

RESEARCH ARTICLE

Aloe Vera gel extract and sunlight mediated synthesis of silver nanoparticles with highly effective antibacterial and anticancer activity

Noha Mohamed^{1*}, H.M. El-Masry²

¹ Biophysics Department, Faculty of Science, Cairo University, 12613 Giza, Egypt.

² Biological Evaluation of Natural and Microbial Products Unit, National Research Centre, Dokki, Giza, Egypt

ARTICLE INFO

Article History:

Received 14 Nov 2019

Accepted 21 Jan 2020

Published 1 Feb 2020

Keywords:

Aloe Vera

Sunlight

green

silver nanoparticles

anticancer

antibacterial

ABSTRACT

In this study, a single-step method for green synthesis of silver nanoparticles using Aloe Vera gel extract and sunlight was investigated. The Aloe Vera gel extract is composed of pectins, lignin, and hemicellulose, which can be used in the reduction of silver ions to produce colloidal silver nanoparticles (AgNPs-AV). The preliminary preparation of silver nanoparticles was observed by an immediate color change to brown. The prepared silver nanoparticles were characterized by transmission electron microscopy, Fourier transform infrared spectroscopy, dynamic light scattering, and zeta potential measurements. Additionally, the cytotoxicity and antimicrobial activity of AgNPs-AV was tested. Results indicated the formation of stable spherical 90±40nm with a strong absorption peak appears between 400 and 500 nm. 28.7 ±0.781% of breast cancer cells survived after incubation with AgNPs-AV invitro for 72 hrs. In addition to AgNPs-AV inhibited the growth of the most important human skin pathogens (candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus). The largest inhibition zone was observed for candida albicans. This method for synthesis is very fast, produces spherical colloidal silver nanoparticles having an excellent antioxidant activity, high anticancer therapeutic index and very promising antibacterial activity. This green synthesized nano-formulation has a great potential to be explored in many different aspects.

How to cite this article

Mohamed N., El-Masry H.M. Aloe Vera gel extract and sunlight mediated synthesis of silver nanoparticles with highly effective antibacterial and anticancer activity. J. Nanoanalysis., 2020; 7(1): 73-82. DOI: 10.22034/jna.2020.1884807.1176.

INTRODUCTION

Silver nanoparticles (AgNPs) have been studied extensively due to their wide range of applications and unique features. AgNPs is very promising in many uses include catalysis, antibacterial activity, drug delivery, anticancer effect, biosensing, imaging, antioxidant activities, anti-proliferative agents, textile fabrics and plastics to eliminate microorganisms [1-10]. The green synthesis methods for synthesis of silver nanoparticles have been proposed to become eco-friendly alternative and cost-effective compared to chemical and physical preparation methods [11-18]. Silver nanoparticles were synthesized

previously by green methods from various plant extracts including Talinum triangular leaves, blackberry fruit extract, Elicostemma axillare, Panax ginseng fresh leaves, Geranium leaves, Origanum vulgare L. Extract, Acalypha indica, Pelargonium graveolens and Aloe Vera leaves extract [19-24]

Aloe Vera leaves extract (AV) is a medicinal agent contains more than 200 active ingredients, and 75 nutrients including 20 minerals, 12 vitamins, and 18 amino acids. Aloe Vera is a natural anti-microbial, antifungal, anti-biotic, anti-septic, anti-viral, antiallergic, anti-bacterial, anti-inflammatory, immunostimulant, anti-tumor, and extremely effective anti-oxidant

* Corresponding Author Email: nmsayed@sci.cu.edu.eg

[25-27]. Also, it is a rich source of secondary metabolites including lignin, hemicellulose, and pectins which can easily reduce silver nitrate to silver nanoparticles. Aloe Vera gel extract can be used as surfactant to prevent nanoparticles aggregation through decreasing the net surface energy due to containing various chemical compositions such as amino acids, minerals, enzymes, vitamins (A, C, and E), anthraquinones, lignin, aloin, monosaccharide, polysaccharides, salicylic acid, saponins, sterols, and minerals (zinc, calcium, phosphorous, potassium, manganese, iron, copper, sodium, chromium, and magnesium) [28-30]

This study aims to efficiently prepare silver nanoparticles with large scale and low cost using plant extract and sunlight. Aloe Vera gel extract not only used as reducing agent, but also it acts as a stabilizing agent. The successful preparation of silver nanoparticles was confirmed using different characterization techniques such as UV-Vis spectroscopy, transmission electron microscopy, FTIR, zeta potential measurement, and dynamic light scattering. Additionally, the cytotoxicity and antimicrobial activity of AgNPs-AV was tested.

MATERIALS AND METHODS

Materials

Silver Nitrate (AgNO_3), 2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene BHT were purchased from Sigma-Aldrich. Fresh Aloe Vera leaves were obtained from faculty of Agriculture Cairo University.

Extraction of Aloe Vera gel

Fresh Aloe Vera leaves were collected and washed several times with distilled water in order to remove the dust particles. The gel or mucilage extracted from the fresh leaves, digested, filtered and centrifuged at 3000 rpm for 30 min, then boiled. The Aloe Vera gel extract (AV) stored at -15°C for use within one week

Preparation of silver nanoparticles

Twenty mg of AgNO_3 was added to 50ml distilled water, then 20 ml of the AV were added then exposed to sunlight for 10 min.

The final solution containing silver nanoparticles was centrifuged at 8000 rpm for 30 min and washed twice with distilled water then resuspended in 50 ml distilled water.

Characterization of the prepared nanoparticles

The morphology and size of AgNPs-AV were determined using transmission electron microscopy (TEM)(Jeol JEM1230, USA). The absorption spectrum of AgNPs-AV was measured using UV-Vis spectrophotometer (Jenway UV-6420; Barloworld Scientific, Essex, UK) in the wavelength range from 300 to 700 nm. The zeta potential and particles size distribution of the prepared AgNPs-AV was measured using (Zeta Potential/Particle Sizer Malvern panalytical, Zetasizer Nano ZS90,USA).

Fourier Transform Infrared (FTIR) was performed using (A Basic Vector, FT/IR-4100 type A, Germany) for AgNPs-AV and AV in the range of $4000-400\text{ cm}^{-1}$.

Antioxidant activity of silver nanoparticles

The antioxidant activity of silver nanoparticles was determined using DPPH radical method [31]. Briefly, 0.1 ml of AgNPs-AV was added to 1 ml of 0.1 mM of freshly prepared DPPH solution in ethanol. Then, incubated 20 min at room temp in the dark. The solutions absorbency of various tubes was measured at 517 nm. The control sample was prepared without silver nanoparticles. The positive control is 0.1 ml of BHT with the same concentration as silver nanoparticles. The free radical scavenging activity was measured as a decreasing the DPPH absorbance and calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{\text{Abs c} - \text{Abs S}}{\text{Abs C}} \times 100$$

Where, Abs c is the absorbance of the control, and Abs s is the absorbance of the sample or standard.

In vitro cytotoxicity against MCF7 breast cancer cell line

The antitumor activity of AgNPs-AV was tested against MCF7 breast cancer cell line as an example of most common human disease. MCF7 cells were plated in 96-well plates (10^6 cells per well) for 24 hrs at 37°C in 200 μl of RPMI 1640 medium containing 10% FBS to allow the attachment of the cell to the wall of the plate. (20, 40, 60, 80, 100) μl of the culture medium was discarded, then the monolayer cells were treated with (20, 40, 60, 80, 100) μl of AgNPs-AV (200 $\mu\text{g/ml}$) and AV for 72 hrs at 37°C and atmosphere of 5% CO_2 . After 72 hrs, Cells were fixed, washed and stained with Sulfo-

Rhodamine – B stain. Acetic acid was added to remove the excess stain and the attached stain was recovered using Tris EDTA buffer. The intensity of the color was measured using an Elisa reader. Each concentration was repeated five times. The cytotoxicity was expressed as the percentage of the cell viability compared to the untreated cells. The cell viability was calculated as:

$$\text{Cell Viability (\%)} = \frac{\text{Abs S}}{\text{Abs C}} \times 100$$

Where Abs S is the absorbance of the treated cells and Abs C is the absorbance of control.

Antimicrobial activity of biosynthesized nanoparticles

Twenty eight grams of the nutrient agar medium were dissolved in 1 liter of distilled water. Then bring to boil to be completely dissolved and sterilized by autoclaving at 121°C for 15 min. Qualitative evaluations were carried out in nutrient agar plates according to Mostafa et al., 2016 to investigate the antimicrobial activity of AgNPs-AV nanoparticles followed by calculating the minimal inhibitory concentration (MIC)[32-33]. Different types of Bacteria used in this study such as Gram positive bacteria (Staphylococcus aureus ATCC 6538), pathogenic yeast (Candida albicans ATCC 10231) and Gram negative bacteria (Pseudomonas aeruginosa ATCC 27853). The inoculation of all microorganisms was prepared from fresh overnight broth cultures that were incubated at 37°C [34]. The spore suspension of pathogenic strains was prepared and adjusted to approximately 0.5 McFarland standard (1×10^8 spores-ml). Then, 250.0 µL of spore suspensions were inoculated into each plate containing 50 ml of the sterile nutrient agar medium. After the media cooled and solidified, 100 µL of the prepared sample was applied to the wells (well diameter = 0.6 mm) that were prepared previously on the inoculated agar plates in triplicated times. These seeded plates were placed in the refrigerator for two hour, followed by incubation at 37 °C for 24 hrs and zones of inhibition (ZI) were measured in mm [33]. The antimicrobial activity of AgNPs-AV nanoparticles was tested with different concentrations as follows (100.0, 50.0, 25.0, 20.0, 10.0, 5.0 µg/ml) in duplicate times.

RESULTS AND DISCUSSION:

Preparing nanoparticles using physical methods require high consumption of energy on

the other hand chemical methods usually leads to remaining of some toxic substances which limit the biological applications of the prepared nanoparticles. Now many researchers synthesis different types of nanoparticles by fungi, bacteria, and plants to form biocompatible and green nanomaterials. Using plant extracts of can have a great potential over the common chemical methods of nanoparticle production due to safety, non-toxic, low costs, and compatible with the environment [12-21]

In this method, silver nanoparticles were prepared very fast using the reducing power of sunlight in addition to the reducing power of AV gel.

When silver nitrate was added to the AV gel extract in the presence of sunlight the colorless solution was turned into white. The successful preparation was observed by the color change from white to reddish brown. As the sunlight exposure time increases the intensity of color increase (Fig. 1). Which indicate the reduction of Ag⁺ ions successfully to Ag⁰ forming silver nanoparticles.

To obtain complete information about the morphology and shape of nanoparticles, TEM was used. The TEM image revealed that the prepared AgNPs-AV was predominantly spherical (Fig. 2a). TEM images reveal the successful preparation of spherical silver nanoparticles. The electron diffraction pattern (Fig. 2b) shows that AgNPs-AV nanoparticles are polycrystalline. UV-Vis absorption spectrophotometer was used to measure the absorption spectrum which shows a peak between 400-500 nm (Fig. 2c).

The average particle size distribution of AgNPs-AV was recorded to be 88.78 ± 40 nm (Fig. 3a) with a zeta potential of (-29.8) (Fig. 3b). This value of zeta potential indicates the production of stable silver nanoparticles. The aloe Vera gel extract has various molecules which lead to steric repulsion between individuals preventing nanoparticles from aggregation [28]. FTIR spectroscopy is performed to confirm the association of Aloe Vera gel active components such as phenols, proteins, and sterols with silver and their ability to act as reducing and stabilizing agent.

The AgNPs-AV exhibited absorption peaks at 1059, 1383, and 1632, 2373, 2929, 3407 cm⁻¹ due to having cyclic OCH₃, C=O, C–OOH, S-H, CH₂ and OH functional groups, respectively (Fig.4b).

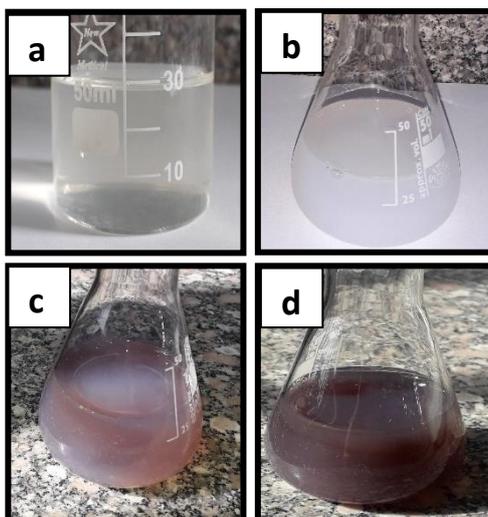


Fig. 1: Photographic image of a) AV gel extract, b) The reaction mixture after the addition of silver nitrate solution, c) the reaction mixture after 5 min of reaction in sunlight, and d) the final solution after 10 min exposure to sunlight.

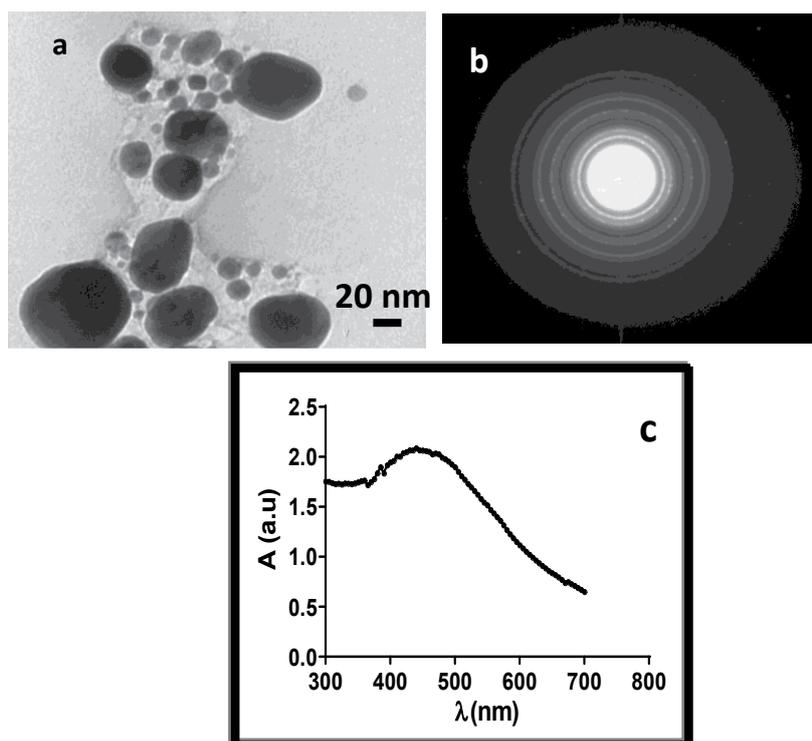


Fig. 2: a) Transmission electron microscope image of (AgNPs-AV), b) SAED pattern of AgNPs-AV and c) its UV-Vis absorption spectrum.

These characteristic peaks were compared with AV gel extract (Fig. 4a) showed bands at wavenumber (cm^{-1}) 1069, 1253, 1578, 2375, 2928, 3423 and slight shift were seen in the case

of silver nanoparticles. From the FTIR spectra, it was confirmed that Aloe Vera gel extract act as a reducing agent for the synthesis of colloidal silver nanoparticles. Further, the Aloe Vera gel extract

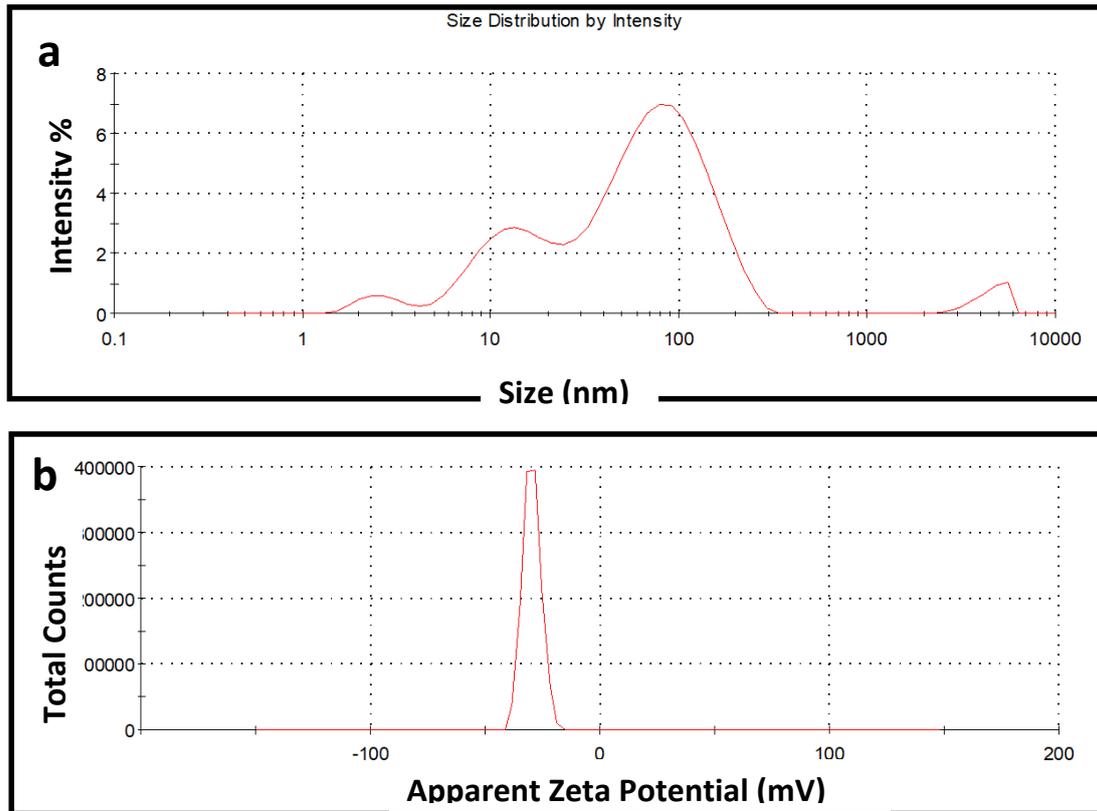


Fig. 3: Dynamic light scattering size distribution of the prepared AgNPs-AV (a) and its Zeta potential (b).

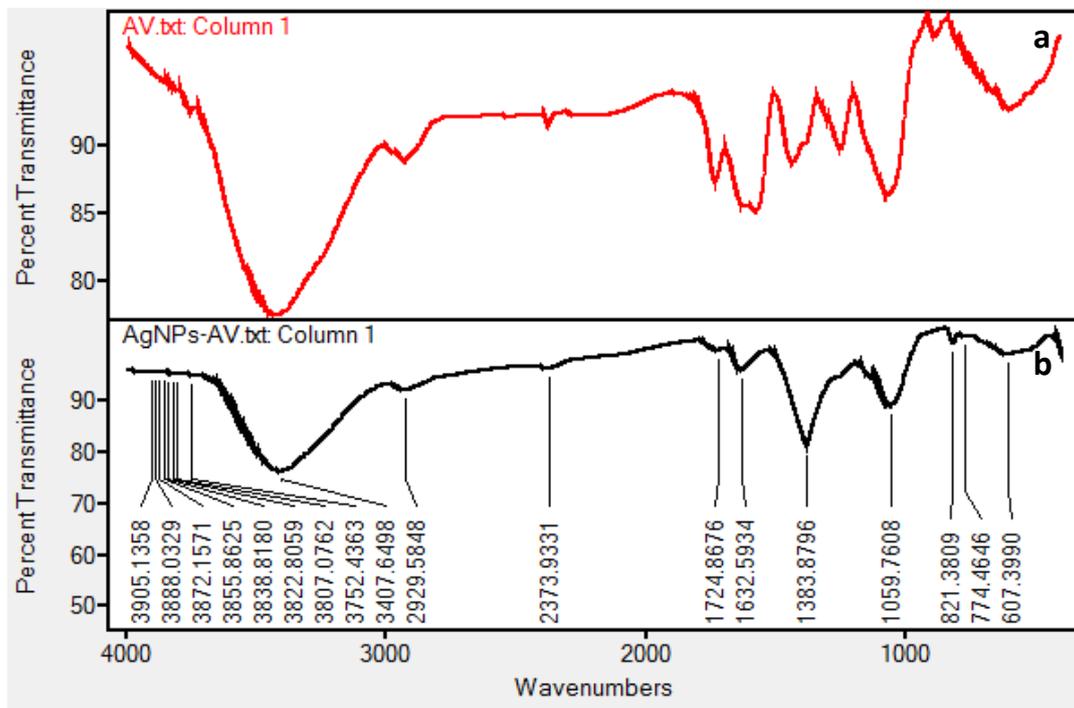


Fig. 4: FTIR spectrum of AV a) and AgNPs-AV b).

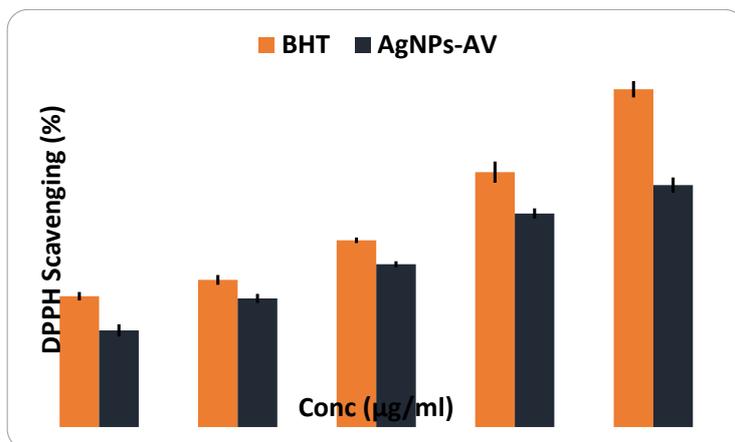


Fig. 5: Measurement of antioxidant activity of AgNPs-AV and BHT data represent as mean \pm standard deviation.

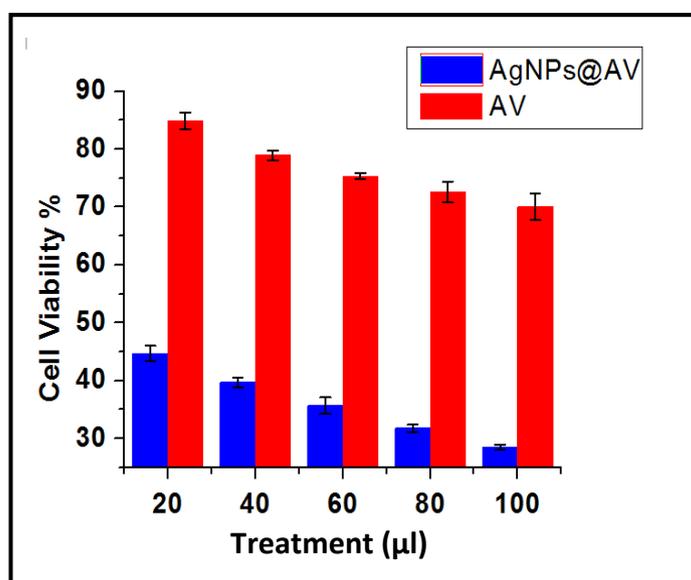


Fig. 6: Cell viability percent of MCF7 breast cancer cell line treated with different volumes of AgNPs-AV (200 µg/ml) and AV gel extract.

forms biological capping layer around silver nanoparticles that prevented the agglomeration of the nanoparticles [30].

The effect of various concentrations of AgNPs-AV on DPPH free radical scavenging activity is shown in Fig. 5. Both AgNPs-AV and standard BHT has inhibitory activity against DPPH radical. As the concentration of silver nanoparticles increases the free radical scavenging activity increases. AgNPs-AV exhibit antioxidant activity of 48.99% at 200 µg/ml. However, the BHT showed 68% inhibition in the same concentration. This DPPH scavenging

activity of AgNPs-AV is due to the donation of hydrogen of antioxidant molecules coating the green synthesized silver nanoparticles.

The in-vitro antitumor activity of silver nanoparticles was tested for against human MCF7 breast cancer cell line. The results indicate that both AV and AgNPs-AV showed anticancer activities against the breast cancer cell line. This potential anticancer therapeutic agent AgNPs-AV can inhibit cancer cells growth in vitro (Fig. 6). At the highest volume 100 µl of AgNPs-AV (200µg/ml) 28.7 \pm 0.781% of the cells survive. Previous studies reported that silver

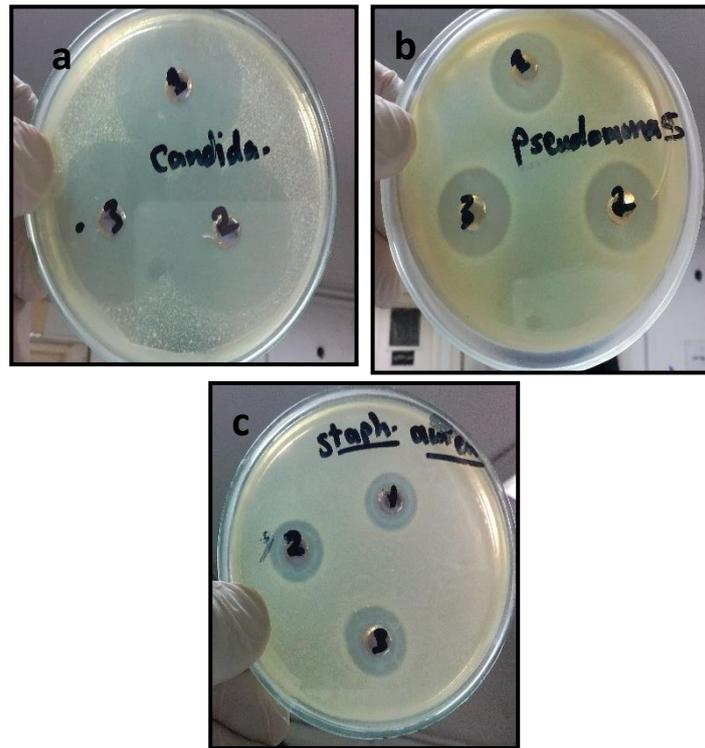


Fig. 7: Antimicrobial screening of AgNPs-AV against *Candida albicans* (a), *Pseudomonas aeruginosa* (b) and *Staphylococcus aureus* (c).

nanoparticles induce DNA damage in MCF7 cells by generating ROS. The generated ROS promote cell damage by attacking cellular components, including proteins, DNA, and lipids, to induce oxidative stress which in turn causes damage to cancer cells. Apoptosis signal is induced by the silver nanoparticles via a caspase-dependent pathway along with the involvement of mitochondria. Caspase 9/3 can be activated by silver nanoparticles by a time-dependent effect that stimulated by the disruption of the membrane of mitochondrial [35-36]. On the other hand, this anticancer activity of silver nanoparticles may be due to Ag^+ ions is similar to Ca^{+2} ions this means that high amount of Ag^+ ions can inhibit the sarcoplasmic reticulum which leads to cell death [37].

Silver nanoparticles were known to have a strong antimicrobial effect, it is very important to assess the antimicrobial effect of this green synthesized formulation against important microbes which cause severe infections in human skin during injuries or burns. We select 3 species of microbes for the antimicrobial screening of silver

nanoparticles. *Candida albicans* was selected as pathogenic yeast, which causes a series of health threat. *Staphylococcus aureus* was chosen as gram-positive bacteria which can infect the skin and respiratory tract. In addition to *Pseudomonas aeruginosa* was chosen as a gram a negative bacterium which is common in skin infections. The results confirmed that the antibacterial activity was present in AgNPs-AV. An inhibition zone of 40mm was observed for *Candida albicans* (Fig. 7a). In case of *Pseudomonas aeruginosa* and *Staphylococcus aureus* the observed inhibition zones were 25 ± 1 mm (Fig. 7b) and 17.6 ± 0.57 mm (Fig. 7c) respectively.

The difference in the antibacterial effect of the preparation AgNPs-AV in different types of pathogenic bacteria was attributed to the difference in the structure and compositions of the bacterial cell walls. Previously reported works describe that the released silver ion from colloidal silver nanoparticles was responsible for its excellent antimicrobial activity [38]. The released silver ions will bind with the thiol groups of enzymes, which caused enzymes inhibition [39]. As the concentration of AgNPs-AV nanoparticles

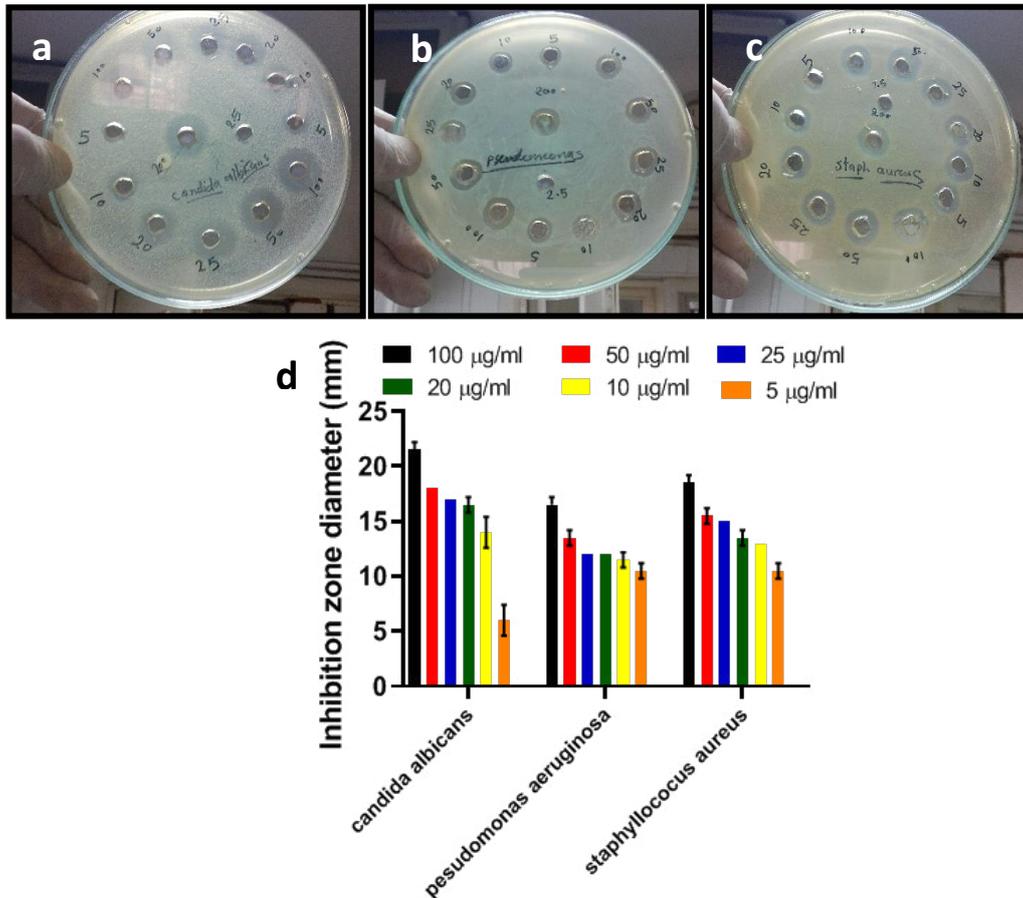


Fig. 8: photographic image of different concentrations of AgNPs-AV (100.0, 50.0, 25.0, 20.0, 10.0, 5.0 and 2.5µg/ml) in duplicated times for *Candida albicans* (a), *Pseudomonas aeruginosa* (b) and *Staphylococcus aureus* (c) and graphical representation of the obtained results (d).

increased the diameter of inhibition zone increased (Fig. 8). Due to the distinctive physicochemical and antimicrobial properties AgNPs-AV nanoparticles, they can be used for treatment of skin burns or injury.

CONCLUSION

The above study presents a novel and easy procedure to prepare AgNPs-AV. The aloe Vera gel extract was acting as a reducing agent and capping legend under the exposure of sunlight. AgNPs-AV provides a high therapeutic index for the treatment of breast cancer. Besides, it has very promising antimicrobial activity against different human pathogens such as gram positive (*Staphylococcus aureus*), pathogenic yeast (*Candida albicans*) and gram negative bacteria

(*Pseudomonas aeruginosa*). So by applying this new greenly synthesized formulation, we can reduce the use of chemotherapeutic agents for the treatment of breast cancer. In addition, applying this formulation to injured skin or burned skin can induce very high therapeutic efficacy after localized application. That may replace the use of antibiotics due to the increase in the number of resistant bacterial strains every year.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Biophysics faculty of science Cairo University for providing chemical and give all the lab facility do this research work and characterization of all the sample also.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

There is no funding source

This article doesn't contain any studies with human participants or animals performed by any of the authors

CONTRIBUTORS

Noha Mohamed prepares, characterizes silver nanoparticles and writes the manuscript. H.M. El-Masry performs the antimicrobial experiments. All authors have approved the final article.

REFERENCES

- [1] Xiao-Yun Dong, Zi-Wei Gao, Ke-Fang Yang, Wei-Qiang Zhang, Li-Wen Xu. Nanosilver as a new generation of silver catalysts in organic transformations for efficient synthesis of fine chemicals. *Catalysis Science & Technology*. 2015, 5 (5): 2554-2574.
- [2] Ning Wang, Yunxia Hu, Zongren Zhang. Sustainable catalytic properties of silver nanoparticles supported montmorillonite for highly efficient recyclable reduction of methylene blue. *Applied Clay Science*. 2017, 150: 47-55.
- [3] Zenon Foltynowicz, Daniela Gwiazdowska, Dorota Rodewald, Agnieszka Nowaczyk, Marian Filipiak. Antimicrobial properties of socks protected with silver nanoparticles. *Fibres & Textiles in Eastern Europe*. 2013, 5 (101): 91-96.
- [4] Ardhendu Mandal. Silver Nanoparticles as Drug Delivery Vehicle against Infections. *Global Journal of Nanomedicine*. 2017 3(2): 1-4.
- [5] Benyettou, F., R. Rezugui, F. Ravaux, T. Jaber, K. Blumer, M. Jouiad, L. Motte et al. Synthesis of silver nanoparticles for the dual delivery of doxorubicin and alendronate to cancer cells. *Journal of Materials Chemistry B*. 2015, 3(36): 7237-7245.
- [6] Paige K Brown, Ammar T. Qureshi, Alyson N. Moll, Daniel J. Hayes, W. Todd Monroe. Silver nanoscale antisense drug delivery system for photoactivated gene silencing. *ACS nano*. 2013, 7(4): 2948-2959.
- [7] Parisa Sistan, Leila Sofimaryo, Zahra Rakhshan Masoudi, Arezou Sayad, Roya Rahimzadeh, and Bahareh Salehi. A penicillin biosensor by using silver nanoparticles. *International Journal of Electrochemical Science*. 2014, (9): 6201-6212.
- [8] Maharashtra K. Rai, S. D. Deshmukh, A. P. Ingle, A. K. Gade. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *Journal of applied microbiology*. 2012, 112(5): 841-852.
- [9] Asharani, P. V., M. Prakash Hande, Suresh Valiyaveetil. Anti-proliferative activity of silver nanoparticles. *BMC cell biology*. 2009, 10(1): 65.
- [10] Mahendra Rai, Alka Yadav, Aniket Gade. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*. 2009, 27(1): 76-83.
- [11] Xiangqian Li, Huizhong Xu, Zhe-Sheng Chen, Guofang Chen. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials* 2011, 2011.
- [12] Fouad K. Alsammaraie, Wei Wang, Peng Zhou, Azlin Mustapha, Mengshi Lin. Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. *Colloids and Surfaces B: Biointerfaces*. 2018, 171: 398-405.
- [13] Naumih Noah. Green synthesis: Characterization and application of silver and gold nanoparticles. In *Green Synthesis, Characterization and Applications of Nanoparticles*, Elsevier, 2019: 111-135.
- [14] Shakeel Ahmed, Mudasir Ahmad, Babu Lal Swami, Saiqa Ikram. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of advanced research*. 2016, 7(1): 17-28.
- [15] Elias de Barros Santos, Natiara Vaughn Madalossi, Fernando Aparecido Sigoli, Italo Odone Mazali. Silver nanoparticles: green synthesis, self-assembled nanostructures and their application as SERS substrates. *New Journal of Chemistry*. 2015, 39(4): 2839-2846.
- [16] Ramachandran Rajan, Krishnaraj Chandran, Stacey L. Harper, Soon-Il Yun, P. Thangavel Kalaiichelvan. Plant extract synthesized silver nanoparticles: an ongoing source of novel biocompatible materials. *Industrial Crops and Products*. 2015, 70: 356-373.
- [17] Sista Kameswara Srikar, Deen Dayal Giri, Dan Bahadur Pal, Pradeep Kumar Mishra, Siddh Nath Upadhyay. Green synthesis of silver nanoparticles: a review. *Green and Sustainable Chemistry*. 2016, 6(1): 34.
- [18] Morteza Yousefzadi, Zohreh Rahimi, Vahid Ghafari. The green synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized from green alga *Enteromorpha flexuosa* (wulfen) J. Agardh. *Materials Letters*. 2014, 137 : 1-4.
- [19] Oluwafemi Adeleke Ojo, Babatunji Emmanuel Oyinloye, Adebola Busola Ojo, Olakunle Bamikole Afolabi, Omolara Abosede Peters, Oluranti Olaiya, Adewale Fadaka, Johnson Jonathan, Oluronke Osunlana. Green synthesis of silver nanoparticles (AgNPs) using *Talinum triangulare* (Jacq.) Willd. leaf extract and monitoring their antimicrobial activity. *Journal of Bionanoscience*. 2017, 11(4) : 292-296.
- [20] Brajesh Kumar, Kumari Smita, Luis Cumbal, Alexis Debut. Green synthesis of silver nanoparticles using Andean blackberry fruit extract. *Saudi Journal of Biological Sciences*. 2017, 24(1): 45-50.
- [21] Shani Raj, Suresh Chand Mali, Rohini Trivedi. Green synthesis and characterization of silver nanoparticles using *Enicostemma axillare* (Lam.) leaf extract. *Biochemical and Biophysical Research Communications*. 2018, 503(4): 2814-2819.
- [22] Verónica Castro-Aceituno, Sungeun Ahn, Shakina Yesmin Simu, Priyanka Singh, Ramya Mathiyalagan, Hyun A. Lee, Deok Chun Yang. Anticancer activity of silver nanoparticles from *Panax ginseng* fresh leaves in human cancer cells. *Biomedicine & Pharmacotherapy*. 2016, 84: 158-165.
- [23] Mohammed Rafi Shaik, Mujeeb Khan, Mufsir Kuniyil, Abdulrahman Al-Warthan, Hamad Z. Alkhatlan, Mohammed Rafiq H. Siddiqui, Jilani P. Shaik et al. Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities. *Sustainability*. 2018, 10(4): 913.
- [24] Patcharaporn Tippayawat, Nutthakritta Phromviyo,

- Parichart Boueroy, Apiwat Chompoosor. Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. *PeerJ*.2016, 4: e2589.
- [25] Rafael Minjares-Fuentes, Antoni Femenia. Aloe vera. In *Nonvitamin and Nonmineral Nutritional Supplements*, pp. 145-152. Academic Press, 2019.
- [26] Dalia I. Sánchez-Machado, Jaime López-Cervantes, Raquel Sendón, Ana Sanches-Silva. Aloe vera: Ancient knowledge with new frontiers. *Trends in Food Science & Technology*. 2017, 61: 94-102.
- [27] Reynolds, T., A. C. Dweck. Aloe vera leaf gel: a review update. *Journal of ethnopharmacology*. 1999, 68 (1-3): 33-37.
- [28] Vélez, Ederley, Gloria Campillo, Gladis Morales, César Hincapié, Jaime Osorio, Oscar Arnache. Silver nanoparticles obtained by aqueous or ethanolic aloe Vera extracts: An assessment of the antibacterial activity and mercury removal capability. *Journal of Nanomaterials*. 2018, 2018.
- [29] Rubina Lawrence, Priyanka Tripathi, Ebenezer Jeyakumar. Isolation, purification and evaluation of antibacterial agents from Aloe vera. *Brazilian Journal of Microbiology*. 2009, 40 (4): 906-915.
- [30] Khan, A., A. Shaheen, T. Mahmood, W. Rehman. Yaqoob. Novel Synthesis and Characterization of Silver Nanoparticles from Leaf Aqueous Extract of Aloe Vera and their Anti-Microbial Activity. *Journal of Nanoscience and Nano-technology Applications*. 2017, 1: 103.
- [31] Arumugam Sudha, Jeyaraman Jeyakanthan, and Pappu Srinivasan. Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. *Resource-Efficient Technologies*. 2017, 3 (4): 506-515.
- [32] Anand Kumar Keshari, Ragini Srivastava, Payal Singh, Virendra Bahadur Yadav, Gopal Nath. Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *Journal of Ayurveda and Integrative Medicine*. 2020, 11(1):37-44.
- [33] Faten A. Mostafa, Abeer A. Abd El Aty, Eman R. Hamed, Basma M. Eid, Nabil A. Ibrahim. Enzymatic, kinetic and anti-microbial studies on *Aspergillus terreus* culture filtrate and *Allium cepa* seeds extract and their potent applications. *Biocatalysis and Agricultural Biotechnology*. 2016, 5: 116-122.
- [34] Weam S El-Serwy, Neama A. Mohamed, Walaa S. El-Serwy, Emad MM Kassem, Abeer A. Abd El Aty. Synthesis of new benzofuran derivatives and evaluation of their antimicrobial activities. *Research Journal of Pharmaceutical Biological and Chemical Sciences*. 2015, 6(3): 213-224.
- [35] Rajesh Kotcherlakota, Sourav Das, Chitta Ranjan Patra. Therapeutic applications of green-synthesized silver nanoparticles. In *Green Synthesis, Characterization and Applications of Nanoparticles*, pp. 389-428. Elsevier, 2019.
- [36] Chun Gon Kim, Verónica Castro-Aceituno, Ragavendran Abbai, Hyun A. Lee, Shakina Yesmin Simu, Yaxi Han, Joon Hurh, Yeon-Ju Kim, Deok Chun Yang. Caspase-3/ MAPK pathways as main regulators of the apoptotic effect of the phyto-mediated synthesized silver nanoparticle from dried stem of *Eleutherococcus senticosus* in human cancer cells. *Biomedicine & Pharmacotherapy*. 2018, 99: 128-133.
- [37] Khan, Y., M. Numan, M. Ali, A. T. Khali, T. Ali, N. Abbas, and Z. K. Shinwari. Bio-synthesized silver nanoparticles using different plant extracts as anti-cancer agent. *Journal of Nanomedicine Biotherapeutic Discovery* 2017, 7(154): 2.
- [38] Qing Ling Feng, Jian Wu, G. Q. Chen, F. Z. Cui, T. N. Kim, J. O. Kim. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Materials Research*. 2000, 52(4): 662-668.
- [39] Yongqiang Zhang, Xinfeng Cheng, Yucang Zhang, Xinghua Xue, Yunzhi Fu. Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2013, 423 : 63-68.