Venis R. Synthesis of eco-friendly copper nanoparticles for augmentation of catalytic degradation of organic dyes. *Journal of Molecular Liquids*. 2018;260:1-8.
9. Sharma P, Bhatt D, Zaidi M, Saradhi PP, Khanna P, Arora S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl Biochem Biotechnol*. 2012;167(8):2225-33.
10. Zhang J, Si G, Zou J, Fan R, Guo A, Wei X. Antimicrobial effects of silver nanoparticles synthesized by *Fatsia japonica* leaf extracts for preservation of citrus fruits. *J Food Sci*. 2017;82(8):1861-6.
11. Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. *Int J Nanomedicine*. 2012;7:2767-81.
12. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci*. 2014;9(6):385-406.
13. Teng Z, Luo Y, Wang Q. Carboxymethyl chitosan–soy protein complex nanoparticles for the encapsulation and controlled release of vitamin D3. *Food Chem*. 2013;141(1):524-32.
14. Cai X, Lee A, Ji Z, Huang C, Chang CH, Wang X, et al. Reduction of pulmonary toxicity of metal oxide nanoparticles by phosphonate-based surface passivation. *Part Fibre Toxicol*. 2017;14(1):13.
15. Yadi M, Mostafavi E, Saleh B, Davaran S, Aliyeva I, Khalilov R, et al. Current developments in green synthesis of metallic nanoparticles using plant extracts: a review. *Artif Cells Nanomed Biotechnol*. 2018;46(sup3):S336-S43.
16. Salunke BK, Sawant SS, Lee SI, Kim BS. Microorganisms as efficient biosystem for the synthesis of metal nanoparticles: current scenario and future possibilities. *World J Microbiol Biotechnol*. 2016;32(5):88.
17. Shanmuganathan R, MubarakAli D, Prabakar D, Muthukumar H, Thajuddin N, Kumar SS, et al. An enhancement of antimicrobial efficacy of biogenic and ceftriaxone-conjugated silver nanoparticles: green approach. *Environmental Science and Pollution Research*. 2018;25(11):10362-70.

18. Pugazhendhi A, Prabakar D, Jacob JM, Karuppusamy I, Saratale RG. Synthesis and characterization of silver nanoparticles using *Gelidium amansii* and its antimicrobial property against various pathogenic bacteria. *Microb Pathog*. 2018;114:41-5.
19. Latha M, Sumathi M, Manikandan R, Arumugam A, Prabhu N. Biocatalytic and antibacterial visualization of green synthesized silver nanoparticles using *Hemidesmus indicus*. *Microb Pathog*. 2015;82:43-9.
20. Mariselvam R, Ranjitsingh A, Nanthini AUR, Kalirajan K, Padmalatha C, Selvakumar PM. Green synthesis of silver nanoparticles from the extract of the inflorescence of *Cocos nucifera* (Family: Arecaceae) for enhanced antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2014;129:537-41.
21. Saha J, Begum A, Mukherjee A, Kumar S. A novel green synthesis of silver nanoparticles and their catalytic action in reduction of Methylene Blue dye. *Sustainable Environment Research*. 2017;27(5):245-50.
22. Vinay SP, Udayabhanu, Nagaraju G, Chandrappa CP, Chandrasekhar N. Novel Gomutra (cow urine) mediated synthesis of silver oxide nanoparticles and their enhanced photocatalytic, photoluminescence and antibacterial studies. *Journal of Science: Advanced Materials and Devices*. 2019;4(3):392-9.
23. Philip D. Honey mediated green synthesis of silver nanoparticles. *Spectrochim Acta A Mol Biomol Spectrosc*. 2010;75(3):1078-81.
24. Phae-ngam W, Thamaphat K, Chutrakulwong F, Oopathump C. One-step green synthesis of chitosan-silver nanoparticles. *Suan Sunandha Sci Technol J*. 2017;4:19-23.
25. Das S, Dhar B. Green Synthesis of Noble Metal Nanoparticles Using Cysteine Modified Silk Fibroin: Catalysis and Anti-bacterial Activity. *RSC Advances*. 2014;4.
26. Shafiee HA, Motamedi MH, Mina M, Taheri JB, Azimi S, Joharchi K, et al. Evaluation of cytotoxic effects of Anbarnesa on fibroblast L929: Can it be used as a mouthwash? *Anc Sci Life*. 2014;33(4):203-7.
27. Parvin N, Validi M, Banitalebi M, Mobini G, Ashrafi K, Farrokhi E, et al. Effect of medicinal smokes on some nosocomial infection factors. *Shahrekord-University-of-Medical-Sciences*. 2010;12(2):76-83.
28. Gao X, Lu Y, Fang L, Fang X, Xing Y, Gou S, et al. Synthesis and anticancer activity of some novel 2-phenazinamine derivatives. *Eur J Med Chem*. 2013;69:1-9.
29. McGuire S. World cancer report 2014. Geneva, Switzerland: World Health Organization, international agency for research on cancer, WHO Press, 2015. Oxford University Press; 2016.
30. Jang SJ, Yang IJ, Tettey CO, Kim KM, Shin HM. In-vitro anticancer activity of green synthesized silver nanoparticles on MCF-7 human breast cancer cells. *Materials Science and Engineering: C*. 2016;68:430-5.
31. Devi JS, Bhimba BV, Ratnam K. In vitro anticancer activity of silver nanoparticles synthesized using the extract of *Gelidiella* sp. *Int J Pharm Pharm Sci*. 2012;4(suppl 4):710-5.

32. Sadeghi-aliabadi h, Saremi m, Mirian m, Ghannadi a. Cytotoxic evaluation of medicinal smoke "Anbar Nasara" against cancer cells (Hela & KB) and a normal cell line (L929), using MTT assay. *Yafteh*. 2013;15(3):87-94.
33. Ahmadian-Attari MM, Amrollahi Z, Momeni PS, Khodaii Z, Bitab A, Bozorgi AH. Chemical Constituents of Donkey Dung (Anbarnasara): Questioning the Recent Claims Concerning Therapeutic Effects. *International Journal of Enteric Pathogens*. 2019;7(1):19-22.
34. Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari M. Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. *International journal of nanomedicine*. 2018;13:8013.
35. Mousavi-Khattat M, Keyhanfar M, Razmjou A. A comparative study of stability, antioxidant, DNA cleavage and antibacterial activities of green and chemically synthesized silver nanoparticles. *Artif Cells Nanomed Biotechnol*. 2018;46(sup3):S1022-S31.
36. van der Zande M, Undas AK, Kramer E, Monopoli MP, Peters RJ, Garry D, et al. Different responses of Caco-2 and MCF-7 cells to silver nanoparticles are based on highly similar mechanisms of action. *Nanotoxicology*. 2016;10(10):1431-41.
37. Lu J, Liong M, Zink JI, Tamanoi F. Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs. *Small (Weinheim an der Bergstrasse, Germany)*. 2007;3(8):1341-6.
38. Jensen TR, Malinsky MD, Haynes CL, Van Duyne RP. Nanosphere lithography: tunable localized surface plasmon resonance spectra of silver nanoparticles. *The Journal of Physical Chemistry B*. 2000;104(45):10549-56.
39. Tsuji T, Kakita T, Tsuji M. Preparation of nano-size particles of silver with femtosecond laser ablation in water. *Applied Surface Science*. 2003;206(1-4):314-20.
40. Iyer AK, He J, Amiji MM. Image-guided nanosystems for targeted delivery in cancer therapy. *Current medicinal chemistry*. 2012;19(19):3230-40.
41. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. *Advanced drug delivery reviews*. 2011;63(3):170-83.
42. Elavazhagan T, Arunachalam KD. Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. *Int J Nanomedicine*. 2011;6:1265-78.
43. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnol Prog*. 2006;22(2):577-83.
44. Dubey SP, Lahtinen M, Sillanpää M. Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2010;364(1-3):34-41.
45. Bindhu MR, Umadevi M. Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013;101:184-90.

46. Eastman A, Perez RP. New targets and challenges in the molecular therapeutics of cancer. *Br J Clin Pharmacol.* 2006;62(1):5-14.

Figures

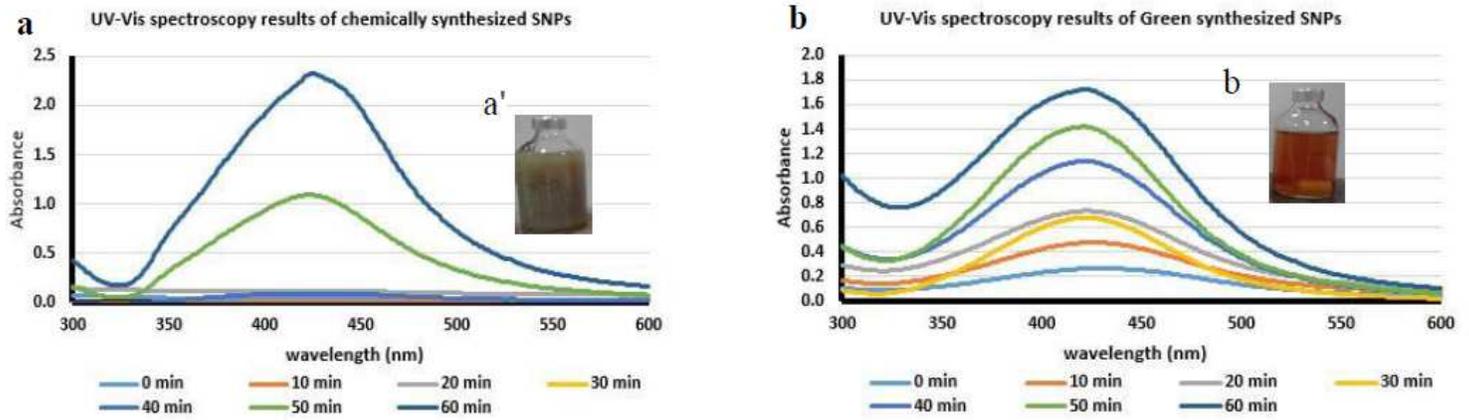


Figure 1

UV-Vis spectra of the silver nanoparticles synthesized through (a) chemical and (b) green methods. Color changes in chemical and green methods was demonstrated in a' and b' figures respectively.

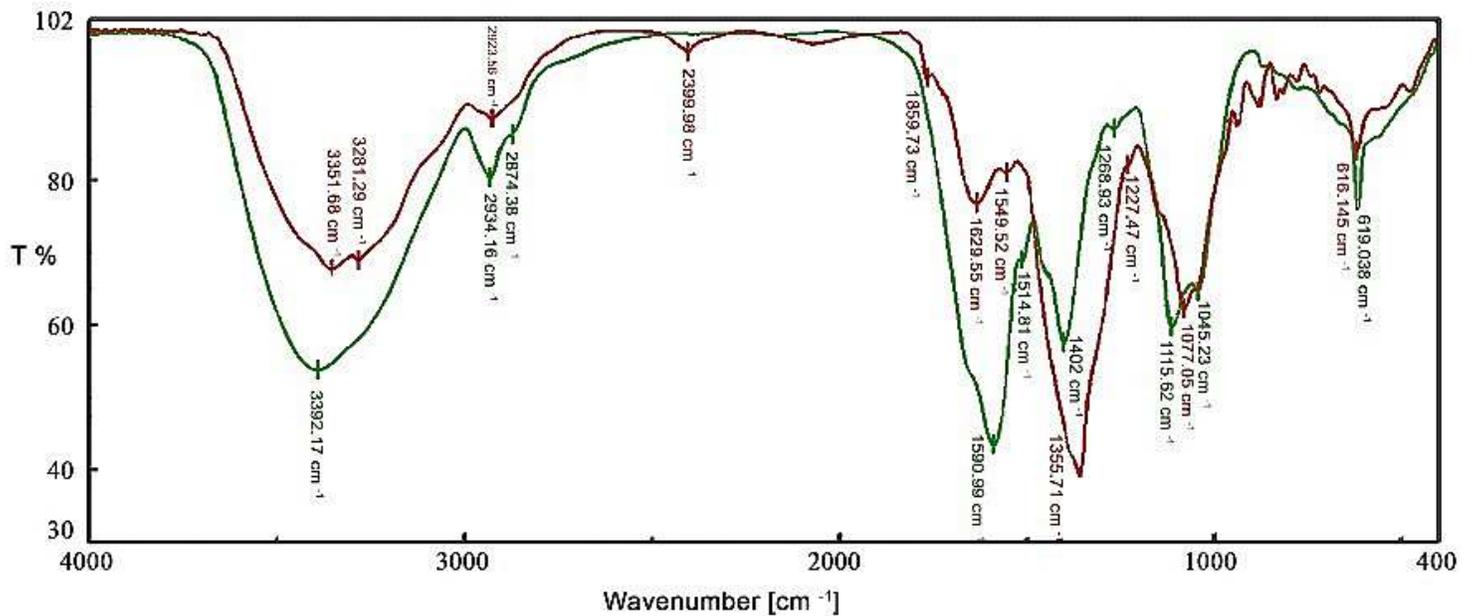


Figure 2

FTIR spectra of Anbarnesa extract (green curve) and silver nanoparticles synthesized by Anbarnesa extract (red curve).



Figure 3

(a) DLS histograms and (b) Zeta potential histograms of green synthesized AgNPs



Figure 4

X-Ray diffraction spectra of (a) chemically and (b) green synthesized AgNPs



Figure 5

TEM micrographs and particle size distribution histogram of AgNPs synthesized using Tulsi extract

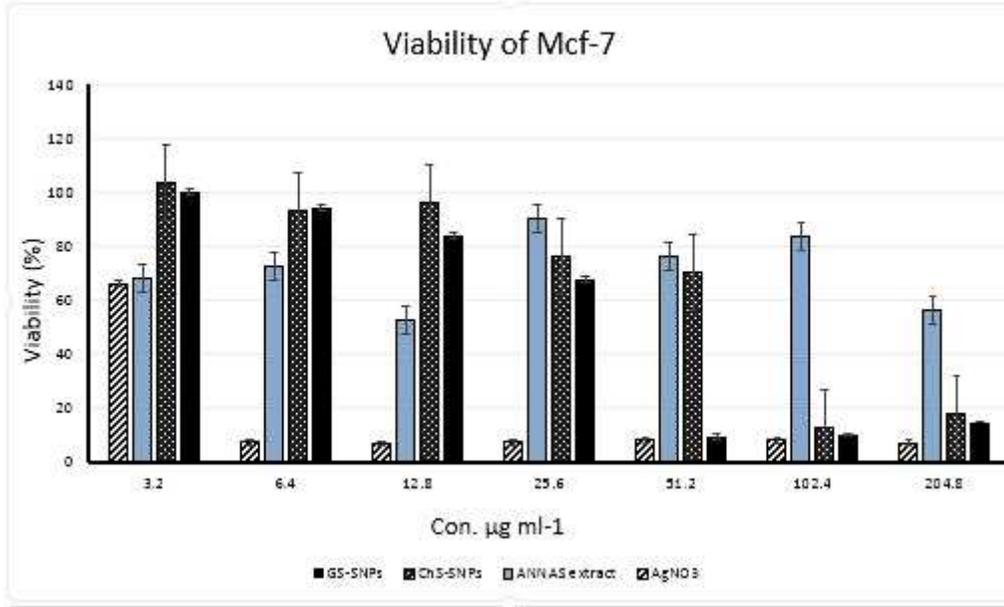


Figure 6

the viability percentage of the MCF-7 cells after incubation for 24 hours with different concentrations (3.2 $\mu\text{g.ml}^{-1}$ to 204.8 $\mu\text{g.ml}^{-1}$) of the green/chemically synthesized silver nanoparticles, Anbarnesa extract and silver nitrate solution.



Figure 7

Morphological changes of MCF-7 cells after incubation with silver nanoparticles at the concentrations of 3.2, 25.6 and 204.8 $\mu\text{g.ml}^{-1}$ for (a-c) silver nitrate solution, (d-f) Anbarnesa extract, (g-i) chemically synthesized nanoparticles and (j-l) green synthesized nanoparticles. The standard medium was prepared with no more material than culture media and the cells (m).