Digest Journal of Nanomaterials and Biostructures Vol. 6, No 2, April - June 2011, p. 869 - 877

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM THE LEAF EXTRACTS OF EUPHORBIA HIRTA AND NERIUM INDICUM

M. MANO PRIYA, B. KARUNAI SELVI[a], J.A. JOHN PAUL[*]
Department of Biotechnology, Sri Kaliswari College, Sivakasi-626 130, Tamil Nadu, India.
[a]*Department of Botany, V.V.Vanniaperumal College for Women, Virudhunagar – 626 001, Tamil Nadu, India.*

Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. In this study, we report the synthesis of silver nanoparticles using the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. Synthesized particles are characterized by UV – Spectrophotometer, SEM, FTIR and X- ray diffraction analysis. The Debye-Scherrer equation was used to calculate particle sizes and the average size of the silver nanoparticles synthesized by *E.hirta* was 31nm and by *N.indicum* was 29nm. Further the synthesized silver nanoparticles were tested against common bacterial pathogens.

(Received March 16, 2011; Accepted May 3, 2011)

Keywords: Silver nanoparticles, *Euphorbia hirta*, *Nerium indicum*, *Antimicrobial activity*

1. Introduction

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. In the synthesis of metal nanoparticle by the reduction of the corresponding metal ion salt solutions. Nanoparticles are often referred to as clusters, nanospheres, nanorods and nanocups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Nanoparticles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects [1].

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis [2]. A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants [3-4].

The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nanoparticles has been reported. The shape and size of the nanoparticles synthesized using plants can be controlled and modulated by changing the pH [5].

The reduction of silver ions (Ag+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Medicinal herbs are the local heritage with global importance. Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite in one or more parts of these plants. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oils etc.

E.hirta belongs to the family *Euphorbiaceae* which is found in many parts of the world. The plant has been reported to treat wide variety of diseases [6]. *N.indicum* Linn. belongs to family

[*] Corresponding author: jajpaul@gmail.com

Apocynaceae. All parts of the plants are poisonous and used in eastern system of medicine. It is anti-helimenthic, diaphoretic, carminative, febrifuge, ophtholic, powerful heart poison and repellant [7].

The antibacterial effects of Ag salts have been noticed since antiquity and Ag is currently used to control bacterial growth in a variety of application, including dental work, catheters, and burn wounds. In fact, it is well known that Ag ions and Ag – based compounds are highly toxic to microbes, showing strong biocidal effects. Many synthetic procedures for silver nanoparticles are available, but a narrow and controlled size preparation seems to be difficult to obtain because that is depend on the concentration of reacting chemical and controlled reaction environment. Colloidal metal particles can be obtained by chemical synthesis but this method use toxic chemicals in the synthesis protocol, which raises great concern for environmental reasons [8]. Hence the present study is aimed to synthesis silver nanoparticles from two plants using silver nitrate. Further the synthesized silver nanoparticles were applied to act against common pathogens.

2. Materials and methods

2.1 Preparation of leaf extracts

Fresh leaves of two different plants i.e. *E.hirta* and *N.indicum* free from diseases were collected, washed thoroughly 2-3 times with tap water and with sterile water, shade-dried, powdered and used for extraction. 20 grams of dried leaf powders were continuously extracted with 250ml of chloroform followed by diethyl ether. The extract was stored at 4°C for further analysis.

2.2 Synthesis of silver nanoparticles

A 10mL of 10 ppm plant extract was added into 90ml of aqueous solution of 1mM silver nitrate **(**$AgNO_3$) for reduction of silver nitrate into Ag^+ ions and kept at room temperature for up to 7 hours. After 10 minutes the color of the solution changed into yellowish black indicating the formation of silver nanoparticles. The bio reduced silver nanoparticle solution was filtered through Whatmann No.1 filter paper and the filtrate was measured using UV-Visible absorbance.

2.3 UV-Visible spectra analysis

UV-Visible spectroscopy analysis was carried out on a Systronic UV-Visible absorption spectrophotometer 117 with a resolution of ±1nm between 200-1000nm processing a scanning speed of 200nm/min. The progress of the reaction between metal ions and the leaf extracts were monitored by UV-Visible spectra of silver nanoparticles in aqueous solution after diluting a small aliquot of 100 µL of the sample with 1 ml deionized water in different wavelengths i.e. 340, 380, 420, 460, 500, 540, 580 and 620nm and in different reaction times i.e., 1, 2, 3, 4, 5, 6 and 7 hours at 380 nm.

2.4 SEM analysis of silver nanoparticles

The pellet was subjected for SEM analysis. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis.

2.5 FTIR analysis of silver nanoparticles

For FTIR measurements, the synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 minutes. The pellet was washed thrice with 5 ml of deionised water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. This was analyzed by FTIR.

2.6 XRD analysis

A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks, using the Debye-Scherrer formula

$$D = 0.94\lambda/\beta\cos\theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes. λ is the X ray wave length**.** β is the full width at half maximum and θ is the diffraction angle.

2.7 Anti bacterial Assays

The antibacterial assays were done on bacterial organisms like *Escherichia coli*, *Streptococcus pyrogens*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, and *Citrobacter* sp. by standard disc diffusion method. Luria Bertani (LB) broth/agar medium was used to cultivate Bacteria. Fresh overnight culture of inoculums (100µl) of each culture was spread on to Muller Hinton Agar (MHA) plates. Sterile paper disc of 5mm diameter containing 10mg/liter silver nanoparticles and standard antibiotic chloroemphenicol (100µg/ml) containing discs were placed in each plate as control. The plates were incubated at 37^{o}C for overnight. Next day the inhibition zones around the discs were measured.

3. Results and discussion

3. 1. UV-Visible spectra analysis

The synthesized Ag nanoparticles were confirmed by visual observation. The color was changed into reddish brown due to reduction of silver ions. It was well known that Ag nanoparticles exhibits reddish brown color in aqueous solution due to excitation of surface plasmon vibrations. The synthesized Ag nanoparticle using *E.hirta* and *N.indicum* plant extracts were detected by UV-Vis spectrophotometer at various nm. Absorption spectra of silver nanoparticles formed in the reaction mixture at different nm. i.e. 340,380,420,460,500,540,580 and 620nm, the particle has increasingly sharp absorbance maximum peak at 380nm and gradually decreased while nanometer increased (Fig. 1). Absorption spectra of Ag nanoparticles formed in the reaction mixture at different time intervals at 380nm showed the particle has increasingly sharp between 2nd and 3rd hour and gradually decreased while hours increased (Fig. 2).

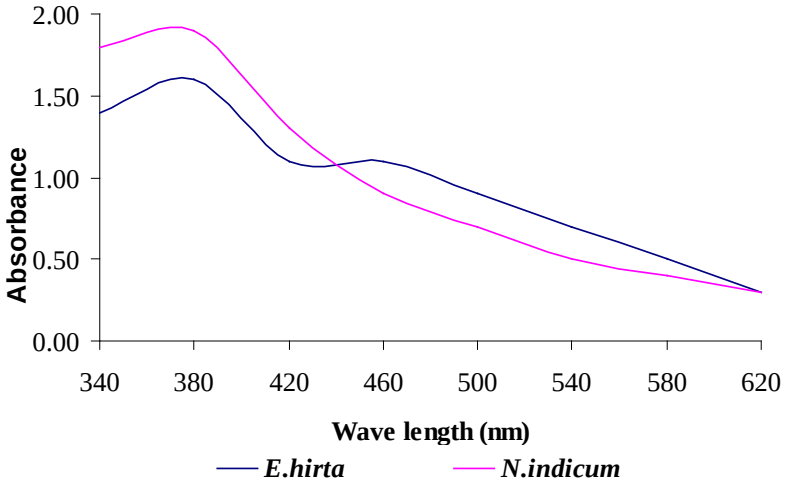


Fig. 1. UV–vis spectra recorded as a function of time of reaction by leaf extracts in the ranges 340–620 nm

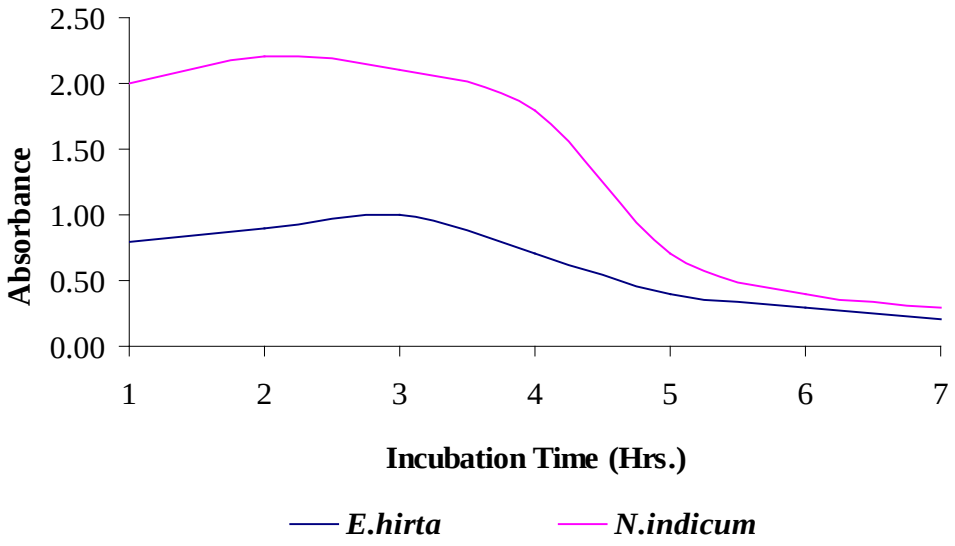


Fig. 2. Intensity absorbance of the plasmon resonance (380 nm) in function of time of reaction in an aqueous solution of 10−3 M AgNO3 with the plants extract.

The nanoparticles were primarily characterized by UV–Visible spectroscopy, which was proved to be a very useful technique for the analysis of nanoparticles. Reduction of Ag+ ions in the aqueous solution of silver complex during the reaction with the ingredients present in the plant leaf extracts were observed by the UV-Vis spectroscopy revealed that silver nanoparticles in the solution may be correlated with the UV-Vis spectra. As the leaf extracts were mixed with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated that the formation of Ag nanoparticles [9-10]. UV-Vis spectrograph of the colloid of Ag nanoparticles has been recorded as a function of time by using a quartz cuvette with silver nitrate as the reference.

In the UV- spectrum, the broadening of peak indicated that the particles are poly dispersed. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 2 hours of reaction making it one of the fastest bioreducing methods to produced Ag nanoparticles [11]. The surface plasmon band in the silver nanoparticles solution remains close to 380nm throughout the reaction period indicates that the particles are dispersed in the aqueous solution, with no evidence for aggregation. It was observed that the nanoparticles solution was stable for more than six months with little signs of aggregation [12].

3.2 SEM analysis

The scanning electron microscopic (SEM) image shown high density Ag nanoparticles synthesized by *E.hirta* and *N.indicum* plant extracts further confirmed the presence of Ag nanoparticles (Figs. 3a and 3b). It was shown that relatively spherical and uniform Ag nanoparticles were formed with diameter of 13 to 61 nm. The SEM image of silver nanoparticles were synthesized from two different plant extracts were assembled on to the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles. It was shown that relatively spherical and uniform silver nanoparticles were formed. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [13].

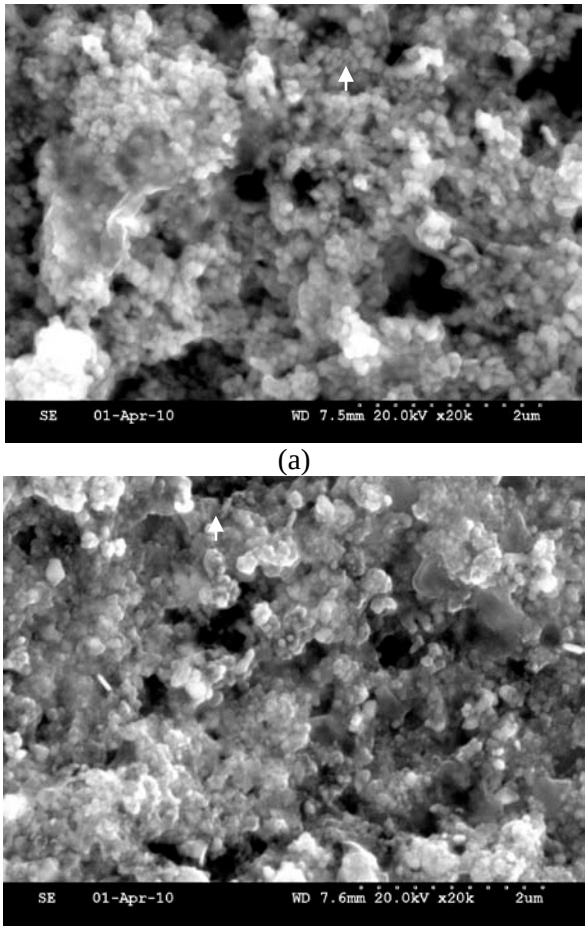
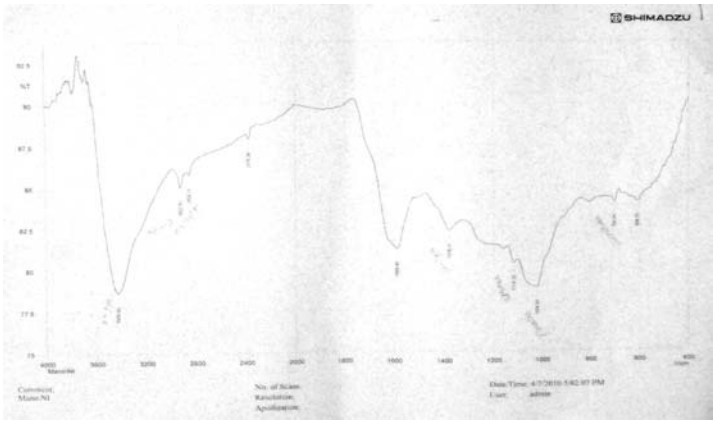


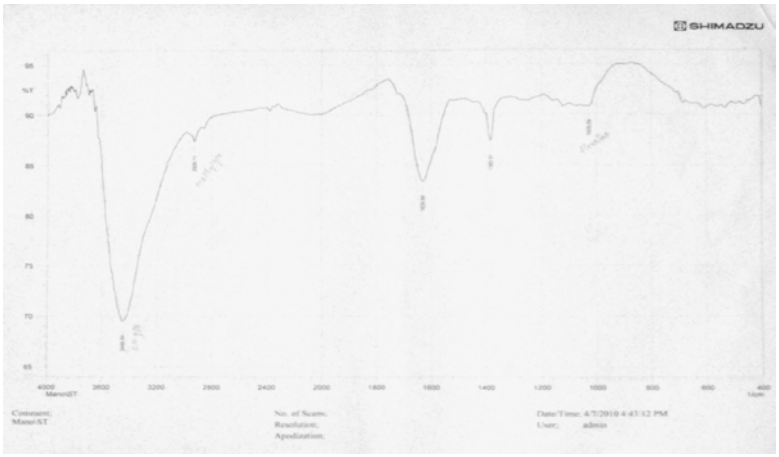
Fig. 3. SEM micrograph of silver nanoparticles synthesized by plant extracts (particles shown by arrow of 30 nm)- a) E.hirta b) N.indicum

3.3 FTIR analysis

FTIR spectrum of Ag nanoparticles synthesized from *E.hirta* and *N.indicum* extracts were shown in (Figs. 4a and 4b). FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by leaf broth. The Peaks near 3440 cm^{-1}, 2924cm^{-1} and 2854^{-1} assigned to OH stretching and aldehydic C-H stretching respectively. The weaker band at 1629 cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1041 cm^{-1} corresponds to C-N stretching vibration of the amine. The peak near 1741 cm^{-1} corresponds to C=C stretching (non conjugated). The peak near 833 cm^{-1} assigned to $–C=CH_2$. the peak near 677cm^{-1} and 651.96 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems -CH=CH(cis).



(a)



(b)

Fig.4. FTIR spectra of silver nanoparticles synthesized by plant extracts - a) E.hirta b) N.indicum

3.4 XRD analysis

The synthesized Ag nanoparticles using *E.hirta* and *N.indicum* plant extracts was further confirmed by the characteristic peaks observed in XRD image (Figs. 5a and 5b). The XRD pattern showed nine and eight intense peaks in the whole spectrum of 2θ values ranging from 10 to 80 for *E.hirta* and *N.indicum* respectively. An average size of the particles synthesized by *E.hirta* was 31nm with size ranging from 13 to 53 nm and an average size of the particle synthesized by *N.indicum* was 29nm with size ranging from 20 to 61nm. The typical XRD pattern revealed that the sample contains a mixed structure of silver nanoparticles. The average estimated particle sizes of the samples were calculated using the Debye-Scherrer formula. A number of Bragg reflections corresponding to the (111), (200), (220) and (311) sets of lattice planes are observed which may be indexed based on the face centered cubic (fcc) structures of silver, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles [14].

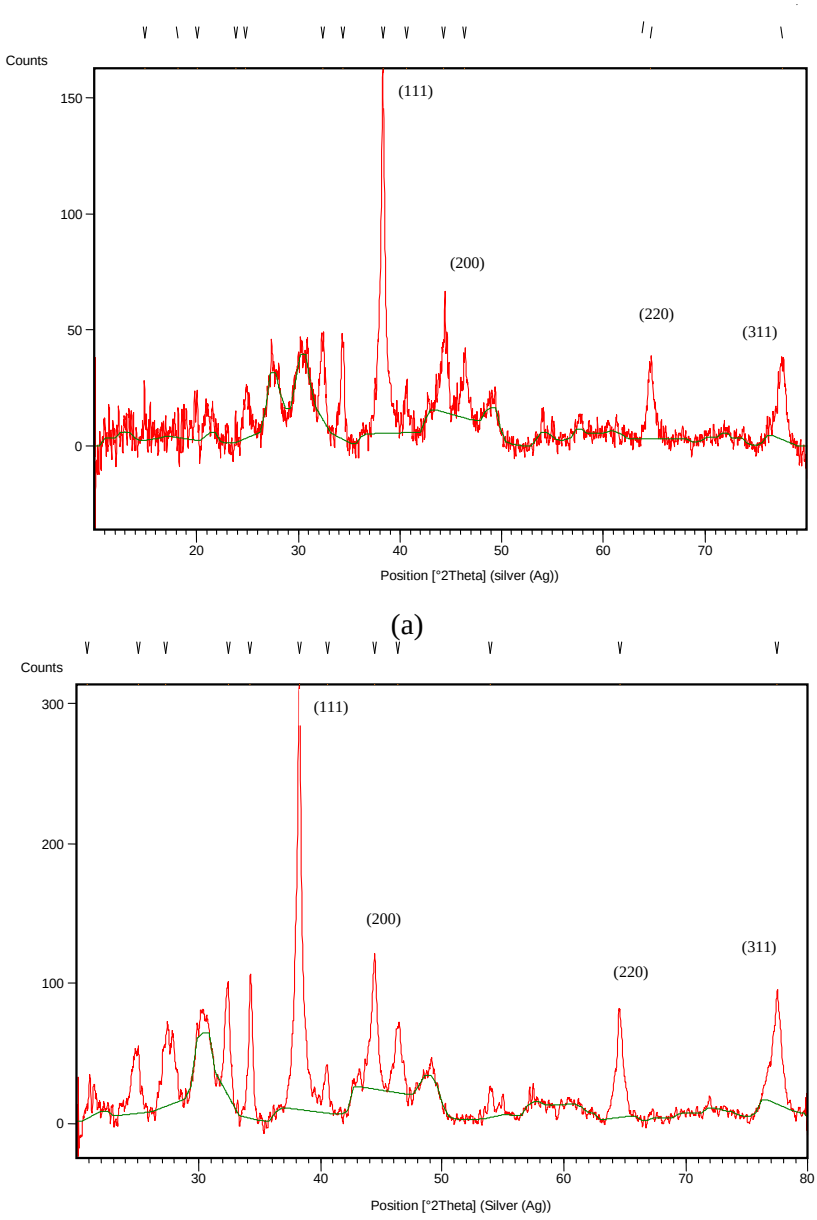


Fig.5 . XRD pattern of silver nanoparticles formed after reaction of plant extracts - a) E.hirta b) N.indicum

3.5 Anti bacterial Assays

Fig. 6. shows the antimicrobial activity of synthesized Ag nanoparticles against six different bacteria such as *E.coli, S.pyrogens, S.auereus, B.Subtilis, S.typhi and Citrobacter* sp. As it showed a clear inhibition zone (Table 1), the synthesized Ag nanoparticles were highly effective in their activity against *S.pyrogens, B.Subtilis, S.typhi and Citrobacter* sp. than antibiotics. Standard antibiotic disc (100µg/ml) chloremphenicol was used as control. Bacterial membrane proteins and DNA makes preferential sites for silver nanoparticles interaction as they possess sulphur and phosphorus compounds and silver have higher affinity to react with these compounds. Antibacterial effect of silver nanoparticles obeyed a dual action mechanism of antimicrobial activity, (i.e.) the bactericidal effect of Ag+ and membrane disrupting effect of the polymer subunits [10].

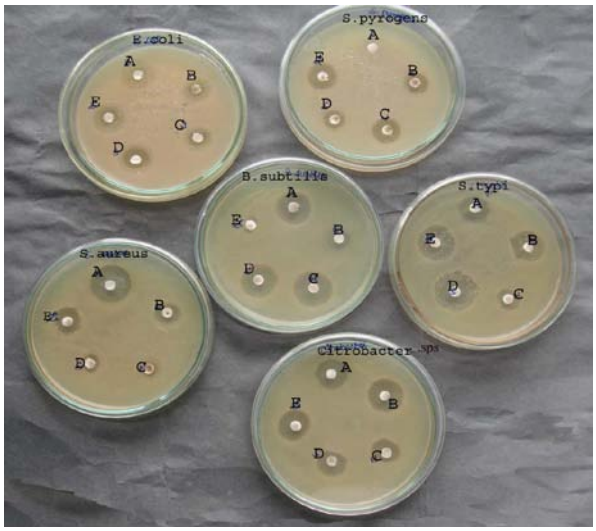


Fig. 6. Photograph of antimicrobial activity of antibiotics and silver nanoparticles against E.coli, S.pyrogens, S.aureus, B.subtilis, S.typhi and Citrobacter sp.

Table 1. Antimicrobial activities (Zone of inhibition in cm) of synthesized silver nanoparticles against six different common pathogens.

Treatments	Zone of Inhibition (cm)					
	E.coli	*S.pyrogens*	*S.aureus*	*B.subtilis*	*S.typhi*	*Citrobacter* sp.
A	1.7	0.6	1.6	1.5	1.3	1.4
B	0.9	1.0	0.5	0.8	1.4	1.6
C	0.7	1.3	0.3	1.6	0.5	1.2
D	1.1	1.0	0.4	1.1	1.6	1.1
E	0.9	1.4	0.6	0.3	1.5	1.3

A) Antibiotics as control (Chloroemphenicol-100µg/ml)
B) Ag NPs from chloroform extract of *E.hirta*
C) Ag NPs from diethyl ether extract of *E.hirta*
D) Ag NPs from chloroform extract of *N.indicum*
E) Ag NPs from diethyl ether extract of *N.indicum*

4. Conclusions

The bio- reduction of aqueous Ag+ ions by the leaf extracts of the plant has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. But the capability of other plant parts such as fruit and root as a capping and reducing agent is not tested and not well defined. In the present study we found that leaves were good source for the synthesis of silver nanoparticles has many

advantages such as, ease with which the process can be scaled up, economic viability and to obtain smaller particle size. This study demonstrated the possibility of use of biologically synthesized silver nanoparticles and their incorporation in materials, providing them sterile properties. The cotton fabrics incorporated with these silver nanoparticles exhibited antibacterial activity against the common pathogens. Prepared nanoparticles can be used as bactericidal and in wound healing, water purification and also in the field of medicine.

Acknowledgments

The authors gratefully acknowledge the Central Electro Chemical Research Institute, Karaikudi, Tamil Nadu for their help in SEM and XRD analysis.

References

[1] Armendariz, V., J. L., Gardea- Torresdey, I.., Herrera, M., Jose-Yaceman, J.R. Peralta-Videa and P. Santigo. Journal of Nanoparticles Research, **6**(4), 377 (2004).
[2] Bhattacharya, D and R.K. Gupta. Nanotechnology and potential of microorganisms. Critical Reviews in Biotechnology, **24**(4), 199 (2005).
[3] Mohanpuria, P., N.K. Rana and S.K. Yadav. Biosynthesis of nanoparticle: technological concepts and future applications. Journal of Nanoparticles Research **7**, 9275 (2007).
[4] Farooqui, M.A., P.S. Chauhan., P.Krishnamoorthy, J. Shaik. Digest Journal of Nanomaterials and Biostructures,. **5**(1), 43 (2010).
[5] Gardea-Torresedey, J.L., G. Gombez., M. Jose-Yaceman,, J. G. Parsons, J.R. Peralta- Videa, H. Tioani. Journal of Hazardous Substance Reseach **4,** 1 (2003).
[6] Chikaogueke. C., N. Jude., C. Ifeanyi Okoli., N. Beatrice and K. Anyanwu. Antibacterial Activities and Toxicological potentials of Crude Ethanolic Extracts of Euphorbia hirta, Journal of American Science **3**(3), 11 (2007).
[7] Baquer. S.R and M.Tasnif. Medicinal Plants of southern West Pakistan. Bulletin monograph: Nat Matt **3**, 68 (1967).
[8] Lee. H. J. and S. H. Jeon. Bacteriostasis of nanosized colloidal silver on polyester nonwovens. Text .Res. J.**74**, 430 (2004).
[9] Shankar,S.S., A. Ahmed., B. Akkamwar., M. Sastry., A. Rai and A. Singh. Biological synthesis of triangular gold nanoprism. Nature **3,** 482 (2004).
[10] Jain. D. H., D. Kumar., S. Kachhwaha and S.L. Kothari, Digest J. of Nanomaterials and Biostructures, **4,** p.557 (2009).
[11] Kim. D.V.M., M.S.E. Kuk., J. Kyeong Nam, M.S.Yu. Biol and Med **3,** 95 (2007).
[12] Saifuddin. N. C., W. Wong and A.A. Nur Yasumira. J. of chem. **6**(1), 61 (2009)
[13] Song, J.Y. and B. S. Kim. Biological synthesis of bimetallic Au/Ag using persimmon (Diopyros kaki) Leaf extract. Korean. J. chem. Eng.,. **25**(4): p. 808 (2008).
[14] Sathyavathi, R., M. Balamurali Krishna., S. Venugopal Rao., R. Saritha and Narayana Rao. Biosynthesis of Silver nanoparticles using coriandrum sativum leaf extract and their Application in Nonlinear Optics. Adv. Sci Letters,. **3**, 1 (2010).