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Original Research Article

# GREEN SYNTHESIS AND OPTOELECTRONIC CHARACTERIZATION OF SILVER NANOPARTICLE PREPARED USING AQUOUS *HOUTTUNIA CORDATA* LEAF EXTRACT AND ITS POTENTIAL APPLICATION AS ANTIBACTERIAL AGENT

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**Abstract:** Green synthesis of silver nanoparticles and its activity on bacterial pathogens have attracted a lot of attention due to possibility of their application in water treatment. The green synthesis method is a simple biological, environment-friendly and cost effective approach. In the present study, silver nanoparticles were rapidly synthesized using aqueous leaf extract of *Houttuynia cordata* as reducing and stabilizing agents and UV-Vis absorption spectroscopy, X-ray diffraction analysis (XRD) and FTIR were used to monitor the quantitative formation of silver nanoparticles. The Transmission Electrone Microscopy (TEM) analysis reveals spherical shape of the synthesized nanoparticles and the size of Ag nanoparticles are in range 15 to 20 nm. The antibacterial potential of synthesized nanoparticles were investigated against cultures of gram negative bacteria *Escherichia coli* and *Klebsiella pneumonia* and gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis.* The result showed an enhanced antibacterial efficacy.

**Keywords:** *Houttuynia cordata;* Silver nanoparticle; Biosynthesis; Antibiotic; Control; Optimisation ; UV-Vis; XRD; TEM

## Introduction

The biosynthesis of nanoparticles using various plant materials is considered as green technology. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy due to their extremely large surface area which provide better contact with microorganisms [1]. The biological method for the synthesis of silver nanoparticles using plant extracts as a reducing agent is widely studied and adopted due to its advantages over others. Though a number of approaches are available for the synthesis of silver nanoparticles like reduction in solutions[2], chemical and photochemical reactions in reverse micelles[3], thermal decomposition of silver compounds[4], radiation assisted[5], electrochemical[6], sonochemical[7], microwave assisted process[8] etc, yet environment friendly green synthesis approaches has been focused recently to avoid using hazardous materials.

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*Houttuynia cordata* is a medicinal herb that is found throughout Eastern Asia. In the North-East region of India, whole plant of *H. cordata* is eaten raw as a medicinal salad for lowering the blood sager level [9]. Moreover, leaf juice is taken for the treatment of cholera, dysentery, curing of blood deficiency and purification of blood[10]. Previous studies showed that *H. cordata* extracts had antiviral and antibacterial [11,12], antiallergic [13], antioxidant and antimutagenic activities [14], anti-leukemic, anti-inflammatory and immunomodulatory effects [15-17]. A variety of components were shown existing abundantly in *H. cordata* such as alkaloids and flavonoids [18-19].

In the present we have synthesized silver nanoparticles using aqueous leaf extract of *Houttuynia cordata* and its antibacterial efficacy was estimated.

**Materials and Methods**

*Plant material*

Mature leaves of *H. cordata* were collected from natural populations growing in assam.

*Preparation of Extract*

Freshly collected *H. cordata* leaves were washed thoroughly , shade dried for 15 days and ground to get the fine powder. Exactly 15 g of sterilize *H.cordata* leaf powder was taken and mixed with 150 ml of Milli Q water and kept in boiling water bath for 30 min. The resulting crude extract was filtered with Whatman No1 filter paper. The filtrate was collected in brown bottle and stored in refrigerator for further studies. The filtrate was used as reducing and stabilizing agent for 1 mM of $AgNO_3$($AgNO_3$, 99.99%, Sigma-Aldrich). All the chemicals and reagents used in the present study were of high analytical grade.

*Optimisation of synthesis of silver nanoparticles*

Silver nanoparticles were synthesized by treating leaf extracts with 1mM silver nitrate solution in ratio 3:10 (v/v) and kept in magnetic stirrer for 30minutes at room temperature. The reaction mixture was then exposed to different conditions like sunlight irradiation, UV irradiation and room temperature. The colour change of the solution was checked periodically and the conical flasks were incubated at room temperature for 24 h.

*Antimicrobial activity of silver nanoparticles synthesized using H.cordata leaf extract:*

*Test microorganisms:*

*Escherichia coli* (MTCC 739), *Klebsiella pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441) were used for antimicrobial screening. The microbial cultures we used were produced from the "Microbial Type Culture Collection and Gene Bank" (MTCC), Chandigarh, India. We maintained the bacterial culture on nutrient agar slants and were stored at -4°C.

*Antibacterial assay:*

Antibacterial assay of silver nanoparticles were studied by Well Diffusion Method [20]. The Mueller Hinton agar was poured on to sterile Petri plates and plates were inoculated with 2.0 ml of inoculum by spreading the swab over the plate. Cultures of *Escherichia coli*, *Klebsiella pneumonia***,** *Staphylococcus aureus* and *Bacillus subtilis* were swabbed on to the agar plates. With the help of sterile borer, wells of 8mm diameter were cut on the agar plates and loaded with silver nanoparticle solution and a standard antibiotic (Tetracyclin). 1mM silver nitrate ($AgNO_3$) were used as a control. All plates were incubated at $37^0$C for 24 hrs. After incubation period, the inhibition diameters were measured with Hi-Media scale. The experiments were performed in triplicate [21, 22].

*Data analysis:*

The experiments were conducted in a completely randomized design and repeated three times. The data were analyzed using origin graphic software and SPSS software version-16.

**Results and Discussions**

The synthesis of silver nanoparticles using *H. cordata* leaf extract was found to be significant in leaf extract exposed to sunlight irradiation method compared to other methods. The colour of the reaction medium gradually changed to dark brown because of the surface plasmon resonance.

*Characterization of silver nanoparticles UV-Vis Spectra analysis:*

The UV-VIS absorption spectra of silver nanoparticles are shown in Fig.1 .Change in the colour was observed in the silver nitrate

solution incubated with the leaf extract. The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction using UV- Vis spectrophotometer119(Systronics) after different time intervals (0h, 24h, 48h, 72h) taking 1ml of the diluted sample, compared with 1 ml of distilled water used as blank. UV-Vis spectral analysis has been done by using spectrophotometer at a resolution of 1 nm from wave length 250to 800 nm. A control reaction mixture was also maintained without plant leaf extract.

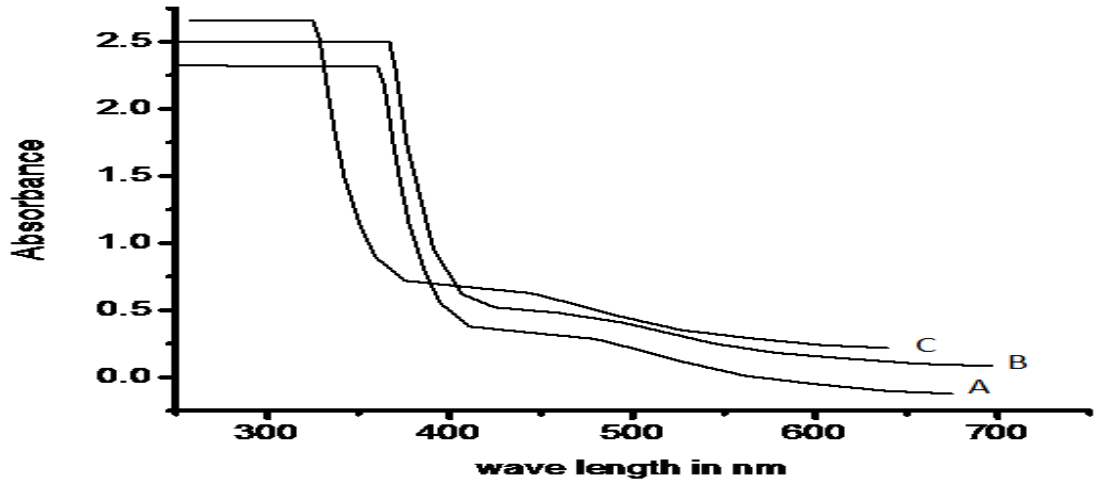


Fig.1 Absorbance vs. Wavelength plot of nanocrystalline silver particle for different condition [The graph A is the condition of irradiation at room temperature after 24 hrs and B is UV treatment and C sun light treatment]

The graph A is the condition of irradiation at room temperature after 24 hrs and B represent UV treatment and C sun light treatment. It is observed that in presence of sunlight the peaks are blue shifted indicating nanoparticle formation.

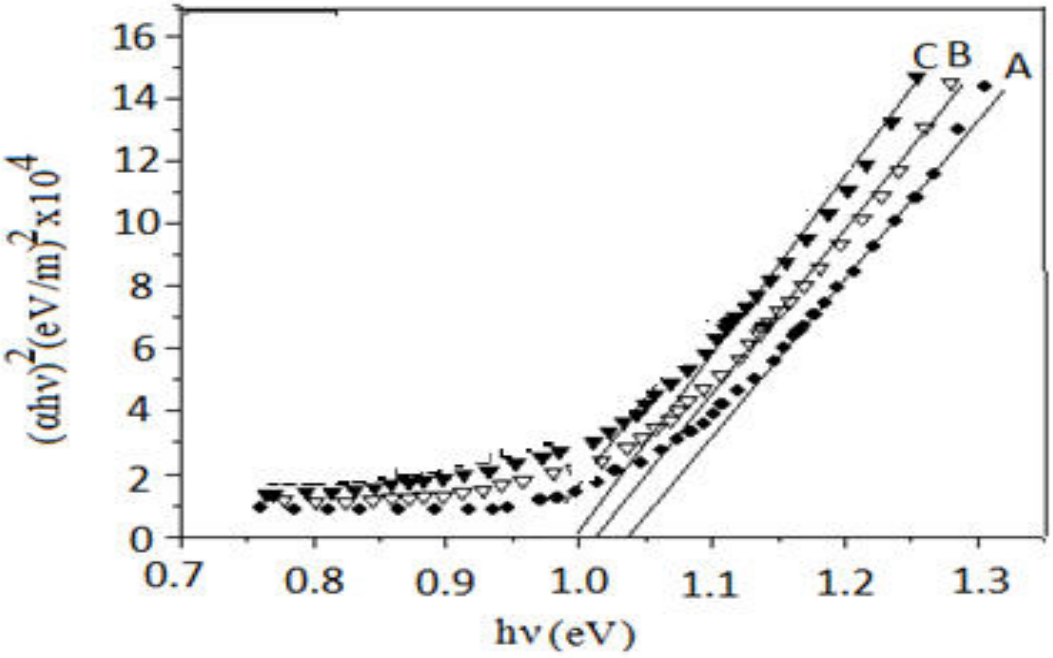


Fig.2 Plot of $(\alpha h\nu)^2$ vs hν of silver nanocomposite
[The graph A is the condition of irradiation at room temperature after 24 hrs and B is UV treatment and C sun light treatment]

Further band gap energy $E_g$ has been calculated by relating with absorption coefficient (α) and the incident photon energy (hν). The calculated band gap energy found to be in between 1 to 1.09 eV.

*FTIR analysis:*

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range 4000–400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ was used. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform InfraRed [FTIR] for the analysis of the nanoparticles.

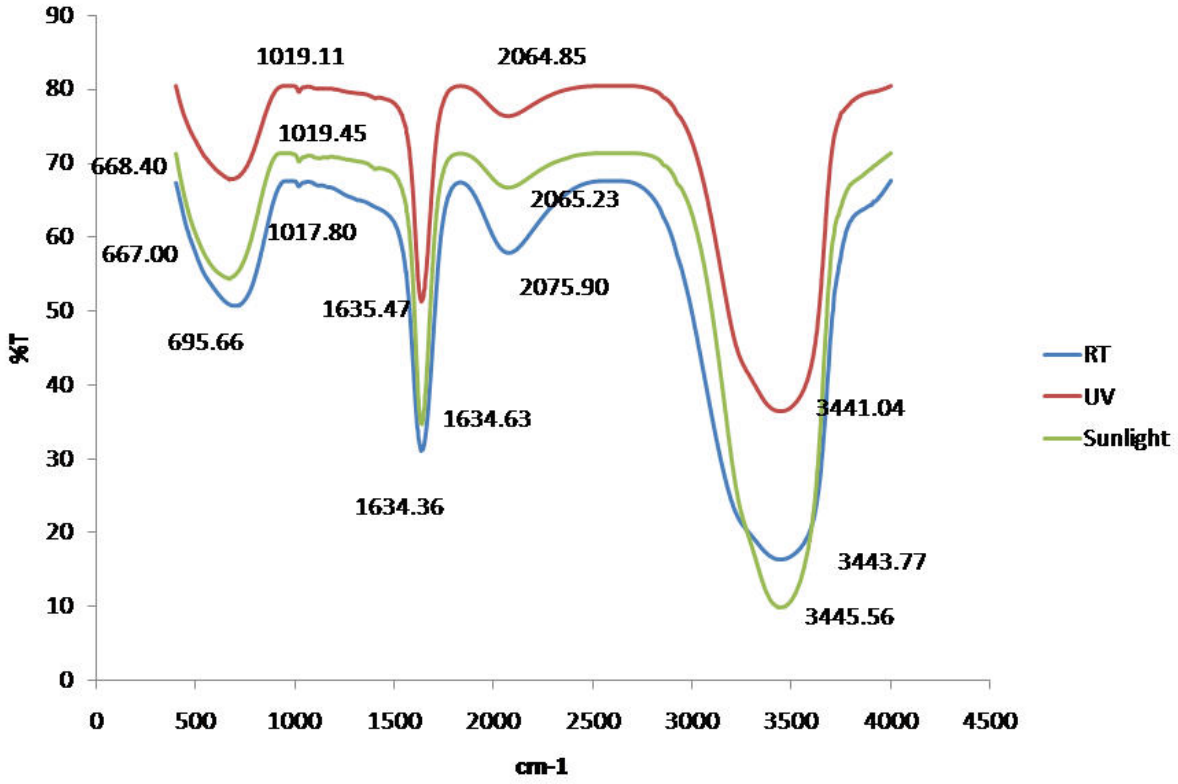


Fig 3 FTIR spectra of nanocomposite silver particles.

The FTIR analysis of silver nanoparticles were recorded for different conditions ( Fig-3). The AgNP solutions displayed five absorption peaks, reflecting its complex nature. A peak at 3441-3445 $cm^{-1}$ results due to the stretching of the N– H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The absorption peak at 2064-2075 $cm^{-1}$ could be assigned to –CH stretching vibrations of –CH3 and –CH2 functional groups. The shoulder peak at 1638 $cm^{-1}$ assigned for C=O group of carboxylic acids. The peak at 1634 $cm^{-1}$ indicates the fingerprint region of CO, C–O and O–H groups, which exists as functional groups of bulbil extract. The weak band at 1017 $cm^{-1}$ can be assigned to the C-N stretching vibrations of aliphatic amines. FTIR study indicates that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of bulbil extract are mainly involved in reduction of $Ag^+$ to $Ag^0$ nanoparticles. The FTIR values showed reduction and capping of silver ions which may be due to the presence of alkaloids.

*XRD analysis*:

X-ray diffraction (XRD) analysis of drop-coated films of silver nanoparticles was prepared for the determination of the formation of silver nanoparticle by an X-ray diffractometer operated at a voltage of 40kv and a current of 30mA with Cu Kα radiation.

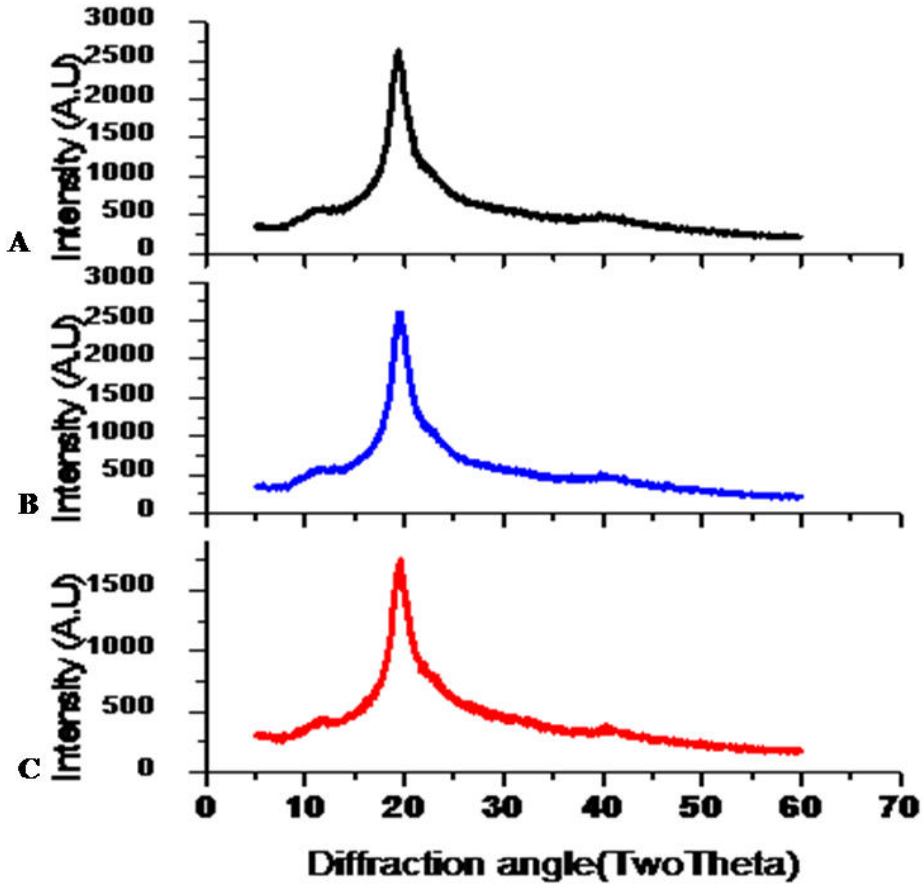


Fig 4. XRD patterns of silver films deposited on glass substrates for different expose condition [The graph A is the condition of irradiation at room temperature and B represent under UV treatment and C represent treatment with sunlight]

XRD patterns silver nanocrystalline films deposited on glass substrate for different conditions like sunlight irradiation, UV irradiation and room temperature are shown in the Fig.4. The peak observed near $2\theta = 20.5^0$ is due to the crystalline phase of matrix with a shallow shoulder corresponding to the amorphous part of the polymer . The diffraction peaks at angles near $2\theta = 30.2^0$ and $2\theta = 41.15^0$ corresponds to (111) and (220) planes of the mix cubic phase of silver. X-Ray peak intensities are weak and broad compared to bulk counterpart suggesting small crystallite size. The X-ray peaks are also found to shift to higher diffraction angle when sample irradiate with room temperature to sunlight. The graph A is the condition of irradiation at room temperature and B represent under UV treatment and C represent treatment with sunlight. The lattice contraction is expected to occur because of higher surface to volume ratio with decreasing crystallite size and increase in strain. The shifting of peak position to higher diffraction angle due to strain was confirmed by calibrating the XRD prior to each observation using standard silicon sample. The size of the nanocrystal was determined using Scherer formula [23-25].

$$D = \frac{kl}{Vw_{2q}\cos q_B} \quad \text{---------- (1)}$$

Where $q_B$ is the Bragg angle and K=0.9 for spherical shape (from TEM).

*Transmission Electron Microscope analysis (TEM):*

The sophisticated machine, we have used was Transmission electron microscope (JEOL JEM-100cx). The accelerated voltage 80keV and beam current is 80mA.The resolution is up to 450000.

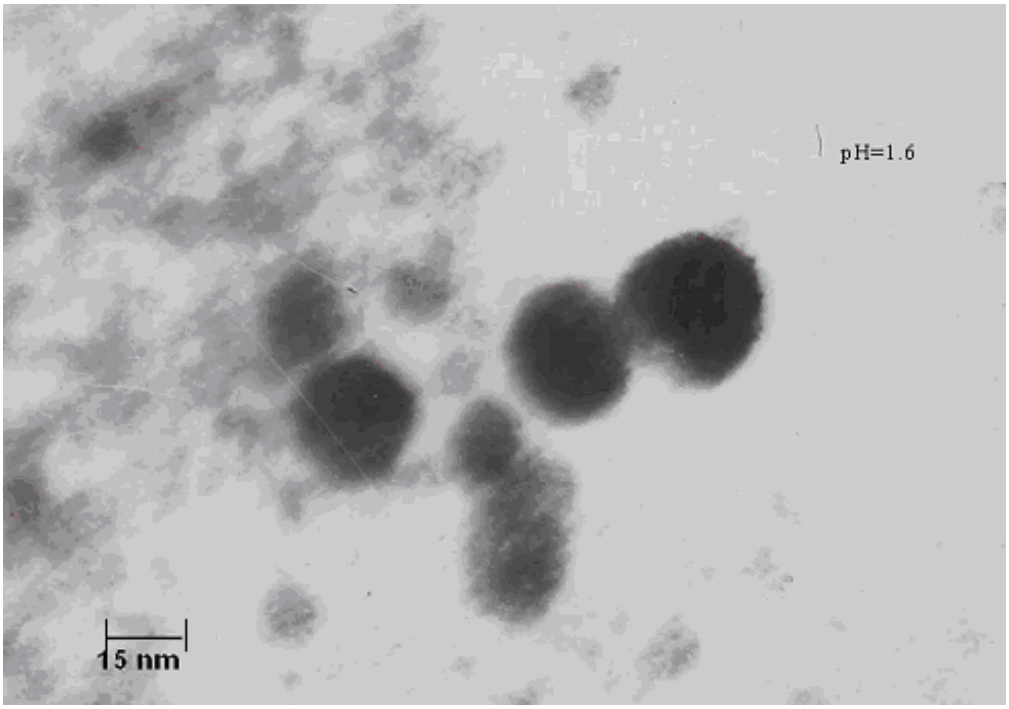


Fig 5. TEM image of silver nanoparticles synthesized using *Houttunia cordata* leaf extract

Considering the SEM image ( not shown) we say Ag nanoparticles are densely packed, but at higher magnification (200000) image revels the size of Ag nanoparticles are in range 15 to 20 nm. Again from the fig-5 it is observed that synthesized nanoparticles are nearly spherical in shape. The average particle size obtained from TEM observations were found to be nearly similar as those obtained from XRD and EMA model from UV- VIS absorption spectra.

*Antimicrobial activity :*

Antibacterial activity of the synthesized silver nanoparticles was studied for some pathogenic bacterial species. The antimicrobial activity was determined in-vitro by measuring zone of inhibition in mm using 50µl of sample and well size of 8mm diameter. Tetracyclin of 1mg/ml, concentration was used as a control antimicrobial agent. The silver nanoparticles synthesized showed inhibition zone against the studied bacterial species. The results shown in the Table-1 depict that silver nanoparticles are efficient giving a zone of inhibition.

**Table 1: Antibacterial efficacy of silver nitrate, leaf extract, silver nanoparticle, and standard antibiotic against four bacterial strains**

| Bacterial Strains | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | $AgNO_3$ 1mM | Leaf Extract | AgNP | Tetracyclin ($1mgml^{-1}$) |
| ***E. coli*** | 9±0.14 | 10±0.11 | 14±0.13 | 35±0.08 |
| ***K. pneumonia*** | 9±0.11 | 11±0.16 | 16±0.15 | 32±0.09 |
| ***B. subtilis*** | 9±0.15 | 13±0.14 | 17±0.11 | 31±0.09 |
| ***S. aureus*** | 9±0.11 | 12±0.08 | 16±0.11 | 34±0.11 |

Results were expressed as mean ± standard deviation

The antibacterial efficacy of the biological silver nanoparticles reported the present study may be described by the mechanism of anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues [26].

**Conclusions**

The present study reports a reliable, eco-friendly and simple biological approach for preparation of stable metallic nanoparticles which is very necessary in the field of nanotechnology. The obtained silver nanoparticles were characterized using UV-Vis, FTIR and XRD techniques. Biologically synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antibacterial and antimicrobial properties [27].The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity.

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**References**

[1] Gong P, Li H, He X, Wang K, Hu J and Tan W, () Preparation and antibacterial activity of Fe O @Ag nanoparticles. *Nanotechnol*, 2007,34(18): 604-611.

[2] Goia D.V. and Matijevic, E.N., Preparation of Monodispersed Metal Particles. *J Chem*, 1998,22: 1203-1215.

[3] Taleb C, Petit M and Pileni P, Synthesis of Highly Monodisperse Silver Nanoparticles from AOT Reverse Micelles:A Way to 2D and 3D Self-Organization. *Chem Mater*, 1997, 9(4): 950-959.

[4] Esumi K, Takafumi T, Kanjiro T and Kenjiro M, Preparation and characterization of bimetallic palladium-copper colloids by thermal decomposition of their acetate compounds in organic solvents, *Chem Mater*, 1990, 2(5): 564– 567.

[5] Henglein A, Reduction of Ag (CN)-2 on Silver and Platinum Colloidal Nanoparticle, *Langmuir*, 2001,17: 2329- 2333.

[6] Rodriguez-Sanchez L, Blanco M.C. and Lopez-Quintela MA, Electrochemical Synthesis of Silver Nanoparticles, *J Phys Chem B* , 2000,104(41): 9683–9688 .

[7] Zhu JJ, Liu SW, Palchik O, Koltypin Y and Gedanken A, Shape- Controlled Synthesis of Silver Nanoparticles by Pulse Sonoelectrochemical Methods, *Langmuir*, 2000,16(16): 6396-6399.

[8] Pastoriza-Santos and Liz-Marzan LM, Formation of PVP-protected metal nanoparticles in DMF, *Langmuir*,2000, 18: 2888–2894.

[9] Frlht.Org.in. Medicinal Plants Conservation and Sustainable Utilisation-Meghalaya, India. Annexure-C. 72-5. 2003. Available at http://frlht.org.in/html/reports/meghalayaslpc.pdf .

[10] Hynniewta SR, Kumar Y, Herbal remedies among the Khasi traditional healers and village folks in Meghalaya, *Indian J Tradit Knowl*, 2008, 7:581–586.

[11] Hayashi K, Kamiya M, Hayashi T, Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV, *Planta Med* , 1995,61: 237-241.

[12] Lu H, Wu X, Liang Y, Zhang J, Variation in chemical composition and antibacterial activities of essential oils from two species of *Houttuynia* THUNB, *Chem Pharm Bull(Tokyo)*, 2006, 54:936-940.

[13] Lee JS, Kim IS, Kim JH, Kim JS, Kim DH, Yun CY, Suppressive effects of *Houttuynia cordata* Thunb (Saururaceae) extract on Th2 immune response, *J Ethnopharmacol*, 2008, 11:734-40.

[14] Chen YY, Liu JF, Chen CM, Chao PY, Chang TJ, A study of the antioxidative and antimutagenic effects of *Houttuynia cordata* Thunb. using an oxidized frying oil-fed model, *J Nutr Sci Vitaminol (Tokyo)*,2003, 49: 327-333.

[15] Yang L, Jiang JG, Bioactive componrnts and functional properties of *Houttuynia cordata* and its application, *Pharmaceutical Biology*, 2009, 47:1154-1161.

[16] Wu LS, Si JP, Yuan XQ, Shi XR, Quantitative variation of flavanoids from *Houttuynia cordata* from different geographic origins in China, Chinese *Journal of Natural Medicines*, 2009, 7(1):40-46.

[17] Cho EJ, Yokozawa T, Rhyu DY, Kim HY, Shibahara N, Park JC, The inhibitory effect of 12 medicinal plants and their component compounds on lipid peroxidation, *Am J Chin Med*, 2003,31(6): 907-917.

[18] Kim SK, Ryu SY, No J, Choi SU, Kim YS, (2001) Cytotoxic alkaloids from *Houttunia cordata*, *Arch Pharm Res.* Vol.24, No.6, p 518-521.

[19] Meng J, Leung KS, Dong XP, Zhou YS, Jiang ZH, Zhao ZZ, , Simultaneous quantification of eight bioactive components of *Houttuynia cordata* and related Soururaceae medicinal plants by on-line high performance liquid chromatography diode array detector-electrospray mass spectrometry, *Fitoterapia*, 2009,80(8): 468-474.

[20] Perez C, Paul M, Bazeraue P, Antibiotic assay by agar well diffusion method, *Acta Biol Med Exp.*,1990,15:113-115.

[21] Nakamura CV, Ueda-Nakamura T, Bando E, AFN Melo, DAG Cortez, BPD Filho, Antibacterial activity of *Ocimum gratissimun* L. essential oil, Memo´ rias do Instituto Oswaldo Cruz, 1999, 94(5):675-678.

[22] Saikia M and Handique PJ, Antioxidant and antibacterial activity of leaf, bark, pulp and seed extracts of seabuckthorn (*Hippophae salicifolia* D. Don) of Sikkim Himalayas, *J. Med. Plant Res*., 2013, 7:1330-1338.

[23] Barman J, Sarma KC, Sarma M, Sarma K, Structural and optical studies of chemically prepared nanocrystalline thin films, *Indian Journal of Pure and Applied Physics*., 2008, 46: 339.

[24] Bora JP, Barman J, Sarma KC, Structural and optical properties of CdS nanoparticles, *Chalcogenide Letters*, 2008, 5(9): 201.

[25] Barman J, Bora JP, Sarma KC, Optical properties of chemically prepared CdS quantum dots in polyvinyl alcohol, *International Journal of modern physics.*, 2009, 23(4):545-555.

[26] Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D, Characterization of enhanced antibacterial effect of novel silver nanoparicles, *Nanotechnology*, 2007, 18(22) 225103–225111.

[27] Shahverdi AR, Mianaeian S, Shahverdi HR, Jamalifar H and Nohi AA, () Rapid Synthesis of Silver Nanoparticles Using Culture Supernatants of *Enterobacteria*: A Novel Biological Approach, Process Biochem., 2007,42: 919-923.