Scholarly Research Journal for Humanity Science & English Language
Online ISSN 2348-3083, SJ IMPACT FACTOR 2021: 7.27
UGC Approved Sr. No.48612, OCT- NOV 2017, Vol-4/24

ANTIMICROBIAL PROPERTIES OF MORINGA OLEIFERA SECONDARY METABOLITES, A STUDY WITH THE HELP OF SILVER NANOPARTICLES

Kanumalla Raghu Kranti Kumar

Lecturer in Zoology, Dept. of Zoology Sri Vivekananda Degree College, Podili, A.P

INTRODUCTION

One of the most important areas of nanotechnology is biomedicine, where metallic nanoparticles have been used for different applications such as controlled release of drugs, synthesis of new medicines, tumor cells detection,among others (Ghazwani, 2015). AgNPs are gaining particular attention dueto their desirable properties like optical, magnetic, electronic, biological, catalytic and antibacterial activity. Chemical and physical methods have been used to synthetize silver nano-particles but most of these techniques are not environmentally friendly (Suarez-Cerda et al., 2015). Biological methods currently employ microorganisms, plants and their extracts (Kuppusamy et al., 2014). Many studies have proven that the plant extracts act as potential precursors for the synthesis of nanomaterials in a non-hazardous way. Plant extracts contain metabolites which act as reducing and stabilizing agents for bio-reduction of metallic NPs (Anjum and Abbasi, 2016; Kuppusamy et al., 2014). Recent reports reveal green synthesis of silver nanoparticles usingprimary metabolites of the plants extracts as reducing agents, for example leafextract of *Moringa* (Prasad and Elumalai, 2011), extracts of *Prosopis glandulosa* (Abdelmoteleb et al., 2016), and of *Vitex negundo* plants. Antimicrobial activity against human pathogens has also been found suitable for the biosynthesis of AgNPs (Kathireswari et al., 2014). However, most of these techniques do not use secondary metabolites as reducing agents. Silver nanoparticles can acts as effective and alternative bactericide agents for combating bacterial drug resistance problems (Ahmed et al., 2015). The surface area of the NPs ensemble becomes larger as the particle size decreases, which in turn increases the total surface available for contact with bacteria, increasing the antibacterial efficiency of AgNPs (Ghazwani, 2015).

The aim of this paper was to synthesize AgNPs by the reducing effect of secondary metabolites produced by the activity of fungi and bacteria, particularly the *Nigrospora* sp. fungus from *M. oleifera* stem, in an aqueous solution of silver nitrate. The AgNPs formation was evaluated by UV-Vis,fourier transformed infrared (FTIR) spectroscopy, dynamic light

scattering (DLS), scanning electron microscopy (SEM) and electron dispersive X-ray (EDS). The synthetized AgNPs exhibit antibacterial properties against different pathogenic microorganisms.

MATERIALS AND METHODS

Stem and reagents/reactants

The chemicals used in this study were from Sigma (Bangalore, India) and Merck (Mumbai, India) with specifications of the American Chemical Society (ACS). *M. oleifera* stem samples were harvested from trees cultivated in Mexicali, Baja California, Mexico. The stem samples were previously washed with 1% v/v hypochlorite aqueous solution and distilled water to remove the dust. The sample was macerated in a mortar and dried at room temperature.

Preparation of the reductant precursors

Plant extract was prepared according to the methods of Prasad and Elumalai (2011) with some modifications. Ten grams of fresh-dried macerated *M. oleifera* stem in 1 L of water were heated for 20 min at 60°C to extract the active ingredients into the aqueous phase. After that, the solution was kept in incubation to permit the growing of microorganisms contained in the plant; endophytic bacteria and fungus. The main developed microorganism in the culture was the *Nigrospora* sp. fungus, which has been reported to produce bioactive secondary metabolites from *M. oleifera* stem extracts, mainly griseofulvin, dechlorogriseofulvin, 8-dihydroamulosin and mullein (Zhao et al., 2012). The culture was grown during 10 days and then the supernatant with metabolites was employed for the synthesis.

Biosynthesis of AgNPs

Working solutions with different v/v ratios of supernatant from *M. oleifera* stem ferments were prepared using 1 mM silver nitrate. The synthesis of AgNPswas conducted at room temperature with ratios of 1:1 (A), 1:5 (B) and 1:10 (C) between the silver nitrate and the supernatant. The reaction was carried out during 10 min. The bioreduction of Ag^+ ions was observed by color change from faint yellow to yellow-brown, indicating the formation of AgNPs (Bello et al., 2015; Netala et al., 2016). The reaction mixtures were poured into a test tube and the separation was carried out using a centrifuge (10000 rpm/10 min). The supernatant was extracted with a micropipette and the pellet was suspended in 10 mL acetone and then in distilled water. This centrifugation and resuspension processes were repeated 3 to 4 times. The resulting AgNPswere air dried to evaporate excessive liquid and these were used for further characterization.

Characterization of AgNPs

The progress of reaction was followed spectrophotometrically using a Perkin- Elmer UV-Vis Lambda 25 spectrophotometer. UV-visible spectroscopy is an important technique to establish the formation and stability of NPs in solution (Dhand et al., 2016). The scan was recorded from 300 to 600 nm, showing a characteristic peak appearing within the range of 440 to 450 nm. FTIR was carried out to identify the molecules corresponding to the metabolites involved in reduction, capping and stabilization of the synthetized AgNPs (Haghighi Pak et al., 2016). AgNPs were recorded using a Perkin-Elmer Spectrum Onein ATR mode in the 4000 to 500 cm^{-1} range. The presence of AgNPs was confirmed by EDS, and the surface characterization was performed by SEM analysis. This was done using a JEOL JSM-6010L, with an accelerating voltage of 10 kV and a STEM support. Zeta potential and hydrodynamic sizes of the synthesized NPs were determined by introducing 3 mL of sample in the Nanotrac Wave instrument.

Determination of antimicrobial activity by well-diffusion method

The antimicrobial activities of the NPs were confirmed by well diffusion method against pathogenic microorganisms (Kim et al., 2007). Pure culturesof *Escherichia coli* (ATCC-25922), *Klebsiella cloacae* (ATCC-23355) and *Staphylococcus epidermidis* (ATCC-12228), Manassas, VA, USA wereused for antibacterial analysis. Approximately, 80 mL of trypticase soy were dispersed on sterilized Petri dishes. One hundred micro liter (about 100 CFU/mL) of each bacterium was spread uniformly onto the individual plates using sterile cotton swabs. The dried AgNPs were dissolved in distilled water and used immediately. 30 µL of different ratios of AgNPs, 1:10 (A), 1:5 (B) and1:1 (C) were loaded to each well through a micropipette (positive control), 30 µL of $AgNO_3$ solution and a blank was prepared without AgNPs; all sets were incubated at 37°C for 24 h. Afterwards the zone of inhibition (ZOI) was measured (Haghighi Pak et al., 2016; Lokina et al., 2014). The antimicrobial activity was realized for triplicate for each microorganism.

RESULTS AND DISCUSSION

The collaborative action of biomolecules in the supernatant was visualized through change of the color of the solutions. The supernatant presented the usual faint yellow color of media before reacting with $AgNO_3$. The color tones changed, turning from yellow to brown, indicating that NPs with different sizes were formed. This result indicates that the secondary metabolites obtained from the metabolic activity of *Nigrospora* sp. fungus in *M. oleifera* stem

aqueous extracts were able to reduce the silver nitrate precursor to AgNPs. AgNPs have free electrons, which give an increment of the surface plasmon resonance (SPR) absorption band due to the interaction of electrons of metal nanoparticles with the light wave (Elumalai et al., 2015; Ghaedi et al., 2015; Gurunathan et al., 2009). A shift of the band maximum from 440 to 436 nm is observed in the UV-Vis spectra depending on the supernatant/AgNO3 ratio. The shift corresponds to change of color, which is due to reduction of Ag^+ and formation of AgNPs. The results show that the ratio of supernatant plays animportant role in the control of nanoparticle formation. The narrow peak was observed when observed when supernatant was mixed with AgNO3 solution in a 1:10 ratio indicating ferment in the *Nigrospora* sp. fungus were responsible for the acceleratedreduction and stabilization of Ag-NPs. The FTIR spectrum of colloid suspension with AgNPs synthesized using *M. oleifera* stem ferment. The broad band at 3593 cm^{-1} may be related to OH- groups present in biomolecules. The band observed at 2917 to 2843 cm^{-1} was assigned to the aliphatic C—H group. The peak around 1624 cm^{-1} corresponds to C=O stretching vibration, while the peaks observed at 1541 and 1341 cm^{-1} correspond to secondary amine group and to symmetric bending of CH3, respectively. The peak at 1274 cm^{-1} is due to $SO3^-$ stretching vibration and the one at 1077 $cm^{—1}$ corresponds to C=O bonds of ether, ester or phenol. Comparison with standard library reveals the presence ofcharacteristic peaks of β-carotenes, flavonoids and tannins, which are actively involved with enzymes in the reduction and stabilization of the AgNPs.

Analysis of AgNPs size distribution

Samples A and B present size distributions with maximums of ~15 and 6.5%, respectively, centered at approximately 35 nm. The distribution maximum of sample C is ~38% at ~3 nm. In addition, the nanoparticles in sample C exhibit better uniformity compared to samples A and B seen as much narrow distribution. The average size of the AgNPs in samples A, B and C was 70, 25 and 3 nm, respectively. The obtained results demonstrate that the supernatant/AgNO3 ratio has an important role in controlling the growth and size of AgNPs during the green synthesis.

Antimicrobial activity of AgNPs against pathogenic microorganism

Zone of inhibition found for *E. coli* (*E. coli*), *S. epidermis* (*S. epidermidis*) and *K. cloacae* (*K. cloacae*) were 19.5, 19 and 18 mm respectively (Figure 6). Results in Table 1 shows minimal level bacterial inhibition of AgNO3 solutions and blank (ferments alone) in

comparison to obtained silver nanoparticles in this study. Results confirm that biosynthesized AgNPs presents antibacterial properties. The antibacterial activity is similar to the reference antibiotic reported (Prasad and Elumalai, 2011). The mechanism bactericide of AgNPsis not fully explored. Maybe antibacterial activity is due to the AgNPs bind of the cell membrane through covalent and ionic bonding, causing changesin its permeability which affects their respiratory functions. They penetrate bacteria, damaging structures containing sulfur and phosphorus based functional groups such as the DNA chains; it also contributes to the bactericidal effect of the Ag^{+} ions emerging from the surface of the AgNPs (Abdelmoteleb et al., 2016).

CONCLUSION

This study demonstrated the synthesis of AgNPs by the reducing effect of secondary metabolites produced by the activity of *Nigrospora* sp. fungus, based on a simple, safe and green method. AgNPs synthesis was achieved in a short time after mixing the supernatant with the silver nitrate solution. The NPs obtained were characterized by UV-Vis, DLS and SEM-EDS spectroscopy. The characteristic signal in the colloidal suspensions is proven to be sensitive to the detection of AgNPs because they showed a strong absorption peak because the silver nanoparticles exhibit excitation of SPR. UV-Visible analysis showed that the absorption peaks depends on the ratios employed due to the absorbance peaks that decreased as the size of the nanoparticles increased. SEM images shows that sizes of particles observed were well-defined in the 3 to 70 nm range with spherical shapes. EDS analysis of AgNPs confirmed the presence of elemental silver. AgNPs obtained showed antibacterial activity; inhibiting the growth of *E. coli*, *K. cloacae* and *S. epidermidis.* Results contribute to the development of new routes of synthesis utilizing secondary metabolites. Bioactive compounds by *Nigrospora* sp.,fungus from *M.* oleifera stem stands as potential candidates for biosynthesis and stabilizer of AgNPs in biomedical Applications.

REFERENCES

Abdelmoteleb A, Valdez-Salas B, Cece-a-Duran C, Tzintzun-Camacho O, Gutiérrez-Miceli F, Grimaldo-Juarez O, González-Mendoza D (2016). Silver nanoparticles from Prosopis glandulosa and their potential application as biocontrol of Acinetobacter calcoaceticus and Bacillus cereus. Chem. Spec. Bioavailab. 29(1):1-5.

Ahmed MJ, Murtaza G, Mehmood A, Bhatti TM (2015). Green synthesis of silver nanoparticles using leaves extract of Skimmia laureola: characterization and antibacterial activity. Mater. Lett. 153:10-13.

Anjum S, Abbasi BH (2016). Biomimetic synthesis of antimicrobial silver nanoparticles using in vitro-propagated plantlets of a medicinally important endangered species: Phlomis bracteosa. Int. J. Nanomed. 11:1663.

Bello OS, Adegoke KA, Akinyunni OO (2015). Preparation and characterization of a novel adsorbent from Moringa oleifera leaf. Appl. Water Sci. pp. 1-11.

Dhand V, Soumya L, Bharadwaj S, Chakra S, Bhatt D, Sreedhar B (2016). Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Mater. Sci. Eng. C 58:36-43.

Elumalai K, Velmurugan S, Ravi S, Kathiravan V, Ashokkumar S (2015). Green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity. Spectrochim. Acta Mol. Biomol. Spectrosc. 143:158-164.

Ghaedi M, Yousefinejad M, Safarpoor M, Khafri HZ, Purkait MK (2015). Rosmarinus officinalis leaf extract mediated green synthesis of silver nanoparticles and investigation of its antimicrobial properties. J. Ind. Eng. Chem. 31:167-172.

Ghazwani AA (2015). Biosynthesis of silver nanoparticles by Aspergillus niger, Fusarium oxysporum and Alternaria solani. Afr. J. Biotechnol. 14(26):2170-2174.

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, Hariharan N, Eom SH (2009). Biosynthesis,

purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf. B 74(1):328-335.

Haghighi Pak Z, Abbaspour H, Karimi N, Fattahi A (2016). Eco-Friendly Synthesis and Antimicrobial Activity of Silver Nanoparticles Using Dracocephalum moldavica Seed Extract. J. Appl. Sci. 6(3):69.

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine 3(1):95-101.

Kuppusamy P, Yusoff MM, Maniam GP, Govindan N (2014). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–An updated report. Saudi Pharm J. 24(4):473-484.

Lokina S, Stephen A, Kaviyarasan V, Arulvasu C, Narayanan V (2014). Cytotoxicity and antimicrobial activities of green synthesized silver nanoparticles. Eur. J. Med. Chem. 76:256-263.

Netala VR, Bethu MS, Pushpalatha B, Baki VB, Aishwarya S, Rao JV, Tartte V (2016). Biogenesis of silver nanoparticles using endophytic fungus Pestalotiopsis microspora and evaluation of their antioxidant and anticancer activities. Int. J. Nanomed. 11:5683-5696.

Prasad TNVKV, Elumalai EK (2011). Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. Asian Pac. J. Trop. Biomed. 1(6):439-442.

Suarez-Cerda J, Alonso-Nunez G, Espinoza-Gomez H, Flores-Lopez LZ (2015). Synthesis, kinetics and photocatalytic study of "ultra-small" Ag- NPs obtained by a green chemistry method using an extract of Rosa 'Andeli' double delight petals. J. Colloid Interface Sci. 458:169-177.

Zhao JH, Zhang YL, Wang LW, Wang JY, Zhang CL (2012). Bioactive secondary metabolites from Nigrospora sp. LLGLM003, an endophytic fungus of the medicinal plant Moringa oleifera Lam. World J. Microbiol. Biotechnol. 28(5):2107-2112.