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Green synthesis of silver nanoparticles using *Portulacaria afra* plant extract: characterization and evaluation of its antibacterial, anticancer activities

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Abstract

Applications of nanotechnology in different areas of research have expanded over the last years. Silver nanoparticles (AgNPs) have beneficial effects as antimicrobials, antioxidants and/or anticancer. Yet, one of the major limitations of their use was employing toxic chemicals as reducing agents. Biosynthesis was advantageous over the physical and chemical synthesis. The obtained nanoparticles were characterized using the High-Resolution Transmission Electron Microscope. Disc diffusion method was used to evaluate the antibacterial activity of AgNPs against *Bacillus subtilis*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis*. Cytotoxic activity of biosynthesized AgNPs was tested against humane breast cancer cell line (MCF-7). Results of characterization showed that AgNPs were irregular spherical in shape, with average diameter of 27.41nm, and width of 4.36nm. The antibacterial assay showed that *Portulacaria afra* extract had no inhibitory potential against the tested bacteria. However, both $AgNO_3$ and AgNPs exhibited recognized inhibitory potency against all tested bacteria. AgNPs exhibited wider inhibition zones than $AgNO_3$. Cytotoxicity test revealed that green synthesized AgNPs had inhibitory activity against cancer cell line (MCF-7) which was concentration dependent, with IC_{50} of 75.40 µmole. The aims of the present work were to study the possible green synthesis of AgNPs using *P. afra* aqueous leaf extract as a reducing agent; to characterize them, to investigate the antibacterial potency and cytotoxic potential of these biosynthesized AgNPs.

Keywords: Silver nanoparticles, *Portulacaria afra*, Biosynthesis, Antibacterial, Anticancer activity

1. Introduction

Applications of nanotechnology in different areas of research have expanded over the last years, and scientists are continuously exploring all news about this technology. Nanomaterials have been reported to have unique characteristics including; their size, shape distribution, and morphology,

which allowed them to have several applications. Among different types of nanomaterials; silver nanoparticles (AgNPs) were interestingly studied for their beneficial effects when used as antimicrobials, antioxidants and/or anticancer compounds. In addition to their medical uses, AgNPs were also used in clothing, food industry, paints, electronics and other fields (Abdel-Aziz *et al*., 2014).

AgNPs can be synthesized by several physical and chemical methods (Roopan *et al*., 2013) such as; UV or microwave irradiation, chemical reduction, photochemical method, electron irradiation and sonoelectrochemical method (Suman *et al*., 2013). However, the major limitations of these methods include high cost, employ of toxic chemicals as reducing and stabilizing agents, use of organic solvents or non-biodegradable agents (Zhang *et al*., 2013). According to Jagtap and Bapat, (2013), biosynthesis of nanoparticles was advantageous over the physical and chemical methods; as it was clean, non-toxic, involve the use of an ecofriendly renewable high energy efficient materials, benign reaction media, non-hazardous as well as non-toxic solvents. In last few years, the potential use of various plants extracts such as; banana, hibiscus, geranium leaves, Cinnamomum, *Aloe vera*, basil tansy fruits, and sweet pepper for the green synthesis of AgNPs were investigated (Malabadi *et al*., 2012).

Remya *et al*., (2015) pointed out that plants serve as readily available sources of bioactive compounds including; alkaloids, amino acids, flavonoids, terpenoids and other phenolic intermediates; that could act as effective reducing agents for the bio-reduction of metals into nanoparticles (NP), which in turn have a wide range of biological applications. *P. afra* L. plant is a succulent member of the Didereaceae (formerly of the Portulacaceae), which was native to South Africa and commonly found in semi-arid areas. *P. afra* called elephant's food; has small succulent leaves. This plant can grow up to 2–5m in height as a large woody shrub, or small tree (Guralnick and Gladsky, 2017).

2. Materials and methods

2.1. Collection of *P. afra* leaf samples and chemicals

Fresh leaves of *P. afra* (Fig. 1) were collected from local garden in Giza governorate, Egypt. The leaves were washed with deionized water, and then allowed to dry at room temperature. In order to prepare the extract, fresh leaves were cut into small pieces. All chemicals used were of analytical grade purchased from Sigma-Aldrich (USA).

Fig. 1. Leaves of *P. afra* plant

2.2. Preparation of plant leaf extract

The extract was prepared by weighing 2.5 g of fresh leaves in 250 ml of deionized water, and then boiling them for 30 min. on a hot plate with magnetic stirrer. The resulting solution was cooled and filtered using a filter paper Whatman No. 1. The extract was colorless and clear, with pH 4.5. The extract solution was stored at 4°C until further use as a reducing agent (Suman *et al*., 2013).

2.3. Biosynthesis of AgNPs

The biosynthesis of AgNPs was performed by adding 1ml of *P. afra* extract to 30 ml of $AgNO_3$ (1mM) solution according to the method described by El-Chaghaby and Ahmad, (2011) with some modifications. This mixture was boiled with continuous stirring for 5 min. Formation of AgNPs was monitored using a UV–vis spectrophotometer (SpecorD 250 plus- Analytik Jena) in the wavelength range of 200–800 nm.

2.4. Characterization of biosynthesized AgNPs

AgNPs biosynthesized using *P. afra* extract was characterized using High-Resolution Transmission Electron Microscope (HR-TEM, Tecnia G20, FEI, Netherlands), and particles size distribution was determined by laser diffractometer using Zeta Sizer nano-series (Nano ZS).

2.4. Antibacterial potential of AgNPs

Disc diffusion method was adopted to evaluate the antibacterial activity of biosynthesized AgNPs. The assay was done following the procedure described by Malabadi *et al*., (2012). Six bacterial species were tested including *B. subtilis*, *E. coli*, *N. gonorrhoeae*, *P. aeruginosa*, *S. aureus* and *Strept. faecalis*, obtained from Microbiology unit at Microanalytical center, Cairo University. In this experiment, filter paper discs (1 cm) were soaked separately in one of the following solutions: 1-*P. afra* extract (10 mg/ ml), 2-$AgNO_3$ (1mM), and 3-AgNPs (1 mM). 0.1 ml culture suspension of each bacterium adjusted to 10^8 cells/ ml, was seeded separately into Muller-Hinton agar medium, and then poured into Petri plates. One filter paper disc from each treatment was placed aseptically at the center of each of these plates. Plates were incubated at 37°C for 24 h. Three replicate plates were used for each treatment. Zones of inhibition of bacterial growth were measured around each disc and recorded in mm. (El-Chaghaby *et al*., 2014).

2.5. Cytotoxic activity of AgNPs

The human breast cancer cell line (MCF-7) was obtained from Egyptian national cancer institute, Cairo University, Egypt. The cytotoxic activity of AgNPs against this breast cancer cell line (MCF 7) was measured using 3-[4,5-dimethylthiazol-2-yl]2,5-di-phenyltetrazolium bromide (MTT) assay as described by (Chandrasekaran *et al*., 2016). The % cell inhibition and % cell viability were determined using the following formulas:

% cell inhibition = 100 – Abs_{570nm} (sample)/ Abs_{570nm} (control) × 100.

% cell viability = (Abs_{450nm}treatment− Abs_{450nm}blank)/ (Abs_{450nm}control − Abs_{450nm}blank) × 100%

The concentration of AgNPs showing 50% inhibition of cell viability (IC_{50} values) was obtained from the plot of viability percentage against AgNPs concentrations (µmole).

2.6. Statistical analysis

The data were statistically analyzed using one way ANOVA and the means were compared by Duncan's test at ($p < 0.05$).

3. Results and Discussion

3.1. Biosynthesis of AgNPs

Reduction of $AgNO_3$ solution after 5 min. from adding *P. afra* extract was visually noticed; where the colorless solution turned to yellowish brown in color which indicated extracellular synthesis of NP in accordance with Husseiny *et al*., (2015). To confirm the formation of AgNPs; UV-visible absorption pattern (Fig. 2) showed the characteristic surface plasma resonance of colloidal AgNPs which ranged between 300-500 nm, and exact peak was observed at 420 nm. AgNPs exhibited interesting optical properties directly related to their Localized surface plasma resonance (LSPR); which was highly dependent on the morphology of these NP. In a similar earlier study of El-Chaghaby and Ahmad, (2011), a typical plasma resonance band of AgNPs was confirmed by an absorption band in the range of 400- 450 nm.

3.2. Characterization of biosynthesized AgNPs

HR-TEM image of the biosynthesized AgNPs were shown in Fig. (3a). This technique was employed to visualize the morphology and shape of the synthesized AgNPs. The AgNPs were irregular, spherical in shape and with different sizes.

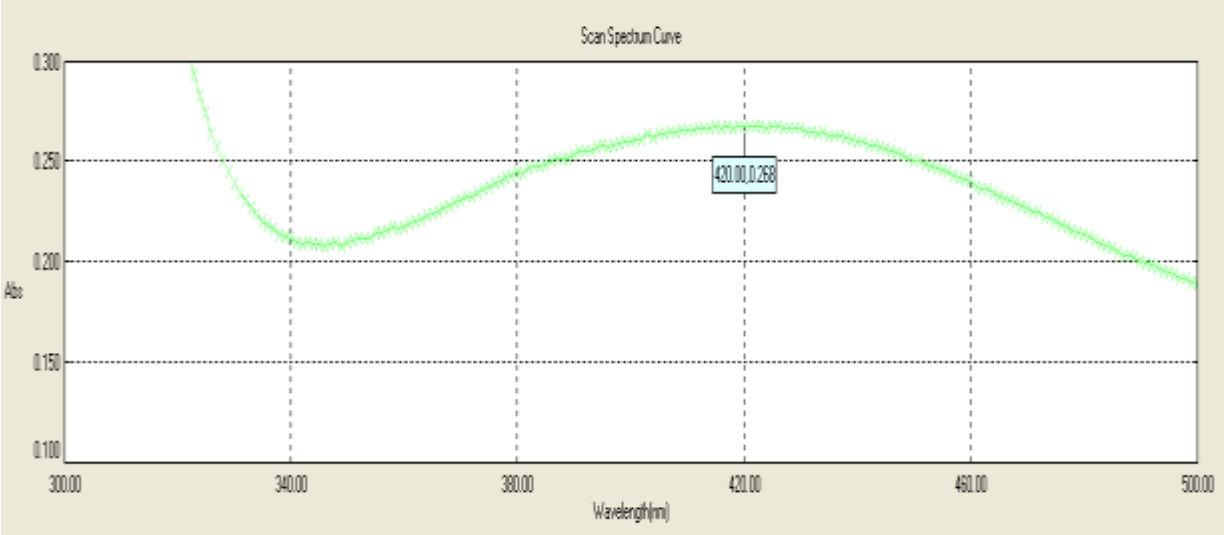
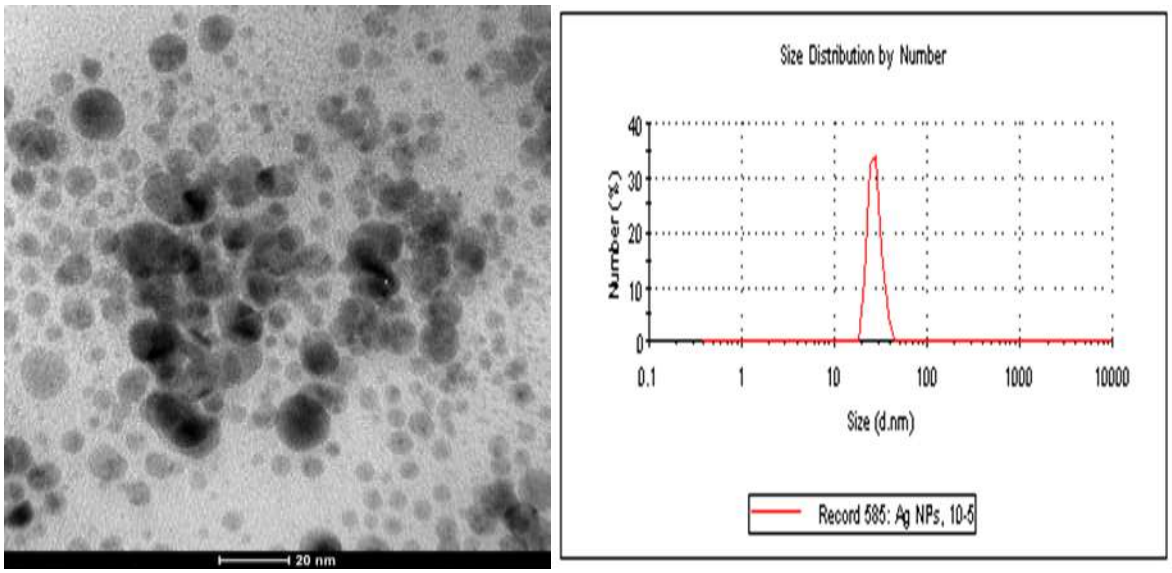


Fig. 2. UV-absorption spectra of AgNPs biosynthesized using *P. afra* leaf extract



(a) (b)

Fig. 3: (a) TEM image, (b) Particle size distribution of AgNPs

The AgNPs size distribution was shown in Fig. (3b); where, 99.8% of these NP had average diameters of 27.41 nm, and width of 4.36 nm.

3.3. Antibacterial potency of AgNPs

Results of detecting the antibacterial potential of AgNPs were shown in Table (1). The *P. afra* leaf extract had no inhibitory effect against the six studied bacterial spp. On the other hand, $AgNO_3$ and AgNPs exhibited significant inhibition against all tested bacteria. The inhibition zones observed due to activity of AgNPs were found to be wider than those caused by $AgNO_3$. In reference to Zhang *et al*., (2013), this was attributed to the fact that the surface of NP could form a layer of water,

Table 1: Antibacterial potential of plant extract, $AgNO_3$ and AgNPs against some bacterial spp.

Bacterial isolates (Gram reaction)	Inhibition zones (mm)		
	***P. afra* extract** (10 mg/ ml)	**$AgNO_3$** (1 mM)	**AgNPs** (1 mM)
B. subtilis **(G^+)**	none	12^{c}	21^{ab}
S. aureus **(G^+)**	none	11^{d}	20^{bc}
Strept. faecalis **(G^+)**	none	12^{c}	19^{c}
E. coli **(G^-)**	none	14^{ab}	22^{a}
N. gonorrhea **(G^-)**	none	15^{a}	22^{a}
P. aeruginosa **(G^-)**	none	13^{bc}	20^{bc}

-Data presented were mean of three replicates, and different superscripts within same column were significantly different ($p<0.05$)

thus Ag ions could easily be released into the water. The bacterial cell-membrane was composed mainly of phospholipid bilayers and protein molecules; therefore, the whole cell membrane owned negative charge due to the presence of this phosphate group. The Ag ions had positive charge, thus were able to bind to the bacterial cell membrane quickly, causing deformation of this bacterial structural membrane. Moreover, Ag ions could also be strongly attracted to the negatively charged sulfhydryl group (SH) of the bacterial enzymes, causing their deactivation and subsequent inhibition of cell growth.

It could be also observed that the inhibitory effect of AgNPs was significantly ($p<0.05$) higher against some Gram negative compared to Gram positive bacteria. This might be because Gram positive bacterial cell wall was thicker; composed of thick peptidoglycan layer to provide structural strength to the cell, thus become able to counteract the cytoplasmic osmotic pressure (Zafar *et al*., 2016).

3.4. Cytotoxic activity of AgNPs against MCF-7 cell line

% Cell inhibition assay was an important test to measure the cellular response to drugs/toxicant, and also to provide information about cell survival, death and metabolic activities (Chandrasekaran *et al*., 2016). In the current work, it was noticed that AgNPs showed no cytotoxic effect against MCF-7 cell line at low concentrations up to 40 µmole. This was attributed to the green synthetic approach of AgNPs followed in this work; which might result in reducing its toxicity especially at low concentration, compared to AgNPs synthesized chemically. Above 40 µmole, the cytotoxicity of AgNPs against MCF-7 cell line was found to be concentration dependent, as the percentage of cell viability was decreased with increasing corresponding concentrations of the AgNPs (Table 2).

The IC_{50} is the dose required to kill 50% of the cultured breast cancer cells population, and it was estimated from the dose response curve plotted (Fig. 4). Currently, the IC_{50} of AgNPs was recorded to be 75.40 µmole. Previous study of Valodkar *et al*., (2011) reported that NP induced cytotoxicity in a variety of mammalian cell lines (hepatocytes and

Table 2: Effect of AgNPs on viability % of MCF-7 cell line

AgNPs (µmole)	Viability of cells (%)
100	4.169127
60	91.95701
40	100
20	101.1929
10	94.60257
4	91.24838
2	93.3979
1	93.69316

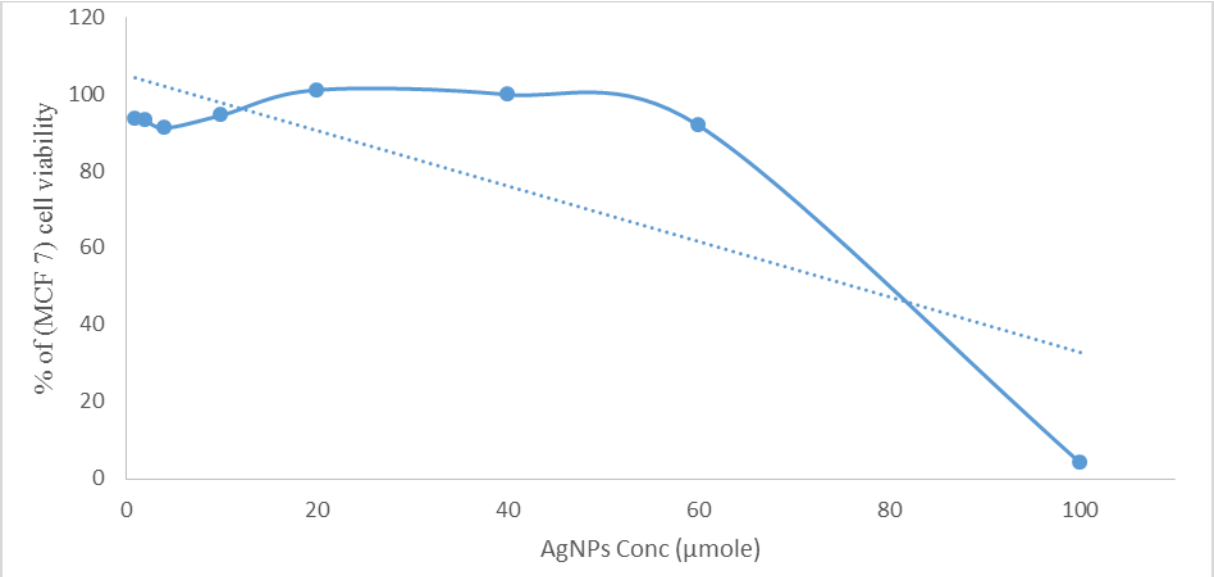


Fig. 4.: Plot of % breast cancer cell (MCF 7) viability against AgNPs concentrations

fibroblasts), due to oxidative damage to the cell membrane and mitochondrial dysfunction. NP induced toxicity was exerted in the form of transferring electrons from molecular oxygen or by blockade of electron transport chain.

Conclusion

It could be concluded from the present findings that, AgNPs biosynthesized using *P. afr*a leaf extract demonstrated potential antibacterial potency against both Gram positive and Gram negative bacteria. Moreover, it exhibited anticancer activity against breast cancer cell line (MCF 7). Therefore, these AgNPs have several advantages such as; cost effectiveness and eco-friendly technique for their green synthesis, which will help in their wide scale commercial production.

Conflict of interests

The authors declare no conflict of interests regarding publication of this article.

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4. References

Abdel-Aziz, M.S.; Shaheen, M.S.; El-Nekeety, A. A. and Abdel-Wahhab, M.A. (2014). Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. Journal of Saudi Chemical Society. 18: 356-363.

Chandrasekaran, R.; Gnanasekar, S.; Seetharaman, P.; Keppanan, R.;Arockiaswamy, W. and Sivaperumal, S. (2016). Formulation of *Carica papaya* latex-functionalized silver nanoparticles for its improved antibacterial and anticancer applications. Journal of Molecular Liquids. 219: 232-238.

El-Chaghaby, G.A.; Ahmad, A.F. and Ramis, E.S. (2014). Evaluation of the antioxidant and antibacterial properties of various solvents extracts of *Annona squamosa* L. leaves. Arabian Journal of Chemistry. 7: 227-233.

El-Chaghaby G.A. and Ahmad, A.F. (2011). Biosynthesis of silver nanoparticles using *Pistacia lentiscus* leaves extract and investigation of their antimicrobial effect. Oriental Journal of Chemistry. 27(3): 929-936.

Guralnick, L.J. and Gladsky, K. (2017). Crassulacean acid metabolism as a continuous trait: variability in the contribution of Crassulacean acid metabolism (CAM) in populations of *Portulacaria afra*. Heliyon.

Husseiny, S.M.; Salah, T.A. and Anter, H.A. (2015). Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities. Beni-Suef University Journal of Basic and Applied Sciences. 4: 225-231.

Jagtap, U.B. and Bapat, V.A. (2013). Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. seed extract and its antibacterial activity. Industrial Crops and Products. 46: 132-137.

Malabadi, R.B.; Mulgund, G.S.; Meti, N.T. and Nataraja, K. (2012). Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoria ternatea*. Research in Pharmacy. 2: 10-21.

Remya, R.R.; Rajasree, S.R.R.; Aranganathan, L. and Suman, T.Y. (2015). An investigation on cytotoxic effect of bioactive AgNPs synthesized using *Cassia fistula* flower extract on breast cancer cell MCF-7. Biotechnology Reports. 8: 110-115.

Roopan, S.M.; Rohit; Madhumitha, G.; Rahuman, A.A.; Kamaraj, C.; Bharathi, A. and Surendra, T.V. (2013). Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. Industrial Crops and Products. 43:631-635.

Suman, T.Y.; Radhika R.S.R.; Kanchana, A. and Elizabeth, S.B. (2013). Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morinda citrifolia* root extract. Colloids Surfaces B Biointerfaces. 106: 74-78.

Valodkar, M.; Jadeja, R.N.; Thounaojam, M.C.; Devkar, R.V. and Thakore, S. (2011). Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Materials Chemistry and Physics. 128: 83-89.

Zafar, N.; Shamaila, S.; Nazir, J.; Sharif, R.; Rafique, M.S.; Ul-Hasan, J.; Ammara, S. and Khalid, H. (2016). Antibacterial Action of Chemically Synthesized and Laser Generated Silver Nanoparticles Against Human Pathogenic Bacteria. Journal of Materials Science and Technology. 32(8): 721-728.

Zhang, Y.; Cheng, X.; Zhang, Y.; Xue, X. and Fu, Y. (2013). Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 423: 63-68.