Green synthesis of Silver Nanoparticle using *Elettaria Cardamomom* and Assessment of its Antimicrobial Activity

GnanaDhas GnanaJobitha, Gurusamy Annadurai[*1], Chellapandian Kannan*[2]

[1]Environmental Nanotechnology Division, Sri Paramakalyani Centre for Environmental Sciences,

Manonmaniam Sundaranar University, Alwarkurichi – 627412, Tamilnadu, India.

[2]Department of chemistry, Manonmaniam Sundaranar University, Abishekapatti - 627 012, Tamilnadu, India.

Abstract

An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. This study reports the synthesis of silver Nanoparticle by using commercially available spice *Elettaria cardamomom* without the use of toxic chemicals. Visually, the formation of silver Nanoparticle was confirmed by observing the colour changes from pale yellow to dark brown colour and an intense peak was observed in the UV- spectrophotometer at 460 nm. Further, silver nanoparticle was characterized formation was confirmed by XRD, SEM and EDAX analysis. The possible functional groups in the plant extracts were identified by FTIR analysis. Further more the phytosynthesized silver Nanoparticle show the antimicrobial activity against the pathogenic microbial strains (*Bacillus subtilis, Klebsiella planticola*). This novel green approach is a rapid, facile and used for large scale production of metallic Nanoparticle.

Key words: *Elettaria cardamomom,* Silver Nanoparticle, XRD, SEM, FTIR

Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science and technology. It provides the ability to create materials, devices and systems with fundamentally new functions and properties [1]. In the era of nanotechnology, research on nanomaterials is growing day-to-day with increasing demand. This is because metals in nanometer size will exhibit special properties that differs and is more superior from bulk metals [2]. Nanostructured metals are becoming more important in applications of catalysis, sensors, electronics, biotechnology and biomedicine [3]. Recently, research in synthesis of Nanoparticle using microbes and plant extracts gaining more importance due to its eco-friendliness, flexible and main point is the evasion of toxic chemicals [4]. When compare to microbes, plant mediated synthesis is actively practicing by the researchers for its positive advantages like avoidance of maintaining the microbial culture, time-consuming and cost effective [5]. Previously, noble Nanoparticle are synthesized by using various plant materials such as *Gliricidia sepium* [6], *Mucuna pruriens* [7],*Cassia occidentalis* [8], Banana peel [3], *Azadirachta indica* [9],*Desmodium triflorum* [10], *Cassia fistula* [11], *Cinnamomum zeylanicum* [12], *Opuntia ficus-indica* [13],*clerodendrum inerme* [5],*Magnolia kobus* [14], *Coriander* [15] ,*Coriandrum Sativum* [16] etc.

Nowadays, medicinal plants [17], Fruits [18,19, 20] ,weeds [21] and spices [22] has been used for production of Nanoparticle. The spices are the new face in the green synthesis and already Singh *et al.,* [22] have been reported the synthesis of silver Nanoparticle using the clove buds. Due to the presence of aromatic flavor compounds in the clover buds were likely to be responsible for the reduction of silver ions to silver Nanoparticle

[*]**Correspondence to:**

G. Annadurai, PhD, C.Kannan PhD,
Environmental Nanotechnology Division, Department of chemistry
Sri Paramakalyani Centre for Environmental Sciences,
Manonmaniam Sundaranar University,
Alwarkurichi – 627412, Tamilnadu, India
Email: gannadurai@hotmail.com
chellapandiankannan@gmail.com
Tel/Fax: +91-4634-283406

[22]. The Cardamom *(Elettaria cardamomom*) venerated as the 'Queen of Spices' and belongs to the family, Zingiberaceae. It is one of the most important and easily available spices. Seeds of cardamom are the source for its aroma and flavor. Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic compounds and flavonoid compounds [23].

The present work reports the synthesis of silver nanoparticle by using the seed extract of *Elettaria cardamomom*. Herein, we investigated the efficiency of reduction of silver ions (Ag^+) as well as formation of silver-Nanoparticle from the aqueous solution of $AgNO_3$ and extracts of *Elettaria cardamomom.* Further, silver Nanoparticle were characterized by UV-vis spectroscopy, XRD, SEM, EDAX and FTIR.

MATERIALS AND METHODS

Preparation of seed broth: Fresh *Elettaria cardamomom* seeds (Fig.1) were purchased from local market and it was thoroughly washed with distilled water and dried at 37 °C. After that, grind the seeds to make fine powder using modern pestle. About 10g of freshly prepared dry seed powder were suspended in 100 ml of double distilled water and boiled at 60° C temperature for 15 minutes. Then filtered the solution through a whatman filter paper No.1 and the solution was used as stock solution for further experimental use.

Figure 1 - Showing the nature of (a) *E. cardamomom* seeds with coat, (b) *E. cardamomom* seeds

Phyto-assisted Synthesis of silver Nanoparticle: The silver nitrate ($AgNO_3$) was purchased from Himedia Laboratories, Mumbai, INDIA and it was used as a precursor in the silver nanoparticle synthesis process. About 5 ml of *Elettaria cardamomom* seed extract was added to 20 ml of different concentration of $AgNO_3$ (0.16, 0.18, 0.20, 0.22 and 0.24 gm) aqueous solution in conical flask. The mixture was boiled at 80°C for 20 minutes, while heating the colour of solution was changed from pale brown to dark brown. The reduction of Ag^+ ions to Ag^0 was monitored by measuring the UV-vis spectrum of various concentration of reaction mixture (silver nitrate solution + seed extract).

CHARACTERIZATION STUDIES

The reduction of silver metal ions to silver Nanoparticle was preliminary analyzed by using UV-visible Spectrophotometer (Perkin-Elmer) of wavelength about 360-700 nm at regular time intervals. After that, the solution mixture (Seed extract and Silver nitrate) was centrifuged at 15,000 rpm for 20 min and repeated the centrifugation process for 3 to 4 times and filtered the resulting solution through Whatman No.1 filter paper and dry the pellet in hot air oven. Furthermore, the dried particles were characterized by (SEM) were performed using a Philips model CM 200 instrument to study the morphological nature of the nanoparticle. EDAX analysis was performed to verify the crystallinity of the particles Ag Nanoparticle. The X-ray diffraction patterns were recorded on an X-ray diffractometer. The XRD pattern proposes the crystalline nature of the Ag Nanoparticle synthesized from aqueous extracellular *Elettaria cardamomum* seed extract. The FTIR measurements (Perkin-Elmer) were carried out to identify the potential possible biomolecules of *Elettaria cardamomom* seed extract responsible for the reduction, capping of and efficient stabilization of the bio-reduced silver Nanoparticle.

RESULTS AND DISCUSSION

UV–visible spectroscopy is an important technique to determine the formation and stability of metal Nanoparticle in aqueous solution. The reaction mixture changes the colour by adding various concentrations of metal ions. These color changes arise because of the excitation of surface plasmon vibrations in the silver Nanoparticle [24]. It shows yellowish to dark brown in colour (Fig. 2). The dark brown colour of silver colloid is accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromagnetic field [2]. The strong surface plasmon resonance band appears at the range of 440-480 nm and the broadening of peak indicated that the particles are monodispersed.

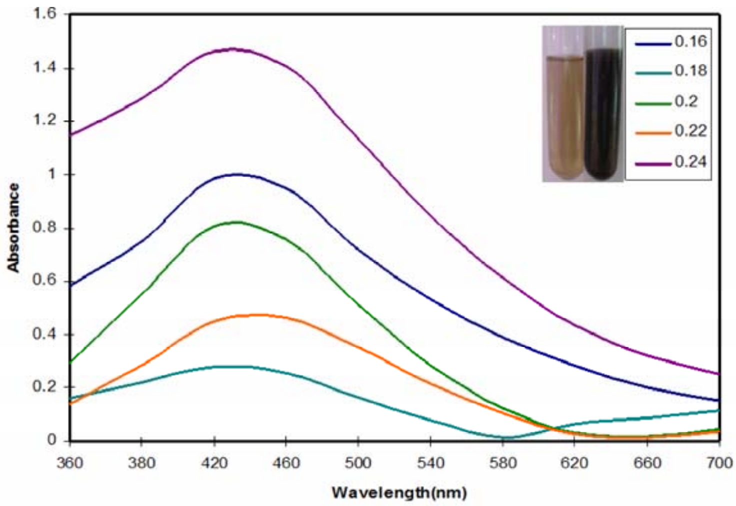

Figure 2 - UV-Visible absorption spectra of biosynthesized silver Nanoparticle from *E.cardamomom* depicting peak at 460 nm. The inset shows extract before (left) and after (right) exposure to silver nitrate.

After the reduction of silver ions by the *Elettaria cardamomom* seed extract, the solution was centrifuged at 10,000rpm for 15minutes. Repeat the centrifugation process to separate silver nanoparticle free from other organic compounds present in solution. The Ag Nanoparticle pellet obtained after centrifugation were redispersed in distilled water and washed for 2 or 3 times. The synthesised Nanoparticles morphology were characterised by scanning electron microscope, this micrograph was taken using a Philips model CM 200 instrument. The silver Nanoparticle formed were predominantly spherical with uniform shape (Fig. 3).It is known that the shape of metal Nanoparticle considerably change their optical and electronic properties [25].The SEM image exposed that the formed nanoparticle was spherical in shape formed with the size range of 40-70 nm.

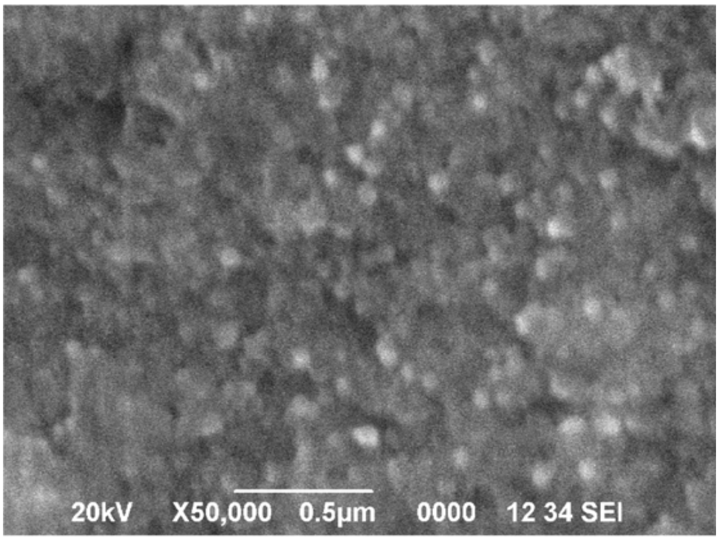

Figure 3 - The SEM images of silver Nanoparticle synthesized from the *E. cardamomom* seed extracts at various magnification.

The EDAX attachment on the SEM provided chemical analysis of the field of view as well as spot analyses of minute particles and confirmed the presence of specific elements. The presence of elemental silver in the reaction mixture was confirmed by EDAX analysis (Fig. 4). The dried silver Nanoparticle were mounted on specimen stubs, and examined under a Philips XL-30. The silver Nanoparticle was exhibited an optical absorption band peaking at 3 keV which is typical of the absorption of metallic silver Nanoparticle. The SEM-EDAX analysis displayed signature spectra for silver and thus convincingly evidenced the presence of this noble metal in the microcubes and microwires These results are consistent with other reports on the EDAX analysis of silver structures synthesized by using extracts derived from the leaves of *Cassia fistula* , *Catharanthus roseus* [11, 26].

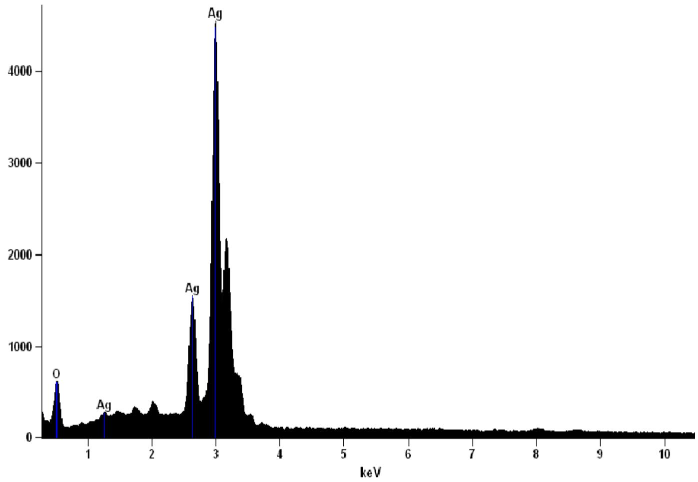

Figure 4 - EDAX spectra of prepared silver Nanoparticle by *Elettaria cardamomom* .

A representative XRD profile of the silver nanoparticle displaying the structural information and crystalline nature of the silver Nanoparticle synthesized from aqueous extracellular *E. cardamomom* seed extract (Fig.5). The XRD measurements of film of the biologically synthesized silver Nanoparticle cast onto glass slides were done and examined on a Phillips PW 1830 instrument. The XRD pattern showed three intense peaks at 38°, 47°, and 77° corresponding to 111, 200 and 311 planes in the whole spectrum of 2θ. The average particle size of the silver Nanoparticle was identified by using the following Debye-Scherrer's equation (1) and it follows [13].

$$D = \frac{K\lambda}{\beta_{1/2} COS\theta} \qquad (1)$$

The equation uses the reference peak width at angle θ, D- Average grain size of crystallite, K is a shape factor which is 0.9 for spherical particles, λ - Incident wavelength, and $\beta_{1/2}$ is the Diffracted Full Width at Half maximum (in radians). The XRD patterns displayed are consistent with earlier reports on microstructures [27, 20].

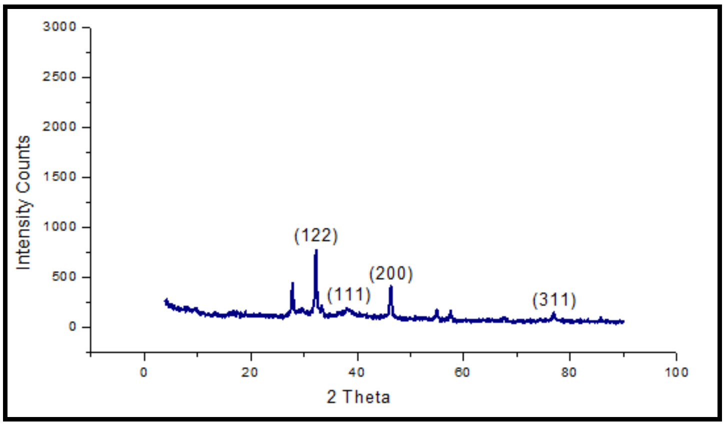

Figure 6 - FTXRD patterns of silver Nanoparticle synthesized using E. cardamomom seed broth.

FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Ag Nanoparticle synthesized using *Elettaria cardamomom.* This spectrum shows lot of absorption bands (Fig. 6) indicates the presence of active functional groups in the synthesized silver Nanoparticles. The intensity peaks are slightly increased for the period of silver nanoparticle synthesis like 3429, 1637, 1382, 595 cm^{-1} as well as some intensity peaks decreased like 1045, 2080, and 2359 cm^{-1}. Fig 5 shows the band at 3429 correspond to N-H, O-H Stretching vibrations of alkanes, amide, alcohol and H–bonded to phenols. The peak at 1637 indicate to C=C, C=O stretching vibrations to alkenes and amide. The peak at 1382 represents to C-H in plane bend to alkenes. The peak at 595 corresponds to C- Cl, C-Br stretching vibrations to alkyl halides. The band at 2080 corresponds to C-N stretching vibration. The weak band at 1045 indicates C-O, C-N stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids, ethers, esters and aliphatic amines in the seed extract. The presence of active functional groups in seed extract results in the swift reduction of silver ions to silver Nanoparticle. To obtain good signal to noise ratio of silver nanoparticle were taken in the range 500–3400 cm^{-1}.

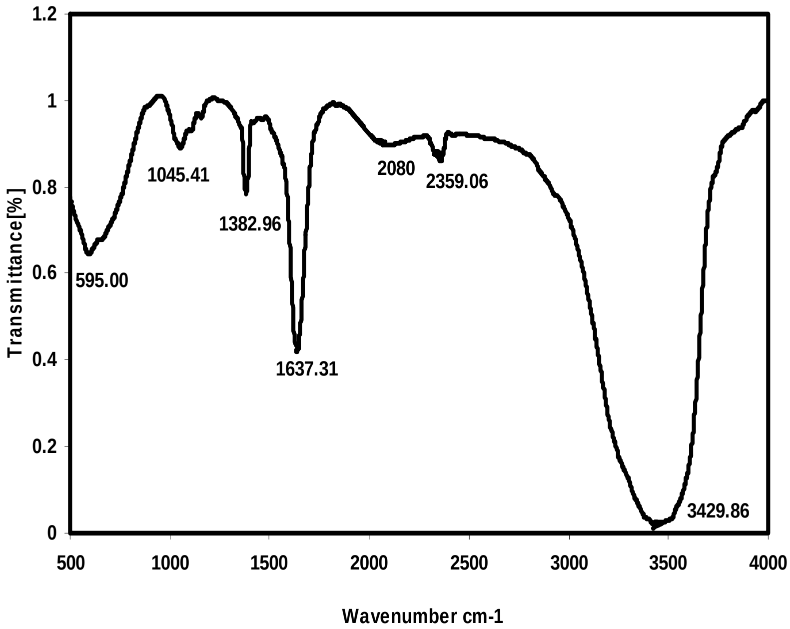

Figure 5 - FT-IR spectrum of silver Nanoparticle synthesized by reacting AgNO3 with E.cardamomom seed extract.

At the very beginning of the biosynthesis, silver ions were reduced to metallic silver by certain components existing in the broth of Fresh *Elettaria cardamomom* seeds extract solution. And silver nuclei were formed at the initial reaction stage. By gathering the surrounding silver atoms, the nuclei gradually grew into relatively small Nanoparticle which may act as the seeds for the growth of larger Nanoparticle. Then, as it was stated in the very beginning of this section, large Nanoparticle with main two distinctive sizes were generated after 1 hrs of reaction. It is well known that the surface energy of larger particles is lower than that of smaller ones, therefore these small Nanoparticle were apt to dissolve into the solution and grow onto larger ones via an Ostwald ripening process [28, 11].

Afterwards, the adjacent large Nanoparticle moved, met and then adhered to form a short chain-shape structure. Zhao *et al* [29] observed similar phenomenon by employing N, N-dimethylformamide to prepare silver nanowires. They proposed that Van der Waals forces induce the aggregation of Nanoparticle through the so-called depletion flocculation. By prolonging the reaction time, the newly formed silver atoms deposit onto the concave regions of the connected Nanoparticle through capillary phenomenon, leading to the formation of long nanorods.

The antibacterial activity of the silver Nanoparticles was examined against *Bacillus subtilis* (MTCC 3053) and *Klebsiella planticolla* (MTCC 2277) by using standard zone of inhibition (ZOI) microbiology assay. The Nanoparticles showed inhibition zone against all the studied bacteria (Fig.7). Maximum zone of inhibition was found to be 23-25 mm for *Bacillus subtilis* (MTCC 3053) and *Klebsiella planticolla* (MTCC 2277). This assay was carried out according to the method of Arya *et al* [8]. The sterile discs (HiMedia-5 mm) were impregnated with different concentrations (1:5diluted) of silver nanoparticle colloids like 10 µl, 20 µl, 30 µl, 40 µl and 50 µl and dried for 10-15 minutes. The dried discs were placed on MH agar surface. Chloromphinicol

(Hi-Media, Mumbai) were used as control and the plates were incubated at 37ºC for 24-48 hours. After incubation, the zone was observed around the Nanoparticles coated discs and measured the zone of inhibition. The seed extract of *E. cardamom* mediated synthesized silver nanoparticle showed minimum inhibition rate against *B. subtilis* and *K. planticola* and it was represented in Table 1.The Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades [30, 31]. Dilute solution of silver nitrate has been used since the 19th century to treat infections and burns [32].

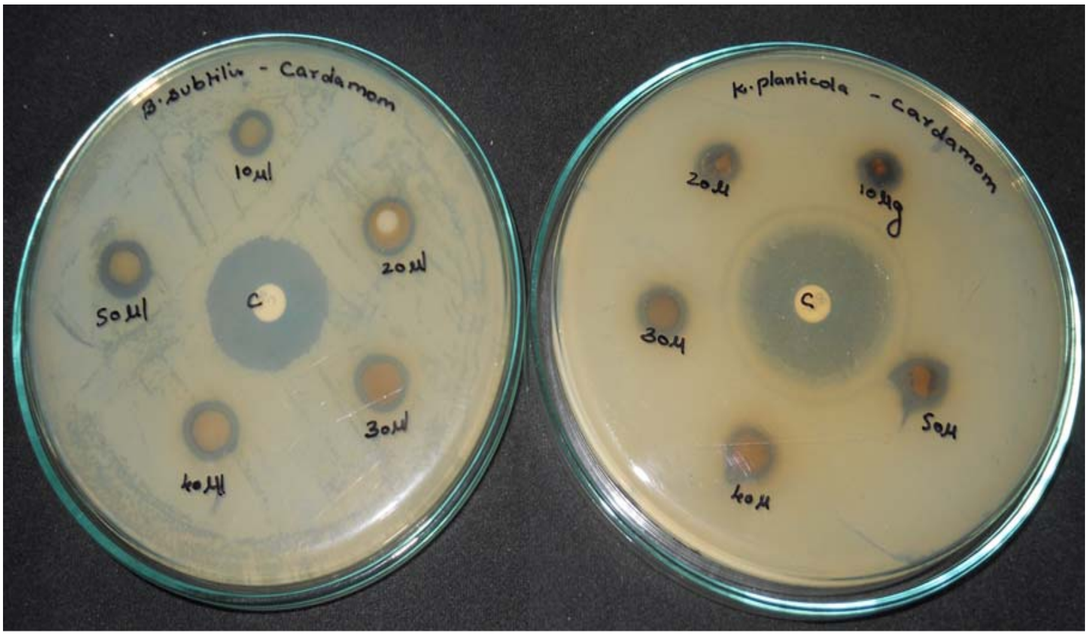

Figure 7 - Antibacterial activity of various concentrations of Ag Nanoparticle against selective bacterial pathogens (*Bacillus subtilis. Klebsiella planticola*).

Table 1
The antimicrobial activity of Ag nanoparticle synthesized using seed extract of *E.cardomomom* (mm).

Silver nanoparticle Concentration (µl)	Bacterial *Sp*	
	Bacillus subtilis (Zone of inhibition-mm)	*Klebsiella planticola* (Zone of inhibition-mm)
10 µl	8	9
20 µl	10	10
30 µl	10	11
40 µl	11	12
50 µl	12	12
Control	23	25

The exact mechanism of the antibacterial effect of silver ions is partially understood. Literature survey reveals that the positive charge on the Ag ion is crucial for its antimicrobial activity. The antibacterial activity is probably derived, through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged Nanoparticles [33, 34, 35, 31]. However, Sondi and Salopek-Sondi [36] reported that the antimicrobial activity of silver Nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag Nanoparticles and was closely associated with the formation of pits in the cell wall of bacteria. The antibacterial efficacy of the biogenic silver Nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the Nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis.

CONCLUSION

In the present investigation, a facile, environmentally benevolent green synthetic route is used for synthesis of silver Nanoparticle. The Phytofabrication of silver nanoparticle by using seed extract of *E. cardamomom* without involvement of any toxic chemicals. The metal ions reduction occurs very rapidly, and the reduction of Ag ions will be completed within 4 hours. The synthesized silver Nanoparticle distinct monodispersity as they show particle size between the range of 40-70nm. The EDAX investigation confirmed a pure phase and high silver content. Water soluble heterocyclic compounds such as flavones were mainly responsible for the reduction and stabilization of the Nanoparticle. Assessment on the antibacterial effect of nanosized silver colloidal solution against *Bacillus subtilis* and *Klebsiella planticola* reveals high efficacy of silver Nanoparticle as a strong antibacterial agent. The present used seeds of *E.cardamomom* as a source which is easily obtainable and extensively useful in biomedical application.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the DST-FIST sponsored programme, Department of Science Technology, New Delhi, India for funding the research development (Ref no S/FST/ESI-101/2010) to carry out this work.

REFERENCE

[1] M. Karkare, Nanotechnology fundamentals and applications. IK International publication, (2008) 2- 4.

[2] J.Y. Song, B.S. Kim, Biological synthesis of bimetallic Au/Ag Nanoparticle using persimmon (*Diopyros kaki*) leaf extract, *Korean journal of chem.Eng*, 25 (2008) 808 - 811.

[3] A. Bankar, B. Joshi , A.R. Kumar, S. Zinjarde , Banana peel extract mediated synthesis of gold Nanoparticle, *Colloids and Surfaces B: Biointerfaces*, 80 (2010) 45 - 50.

[4] S.Mann, Molecular techonics in biomimetic materials chemistry, *Nature,* 365(1993) 499 - 505.

[5] M.D.A. Farooqui, P.S. chauhan, P. krishnamoorthy, and J.shaik, Extraction of silver Nanoparticle from the leaf extracts of *clerodendrum inerme,* Digest *journal of nanomaterials and biostructures,*5(2010) 43 - 49.

[6] W.R. Rajesh, R.L. Jaya, S.K. Niranjan, D.M. Vijay, and B.K. Sahebrao, phytosynthesis of Silver Nanoparticle Using *Gliricidia sepium*, *Current Nanoscience,* 5(2009)117- 122.

[7] S.Arulkumar, and M.Sabesan, Biosynthesis and characterization of gold nanoparticle using antiparkinsonian drug *mucuna pruriens* plant extract. *Int. J. Res. Pharm. Sci*, 4 (2010) 417- 420.

[8] V. Arya, S. Yadav, S. Kumar, J.P. Yadav, Antimicrobial Activity of *Cassia occidentalis* L (Leaf) against various Human Pathogenic Microbes, *Life Sciences and Medicine Research,* 9 (2010) 1-11.

[9] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell Nanoparticle using Neem (*Azadirachta indica*) leaf broth, *J Colloid Interface Sci,* 2(2004) 275- 496.

[10] N .Ahmad, S.Sharma, V.N.Singh, S.F. Shamsi, A.Fatma, and B.R. Mehta, Biosynthesis of Silver Nanoparticle from *Desmodium triflorum*: A Novel Approach towards Weed Utilization, *Biotechnology Research International*, (2010)1 - 8.

[11] L. Lin, W. Wang, J.Huang, Q. Li , D.Sun, X. Yang, H. Wang, N. He, Y.Wang, Nature factory of silver nanowires: Plant-mediated synthesis using broth of Cassia fistula leaf. *Chemical Engineering Journal*, 162 (2010) 852- 858.

[12] S.L. Smitha, D.Philip, K.G.Gopchandrana, Green synthesis of gold Nanoparticle using *Cinnamomum zeylanicum* leaf broth, *Spectrochimica Acta Part A*, 74(2009) 735-739.

[13] A. Gade, S. Gaikwad, V.Tiwari, A. Yadav, A. Ingle, and M. Rai, Biofabrication of Silver Nanoparticle by *Opuntia ficus-indica*: *In vitro* Antibacterial Activity and Study of the Mechanism Involved in the Synthesis, *Current Nanoscience*, 6(2010) 370-375.

[14] J. Y. Song, H.K. Jang, B.S. Kim, Biological synthesis of gold Nanoparticle using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry,* 44 (2009) 1133-1138.

[15] K. B. Narayanan, and N. Sakthivel, *Coriander* leaf mediated biosynthesis of gold Nanoparticle, *Materials Letters*, 62(2008)4588-4590.

[16] R. Sathyavathi, M. B. Krishna, S. V. Rao, R. Saritha, and D. N. Rao, Biosynthesis of Silver Nanoparticle Using *Coriandrum Sativum* Leaf Extract and their application in Nonlinear Optics, *Adv. Sci. Lett*,3(2010) 1-6.

[17] A. Thirumurugan, G.J. Jiflin, G. Rajagomathi, N.A. Tomy, S. Ramachandran, and R. Jaiganesh, Biotechnological synthesis of gold Nanoparticle of *Azadirachta indica* leaf extract, *International Journal of Biological Technology,*1(2010) 75-77.

[18] B. Ankamwar, C. Damle, A. Ahmad, and M. Sastry, Biosynthesis of Gold and Silver Nanoparticle Using *Emblica Officinalis* Fruit Extract, Their Phase Transfer and Transmetallation in an Organic Solution, *Journal of Nanoscience and Nanotechnology* ,5(2005)1665-1671.

[19] D. Jain, H. K. Daima, S. Kachhwaha, S. L. Kothari, Synthesis of plant-mediated silver Nanoparticle using papaya fruit extract and evaluation of their anti microbial activities, *Digest journal of nanomaterials and biostructures,* 4(2009) 557 - 563.

[20] G.S. Ghodake, N.G. Deshpande, Y.P. Lee, E.S. Jin, Pear fruit extract-assisted room-temperature biosynthesis of gold nanoplates, *Colloids and Surfaces B: Biointerfaces* , 75(2010) 584-589.

[21] V. Parashar, R. Parashara, B. sharma, A.C. Pandey, Parthenium leaf extract mediated synthesis of silver Nanoparticle: a novel approach towards weed utilization. *Digest Journal of Nanomaterials and Biostructures,* 4(2009) 45-50.

[22] A.K. Singh, M. Talat , D.P. Singh , O. N. Srivastava, Biosynthesis of gold and silver Nanoparticle by natural precursor clove and their functionalization with amine group, *J. Nanopart Res*, 12(2010) 1667- 1675.

[23] A. Crozier, H. Ashihara, M.N. Clifford, Plant Secondary Metabolites and the Human Diet, Oxford: Blackwell Publishing, 2006.

[24] P. Mulvaney, "Surface plasmon spectroscopy of nanosized metal particles". *Langmuir,* 12(1996) 788-800.

[25] N.R. Jana, Z.L. Wang, T.K. Sau, and T. Pal, Seed-mediated growth method to prepare cubic copper Nanoparticle, *Current Science,* 79(2000)9 -10.

[26] K.S. Mukunthan, E.K. Elumalai, T.N. Patel, V.R. Murty, *Catharanthus roseus*: a natural source for the synthesis of silver nanoparticles, *Asian Pacific Journal of Tropical Biomedicine,* (2011) 270-274.

[27] Z. Li, A. Friedrich and A. Taubert, Gold microcrystal synthesis *via* reduction of $HAuCl_4$ by cellulose in the ionic liquid 1-butyl-3-methyl imidazolium chloride, *J. Mater. Chem*, 18(2008) 1008-1014.

[28] A.R. Roosen, W.C. Carter, Simulations of microstructural evolution: anisotropic growth and coarsening, *Physica A (Amsterdam)*, 261(1998) 232-247.

[29] Q. Zhao, J. Qiu, C. Zhao, L. Hou, and C. Zhu, Synthesis and formation mechanism of silver nanowires by a templateless and seedless method, *Chem. Lett*, 34(2005) 30-31.

[30] A. B. Lansdown, Silver. 1. Its antibacterial properties and mechanism of action, *J. Wound Care*, 11(2002) 125-130.

[31] R.W. Raut, N. S. Kolekar, J. R. Lakkakula, V. D. Mendhulkar and S. B. Kashid, Extracellular synthesis of silver Nanoparticle using dried leaves of *pongamia pinnata* (L) Pierre, *Nano-Micro Letters* ,2(2010)106 -113.

[32] C.L. Fox, Silver sulfadiazine -A new topical therapy for *Pseudomonas* in burns. Therapy of Pseudomonas infection in burns, *Arch Surg*, 96(1968)184-188.

[33] T. Hamouda, A. Myc, B. Donovan, A. Shih, J. D. Reuter and J. R. Baker, A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi, *Microbiol. Res*, (2000) 1-156.

[34] P. Dibrov, J. Dzioba, K.K. Gosink and C. C. Hase, Chemiosmotic Mechanism of Antimicrobial Activity of Ag^+ in *Vibrio cholerae*. *Antimicrob Agents Chemother* , 46(2002) 2668 .

[35] I. Dragieva. S. Stoeva, P. Stoimenov, E. Pavlikianov and K. Klabunde, Complex formation in solutions for chemical synthesis of nanoscaled particles , *Nanostruct. Mater*, 12(1999) 267.

[36] I. Sondi and B.S. Sondi, Silver nanoparticles as antimicrobial agent: a case study on *E.coli* as model for gram negative bacteria, *J.colloid &interface science*, 275(2004)177 -182.