International Journal of Current Engineering and Technology
E-ISSN 2277 – 4106, P-ISSN 2347 – 5161

Available at http://inpressco.com/category/ijcet

Research Article

Biosynthesis of Metallic Nanoparticles towards a Nanodrug Design

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Accepted 16 July 2015, Available online 25 July 2015, **Vol.5, No.4 (Aug 2015)**

Abstract

Objective: *To investigate the antioxidant and antibacterial potential of green synthesized silver nanoparticles (AgNPs) using aqueous leaf extracts of Andrographisechioides (A.echioides).*
Methods: *Green nanoparticle was synthesized using A.echioides leaf extract. The synthesized AgNPs was characterized by Ultraviolet visible (UV-vis) spectroscopy, X-ray diffraction (XRD), Field emission scanning electron microscopy (FESEM) and Fourier transforms infrared spectroscopy (FTIR). The antioxidant activity was assed by DPPH free radical assay and the antibacterial activity was performed using disc diffusion method against B.subtilis, P.aeruginosa and S.aureus.*
Results: *Biosynthesis of AgNPs was determined by UV-vis spectrum that as intense Surface Plasmon Resonance (SPR) band at 440nm. AgNPs showed the XRD peaks at 2θ values of 27.44, 35.90, 37.20, 51.23 and 71.10º were identified with (311), (100), (101), (104) and (006) planes reflection values respectively. The FTIR spectrum of AgNPs exhibited prominent peaks located at 3255, 2930, 1604, 1390 and 1120 cm^{-1}. The peaks were corresponding to the O-H stretching (alcohols and phenols groups), C-H stretching (alkane group), N-H bending (1°amines group), C-H stretching (alkanes and alkyl groups) and C-O stretching (alcohols, carboxylic acids, esters and ethers groups) vibrations respectively. FESEM analysis illustrated the shape in poly-dispersed spherical and size ranged from 17 to 27nm. DPPH free radical scavenging expressed highest percentage of antioxidant activity. The maximum antibacterial activity was noted in P.aeruginosa was 1.9cm of inhibition zone followed by S.aureus and B.subtilis (1.2 and 1cm).*
Conclusion: *The green synthesis of AgNPs using plant extract of A.echioides expressed its antioxidant against DPPH and antibacterial activity against P.aeruginosa; S.aureus; B.subtilis respectively.*

Keywords: *Andrographisechioides, Silver nanoparticles, Antioxidants, Antibacterial Activity, Surface Plasmon Resonance*

Introduction

Green nanotechnology has attained a very big development in bio-manufacturing of nanoparticles (NPs), which is widely applied in the field of science and technology. Green synthesis of NPs using biological resources like plants and micro-organisms provide the range of advantages such as eco-friendly, cost effect, non-hazardous and non-toxic (Albrecht *et al*., 2006; Bar *et al*., 2009; Konga*et al*., 2014; Bhuvaneswari*et al*., 2015). By advancement of green nanotechnology, metal NPs have identified as notable an antioxidant and antibacterial source. Therefore, the biological approach for the AgNPs synthesis becomes essential (Kaushik, *et al*., 2010). The advantages, using plants for the synthesis of AgNPs is that they are easily available, safe to handle and possess a wide range of metabolites, which can aid in the ionic reduction (Singhal*et al*., 2011). Antioxidants are capable of deactivating the free radicals by their unstable atoms or molecules that can cause extensive damage to cells because of imbalance between the generations of Reactive Oxygen Species (ROS) (Hermans*et al*., 2007). The antibacterial activity of AgNPs has been authenticated in the recent years by Michał and Małgorzata, 2013; Mudasir*et al*., 2013;Amit Kumar *et al*., 2014. *A. echioides* is a medicinal plant, belonging to Acanthaceae family, which is rich in flavones, echioidinin and echioidin (Jayaprakasama*et al*., 1999). Bioactive compounds of plants generally like phenol, saponins, alkaloids, amino acids and flavonoids are possessing biological activities including anticancer, antifungal, and anti-inflammatory activities (Nadkarni 1982). The antioxidant ability of this plant has been reported by Premkumar*et al*., 2010. The Present investigation illustrates synthesis of AgNPs using *A.echioides* leaf extracts at room temperature, characterization and its applications in antioxidant and antimicrobial activities. The antibacterial activity of

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AgNPs was investigated against different human pathogenic bacterial strains.

Materials and Methods

Preparation of *A.echioides* leaf extract

Matured fresh leaves of *A.echioides* was collected from Pudukkottai, Tamil Nadu, India. The collected leaves were washed by running water and followed by double distilled water. Cleaned leaves were shade-dried at room temperature and powdered with a sterile electric blender. Two grams of powdered leaves were mixed with 100ml of de-ionized water, boiled to 100ºC for 30min and filtered by Whatman No.1 filter paper. Finally, filtrate was collected in 250ml Erlenmeyer flask and stored at room temperature for further use. For the AgNPs biosynthesis 500µL of extract was mixed with 50mL of 1mM silver nitrate ($AgNO_3$) solution. The reaction flask was covered with aluminium foil and subjected to constant mixing in a rotary shaker at 120rpm. The experiments were conducted in room temperature at 28°C. The color of the solution during the course of the reaction gradually turned from colorless to brown in color.

Characterization of AgNPs

AgNPs formation during the biosynthesis was monitored by UV-vis Spectrophotometer. The UV–vis spectra was recorded at regular intervals by UV–vis spectrophotometer (Lambda25Model) and the sample was measured in the wavelength region of 200–800nm. Synthesized NPs was diluted with deionized water followed by the samples was centrifuged at 10000rpm for 15 min. The residue was dispersed with de-ionized water twice to remove the biological impurities. The purified residues were dried in an oven at 70°C for overnight and the synthesized AgNPs were used for FTIR analysis on a Perkin-Elmer spectrum instrument at a resolution of $4cm^{-1}$ in the transmission mode of $4000\text{-}400cm^{-1}$ in KBr pellets. The particle sizes and nature of AgNPs was determined by XRD using a XPERT PRO model at 30kV, 40mA with Cu K radians at 2θ angle. The shape and size of the formed AgNPs was determined using FESEM and for analysis the sample was provided the crystalline nature.

Antioxidant activity

The scavenging activity of DPPH free radical by *A. echioides* plant leaf aqueous extract and synthesized AgNPs was done according to the method previously reported by Gyamfi*et al*., 1999. 10µL of 0.1mM DPPH (prepared with ethanol) was added to different concentration of *A. echioides* aqueous leaf extract and AgNPs. The reaction mixture was shaken and incubated in the dark for 30min. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm. Ascorbic acid was used as the positive control. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity. The inhibition ratio was calculated from the following equation:

%inhibition = [(control absorbance–sample absorbance)/control absorbance] X 100

Antibacterial activity

The antibacterial activity of AgNPs and aqueous leaf extract of *A.echioides* was assessed by the disc diffusion technique against three human pathogenic bacterial strains namely *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Sterile Whatman no.1 filter paper discs (5mm diameter) were loaded with 20 mg of the sample and placed on nutrient agar plates inoculated with bacterial cultures. The plates were incubated at 37ºC for 24h and the zone of inhibition was measured by using an antibiotic zone scale (Cormican*et al*., 1996). Standard tetracycline antibiotic discs were used as positive control.

Results and Discussion

The UV-vis absorption spectrum analysis is simple and sensitive technique for the observation of NPs synthesis. $AgNO_3$ solution color was changed from colorless to yellow color after mixing of *A. echioides* leaf extract and finally the mixer has changed into brown colour after some incubation time. This color change might be due to the surface plasmon vibrations and stabilizing agents. From fig.1, the highest peak was observed at 440nm, which exhibits crystalline vibration of SPR. This observation was coincident with the aqueous leaf extract of *Citrus unshiu* by Basavegowda*et al*., 2013 and *Cryphonectria sp*. by Mudasir*et al*., 2013. Thus, the green material acts as reducing and stabilizing agents for the generation of metallic NPs. XRD has proven to be a valuable approach to confirm the formation of AgNPs, determining the crystalline structure and to calculate the crystalline size of AgNPs. Fig. 2.a, shows XRD pattern of the biosynthesized AgNPs major peaks occurred at 27.44º, 35.90º, 37.20º, 51.23º and 71.10º values which were corresponding to the planes (311), (100), (101), (104) and (006) of face centered cubic (fcc) crystal structure respectively and also the sharpening of the peak indicated that the crystalline nature of the NPs. The average size of the AgNPs was estimated by using

Scherrer's formula, $D=k\lambda/\beta\cos\theta$

Where 'D' is particle diameter size, k is a constant, 'λ' is wavelength of X-ray source (0.1541nm), 'β' is the full width at half maximum (FWHM) and 'θ' is the diffraction angle. Thus, the size of the AgNPs range from 17 to 46nm and the average size of particles was around 28nm. EDX spectrum analysis indicated strong signal in the silver region and confirms the formation of AgNPs. Table.1 shows the purity of AgNPs and the optical absorption peak is observed approximately at 3keV

Table.1 Inhibition Zones of different bacterial strains with AgNPs and plant aqueous extract

Test sample	Concentration (mg/ml)	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		Diameter of zone (cm)		
Aqueous plant extract	20	1.6	0.7	1.3
AgNPs	20	1	1.9	1.2
Tetracycline	20	2.8	3.5	3.3

which is typical for the absorption of metallic silver nano crystallites due to SPR (Kalimuthu*et al*., 2008). The biogenic AgNPs were characterized by FTIR spectra and this measurement was carried out to identify the available functional molecules of *A. echioides* leaf extract responsible for reduction and stabilization of the bio-reduced AgNPs. Fig.2.b shows prominent bands located at 3255, 2930, 1604, 1390 and 1120 cm^{-1} which are associated with O-H stretching (alcohols and phenol groups), C-H stretching (alkane group), N-H bending (1°amines group), C-H stretching (alkanes and alkyl groups) and C-O stretching (alcohols, carboxylic acids, esters and ether group) vibrations respectively. These results indicated that some proteins and metabolites such as terpenoid or flavonoid present in the *A. echioides* leaf extract, it may be responsible for capping and reduction of AgNPs. This result suggests that the presence of phenolic compound along with the flavonoid present in the plant leaf extract may responsible for the reduction of Ag^{+} to AgNPs (Raut*et al*., 2009), it also proven by Tripathy*et al.,* in *Azadirachtaindica* and in *Ixoracoccinea* by Muthu and Rengasamy., 2013. The phenolic compounds contain hydroxyl and carboxyl groups, which possess the ability to bind metals. The surface morphology of green synthesized AgNPs was observed using FESEM. The presence of AgNPs was confirmed by FESEM, which showed the synthesis of poly-dispersed spherical AgNPs of size that ranged from 17 to 27nm (Fig.3.a). The antioxidant activity of green synthesized NPs was assessed by DPPH scavenging assay by using Rutin as positive control. DPPH was a stable antioxidant compound that receives electrons or hydrogen from AgNPs. Fig.3.b shows DPPH assay that free radical inhibition by green synthesized NPs as well as *A.echioides* leaf aqueous extract.

The average percentage of inhibition of Phytosynthesized AgNPs was higher percentage when compared to plant leaf aqueous extract at various concentrations of green synthesized AgNPs. These results are also co-incidence with DPPH scavenging activity by platinum and AgNPs (Watanabe *et al*., 2009; Saikia*et al*., 2010). The antibacterial activity of aqueous leaf extract and AgNPs was analyzed against *B. subtilis, P. aeruginosa* and *S. aureus* by disc diffusion method. The culture plates were treated with AgNPs, which exhibit higher antibacterial activity than aqueous leaf extract. The zone of inhibition of AgNPs was found to be 1.9, 1.2 and 1.0cm respectively to *P. aeruginosa, S. aureus* and *B. subtilis* as compared to the standard antibiotic tetracycline which produced respective clearance zones in 3.5, 3.3 and 2.8cm at the same concentration of 20mg used in this study (Table.1).

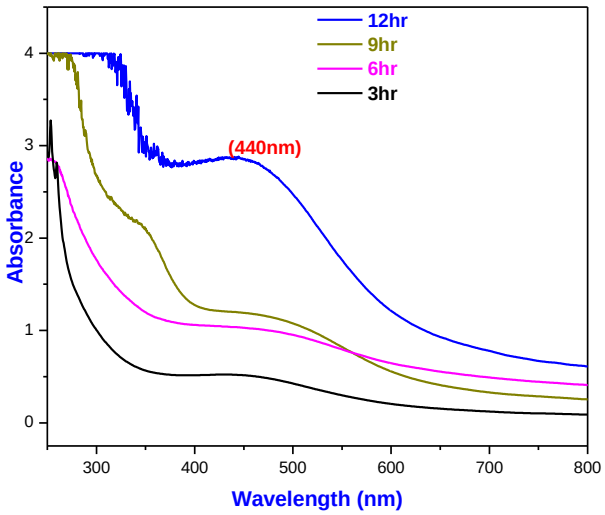
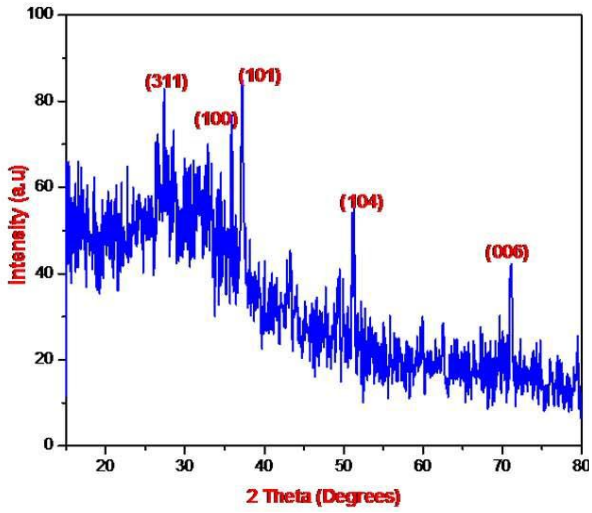


Fig.1UV-visible spectrum of biosynthesized AgNPs and its Plasmon excitation upon the interaction with *A. echioides*leaf extract

The antibacterial activity of AgNPs with respect their effects on bacteria growth were analyzed against *P. aeruginosa, S. aureus* and *B. subtilis* culture. From the fig.4 shows the AgNPs higher better antibacterial activity than the aqueous leaf extract of *A. echioides*. Previous reports also indicated that the green synthesized AgNPs exhibited highest antibacterial activity against *E.coli* and *S.aureus* from *Vitexnegundo* (Mohsen *et al*., 2011); papaya fruit derived AgNPs have been shown to display potent antibacterial activity against *E. coli* and *P. aeruginosa* and the activity was comparable to that of standard antibiotics tetracycline and rifamycine (Jain *et al*., 2009). Though silver has been practiced from the ancient time, recent advancement and reinvention of green AgNPs synthesis has become a popular research area of drug discover. The AgNPs exhibit antibacterial susceptible by attaching to the bacterial cell. Since the bacterial plasma membrane is the site of respiratory chain components, energy transducing systems and for active transport of molecules and ions (Singh *et al*., 2008; Gopinath*et al*., 2012; Jayachandra Reddy *et al*., 2014), any changes in membrane structure would ultimately result in inhibition of bacterial growth.



(a)

Fig. 2(a) XRD pattern of AgNPs synthesized using *A. echioides* leaf extracts

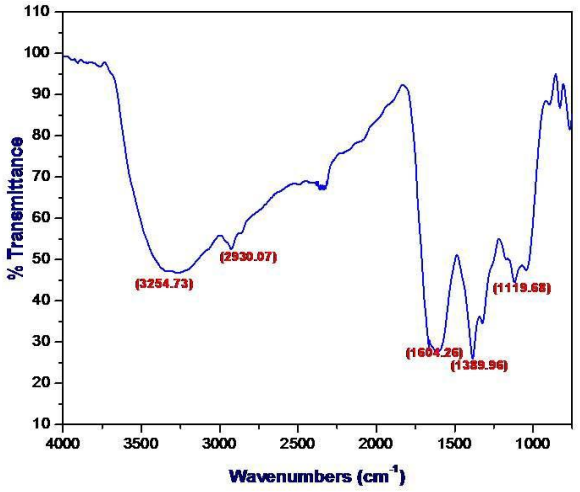


Fig.2(b) FTIR spectrum of green synthesized NPs from *A. echioides* leaf extract

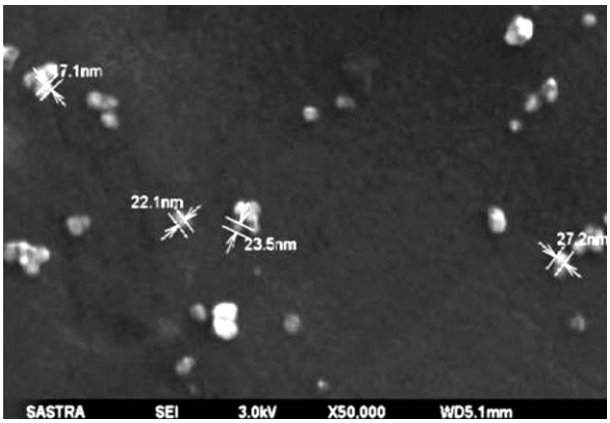


Fig.3(a) FESEM of AgNPs synthesized using *A.echioides* leaf extract

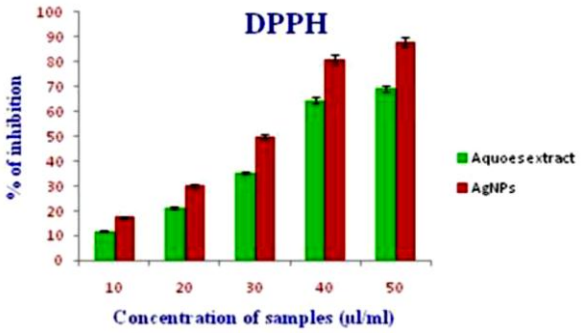


Fig.3(b) Percent scavenging of DPPH free radicals comparison with ascorbic acid (control) and AgNPs

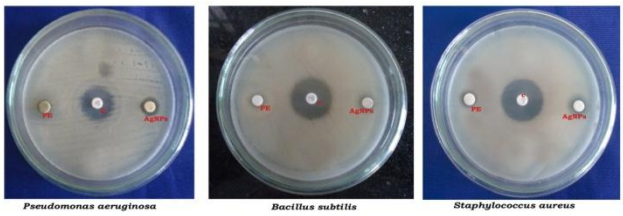


Fig.4 Antibacterial activity of three pathogenic bacteria against plant leaf aqueous extract and AgNPs. PE- Plant Extract; c- Control (Tetracycline); AgNPs- Disc prepared with AgNPs

Conclusion

The results of the present study report that green approach for synthesis of AgNPs using *A.echioides* leaf extract. Data from the studies revealed the advantages of green method for the synthesis of stable AgNPs with biomedical applications. This green approach makes the NPs as cost-effective, high yield, fast and eco-friendly. In addition, the size of the particles produced by the one-step synthesis are large enough for the particles to be used in biological applications. From the antioxidant and antibacterial study, it is established that AgNPs are possible sources for use in nanotherapy.

Acknowledgments

The authors are grateful to Prof. V.Venkatesalu, Professor and Head, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India for his help and provided lab facilities to complete the work and acknowledge their sincere thanks to Prof. S. Navaneethan for spare his time to correct the article.

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