Industrial Crops and Products 41 (2013) 235–240

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Short communication

Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*

M. Vijayakumar[a,*], K. Priya[b], F.T. Nancy[b], A. Noorlidah[a], A.B.A. Ahmed[a]

[a] *Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia*
[b] *Department of Biochemistry, PSG College of Arts and Science, Coimbatore 641014, Tamilnadu, India*

ARTICLE INFO

Article history:
Received 25 January 2012
Received in revised form 5 April 2012
Accepted 9 April 2012

Keywords:
Silver nanoparticles
Green synthesis
Antibacterial activity
Artemisia nilagirica

ABSTRACT

Currently, there is an increasing commercial demand for nanoparticles due to their wide applicability in various markets, such as medicine, catalysis, electronics, chemistry and energy. In this report, a simple and eco-friendly chemical reaction for the synthesis of silver nanoparticles (AgNPs) from *Artemisia nilagirica* (Asteraceae) has been developed. Silver nitrate was used as the metal precursor and hydrazine hydrate as a reducing agent. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDX) were used to characterise the nanoparticles obtained from *A. nilagirica*. The morphology of the AgNPs was determined by SEM and the average diameter of the particles was determined as 70–90 nm. The EDX analysis of the nanoparticles dispersion, using a range of 2–4 keV, confirmed the presence of elemental silver, with no other impurity peaks detected. In addition, the characterised AgNPs has the potential for various medical and industrial applications. The results showed that microbial susceptibility to AgNPs is different for each microorganism.

1. Introduction

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields (Matei et al., 2008). Studying these particular features has always been of great interest to many scientists. In fact, nanoparticles display completely unique properties in comparison with their large-size counterparts (Priyanka et al., 2009). A large number of materials which were considered to be safe actually develop toxicity at nanosize ranges (Reddy et al., 2007), which is mainly related to the increased specific surface area and high reactivity of nanosize materials (Nagarajan and Rajagopalan, 2008). A larger surface area, as in case of nanoparticles, ensures an increased range of probable interactions with bio-organics that are present on the viable cell surface (Rizwan et al., 2010). The considerable antimicrobial activities of inorganic metal oxide nanoparticles, such as ZnO, MgO, TiO_2 and SiO_2, and their selective toxicity to biological systems suggests a potential application as therapeutics, diagnostics, surgical devices and nanomedicine-based antimicrobial agents (Sobha et al., 2010).

The synthesis of metal nanoparticles and nanostructured materials is attracting attention in recent research because of their valuable properties which make them useful for catalysis (Narayanan and El-Sayed, 2004), sensor technology (Gomez-Romero, 2001), and the biological labelling of opto-electronic recorded media and optics (Gracias et al., 2002). Generally, metal nanoparticles can be prepared and stabilised by physical and chemical methods, but the chemical approaches, such as chemical reduction, electro-chemical techniques and photochemical reduction, are most widely used (Chen et al., 2001; Frattini et al., 2005). Studies have shown that the size morphology stability and chemical–physical properties of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of the interaction of metal ions with reducing agents, and the adsorption processes of stabilising agents with metal nanoparticles (Knoll and Keilmann, 1999; Sengupta et al., 2005). Hence, the design of the synthesis method in which the size morphology, stability and properties are controlled has become a major field of interest (Wiley et al., 2007).

Chemical reduction is the most frequently applied method for the preparation of silver (Ag) nanoparticles as stable, colloidal dispersions in water or organic solvents (Tao et al., 2006). The reduction of silver ions (Ag^+) in aqueous solutions generally yields colloidal silver with particle diameters of several nanometres (Wiley et al., 2005). Initially, the reduction of various complexes with Ag^+ ions leads to the formation of silver atoms, which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal particles (Kapoor et al., 1994). When the colloidal particles are much smaller than the wavelength of visible light, the solutions have a yellow colour with an intense band in the 380–400 nm range and other less intense or smaller bands at longer wavelengths in the absorption spectrum (Tessier et al., 2000).

* Corresponding author. Tel.: +60 1 43202066; fax: +60 3 79674178.
E-mail address: drvijay@um.edu.my (M. Vijayakumar).

0926-6690/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2012.04.017

The green synthesis of Ag nanoparticles involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) the selection of the solvent medium, (2) the selection of environmentally benign reducing agents, and (3) the selection of non-toxic substances for the stability of Ag nanoparticles. Therefore, the green chemistry type of Ag nanoparticle synthesis via chemical reaction has been reviewed. The present study highlights (i) the evaluation of silver nanoparticle content in aqueous leaf extracts of *Artemisia nilagirica*, (ii) the methods employed in the synthesis of Ag nanoparticles, characterised through SEM and EDX analysis, and (iii) the role of Ag nanoparticles in antibacterial experiments.

2. Materials and methods

2.1. Plant material

A. nilagirica leaves were collected and authenticated by the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

2.2. Chemicals

Silver nitrate (99.9%), absolute alcohol (99.9%), hydrazine hydrate, sodium citrate and sodium dodecyl sulphate (SDS) were purchase from S.D. Fine-Chem Pvt. Ltd., India. All chemicals were used as supplied. Double-distilled deionised water was used.

2.3. Microorganisms

The assessment of antibacterial activity was carried out using four different strains. The following microorganisms were used: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus subtilis*. The microbial cultures were maintained by the Department of Biotechnology, PSG College of Arts and Science, Tamilnadu, India.

2.4. Preparation of plant extracts

The fresh leaves of *A. nilagirica*, without any infection, were collected and 5 g of the leaves were weighed and washed with double-distilled water before use. The leaves were air-dried for 10 days and were then kept in the hot air oven at 60 °C for 24–48 h. The leaves were cut into fine pieces and 100 ml of double-distilled water was added. The mixture was boiled for 5 min before being decanted, and the mixture was cooled and filtered through Whatman No. 1 filter paper. The boiled extract was refrigerated and used for further experimental procedures.

2.5. Synthesis of silver nanoparticles

For the preparation of Ag nanoparticles, two stabilising agents, sodium dodecyl sulphate (SDS) and sodium citrate were used. For the synthesis of Ag nanoparticles, silver nitrate solution (from 1 mM to 6 mM) and 8% (w/w) SDS were used as a metal salt precursor and a stabilising agent, respectively. Hydrazine hydrate solution with a concentration ranging from 2 mM to 12 mM and sodium citrate solution (1–2 mM) were used as reducing agents. Sodium citrate was also used as stabilising agent at room temperature. The transparent colourless solution was converted to the characteristic pale yellow or pale red colour when citrate of sodium was used as stabilising agent. The occurrence of colour was an indication of the formation of Ag nanoparticles. The Ag nanoparticles were then purified by centrifugation. To remove excess silver ions, the silver colloids were washed at least three times with deionised water under nitrogen stream. A dried powder of the nanosized silver was obtained by freeze-drying. To carry out all of the characterisation methods and the interaction of Ag nanoparticles with bacteria, the silver nanoparticle powder in the freeze-drying cuvette was resuspended in deionised water, following which the suspension was homogenised using a Fisher Bioblock Scientific ultrasonic cleaning container (Guzman et al., 2009).

2.6. SEM analysis

Scanning electron microscopic (SEM) analysis was performed using the Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very small amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min.

2.7. EDX analysis

The particles were isolated by centrifuging 20 ml of suspension in deionised water containing Ag nanoparticles for 20 min at 10,000 rpm. The pellets were collected and were dried in the oven at 50 °C to remove any excess water. The sample was collected in the powder form and was used for EDX analysis. In order to carry out EDX analysis, the leaf extract-reduced AgNPs were dried and drop coated on to carbon film. EDX analysis was then performed using the Hitachi S-3400 N SEM instrument equipped with a Thermo EDX attachment.

2.8. Antibacterial activity study

Antibacterial activity of the synthesised AgNPs was determined using the agar well diffusion assay method (Perez et al., 1990). Approximately 20 ml of molten and cooled nutrient agar media was poured into sterilised petri dishes. The plates were left overnight at room temperature to allow any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells with diameters of 5 mM were prepared with the help of a sterilised stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labelled as A and S. The 'A' well was loaded with 100 μl of antibiotic chloromphenical, which was used as positive control, and the 'S' well was loaded with 100 μl of AgNPs synthesised from an aqueous leaf extract of *A. nilagirica*. The plates containing the bacterial and AgNPs were incubated at 37 °C, and then examined for evidence of zones of inhibition, which appear as a clear area around the wells (Cheesbrough, 2000). The diameter of such zones of inhibition was measured using a metre ruler, and the mean value for each organism was recorded and expressed in millimetres.

3. Results and discussion

The reduction of silver nitrate into AgNPs during exposure to plant extracts is followed by a gradual increase in colour development from clear to yellowish brown, as a result of the surface plasmon resonance phenomenon (Fig. 1). Fig. 2 shows representative SEM images recorded at different magnifications from drop-coated films of the AgNPs synthesised by treating $AgNO_3$ solution with *A. nilagirica*. The SEM images show a high density of AgNPs synthesised by *A. nilagirica* plant extracts, which was further confirmed by EDX. From the EDX spectrum, it is clear that *A. nilagirica* has a recorded weight percent (13%) of the AgNPs (Fig. 3). The antibiotic activity of AgNPs was investigated against various pathogenic organisms such as *S. aureus*, *E. coli*, *B. subtilis* and *P. mirabilis* using well-diffusion method (Fig. 4 and Table 1).

The synthesis of nanoparticles of different shapes and sizes is an emerging area of research due to their use in a variety

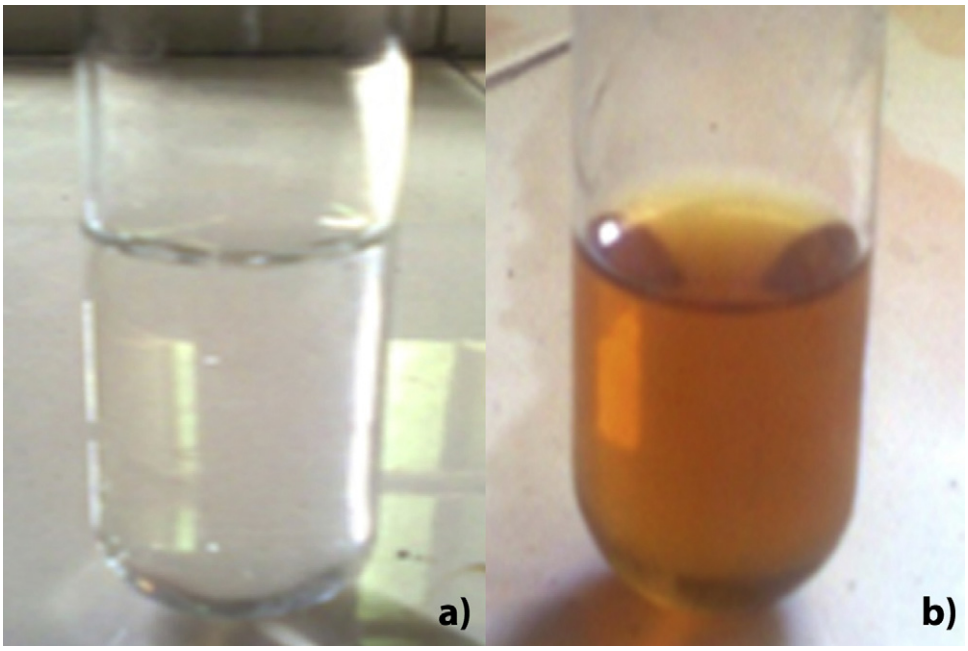

Fig. 1. Colour change of leaf extracts containing silver before and after the synthesis of silver nanoparticles.

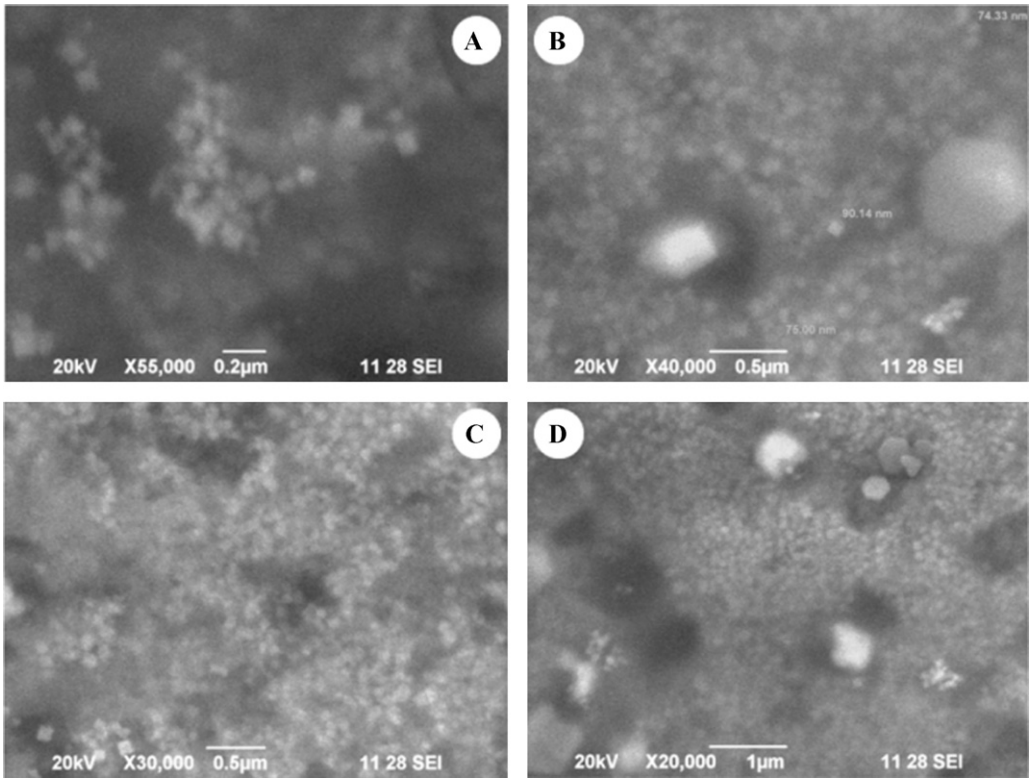

Fig. 2. SEM images of the Ag nanoparticles after bio-reduction of $AgNO_3$ with *Artemisia nilagirica* leaf extract.

Table 1
Antibacterial activity of synthesised Ag nanoparticles using the extract of *A. nilagirica*.

Name of the bacterial strain	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Proteus subtilis*
Ag nanoparticle zone of inhibition in mm	2.8	3.0	2.0	1.9
Reference drug zone of inhibition in mm	3.9	4.6	3.0	2.8

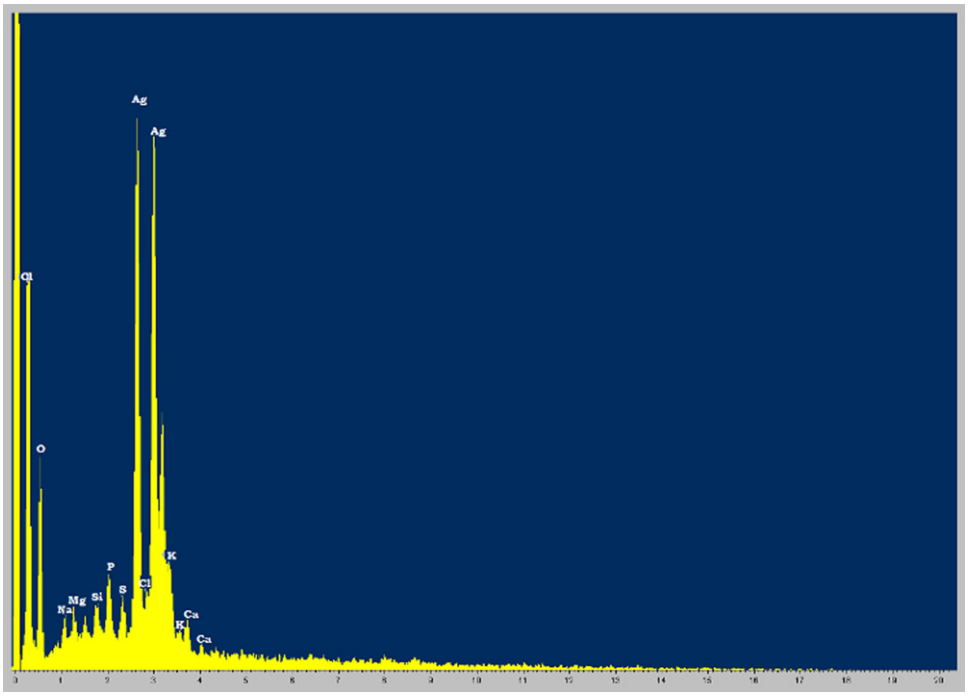

Fig. 3. EDX of the Ag nanoparticles synthesised by *Artemisia nilagirica* leaf extract.

of biological fields. To date, metallic nanoparticles are mostly prepared from noble metals, such as silver, platinum, gold and palladium. The use of metallic nanoparticles in the fields of catalysis, opto-electronics, diagnostics, and display devices has uncovered many significant findings. Among the noble metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine (Parashar et al., 2009). There are various methods for nanoparticle formation such as the sol-process, micelle, sol–gel process, chemical precipitation, the hydrothermal method, pyrolysis, chemical vapour deposition, and bio-based protocols (Leela and Vivekanandan, 2008).

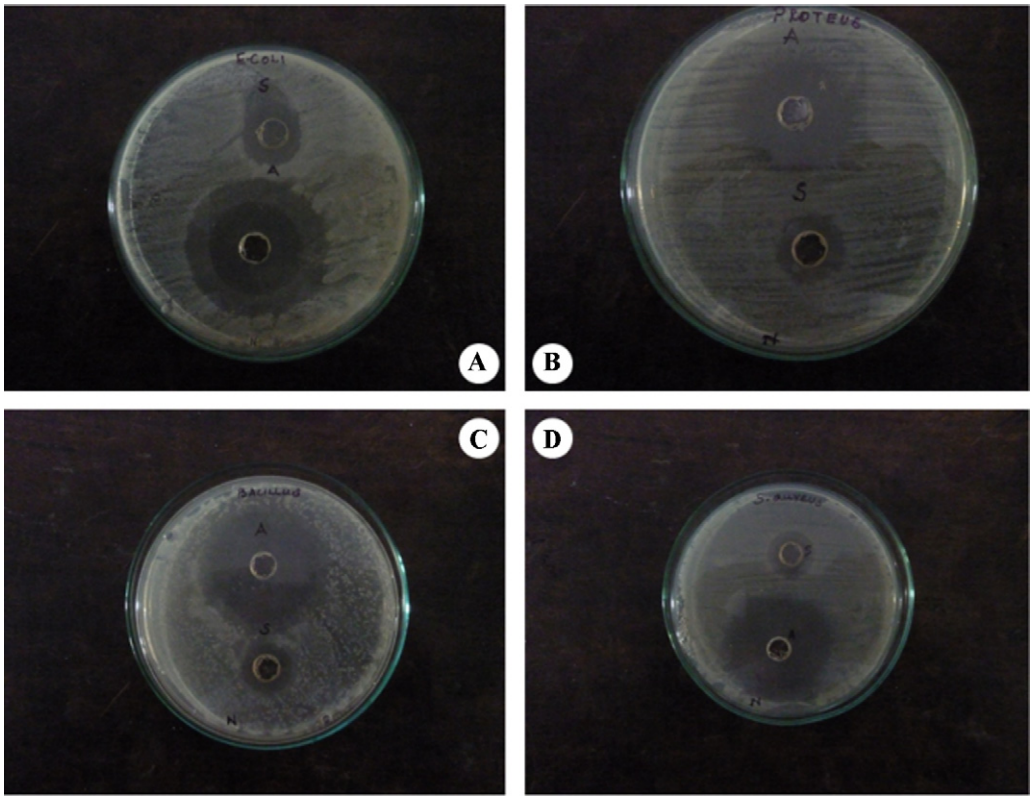

Fig. 4. Antimicrobial activity of Ag NPs against various pathogenic bacterial strains.

3.1. Characterisation of silver nanoparticles by SEM

Fig. 2 shows representative SEM images recorded at different magnifications from drop-coated films of the Ag nanoparticles synthesised by treating $AgNO_3$ solution with *A. nilagirica* leaf extract. The resulting AgNPs were predominantly square and of uniform size. Higher magnification showed the average diameter of these square nanoparticles to be about 70–90 nm. SEM images of biologically synthesised typical silver nanoparticles were obtained from 20 ml of leaf extract, although the exact shape of the nanoparticles was not clearly predicted. A small percentage of resulting nanoparticles were spherical and in the size range 10–45 nm. SEM images of nanotriangles in the same suspension are depicted in Fig. 2. Silver ions were not well separated from each other in the nanotriangles and dimensions in the size range 45–60 nm are capped with smaller particles due to the presence of small crystal and hexagonal particles of approximately 10–25 nm in diameter on the triangular surface.

The SEM analysis of Ag nanoparticles from *A. nilagirica* supports the results of Chandran et al. (2006) in *Aloe vera*. In addition, the rapid biosynthesis of silver nanoparticles of different shapes was observed and the sizes of nanoparticles were increased by high concentrations of *A. Nilagirica* leaf extract. It is interesting to note that the size of the square AgNPs shown in Fig. 2 increased to about 70 nm in size as the dosage of *A. nilagirica* leaf extract increased to 20 ml. According to the nanoparticle size of *A. nilagirica*, results obtained were comparable with silver nanoparticles synthesised using *C. camphora* size, which range from 55 nm to 80 nm.

3.2. Characterisation of silver nanoparticles by EDX

The EDX profile of Ag nanoparticles showed strong signals for silver atoms as shown in Fig. 3. The EDX pattern clearly shows that the Ag nanoparticles are crystalline in nature, which is caused by the reduction of silver ions using *A. nilagirica* leaf extract. The EDX analysis obtained in the present study confirmed the presence of silver nanoparticles of *A. nilagirica* and mostly showed strong signal energy peaks for silver atoms in the range 2–4 keV. In an earlier study, Gardea-Torresdey et al., 2003 obtained formation of individual spherical-shaped silver nanoparticles in the range 2.5–4 keV by using *Alfalfa*.

3.3. The antibacterial efficacy of nanosilver

The mechanism of the inhibitory effects of Ag^+ ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag^+ ion is crucial for its antimicrobial activity through the electrostatic attractions between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles (Dragieva et al., 1999; Hamouda et al., 2001; Dibrov et al., 2002). In contrast, Sondi and Sondi (2004) reported that the antimicrobial activity of AgNPs on Gram-negative bacteria was dependent on the concentration of the Ag nanoparticles used, and was closely associated with the formation of pits in the cell wall of bacteria. Following this, Ag nanoparticles accumulate in the bacterial membrane and cause permeability, resulting in cell death. However, because those studies included both positively charged Ag^+ ions and negatively charged Ag nanoparticles, they are not able to explain the antimicrobial mechanism of positively charged AgNPs (Fig. 4). Therefore, it is thought that there is another mechanism. Amro et al. suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by the progressive release of lipopolysaccharide molecules and membrane proteins (Amro et al., 2000). Also, Sondi and Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with AgNPs (Sondi and Sondi, 2004). Although their inference involved some sort of binding mechanism, the mechanism of the interaction between AgNPs and components of the outer membrane is still unclear (Table 1).

It is well known that Ag^+ ions and Ag-based compounds have strong antimicrobial effects, and many investigators are interested in using other inorganic nanoparticles as antibacterial agents (Crabtreed et al., 2003; Abuskhuna et al., 2004; Furno et al., 2004; Hamouda et al., 2001). These inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with the surface and the metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. To date, antimicrobial agents based on chemicals have been effective for therapy; however, they have been limited to use in medical devices and prophylaxis in antimicrobial facilities. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed, especially in medical devices. Ag^+ ions and Ag salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms. Also, many other researchers have tried to measure the activity of metal ions against microorganisms.

4. Conclusion

A green method to synthesise silver nanoparticles using the *A. nilagirica* plant extract has been developed. In this study, the silver nanoparticle content was from the superior leaf part of *A. nilagirica* and the properties were characterised by SEM and EDX. This characterisation is of use for large scale silver nanoparticle production, and could result in economic viability, as well as being eco-friendly for cancer treatment, drug delivery, sensors and commercial appliances and other medical and electronic applications. The toxicity study of silver nanoparticles on human pathogens opens a door for a new range of antibacterial activity.

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