Research Article

Antibacterial activity, biosynthesis and characterization of Silver nanoparticle from the leaf extract of *Andrographis echioides* (L.) Nees.

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Abstract

Objective: The green synthesis method is eco-friendly, efficient and effective method for the synthesis of silver nanoparticle using the leaf extract of the plant *Andrographis echioides*. It also focuses on antibacterial activity of synthesized nanoparticle. **Materials and methods**: The plant extract acts as a reducing or capping agent in their complex formation reaction. The obtained silver nanoparticle was characterized by using UV, FTIR, FE-SEM and EDAX studies. The nanoparticle was subjected to antibacterial activity of include both gram positive and gram negative bacterial pathogens. The morphology study of nanoparticle elemental analysis of silver and the particle size analyzed for size of the nanoparticle. **Results and conclusion:** The synthesized nanoparticle possesses appreciable size and shape. This was confirmed by the chemical studies. The antibacterial activity of silver nanoparticle showed the average zone of inhibition.

Keywords: *Andrgraphis echioides*, antibacterial , Silver nanoparticles, green synthesis

Introduction

The plant extract of synthesized nanoparticles an adopted method of green, eco-friendly production of nanoparticles and have been special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites (Ankamwar et al., 2005). SNPs have been synthesized from the naturally occurring sources and their products such as, green tea (*Camellia sinensis*), Neem (*Azadirachta indica*) and leguminous shrub (*Sesbania drummondii*) different leaf broth, natural rubber, starch , *Aloe vera* plant extract, lemongrass leaves extract (Vijayaraghavan et al., 2012).

SNPs unique properties such as, size and shape depending optical, electrical, and magnetic properties. It can be incorporated into antimicrobial applications. Several physical and chemical methods have been used for synthesized and stabilized silver nanoparticles (Klaus et al., 1999). Extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants (Mukherjee et al., 2001; Spring, 1995). Green chemistry method acts as natural reducing, capping and stabilizing agents to prepare SNPs with desired morphology and size have become a major focus of researchers. The green synthesis method is used to SNPs without the use of any harmful, toxic and expensive chemical substances (Ahmad et al., 2003; Ankamwar et al., 2005). Green biosynthesis of nanoparticles has a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications (SaSA, 2009). Pure silver has the highest electrical and thermal conductivity of the all metals and the lowest contact resistance. Silver can be present in four different oxidation states: Ag0, Ag2+, Ag3+ (Ramya, 2012).

Materials and Methods

Plant collection and identification

The fresh plant leaves of *Andrographis echioides* (L.) Nees collected from Thuraiyur region Trichy district at month of July. They were identified and authenticated by Dr. S. Soosairaj (SJCBOT 2474), Assistant professor, Department of Botany and with Rapinet Herbarium, St. Joseph college

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Preparations of plant extract

The 10g of dried plant leaves power was taken in 500ml conical flask having 100ml distilled water. The above mixture was heated for 20min at 60ºC. The boiled plant extract filtered using Whatman No1 filter paper. The filtrate was used for the synthesis of nanoparticles (Lekshmi et al., 2012).

Synthesis of silver nanoparticle

The 10ml of filtered plant extract was mixed with 90ml of 1mM solution of silver nitrate. It was kept in dark room through magnetic stirring at 100-120ºC for about 24hrs. The color changes were observed. The silver ions more likely to form complex with secondary metabolites present in the plant extract. The yellow colored solution changes in to dark brown color indicated the formation of silver nanoparticle (Ahmed et al., 2015).

Characterization of silver nanoparticle

The green synthesized silver nanoparticle was characterized by –UVvisible spectrophotometer at (Lambda 35) in the range of 190–1100 nm. The presence of functional groups in *A. echioides* leaves extract synthesized silver nanoparticle was identified by Shimadzu 8400 FTIR Spectroscopy (Perkin Elmer Spectrum) using KBr pellet technique at the range of 4000–400 cm^{-1}. The morphology of SNP was examined by using FE-SEM, and the presence of silver was confirmed by EDAX. The average size analyzed from leaf extract by Particle Size Analyzer technique. Screened antibacterial activity by synthesized nanoparticles (Baer et al., 2012).

Antibacterial activity

The synthesized silver nanoparticle of leaves extract of *A. echioides* was investigated for **antibacterial activity** by standard disc diffusion method (Bauer, 1966) against human pathogenic bacteria including both gram- positive and gram negative bacteria. The sterile disc paper was impregnated with synthesized silver nanoparticle solution and the disc was put on all spread pathogens nutrient agar plates. After which they were incubated at 37 °C for 24 hrs. Incubation, the clear zone appeared and it was measured in triplicate as a zone of inhibition (Mishra and Mishra, 2011; Tharachand et al., 2015). Standard **antibiotic Chloramphenicol** was used as a positive control.

Statistical analysis

The results of the agar disc diffusion activity were implemented in triplicates. Data of all experiments were statistically analyzed and conveyed as Mean ± Standard Deviation by using SPSS software.

Results and discussion

The UV-Vis spectrum of silver nanoparticles was scanned in the wavelength ranging from 190nm-1100nm spectrophotometer (Modal, Lambda 35). The spectrum showed the peak at 433.50nm with the absorption was 0.802824291 (Figure 1) and it was corresponded to the surface Plasmon resonance of SNP. Similarly reported that (Prathna et al., 2011) observed bands in the range 400nm – 420nm in the UV-Vis spectrum; 420nm by sea weed *kappaphycus alvarezeii* (Ganesan et al., 2013) 440nm by leaves of *Merrimia tridendata* and *Citrullus colocynthis* (Ganesan et al., 2013; Satyavani et al., 2011), 412nm by leaves of *Allium cepa* (Antariksh et al., 2012) respectively.

The FTIR measurement was carried out to identify the biomolecules for capping and efficient stabilization of the

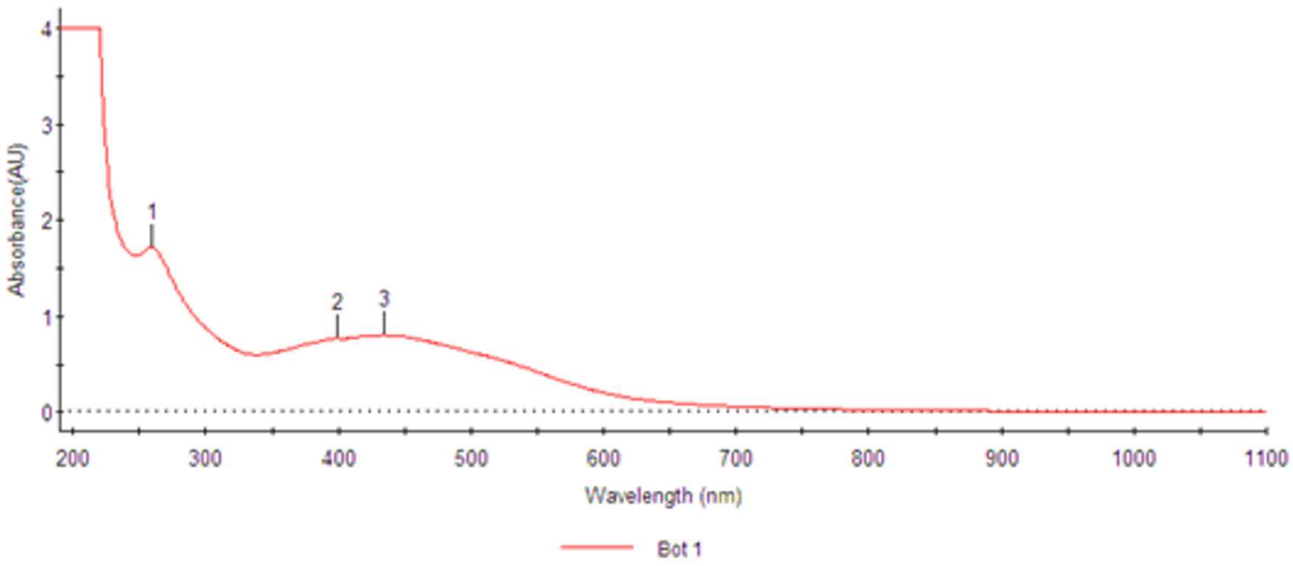

Figure 1. UV-VIS Spectrum of silver nanoparticle using *Andrographis echinoids* leaf extract

Table 1. The results of FT-IR spectroscopy

S. No	Wavelength (Cm-1)	Type of Vibration	Intensity	Functional group
1.	3439.35	N-H (Amine)	Medium (primary amine have two bands secondary have one band often very weak)	Amine
2.	2075.13	C-H (Bending)	Weak	Aromatic Compound
3.	1637.20	C=C (Stretch)	Variable	Alkane
4	1385.16	-C-H (Bending)	Variable	Alkane
5.	1075.36	C-F (Stretch)	Strong	Alkyl halide

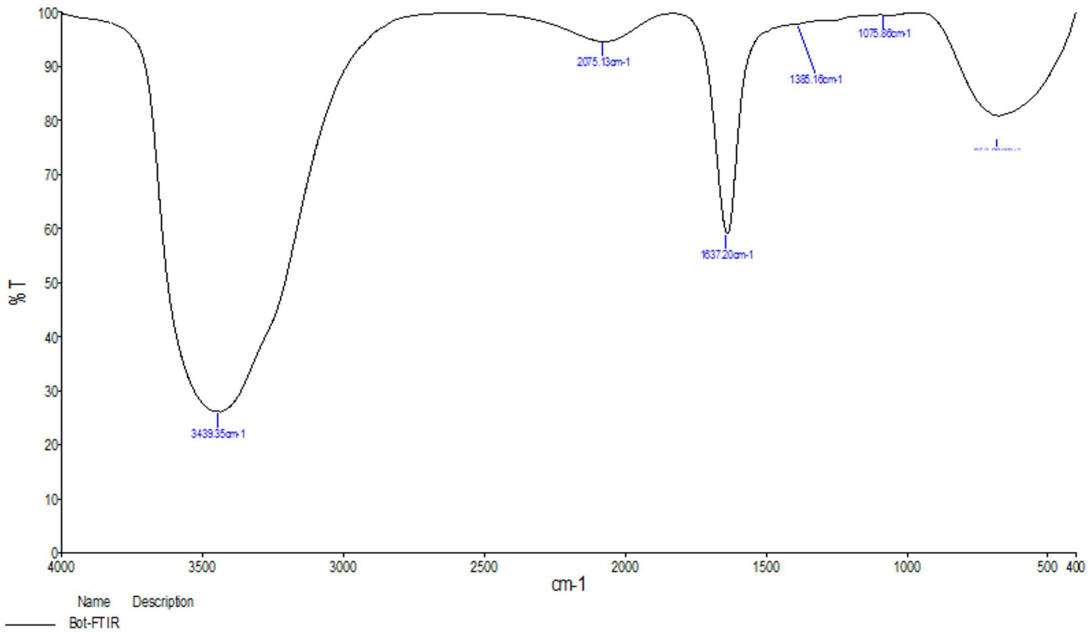

Figure 2. FT-IR Spectrum of silver nanoparticle using *Andrographis echinoids* leaf extract

metal nanoparticles synthesized. It was carried out to characterize the silver nanoparticle obtained from *A. echioides* leaf extract is shown in (Figure 2). In AgNP solution, prominent bands of absorbance were observed at around 1075, 1385, 1637, 2037 and 3439 Cm-1. The observed peak denote C-F (Strong) Alkyl halide, -C-H (Variable) Alkane, C=C (Variable) Alkane, C-H (Weak) Aromatic respectively. Sanmugam et al. (2014) have reported that these bonds could be due to the stretching of –OH in proteins, enzymes or polysaccharides present in the extracts. The small band at 2931.60 was due to the –CH stretching of alkanes the analogs scissoring and bending vibration was observed at 1442.66 and 1380.94 the moderated band noticed at 1720.39 implied of aldehydes ketones and carboxylic acids. The strong peak at 1620.09 denoted the bending vibration of amide I group and proteins present in the extract of *mimusops elengi* Linn. The similarly reported that (Zarnegar and Safari, 2015) the peak at 1635cm-^{1} was the stretching vibration of amide-I C=O group of protein. The band at 1405cm-^{1} could be due to the stretching of C-O group. The band at 1381cm-^{1} corresponded to C-N stretching vibration of aromatic amine. The peak at 1557cm-^{1} was attributed to the bending vibration of N-H group.

The Scanning Electron Microscopy was employed to visualize the size and shape of the silver nanoparticles (Figure 3). The shape of the nanoparticle provides to be

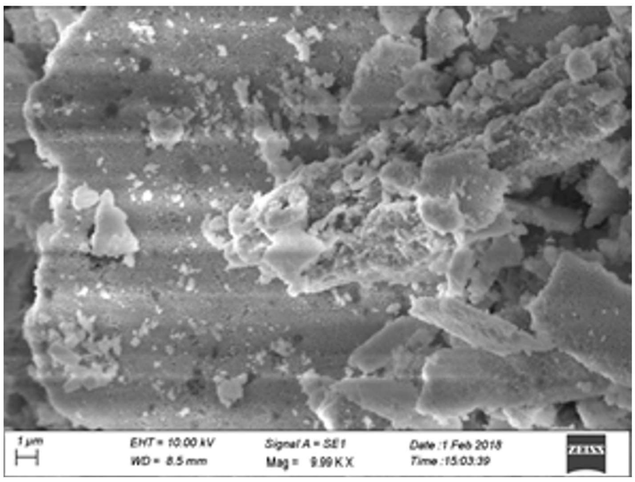

Figure 3. SEM image of Ag_2O

spherical. The formation of silver nanoparticles and their morphological dimensions by SEM study. Particle size analyzer (Figure 4) the various size of the nanoparticle ranging from 43.54nm to 113.28nm an average size of the nanoparticle 83.8nm was determined by the Particle Size Analyzer (PSA). The distribution at lower range of particle size has also in lower range of particle size (Figure 4). The PAS pattern of the suspension of silver nanoparticle synthesized using *Andrographis echioides* aqueous leaf extract. (Ghorbani, 2013) has reported that difference between the large and the smallest size of the nanoparticle. Their average particle size 73.96nm which indicated the narrow distribution of the Synthesized Nanoparticles.

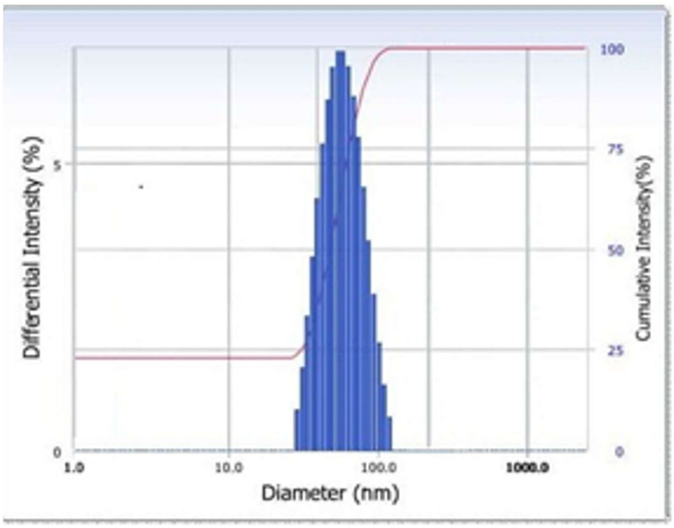

Figure 4. PSA image of Ag_2O

The percentage of SNPs in the sample was analyzed by EDAX for this the synthesized SNPs was characterized by using FE I quanta 200 FEG HR- SEM equipped with EDAX instrument. The EDS spectra showed the two type of elements such as Oxygen (0.56%) and Iron (2.93%) and (3.18%) present in the sample (Figure 5).

Antibacterial activity

An antibacterial activity of silver synthesized nanoparticles by disc diffusion method it was investigated against different human pathogenic bacteria. The synthesized silver nanoparticles exhibited good antibacterial activity against both gram positive and gram negative. Highest zone of inhibition observe on *Brivibacterium paucivorance* (10±0.5) and *Bacillus cerious* (9.6±0.5). The moderated zone of inhibition against *staphylococcus lentus* (8±1) and *Staphylococcus aureus* (8±1) the lowest zone noticed against *Echerichi coli* (7.3±0.5) *Serratia marcescens* (6.6±0.5) and *Klebseilla pneumoniae* (6.6±0.5). No zone of inhibition observed on *Staphylococcus heamolyticus, Entrobacter amnigenus* and *Klebseilla oxytoca* (Table 2). Gao et al. (2014) have reported that SNPs and their antimicrobial properties have been used most widely in the health industry, medicine, food storage, wound dressing, antiseptic creams and a number of environmental applications. Since ancient times, elements silver and its compounds have been used as antimicrobial agents: and it was used to preserve water in form of silver coins/silver vessels (Devi and Joshi, 2015; padalia et al., 2014). Silver nanoparticles enter the bacterial cell it forms a low

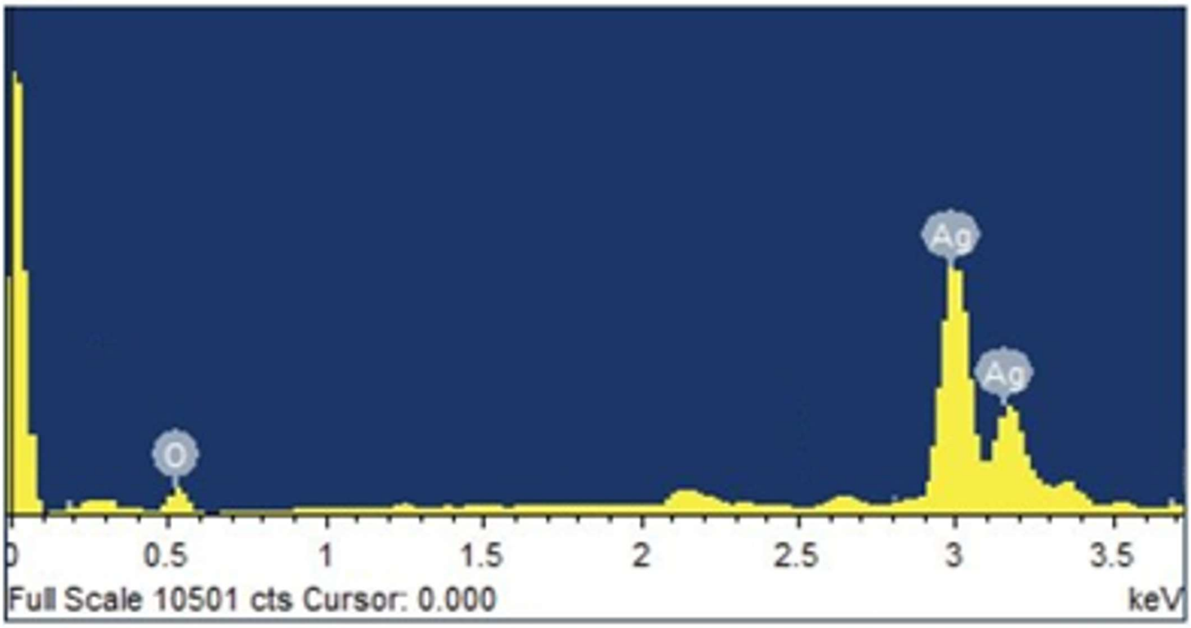

Figure 5. EDAX image of Ag_2O

Table 2. Antibacterial activity of silver nanoparticle

Test bacteria	Inhibition zone diameter in mm (mean ± SD)		
	$AgNO_3$		Positive Control
	Experiment (30µg/disc)	Negative (30mcg/disc)	Chloramphenicol (30mcg/disc)
Gram- positive			
Staphylococcus haemolyticus	-	-	24.3±1.1
Staphylococcus lentus	8±1	-	23±0
Staphylococcus aureus	8±1	-	26.6±0.5
Bacillus cereus	9.6±0.5	-	18.6±0.5
Gram- negative			
Escherichia coli	7.3±0.5	-	25±0
Serratia marcescens	6.6±0.5	-	28±0
Enterobacter amnigenus	-	-	24±1
Klebsiella pneumoniae	6.6±0.5	-	12.3±0.5
Klebsiella oxytoca	-	-	15.3±2.5
Brevibacterium paucivorans	10±0.5	-	30±0

molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cell, which enhance their bactericidal activity (Morones et al., 2005).

Conclusion

The silver nanoparticle was successfully synthesized using green synthetic method having size ranges from 43.54nm to 113.28nm the average size was 83.8nm (Particle Size Analyzer). The shape as spherical (Field emission scanning electron microscopy). The silver nanoparticle exhibit Average antibacterial activity against both positive and gram negative bacterial pathogens.

Conflicts of interest

Not declared.

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