Pak. J. Bot., 51(5): 1853-1857, 2019. DOI: http://dx.doi.org/10.30848/PJB2019-5(37)

PHYTOSYNTHESIS OF SILVER NANOPARTICLES FROM *ARISAEMA JACQUEMONTII* EXTRACT, THEIR CHARACTERIZATION AND ANTIMICROBIAL POTENTIAL

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Abstract

The present study investigates synthesis of silver nano-particles from the methanolic extract of *A. jacquemontii*tubers. Based on visual monitoring of color change from colorless / very light yellow to brown/yellowish brown indicated Ag-metal reduction and synthesis of AgNPs. UV-vis Spectrophotometric analysis indicated that solution containing 1:5 ratios (1ml $AgNO_3$ solution and 5ml extract) produced maximum amount of stableAgNPs. AgNPs were most stable at 1mM NaCl, comparatively moderate stable at 0.5M NaCl and relatively least stable at 1M NaCl concentration. The AgNPs samples heated up to 80-100°C temperature were less stable than the AgNPs collected at 20-40°C temperature range thus suggesting higher stability of AgNPs at lower temperatures. XRD studies revealed that synthesized AgNPs were crystalline in nature with 14.12 nm average size. FTIR analysis suggested the possible involvement of alkenes, ethers and carboxylic acid / phenol functional groups containing compounds in the reduction and capping of Ag-metal and AgNPs. Antimicrobial potential of silver NPs indicated that *P. aeruginosa* was highly susceptible while *K. pneumoniae* was the most resistant microbe. *E. coli, C. albicans, S. aureus* and *X. campestris* showed moderate sensitivity to AgNPs.

Key words: AgNPs, *Arisaema jacquemontii*, UV-vis spectrophotometer, XRD, FTIR, SEM, Antimicrobial potential.

Introduction

Nanotechnology being an interdisciplinary field connects physical, chemical, engineering and biological sciences to design, create and utilize particles having at least one dimension less than 100nm (Dhas *et al*., 2013). Different approaches including chemical and physical methods used to synthesize nanoparticles (NPs) have inherent problems and pose risks and hazards to environment and biological systems (Rajan *et al*., 2015). Green synthesis of NPs from natural biological resources is an efficient, chemicals-free, environmental friendly and sustainable alternative. Over past several years algae (Merin *et al*., 2010), viruses (Pokorski & Steinmetz, 2011), fungi (Raheman *et al*., 2011) and bacteria (Bai *et al*., 2011) have been employed for nontoxic and low-cost metallic NPs (Thakkar *et al*., 2010). Utilizing plant extract for the reduction and fabrication of metals has emerged as a simple, viable, eco-friendly, economical, rapid, efficient and single step technique to synthesize metallic NPs (Logeswari *et al*., 2013).

Different types of materials including tellurium, gold, selenium, alginate, titanium, copper, zinc, silver and many others have been utilized to synthesize NPs from many plants and plant extracts including *Azadirachta indica, R. rugosa, M. paradisiaca, E. guineensis,* Lichens, *N. nucifera, C. camphora, Acalypha indica* (Yallapa *et al*., 2013). The synthesized NPs have shown more effectiveness against many test microbes than their respective extracts and plants (Ahmad *et al*., 2016; Debnath *et al*., 2016). Metallic NPs are widely used for their antimicrobial functionality (Rafi *et al*., 2018; Shehla *et al*., 2018). Silver NPs (AgNPs) have been incorporated into wound dressings, bone cements and implants (Chaloupka *et al*., 2010). Gold NPs (AuNPs) have medically relevant optical and anticancer properties.

Arisaema jacquemontii known as cobra lily belongs to family Araceae. It is a part of some traditional medicines that are used to cure several ailments. It can be used as food (after proper cooking or fermentation), anthelmintic and snakebite antidote. It can be used to treat respiratory infections and dermatitis significantly. A tuber lectin is reported to have anti-proliferative, anticonvulsant and insecticidal properties and affects platelet aggregation. Antioxidant and immune-modulating properties of leaves are also established (Tanveer *et al*., 2013).

Materials and Methods

Plant collection and sample preparation: Tubers of *A. jacquemontii* were collected and thoroughly rinsed with tap water followed by distilled water to remove all the soil and plant debris. Shade dried and finely grinded tuber sample was soaked in methanol for 10 days to yield methanolic extract. After evaporation of all methanol from sample, crude methanolic extract was used for AgNPs synthesis.

Phytosynthesis of AgNPs: For production of bio-inspired AgNPs, *A. jacquemontii* crude methanolic extract (prepared by dissolving 50 mg extract in 100 ml de-ionized water) was used to reduce and fabricate 0.1mM $AgNO_3$ (Silver nitrate) solution. Plant extract and $AgNO_3$ solution were mixed in different ratios followed by UV-vis Spectrophotometric analysis.

Characterization of AgNPs: A high-resolution SEM (Scanning electron microscope) was used to determine the shape and size of synthesized NPs. Synthesis of these nano-particles was confirmed by observing a prominent SPR (Surface Plasmon Resonance) peak in the 400nm-500nm wavelength range using dual beam UV-vis

spectrophotometer. XRD (X-ray diffraction) analysis was carried out to determine the nano-crystallite size of NPs and crystalline nature of AgNPs. FTIR analysis was used to determine the possible functional groups of extract that were involved in silver metal reduction.

Stability of AgNPs: Effects of salt and temperature on stability of AgNPs were checked at different concentrations of sodium chloride (NaCl) salt and at different temperature using UV-vis Spectrophotometric analysis.

Antimicrobial activity: Antimicrobial activity was carried out by following the protocol described by Bakht *et al*., (2017) against different microbes (Table 1).

Statistical analysis

Data was presented as mean values of three replications. MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith, 1983).

Results

In the present study *A. jacquemontii* tuber crude methanolic extract and silver nitrate were used to synthesize AgNPs. Both solutions were mixed in different ratios and continuously stirred. AgNPs synthesis was monitored visually and confirmed by UV-vis Spectrophotometric analysis (Fig. 1). Change in color of the solution from colorless / very light yellow to brown/yellowish brown indicated Ag-metal reduction and synthesis of AgNPs. Increase in the ratios of plant extract and 0.1mM $AgNO_3$ solution resulted in intense color representing the formation of higher quantities of AgNPs. UV-vis spectrum analysis revealed that the sample containing 1ml silver nitrate and 5ml extract (1:5 ratios) reported maximum absorption peak at 432nm wavelength indicating maximum synthesis of AgNPs (Fig. 2).

Stability of the synthesized AgNPs was tested at different concentrations of sodium chloride (NaCl) salt and different temperature using UV-vis spectrophotometric analysis. To check the stability of synthesized AgNPs in saline conditions, 3 different NaCl concentrations (1mM, 0.5M and 1M) were added to the samples separately. UV-vis spectrum analysis revealed decrease in the stability of AgNPs with increase in salt concentration (Fig. 3). Among all the tested NaCl concentrations, AgNPs were most stable at 1mM NaCl, comparatively moderate stable at 0.5M NaCl and relatively least stable at 1M NaCl concentration. The AgNPs samples heated up to 80-100°C temperature range were less stable than the AgNPs collected at 20-40°C temperature range thus suggesting higher stability of AgNPs at lower temperatures. Higher stability of AgNPs sample at temperature range of 20-40°C was indicated by an absorbance peak of relatively sharper than the peak at 80-100°C (Fig. 4).

Analysis of XRD results of AgNPs synthesized from *A. jacquemontii* extract confirmed crystalline nature of AgNPs and demonstrated some characteristic peaks at 38.53°, 40.93° and 44.39° two theta (2θ) values that corresponds to (111), (141) and (200) facets of silver NPs respectively (Fig. 5).. This Bragg's reflections indexing suggested face centered cubic Ag structure. By determining FWHM (Full Width Half Maximum) for intense reflections and applying Sherrer equation suggested average size of nano-crystallite as 14.12nm. Comparison of both spectra reported the disappearance of some bands in silver NPs spectrum at 922.43 cm^{-1},124.45 cm^{-1} and 1171.37cm^{-1} wave numbers and suggested the involvement of possible alkenes, ethers and carboxylic acid/phenol functional group containing compounds in Ag-metal reduction to synthesize AgNPs respectively. Moreover, a small change (±1 to ±100) in wave numbers was observed in rest of the AgNP spectrum absorption bands. A shift of wave numbers in AgNPs spectrum with reference to extract spectrum confirmed AgNPs synthesis (Fig. 6). SEM analysis revealed that an average size of the synthesized AgNPs was 30nm. AgNPs were of uniform shape and spherical morphology (Fig. 7).

Figure 8 shows the antimicrobial potential of AgNPs synthesized from methanolic extract of *A. jacquemontii* tubers against seven different microbial species at 3 different AgNPs concentrations. Analysis of data suggested that AgNPs effectively inhibited growth of all the tested organisms and increase in NPs concentration increased antibacterial activity. Among all the test microbes, maximum reduction was observed in growth of *P. aeruginosa* (67.29 %) at the highest test concentration (1.5mg $disc^{-1}$). Synthesized AgNPs showed relatively moderate growth inhibition of 56.29%, 56%, 52.62% and 52.1% for *E. coli, C. albicans, S. aureus* and *X. campestris,* respectively. *K. pneumonia* was the least susceptible microbe. AgNPs contributed 44.21% growth inhibition of *K. pneumonia at* 1.5mg $disc^{-1}$ concentration.

Table 1. Details of microbial strains that were used during experiment.

Microbial species	Gram stain type	Details
Klebsiella pneumonia	Negative	Clinical isolate, Quaid-E-Azam University Islamabad Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Staphylococcus aureus	Positive	ATCC # 6538
Bacillus subtilis	Positive	Clinical isolate, Quaid-E-Azam University Islamabad Pakistan
Escherichia coli	Negative	ATCC # 25922
Xanthomonas campestris	Negative	ATCC # 33913
Candida albicans	Fungi	ATCC #10231

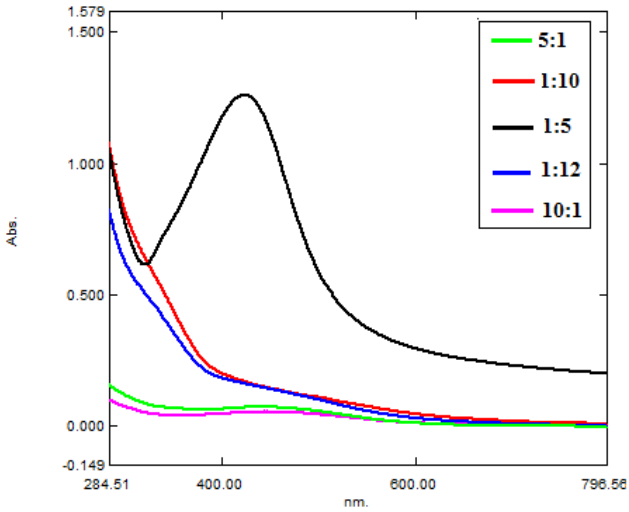

Fig. 1. Comparison of UV-vis spectra of *A. jacquemontii* tubers AgNPs synthesized by combination of methanolic extract and 0.1mM $AgNO_3$ in different ratios.

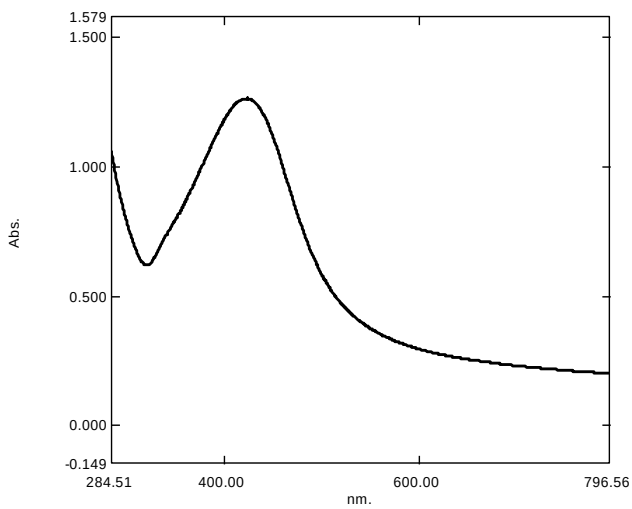

Fig. 2. UV-vis spectrum of *A. jacquemontii* tuber AgNPs showing AgNPs SPR peak at 423nm synthesized by 1:5 ratios.

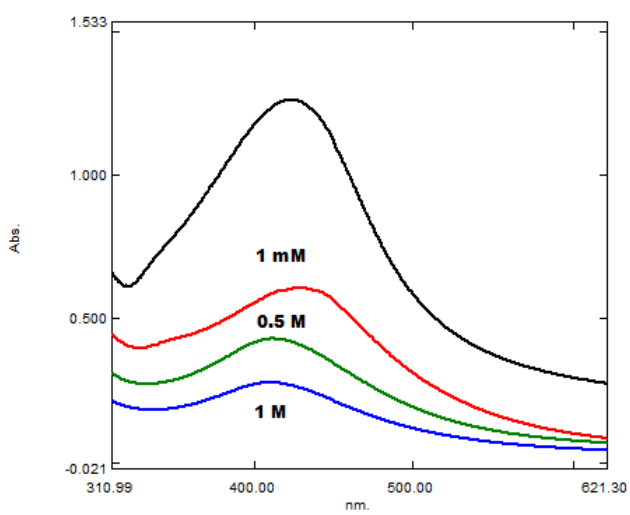

Fig. 3. Comparison of UV-vis spectra of *A. jacquemontii*tuber AgNPs at different salt concentrations.

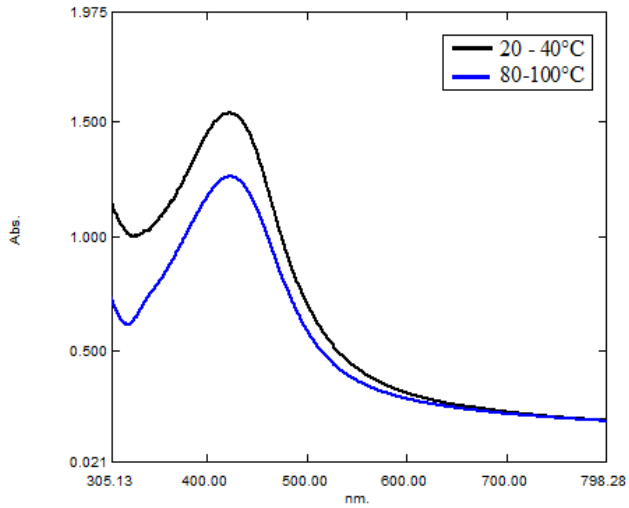

Fig. 4. Comparison of UV-vis spectra of *A. jacquemontii* tuber AgNPs at different temperature range.

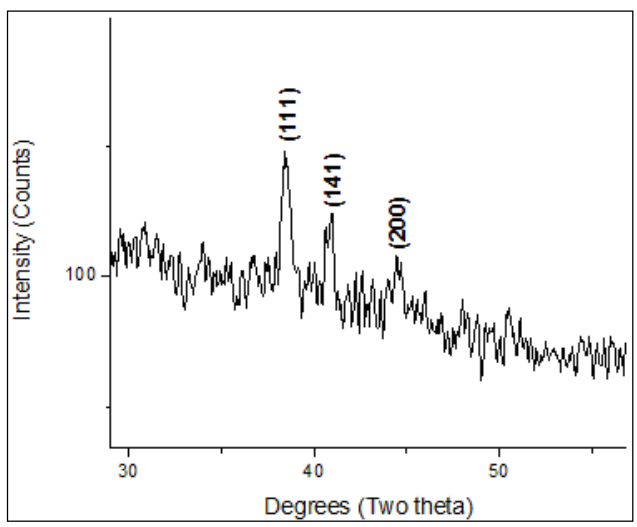

Fig. 5. XRD patterns of *A. jacquemontii* tubers AgNPs.

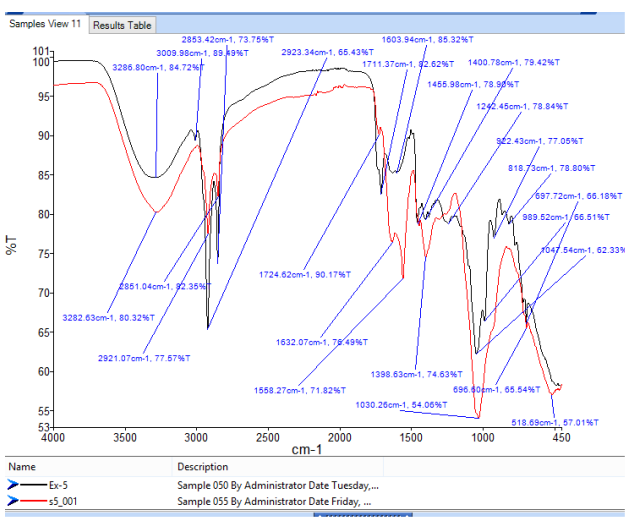

Fig. 6. Comparative FTIR spectra of tuber AgNPs and pure *A. jacquemontii* tuber extract showing absorption bands of AgNPs and extract with % transmittance at corresponding wave numbers (cm^{-1}).

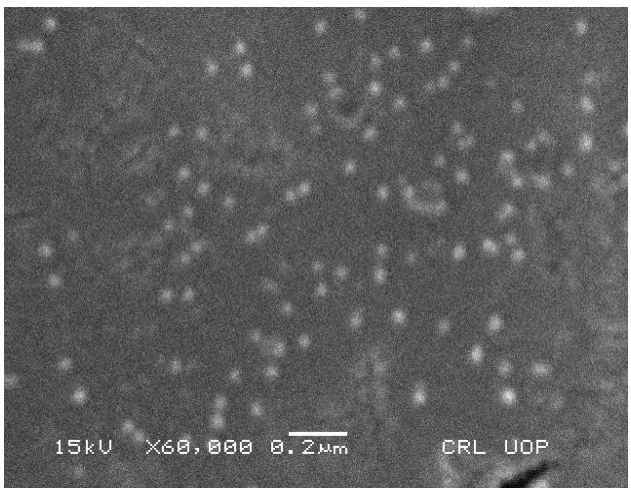

Fig. 7 SEM image of *A. jacquemontii* tuber AgNPs.

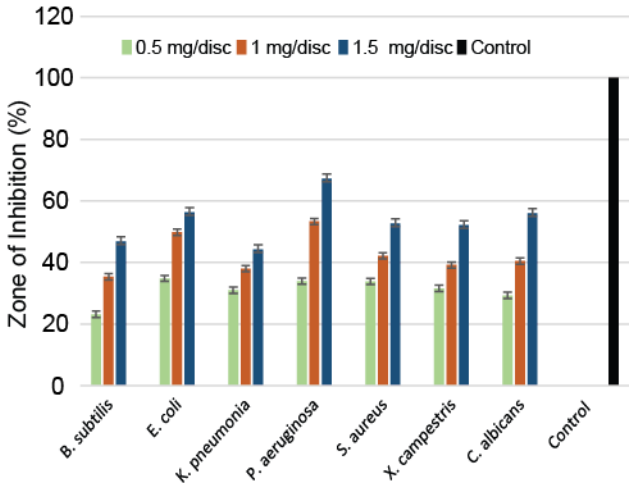

Fig. 8. Antimicrobial activity of AgNPs, with Standard Deviation, against different microbial strains.

Discussion

AgNPs were synthesized from methanolic crude extract of *A. jacquemontii* tuber and 0.1mM $AgNO_3$ solution. Amin *et al*., (2012) also reported the synthesis of NPs from methanolic extract of *S. xanthocarpum* berry. Different ratios of extract and metal solution were mixed and continuously stirred to observe the NPs synthesis. Preliminary observation of silver NPs synthesis was carried out by monitoring color change visually. Color change from colorless/very light yellow to brown/yellowish brown indicated reduction of silver metal and synthesis of AgNPs (Bar *et al*., 2009). AgNPs synthesis was confirmed by UV-vis Spectrophotometric analysis. In some samples, intensity of color of solution increased with the increase in ratios of $AgNO_3$ and tuber extract and represented the formation of higher quantities of AgNPs. Specifically, in some samples, increase in extract ratio with respect to silver nitrate solution resulted in higher amounts of AgNPs. UV-vis spectrum analysis reported maximum absorption and highest absorption peak at 432nm wavelength for the sample containing 1ml solution of silver nitrate and 5ml extract (1:5 ratios) which indicated maximum AgNPs synthesis in the sample among all the tested ratios. Other samples having different ratios reported no or very less AgNPs synthesis. A resonance is created by vibrations of free electrons of silver NPs with light wave resulting in light absorption. This absorption is detected by UV-vis spectrophotometer and an absorption peak is given. A characteristic peak for AgNPs appears in 400nm-500nm range and its intensity depends upon AgNPs concentration. Song & Kim (2009) synthesized AgNPs from five different plants and reported AgNPs synthesis confirmation by UV-vis Spectrophotometric analysis and observed AgNPs peak in 400-500nm range.

Stability of synthesized AgNPs was checked at different salt concentrations and different temperature using UV-vis Spectrophotometric analysis. UV-vis spectra of AgNPs at all the 3 different NaCl salt concentrations (1mM, 0.5M and 1M) revealed that increasing salt concentration affected the NPs and a decrease in the stability of AgNPs. Among the tested salt concentrations, 1M NaCl solution showed least stableAgNPs. It suggested that molar concentrations of salt (1M and 0.5M) affected the synthesized AgNPs more severely than the millimolar (1mM) salt concentration. The AgNPs samples heated up to 80-100°C were less stable and showed an absorbance peak relatively less sharpen than the AgNPs collected at 20-40°C thus suggesting the higher stability of AgNPs at lower temperatures. In agreement of our results, Raveendran *et al*., (2003) reported that AgNPs were reversible at higher temperatures. XRD results confirmed crystalline nature of synthesized AgNPs and reported some characteristic peaks at 38.53°, 40.93° and 44.39° two theta (2θ) values corresponding to (111), (141) and (200) facets of silver NPs respectively suggesting face centered cubic Ag structure. Average nanocrystallite size was calculated as 14.12nm by determining FWHM and applying Sherrer equation. These results are in agreement with Zargar *et al*., (2011).

Comparison of FTIR spectra of tuber methanolic extract and AgNPs revealed disappearance of bands at 922.43 cm^{-1},124.45 cm^{-1} and 1171.37cm^{-1} wave numbers in AgNPs spectrum and suggested the involvement of possible alkenes, ethers and carboxylic acid/phenol functional group containing compounds in the reduction of Ag-metal and synthesis of AgNPs respectively. A small change (±1 to ±100) in wave numbers and a shift of wave numbers in silver NPs with reference to tuber methanolic extract confirmed AgNPs synthesis. Studies of Paosen *et al*., (2017) conducted on AgNPs synthesis from extracts of seven different plants of Myrtaceae family and Elumalai *et al*., (2017) conducted on AgNPs synthesized from extracts of two different plants strengthen our findings. These researchers reported the involvement of phenol, alkene and carboxylic acid groups in AgNPs synthesis. SEM images revealed that synthesized spherical AgNPs were of uniform shape and 30nm average size. Jyoti *et al*., (2016) reported the synthesis of spherical AgNPs from extract of *Urticadioica* leaves that were 20-30 nm in size.

Antimicrobial potential of the synthesized AgNPs was assessed at 3 different AgNPs concentrations against seven different microbial species. Synthesized AgNPs reduced the growth of all the tested microbial species and

increase in AgNPs concentration increased its antibacterial activity against all test microbes. Among all the microbes, the most susceptible was *P. aeruginosa* while minimum inhibition was noted for *K. pneumonia*. Relatively moderate growth reduction was observed for *E. coli, S. aureus, C. albicans* and *X. campestris.* Kaviya *et al*., (2011) also investigated antimicrobial potential of AgNPs against different microbial species including *S. aureus*, *E. coli* and *P. aeruginosa* and reported effective reduction of microbial growth by silver nanoparticles.

Conclusion

It can be concluded from these results that methanolic extract of *A. jacquemontii* can efficiently reduce silver and synthesize stable crystalline AgNPs. Furthermore, synthesized AgNPs were active against some tested microbial species and reduced their growth.

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(Received for publication 19 May 2018)