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Biosynthesized Silver Nanoparticles Using an Aqueous Root Extract of *Iris germanica* as a Reducing Agent and Its Antibacterial Efficacy

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Authors' contributions

This work was carried out in collaboration among all authors. Author VH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PA and VS managed the analyses and characterized data of the samples. Author SR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Biosynthesized silver nanoparticles (AgNPs) are environment friendly, cost-effective, bio-compatible and expanding research area due to their potential applications in medical domain. The present study focuses on biologically synthesized of AgNPs using aqueous root extract of *Iris germanica* as reducing agent as well as capping agent and examined their potential antibacterial efficacy. In reduction reaction it was observed that silver (Ag^{+1}) ions associate in root extract and reduced in solution (Ag^{0}) leads to formation of stable formation of spherical AgNPs. Biosynthesized AgNPs showed an effective and rapid antibacterial activity against both bacterial strain ($gram^{+ve}$ and $gram^{-ve}$). Results exhibited that AgNPs were extremely toxic towards *Bacillus subtilis* and *Escherichia coli* pathogenic bacterial strains and can be utilized for the applications in biomedical science.

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Keywords: Iris germanica root extract as a reducing agent; antibacterial efficacy; $AgNO_3$.

1. INTRODUCTION

Nanotechnology based experimental systems posses significant potential biomedical applications and become a leading research field. When we juxtaposed nanoparticles with bulk materials, it delivers a huge area of chemicals, optional, magnetic characteristics, mechanical and electronic features [1]. Biological methods for the synthesis of silver nanoparticles (AgNPs) gaining medical area due to formation of expected product with low cost without involving any harmful/hazardous chemical reagents. In medical field, a prescribed and unharmed dosage of drug molecules can be administered in the body due to site-specific targeted action of nanoparticles which reduce the undesired toxicity in the body and increase the reactivity of the drug.

Plant extract has capacity to diminish metal ions. In vision of its impeccability, the utilization of fresh plant or whole plant extract and plant tissues to reduce metal salts to NPs has been attracted during the last 30 years [2-5]. Among noble metal nanoparticles, silver nanoparticles with excellent performance plays a significant role in the territories of biology and medicine [6, 7], treatment of disease, food preservation, for the reason of owing higher level of anticancer and antibacterial activities [8,9] and purification of water [10]. Plant extracts seems negligible in toxicity and require minimal purification steps [11]. Biomolecules obtained from plant extract [12-14], bacteria [15], viruses [16], DNA [17] and peptides [18-20] have been used to synthesize AgNPs successfully [21]. Different parts of plants such as fruits, fruit peels, bark, root and callus are utilized in this regard [22-26].

For the synthesis of AgNPs, a variety of methods have been employed such as electrochemical [26], chemical reduction [27-28], laser ablation [29], and hydrothermal methods [30]. But these methods have developed environmental issues by involving use of toxic or hazardous chemical reagents. Hence to prevent these adverse consequences the use of plant extract has been explored to synthesize AgNps and this biological method has found to be cost-effective, nontoxic and environment friendly. In the reduction reaction, plant extract acts as reducer as well as capping agent leading to the production of stable particles with specific/different shapes and dimensions. Recently many plants have been employed for the synthesis of AgNPs such as *Crateva Religiosa* [31], *Bauhinia Variegata* [32], *Moringa pterygosperma* [33], *Cleistanthus collinus* [34], *Morinda citrifolia* [35], *Alternanthera sessilis* [36], *Ceropegia thwaitesii* [37], etc. There are so many medicinal plants used to synthesize metal nanoparticles [38-41].

In the present experiment, we have reported one step procedure for the biological synthesis of Silver nanoparticles using root extract of *Iris germanica*. The root extract was simply intermingled with 1 mm salt solution of $AgNO_3$ and the complete reduction of Ag^{+1} to Ag^{o} was fulfilled after 2 hrs. The biosynthesized AgNPs have been characterized using spectral analysis and imaging microscopic techniques. Antibacterial activity of AgNPs has been investigated against different bacterial strains.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

Plant material was collected from botanical garden of Department of Botany, HNGU, Patan-384265, Gujarat. The voucher specimen of the herbarium *Iris germanica* was deposited to the plant taxonomy of Department of Botany, HNGU, Patan, Gujarat. Silver salt ($AgNO_3$) was purchased from Sigma Aldrich, USA. All over experiment was carried out using double distilled water as a solvent.

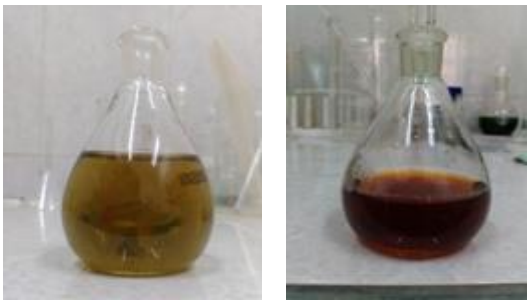
2.2 Preparation of *Iris germanica* Root Extract

The freshly collected plant material was washed several times thoroughly with tape water followed by D.D. water and dried at room temperature under shadow for 1 month. Dried plant material was grinded using grinder. In 100 ml of double distilled water, 10 gm of dried plant powder was boiled at 50°C for 30 min. After cooling, the mixture was filtered using Whatman filter paper no.1 and obtained aqueous filtrate was kept in refrigerator at 4°C for upcoming synthesis of AgNPs.

2.3 Biogenic Synthesis of AgNPs

For the biogenic synthesis of AgNPs, 30 ml of 1 mM $AgNO_3$ (silver salt) solution was slowly added to the 20 ml of prepared plant extract in

conical flask. After adding salt solution put the reaction mixture on magnetic stirrer with hot plate at 50°C for 30 min and color change in reaction mixture was observed from greenish to dark brown (Fig. 1) which confirmed the preliminary confirmation of formation of AgNPs by reducing the ions Ag^{+} to Ag^{0} state. Final confirmation was confirmed using UV-visible spectroscopy with SPR peak at 452 nm. The reduction of the reaction mixture was completed within 3 hrs. The final reaction mixture was subsequently purified using centrifuge for 15 min at 6,000 rpm at 4°C and bottom deposited nanoparticles were collected followed by dried it in oven for 1-2 hr. Obtained crystalline powdered AgNPs was stored in air tight bottle for further characterization and biological screening.



(a) (b)

Fig. 1 (a) Root extract of *Iris germanica* (b) Reaction mixture after completion of reaction

2.4 Characterization of AgNPs

Biosynthesized Silver nanoparticles were initially characterized using UV-vis. Spectrometer (UV-1800, Shimadzu, Japan) in the range of 200-800 nm. X-ray diffraction study was carried using Rigaku D/MAX 40 kV diffractometer equipped with graphite chromatography. The average particle size was deliberated using Debye Scherer's formula,

$$D = \frac{0.9\,\lambda}{\beta \cos\theta}$$

Where, D is the mean crystalline size, β is the FWMH (full width at half maximum), λ is the X-ray wavelength and θ is the diffraction angle. The structural morphology and elemental composition were studied by field electron gun scanning electron microscopy with energy dispersive spectroscopy (FEG-SEM with EDS, JEOL JSM-7600F) and high resolution transmission spectroscopy (HR-TEM, Tecnai G2-F30). Fourier transform infra red spectroscopy (FTIR) (Shimadzu, range of 400-4000 cm^{-1}) showed the functional groups present in the plant extract as a biomolecules which majorly leading the reduction of the metal ions. The antibacterial assay of biogenic AgNPs was inspected against *Bacillus subtilis* MTCC 121 (gram positive) and *Escherichia coli* MTCC 119 (gram negative) pathogenic bacterial strains.

3. RESULTS AND DISCUSSION

3.1 Uv-visible Spectrometer

To conform the formation of AgNPs, the sample was subjected to UV-visible spectra for the analysis. Similarly like gold nanoparticles, the AgNPs also well known to exhibit a Surface Plasmon Absorption band in the visible region. Because of Surface Plasmon Resistance (SPR) the AgNPs exhibited color change green to dark brown at the 400-500 nm visible range. After completion of the reaction, the absorption peak was observed at 452 nm (Fig. 2) which support the strong reducing properties in plant extract.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR analysis was studied using Shimadzu (Range 400-4000 cm^{-1}). This measurement was carried out to indentify the biomolecules present on the biosynthesized AgNPs surface (Fig. 3). To remove extra compounds or free biomolecules (peptides, proteins, amino acids, etc.) which did not act as a reducing or capping agents were centrifuges for 15 min at 6000 rpm using sterile distilled water. Bottom deposited pellets were isolated followed by dried and subjected to FTIR spectroscopy. In result, FTIR peaks were observed at 1100-1580 cm^{-1}, 500 cm^{-1} and 2372 cm^{-1} responsible for the relevant amine (-NH-), Ag^{+} and hydroxyl group (-OH) respectively. The analysis showed that the biomolecules present in the plant extract performed major role to reduce Ag^{+} metal ion in the reaction mixture.

3.3 X-ray Diffraction

The crystalline nature of the AgNPs was revealed by the XRD analysis (Fig. 4). The XRD study revealed the pure crystalline nature of the biologically synthesized AgNPs structure. Lattice plane are (111), (222) and (400) at 2Θ values of 15.87, 29.20 and 38.77 respectively indicate the biogenic AgNPs are face centered cubic (FCC). Due to crystallization of biomolecules present in root extract of *Iris germanica*, other small weak

peaks are obtained which indicate silver as a core material in conjunct. Using Debye Scherrer formula, the obtained average particle size of the biosynthesized AgNPs is 23 nm.

3.4 Field Emission Gun Scanning Electron Microscope (FEG-SEM)

Field Emission Gun Scanning Electron Microscope (FEG-SEM) analysis was performed by JEOL JSM-7600F model to obtain morphological features. With an average particle size around 15 to 50 nm analysis showed irregular shape of the highly aggregated biogenic AgNPs (Fig. 5). By energy dispersive X-ray (EDS), the elemental composition of AgNPs was examined. As a result EDS showed the amount of the AgNPs with strong optical absorption peaks at 1.7, 2.6 and 3.0 keV revealed the purity of the AgNPs (Fig. 6). The elemental amount of silver metal as a mass percentage was 51.52% which evidenced the presence of AgNPs.

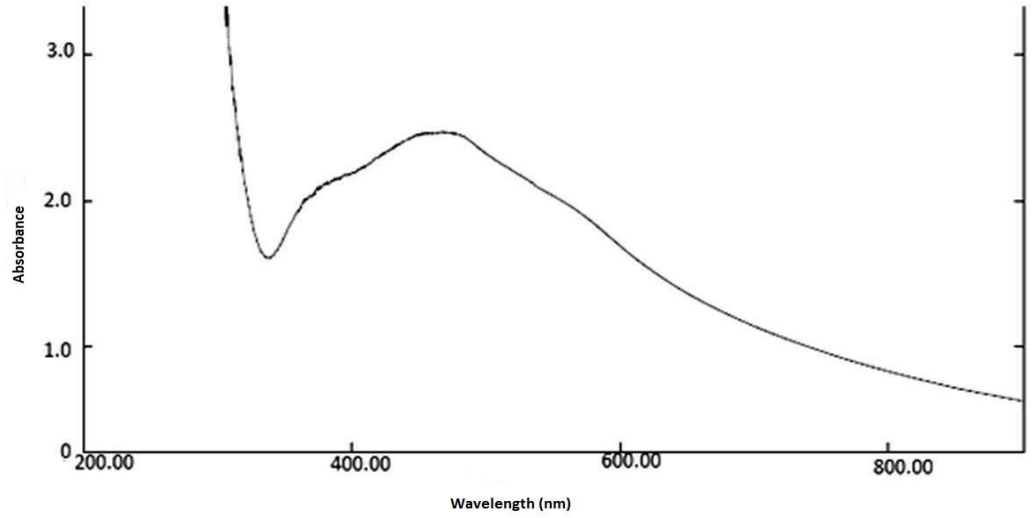


Fig. 2. UV-visible spectra of synthesized silver nanoparticles (452 nm)

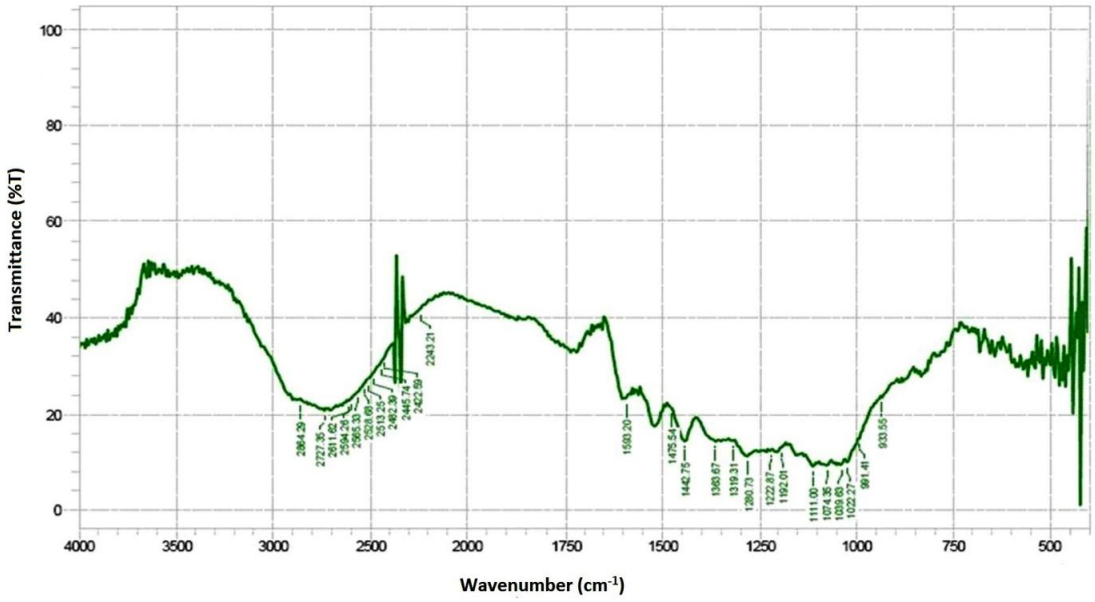


Fig. 3. FTIR data of biosynthesized silver nanoparticles

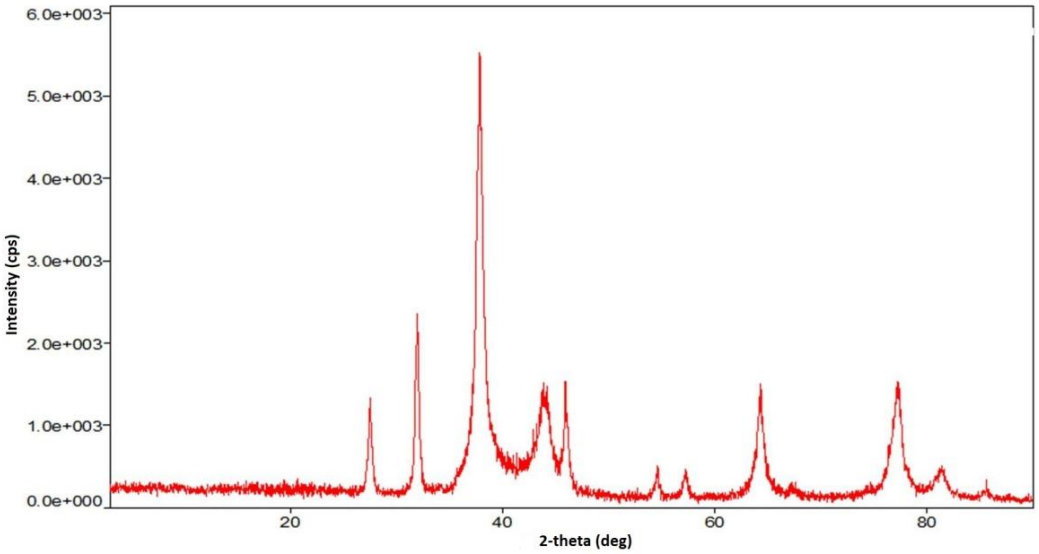


Fig. 4. XRD spectra of crystalline silver nanoparticles

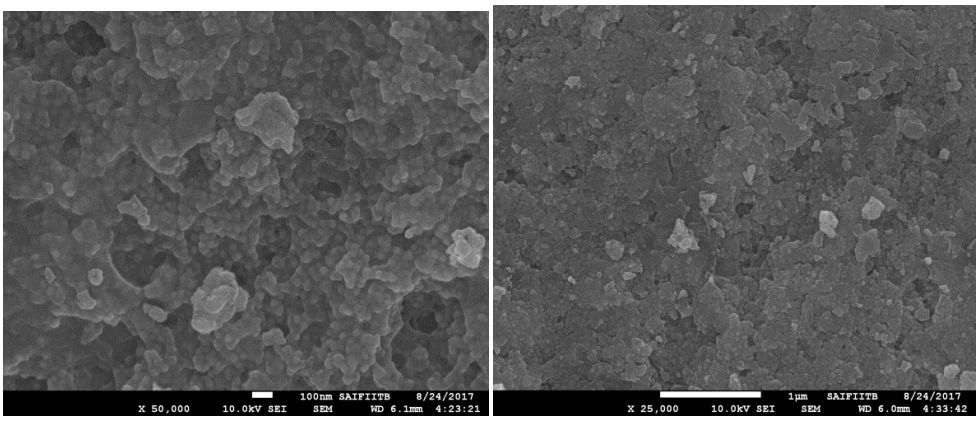


Fig. 5. FEG-SEM images of spherical silver nanoparticles

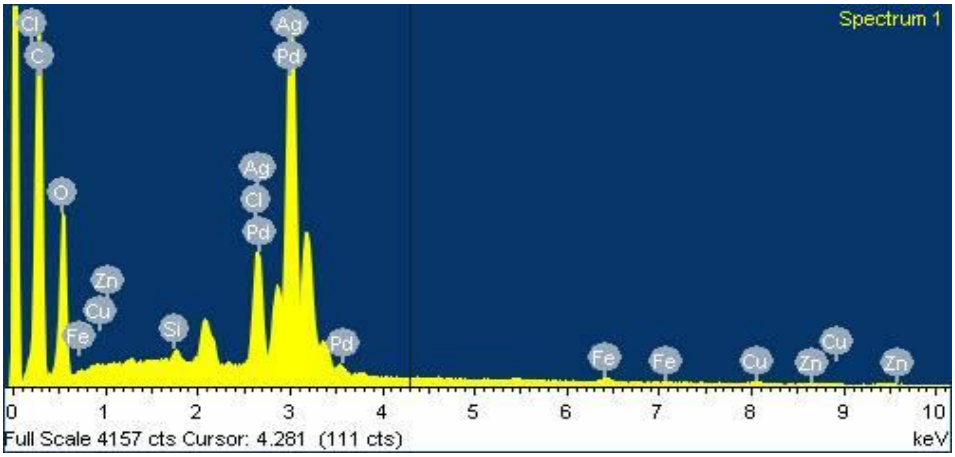


Fig. 6. EDS pattern of biogenic AgNPs

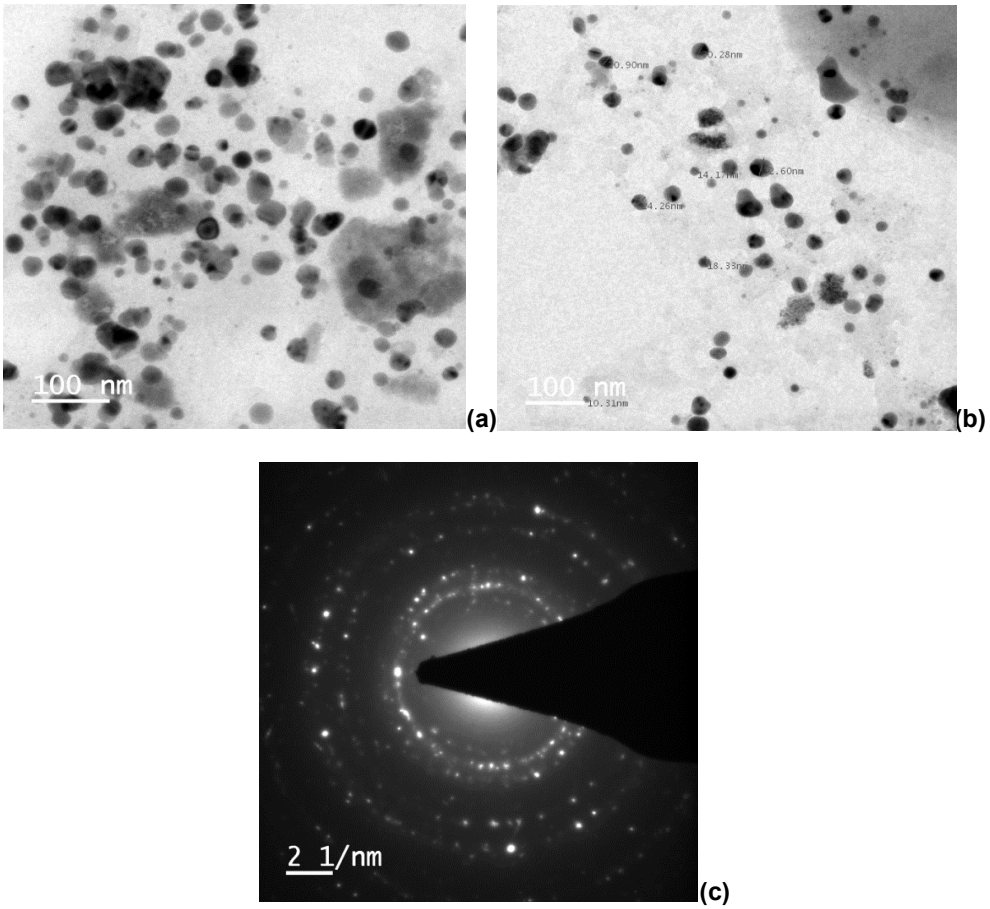


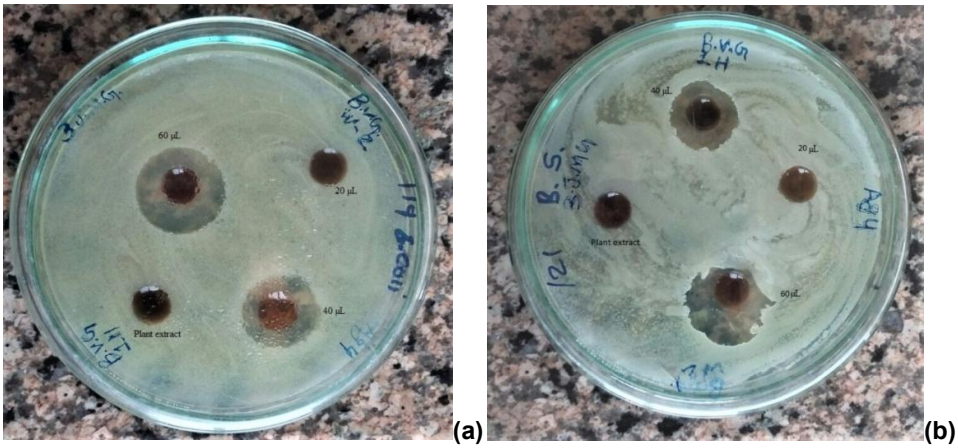
Fig. 7. (a) and (b) are HR-TEM of biosynthesized AgNPs and (c) the selected area electron diffraction (SAED) image of AgNPs

3.5 High Resolution Transmission Electron Microscope (HR-TEM)

High Resolution Transmission Electron Microscopy (HR-TEM) was examined by Tecnai G2-F30 model and obtained the shape morphology and average size of the AgNPs. As a result the crystalline biosynthesized AgNPs obtained with spherical nature (Fig. 7a,b). Accordingly, with a good agreement with XRD data, the average particle size of the biogenic AgNPs was observed about 10 to 30 nm. The crystallinity of the biologically synthesized AgNPs was evidenced by the selected area electron diffraction (SAED) (Fig. 7c).

4. ANTIBACTERIAL ACTIVITY

Biosynthesized silver nanoparticles using root extract of *Iris germanica* examined against gram^{+ve} (*Escherichia coli* MTCC 119) and gram^{-ve} bacteria (*Bacillus subtilis* MTCC 121) with different concentration using agar well diffusion method. Plant extract was also examined using same process. Fresh overnight culture of each strain swabbed uniformly by cotton on plates holding sterile Luria Bertani agar and 4 wells (diameter size - 6 mm) were prepared using cup borer. 50 µL of sample nanoparticles pour into each well and commercial disc of gentamicin was used as positive control. Incubated it for 24 hr at 37^{o}C, after that around the well diameter of inhibition zone was observed in millimeter (Fig. 8) (Table 1). Inhibition zone of bacterial growth is due to inhibitory compounds from the tested sample. We concluded that agar well diffusion method showed good antibacterial activity in both *Bacillus subtilis* and *Escherichia coli* showed excellent inhibition zone. Simultaneously, no result was observed in plant extract. To obtain good results, the experiment was performed thrice. Overall, biosynthesized silver nanoparticles exhibited considerable antibacterial activity against mentioned bacteria.



(a) (b)

Fig. 8. Antibacterial studies of silver nanoparticles (a) *E. coli* (b) *Bacillus subtilis*

Table 1. Antibacterial study of biosynthesized silver nanoparticles against pathogenic bacteria

Bacterial strain	Plant extract	Zone of inhibition of biosynthesized silver nanoparticles with different concentrations			Average zone of inhibition
		20 µL	40 µL	60 µL	
B. subtilis	0 mm	2 mm	8 mm	12 mm	7.3 mm
E. coli	0 mm	1 mm	7 mm	13 mm	7.0 mm

5. CONCLUSION

Silver nanoparticles were successfully synthesized using root extract of *Iris germanica* using aqueous salt solution of $AgNO_3$. At primary stage, the production of AgNPs was observed by dark green to dark brown color change followed by UV spectra analysis with SPR pea at 452 nm. The rapid reduction reaction of Ag^+ to Ag^0 was finally completed within 3 hrs. The biomolecules presents in plant responsible for the reduction of silver metal ion was inspected by FTIR analysis. Biosynthesized AgNPs from *Iris germanica* showed excellent antibacterial activity against both *Bacillus subtilis* (gram positive) and *Escherichia coli* (gram negative) bacterial strains and exhibited significant zone of inhibition. Antibacterial activity raised with increasing dose of AgNPs. To conclude, this investigation explained a cost effective and eco-friendly biological method to synthesize AgNPs for excellent antibacterial activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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