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Biosynthesis of Silver Nanoparticles Using *Drimia indica* and Exploring its Antibacterial Profile

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ABSTRACT

Biological synthesis reflects as an eco-friendly, nontoxic and easy method of nanoparticle preparation. Present investigation deals with biosynthesis of silver nanoparticles using *Drimia indica* leaf extract. Initially the synthesized silver nanoparticles were characterized and confirmed by UV-Vis spectroscopy, X-ray diffraction (XRD) and SEM. The synthesized nanoparticles when used for determination of antibacterial activity, by Microtitre Broth Dilution method exhibited remarkable activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*.

Keywords: *Drimia indica, Klebsiella, Pseudomonas, Escherichia,* Microtitre Broth Dilution

INTRODUCTION

Nanotechnology is the most immerging branch of Science and Technology. It is one of the most promising technologies applied in all areas of Science. Apart from its tremendous applications in Pharmaceuticals, textiles industries, electronics etc, it is ecologically sound and cost effective. Metal nanoparticles have received global attention due to their enormous applications in the biomedical and physiochemical fields. Nanoparticles aqueduct the space between bulky materials and molecular structures [1]. Synthesizing metal nanoparticles using plants has been recognized as green and efficient way for further exploiting plants as convenient nanofactories [2]. Nanoparticles with antimicrobial activity are advantageous in reducing acute toxicity, lowering cost and overcoming resistance as compared to other prevalent antibiotics [3,4].

Antibiotics are used as antimicrobial agents in medical field. Higher doses of antibiotics causes harm to human cells and forms cancerous cells and mutations. Use of nanoparticles as antimicrobial agents is increasing day by day. The over doses of antibiotics harms human cells, causes paralysis with more disabilities.

Continued use of antibiotics for treatment of an array of infections has imposed the danger of developing antibiotic resistance. Widespread bacterial infection treatment regime involves higher initial doses of antibiotics followed by gradual lowering down the doses, which requires a longer span of time. A scenario of increased initial doses is practiced regularly which has developed antibiotic resistance for a specific microorganism or a set of microorganisms. Treatment plans for dreadful infections having selective antibiotics for use are compromised due to development of antibiotic resistance, as single antibiotic is used for treatment of such infections. Control measures may be possible in case of broad spectrum antibiotic used or in organisms that are non-selective to antibiotics. On the other hand there are infections which need specific/selective antibiotics for cure. If resistance for single known antibiotic is developed in organisms, the treatment lines may go unattended or may fail totally. There is a need of promising interventions of multifaceted alternative products to antibiotics which can play a vital role in the field of medical science.

Biogenic nanoparticles are used as replacement over antibiotics to target the microbes [5]. Recently, researchers have showed the antimicrobial potential of biogenic nanoparticles on microorganisms. The use of nanoparticles as antimicrobial agents is increasing exceptionally in the field of medical science

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[6].

Through nanotechnology, highly medicinal plants can be directly used for nanoparticle synthesis. The biologically synthesized nanoparticles have helped in target oriented drug delivery, which has lowered down the drug doses with increased efficacy [7]. The plant based synthesis of nanomaterials can be explored and used for human welfare.

D.indica (*Asparagaceae*) is a highly medicinal plant. Is commonly known as Indian squill or Rankanda or jangali pyaz in India. It is pear-shaped, onion like, scaly bulb that grows up to 30 cm in diameter [8,9]. *D.indica* contains cardiac glycosides, quinones, resins, saponins and steroids [10,11]. It possesses antiprotozoal, hypoglycemic, anticancer, antidiabetic and antimicrobial properties [12,13].

MATERIAL AND METHODS

Materials

The leaves of *D.indica* were collected from Dev Dari, Ambheri, District: Satara, State: Maharashtra (GPS– 17.605087º, 74.279927º). Chemicals used were purchased from Thomas Bakers (C) Pvt. Ltd.

Preparation of Plant Extract

For the preparation of leaf extract, fresh leaves of *D.indica* were washed thoroughly under tap water and bolted dry. Leaves (20 g) were boiled into 100 ml of distilled water at 100℃ for 15 minutes. Extract was passed through Buchner's funnel and volume of filtrate was adjusted to 100 ml with distilled water.

Synthesis of Silver Nanoparticles

Leaf extract (20%) was taken into burette and 3 mM aqueous solution of $AgNO_3$ was taken in conical flask. The flask was placed on magnetic stirrer with hotplate at 60℃. Leaf extract was added drop wise into conical flask containing 3 mM $AgNO_3$ solution with continuous stirring. Colorless $AgNO_3$ solution turned yellowish brown which indicated the formation of silver nanoparticles after reduction of silver ions. After 24 hours the reaction mixture was centrifuged at 10000 RPM. The pellet was taken into beaker and washed with alcohol for 2 times-3 times followed by washes with distilled water. The pellet was dried in microwave oven and further used for characterization process.

Antibacterial Assay

The bacterial cultures were sub cultured on liquid nutrient broth and they were incubated at 37℃ for 24 hours in incubator with continuous stirring. The characterized nanoparticles were used for determination of antimicrobial potential by Microtitre Broth Dilution method on spectrophotometer against pathogenic bacteria *P.aeruginosa*, *K.pneumoniae* and *E.coli*. The antibacterial activity of silver nanoparticles was measured by calculating percent inhibition and minimum inhibitory concentration (MIC)(**Figure 1**).

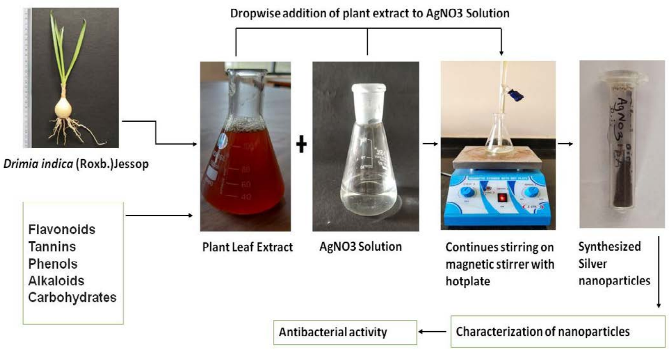

Figure 1: Biological Synthesis of Silver Nanoparticles from Medical Plant Drimia Indica (Roxb.)Jessop

RESULT AND DISCUSSION

UV Visible Spectroscopy

Synthesis of nanomaterials and their applications are having great importance in modern era. Silver nanoparticles were formed by gradual reduction of silver ions during reaction with *D.indica* plant extract. The reaction mixture showed the color change from colorless to light brown to dark brown.

The initial formation of silver nanoparticles was determined by UV visible spectroscopy. The percentage of transmittance light radiation determines when light of certain frequency is passed through the samples. The spectrophotometer analysis records the intensity of absorption (A) or optical density (O.D) as a function of wavelength. The reaction mixture was taken in quartz cuvette and exposed to UV visible radiation (200 nm-800 nm) to MultiscanSky_1530-00496C spectrophotometer. The synthesized nanoparticles were well dispersed in the solution and were stable for long time by showing brown color to reaction mixture. The absorbance peak wasrecorded for nanoparticles which ranged from 2 nm to 100 nm in size. The absorbance maxima were recorded at 421 nm which clearly indicated the formation of silver nanoparticles (**Figure 2**).

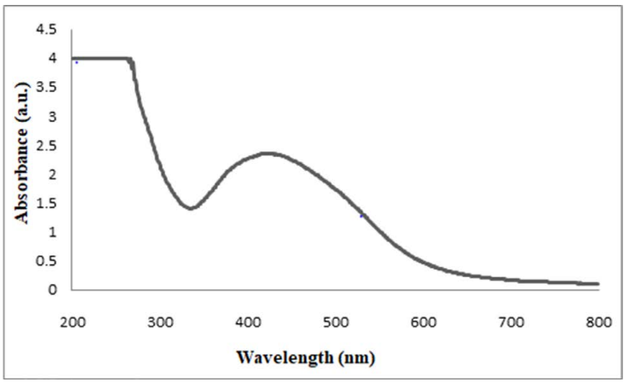

Figure 2: UV-vis absorption spectrum of Silver nanoparticles

SEM

The morphology and size of silver nanoparticles derived from *D.indica* leaf extract were characterized by Scanning Electron Microscope (**Figure 3**). The image magnification was 10,000 X. The studies revealed the average size of silver nanoparticles was between 50 nm-80 nm. These synthesized nanoparticles were highly stable in nature. The nanoparticles were spherical in shape and agglomerated due to isoelectric pH where, the attraction between the particles reaches a maximum, so it is easy to form hard agglomerates.

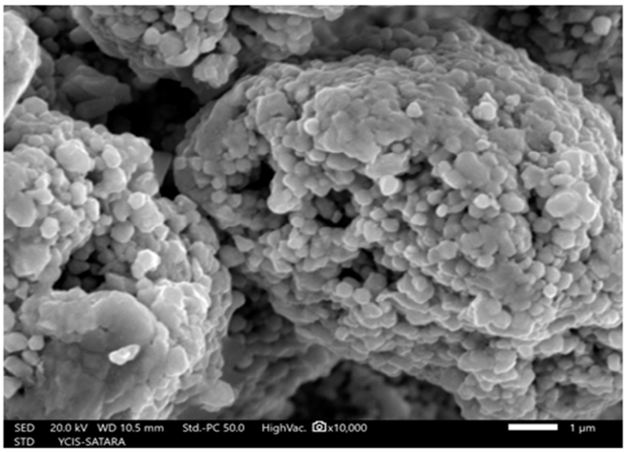

Figure 3: SEM micrograph of Silver nanoparticles.

XRD

To investigate the crystalline nature and structure of Ag NPs, the sample was analyzed on BRUKER-D8 ADVANCE machine at CFC, Shivaji University, Kolhapur. The X-ray diffraction pattern obtained for the silver nanoparticles synthesized from *D.indica* leaf extract showed five distinct diffraction peaks of 2θ values of 38.11° (111), 44.30°(200), 64.22° (220), 77.51° (311) and 81.47° (222) with the assigned planes indicated in the brackets. The free crystalline nature of synthesized nanoparticles was done on the basis of JCPDS file. Analysis was done by using Origin Pro 8 software (**Figure 4**).

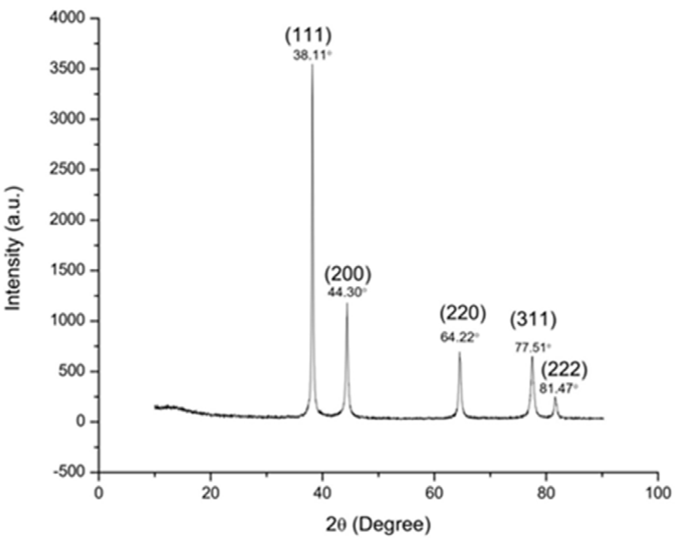

Figure 4: X-ray diffraction pattern of Silver nanoparticles

Antibacterial Assay

The antibacterial activity of silver nanoparticles synthesized from *D.indica* leaf extract was tested on pathogenic bacteria like *P.aeruginosa* [ATCC2021], *Klebsiella* pneumonia [ATCC2075] and *E.coli* [ATCC2065]. Bacterial cultures were maintained in liquid nutrient broth. Calculation of inhibition activity of synthesized nanoparticles on selected pathogenic bacterial cultures was done using different concentrations of Ag NPs. Antibacterial assay was carried out in 96-well microtiter plate. The assay volume was set to 300 µl which contained 260 µl nutrient broth, 20 µl bacterial culture and 20 µl Ag NPs. The Ag NPs were used in varied concentrations (25 µl/ml, 50 µl/ml and 100 µl/ml from mg/ml stock). Replacement of 20 µl Ag NPs with 20 µl distilled water in assay mixture served as negative control. The experiment was performed in triplicates. The 96-well micro-titre plate was incubated for 12 hours. The readings were recorded after time interval of every 30 minutes in MultiscanSky_1530-00496C spectrophotometer. Graph was plotted which indicated sigmoid growth curve. Similar assay was repeated for all bacteria under study. The lowest concentration which inhibits the growth of bacterial strain is taken as MIC of the Ag NPs for the pathogenic bacteria. Broad spectrum antibiotic streptomycin in different concentrations (25 µg/ml, 50 µg/ml and 100 µg/ml) was used to calculate the MIC values for bacterial strains against streptomycin.

When Ag NPs were tested for antimicrobial activity, *P.aeruginosa* showed MIC value of 25 µg/ml, *K.pneumoniae* showed 25 µg/ml and *E.coli* showed 50 µg/ml (**Table 1**).

Table 1: MIC values of Silver nanoparticles with Streptomycin.

Sr. No.	Bacteria	MIC values µg/ml Ag NP's	MIC values µg/ml Streptomycin
1	*P.aeruginosa*	25	100
2	*K.pneumoniae*	25	50
3	*E.coli*	50	100

P.aeruginosa inhibition percentage was recorded to be highest (68%) at 100 µg/ml concentration of streptomycin while it was found to be lowest (45%) at 25 µg/ml Streptomycin concentration. The highest inhibition percentage (65%) at 100 µg/ml and lowest (43%) at 25 µg/ml was recorded for *K.pneumoniae*. While in case of *E.coli* antibiotic Streptomycin showed highest inhibition percentage (69%) at 100 µg/ml and lowest inhibition percentage (47%) at 25 µg/ml (**Figure 5**).

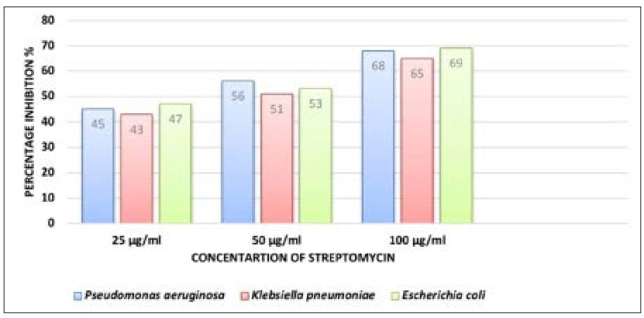

Figure 5: Percentage inhibition of Bacterial species using antibiotic Streptomycin

The highest inhibition percentage of Ag NP's against *P.aeru-*

ginosa was observed to be 54% at Ag NPs concentration 100 µg/ml and lowest 39% at 25 µg/ml. In case of *K.pneumoniae* highest inhibition percentage (49%) was observed at 100 µg/ml of concentration Ag NP's and lowest was 35% at 25 µg/ml. While, highest percentage inhibition for *E.coli* was 51% at 100 µg/ml and lowest was 37% at 25 µg/ml concentration of Ag NPs (**Figure 6**).

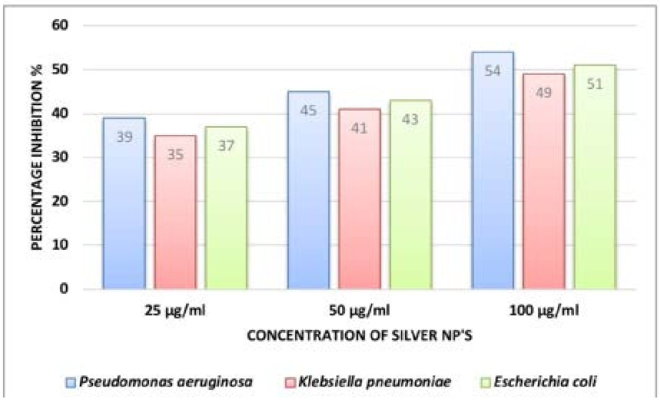

Figure 6: Percentage inhibition of Bacterial species using silver NP's of *D.indica*

Thus, while studying the antibacterial activity of *P.aeruginosa*, highest inhibition percentage (68%) was recorded when Streptomycin (100 µg/ml) was used and when Streptomycin was replaced with same concentration of Ag NPs, the highest inhibition percentage recorded was 54%. In case of *K.pneumoniae*, highest inhibition percentage (65%) was recorded in Streptomycin (100 µg/ml). The same concentration of Ag NPs exhibited 49% inhibition. Highest inhibition percentage (69%) was recorded in *E.coli* for Streptomycin (100 µg/ml). Highest inhibition percentage 5% was recorded for *E.coli* when Ag NP's (100 µg/ml) was used.

The results depicted that the role of antibiotic was remarkable in inhibition of growth in *P.aeruginosa*, *K.pneumoniae* and *E.coli*. Comparative results avoiding antibiotic and replacing it with biologically synthesized Ag NP's showed the presence of inhibition activity in all the bacteria tested. Although inhibition activity of Ag NPs was lower than that of antibiotic, the Ag NPs were able to show antibacterial activity. Further research needs to be refined by designing the protocol in which higher concentrations must be tried to determine antibacterial activity. However, high doses of Ag NPs have advantage over higher doses of antibiotics as Ag NPs are eco-friendly, nontoxic and biologically synthesized. Biologically synthesized nanoparticles can be used as better options over hazardous antibiotics by using them as antibacterial agents.

CONCLUSION

Ag NPs of *D.indica* has shown the lowest antibacterial effect than control in present study, but when the concentration were increased antibacterial effect also increased. It clearly revealed that the inhibition activity of Ag NPs is completely dose dependent and it inhibits the pathogenic bacteria. Thus, they can be widely used as medicines for bacterial diseases. This will eventually help to investigate the problems and mutations caused by high doses of antibiotics.

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CONFLICT OF INTEREST

The author's declared that they have no conflict of interest.

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