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.

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
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 sugar 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°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 a UV-Vis spectrophotometer (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 250 to 800 nm. A control reaction mixture was also maintained without plant leaf extract.

Fig.1 Absorbance vs. Wavelength plot of nanocrystal 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]

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 $h \nu$ 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 ($\alpha$) and the incident photon energy ($h\nu$). 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.

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 –CH$_3$ and –CH$_2$ 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 K$\alpha$ radiation.
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 $\theta = 20.5^0$ 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 $\theta = 30.2^0$ and $\theta = 41.15^0$ 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].

$$D = \frac{kl}{V_{w_{2q}} \cos \theta_q}$$

Where $\theta_q$ 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.
Considering the SEM image (not shown) we say Ag nanoparticles are densely packed, but at higher magnification (200000) image reveals 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**

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>AgNO₃ 1mM</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>9±0.14</td>
</tr>
<tr>
<td><strong>K. pneumonia</strong></td>
<td>9±0.11</td>
</tr>
<tr>
<td><strong>B. subtilis</strong></td>
<td>9±0.15</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>9±0.11</td>
</tr>
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Results were expressed as mean ± standard deviation.
The antibacterial efficacy of the biological silver nanoparticles reported in this 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**


