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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *AZADIRACHTA INDICA* AND *PLECTRANTHUS PURPURATUS* AND THEIR ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Synthesis of nanoparticles is of different types but we have chosen the method of green synthesis which is environmental friendly and non-toxic. All nanoparticles have many advantages and applicability but only the silver nanoparticle has special advantages and numerous amounts of applications. So, in my project I synthesized silver nanoparticles using green synthesis method and explain its application through anti-bacterial studies. The leaf extract of *Azadirachta indica* and *Plectranthus purpuratus* are used to synthesize silver nanoparticles. The reduction of silver ions was confirmed by the color change in the leaf extract. The synthesized silver nanoparticles was characterized using

- UV-Vis spectroscopy
- FTIR Analysis

The antibacterial assays of the synthesized silver nanoparticles were done on *Bacillus subtilis, Escherichia coli and Pseudomonas aerugenosa* by standard disc diffusion method.

INTRODUCTION

Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. New applications of nanoparticles and nanomaterial are emerging rapidly. Silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic and surface enhanced Raman scattering effect. Silver has been used as an antimicrobial agent for centuries; the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance caused by the abuse of antibiotics. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorous and sulfur containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of silver nanoparticles is the release of silver ions from particles.

Generally, silver does not adversely affect viable cells and does not easily provoke microbial resistance. Hence, silver has been incorporated into plastics in various forms (e.g., catheters, dental material, medical devices and implants, and burn dressings) to protect against microbial contamination. Silver-containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganism. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver (Ag+) or metallic silver (Ag) had been developed. Among the synthetic methods used for the preparation of silver nanoparticles, some toxic chemical used as a reducing agent, such as $NaBH_4$, citrate, or ascorbate is most commonly used. Considering that such reducing agents may be associated with environmental toxicity or biological hazards. The development of a green synthesis for silver nanoparticles is desired. Nanoparticle (NP) synthesis using a green chemistry route is a promising avenue of research in nanoscience today. Green chemistry synthetic approaches must be designed for reduced environmental impact waste reduction process safety materials, and energy efficiency. There is still need for economic, commercially viable as well environmentally clean synthetic route to synthesize silver nanoparticles.

The present investigation of our study is to synthesize silver nanoparticles using *Azadirachta indica* and *Plectranthus purpuratus*, to characterize silver nanoparticles by UV-Vis spectrophotometric analysis and FTIR spectrum analysis and to screen out antibacterial activity of silver nanoparticles at different concentrations by disc diffusion method.

MATERIALS AND METHODS

For investigating the synthesis and antibacterial activities of silver nanoparticles, two medicinal plants, Neem plant (*Azadirachta indica)* and Vicks plant (*Plectranthus purpuratus)* werechosen for the study.

SYNTHESIS OF SILVER NANOPARTICLES:

Synthesis of Silver Nanoparticles using *Azadirachtaindica* leaf extract:

The dried leaves of *Azadirachtaindica*plantwere collected andground to a fine powder.3g of fine powder was made upto 100ml with aqueous solution and added 100ml of 1mM silver nitrate solution.This solution was centrifuged for 25 minutes.The collected supernatant was used for the experiment. Different volumes of the supernatant were taken as follows.

S1-10ml of supernatant

S2 (1:1) –5ml of supernatant + 5ml distilled water

S3 (1:4) – 2.5ml of supernatant + 7.5 ml of distilled water and

S4 (1:10) - 1ml of supernatant + 9ml distilled water.

They were heated at 100^{o}C for 5 minutes.

Biosynthesis of Silver Nanoparticles using *Plectranthuspurpuratus* leaf extract:

1g of fresh leaves was washed thoroughly with sterile distilled water.5ml of water was added in the fresh leaves and boiled for 5 minutes. The extract was collected.1.5 ml of leaf extract was added to 30ml of 1mM $AgNO_3$ solution.The solution was used for the experiment.

S5 – 10ml of supernatant

S6(1:1) – 5ml of supernatant + 5ml distilled water.

S7 (1:4) – 2.5 ml of supernatant + 7.5ml distilled water

S8 (1:10) – 1ml of supernatant + 9ml distilled water

After 10 minutes the color of the solution was change.

CHARACTERIZATION OF SILVER NANOPARTICLES:

Ultraviolet Spectrophotometer:

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1 ml of distilled water used as blank.UV-Vis spectral analysis has been one by using An Elico spectrophotometer at a resolution of 1 nm from 200to 1100 nm (Fig.1).

FT – IR analysis:

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range4000–400 cm−1 at a resolution of 4 cm−1was 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 Infra-Red [FTIR] for the analysis of the nanoparticles.

ANTIBACTERIAL ASSAY

Bacterial strains of *Bacillus subtilis, Escherichiacoli, Pseudomonas aerugenas, Staphylococcus aureus*and *Streptococcus anginosus*were selected for the study.

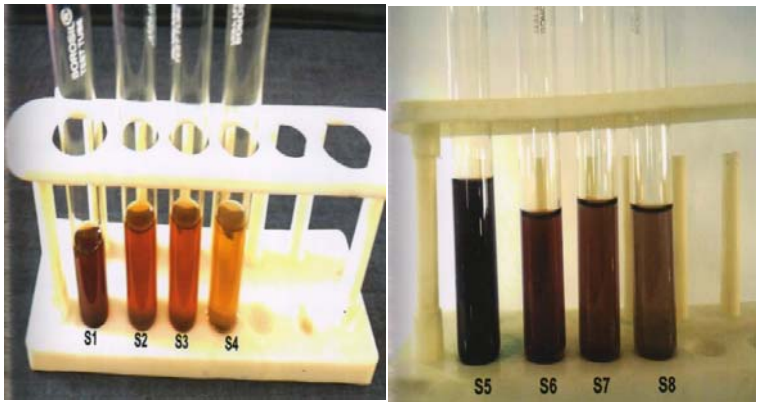
RESULTS AND DISCUSSION

The present study was carried out to access the antibacterial activity of silver nanoparticles synthesized using the leaves of *Azadirachtaindica*and*Plectranthuspurpuratus.*

INDICATION OF COLOR CHANGE

Silver nanoparticles were synthesized using the aqueous extract of *Azadirachtaindica* and *Plectranthuspurpuratus.* The results are shown in (Tube 1 and 2).

Reduction of silver ion into silver nanoparticles during exposure to the plant extract could be followed by color change. Different volumes of aqueous solution of two different plant extracts were added to prepare various samples (1(S1), 1:1(S2), 1:4(S3), 1:10(S4) of *Azadirachtaindica* and 1(S5), 1:1(S6), 1:4(S7) and 1:10(S8) of *Plectranthuspurpuratus.* The solutions were heated at 100^0c. The suspension immediately turned brown in all samples, indicating the formation of silver nanoparticles. The reaction was continued for 5 minutes. The obtained suspension was centrifuged 15, 000 rpm. The silver nanoparticles samples of S1, S2, and S5& S6 exhibit dark yellowish brown color in aqueous solutions due to excitation of surface Plasmon vibrations. The similar results were obtained in the study of extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta*, leaves of *Argemone Mexicana* andCycas leaf.



CHARCTERIZATION OF SILVER NANOPARTICLES

UV – VIS Spectra Analysis

The reduction of pure Ag+ ions was monitored by measuring the UV – Vis spectrum of the reaction medium. The UV – Vis absorption spectrum of silver nanoparticles prepared using

Azadirachta indica and *Plectranthus purpuratus*are shown in figure. The results are given in table 1 and 2.UV – Vis spectra show the characteristic Plasmon absorption peak for the silver nanoparticles at 300nm.

Table: 1 UV-Visible absorption spectrum of silver nanoparticles prepared using *Azadirachta indica*

S.No	Absorbance (nm)	Samples			
		S1(1)	S2(1:1)	S3(1:4)	S4(1:10)
1.	300	3.366	3.358	3.358	1.924
2.	400	3.064	2.003	1.450	1.103
3.	500	3.000	1.730	1.436	0.959
4.	600	2.939	1.078	0.822	0.683
5.	700	2.665	0.679	0.577	0.530
6.	800	2.458	0.525	0.459	0.435

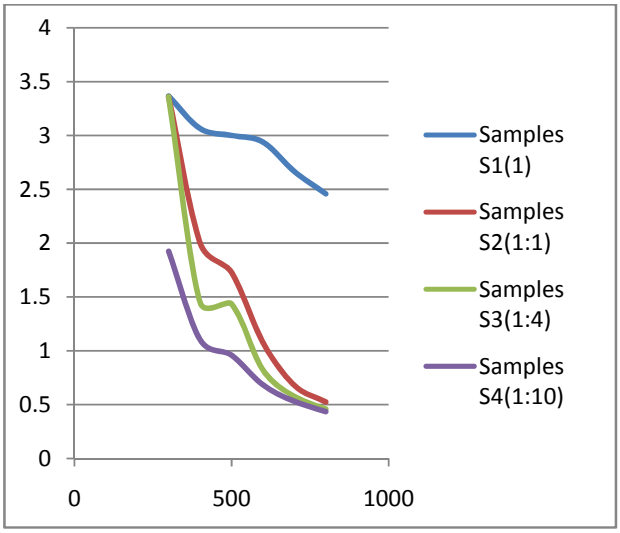
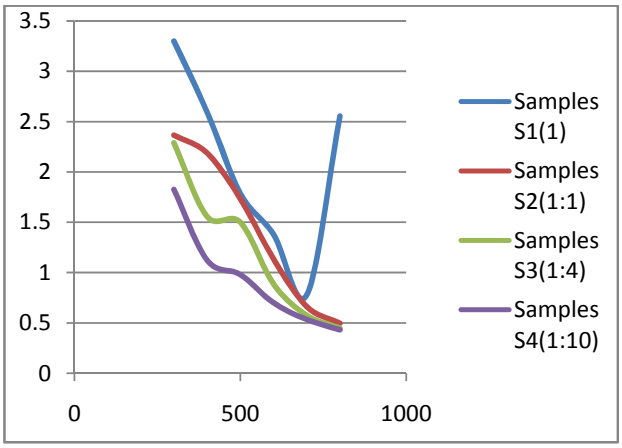


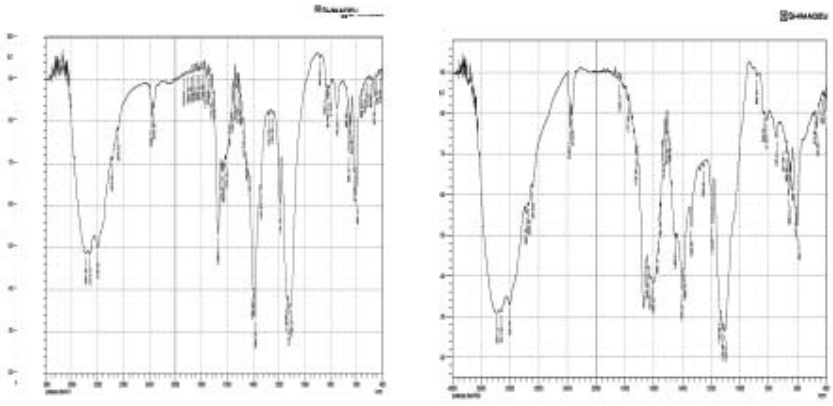
Table: 2 UV - Visible absorption spectrum of silver nanoparticles prepared using *Plectranthus purpuratus*

S.No	Absorbance (nm)	Samples			
		S1(1)	S2(1:1)	S3(1:4)	S4(1:10)
1.	300	3.300	2.365	2.289	1.825
2.	400	2.595	2.187	1.557	1.123
3.	500	1.785	1.734	1.500	0.980
4.	600	1.380	1.135	0.887	0.700
5.	700	0.775	0.662	0.575	0.534
6.	800	2.555	0.497	0.448	0.432

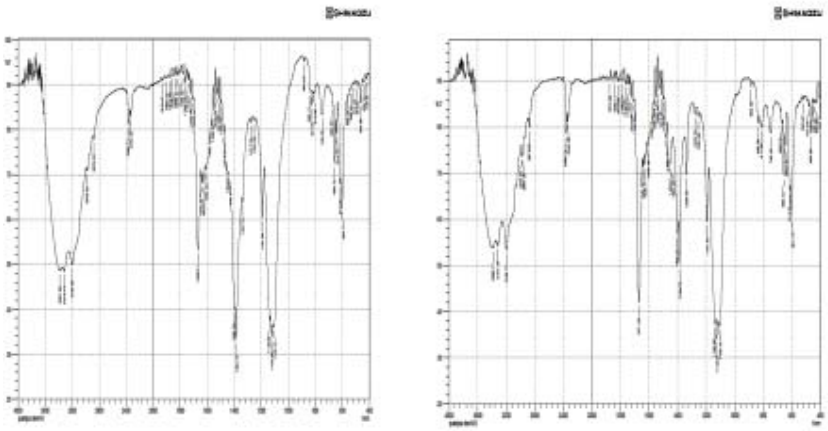


FTIR Analysis

FTIR analysis was used for the characterization of resulting nanoparticle. Compared to Vicks plant (S5 & S6), Neem plant S1 and S2 are having low stretching vibrations in the FTIR spectra. But the peaks were seen in the same pattern for all the samples. The bands seen from 2800cm^{-1} to 2900cm^{-1} and 32cm^{-1} to 3386cm^{-1} were assigned to the stretching vibration of primary and secondary amines (N-H), respectively. The bands seen from16.09cm^{-1} to 1640cm^{-1} and 1513cm^{-1} to 1565cm^{-1} were assigned to primary and secondary amide group (N-H). The peaks at 1739cm^{-1} and 1739cm^{-1} indicates saturated aldehyde and 170592cm^{-1} indicates saturated ketone group. Also bands seen at 1705cm^{-1} to 1723cm^{-1} indicates carboxylic acid and derivatives (C=O, H bonded). 1252cm^{-1} and 1276cm^{-1} peak reflects the presence of acids group. The bands at 1628cm^{-1} and 1532cm^{-1} correspond to the stretch of molecular vibration. The two bands existing at 1450cm^{-1} and 1105cm^{-1} can be assigned to the C-N stretching vibrations of aromatic aliphatic amines. This FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles. IR spectroscopic study has confirmed that amino acid and peptides have formed at coat covering the silver nanoparticles to prevent agglomeration.



(a) (b)



(c) (d)

(a) FTIR spectra of silver nanoparticles synthesized using azadirachta indica at s1 concentration

(b) FTIR spectra of silver nanoparticles synthesized usingazadirachta indica at s2 concentration

(c) FTIR spectra of silver nanoparticles synthesized using plectranthus purpuratus at s5 concentration

(d) FTIR spectra of silver nanoparticles synthesized usingplectranthus purpuratus at s6 concentration

ANTIBACTERIAL ASSAY

The antibacterial assays of the synthesized silver nanoparticles were done on *Bacillus Subtilis, Escherichia Coli, Pseudomonas aerugenosa, Staphylococcus aureus*and *Streptococcus anginosus* standard disc diffusion method. The zone of inhibition of antibiotics against test organisms is shown in table.3. From the results obtained, three antibiotics Ciprofloxacin, Amikacin and Bacitracin were found to have bacterial activities against all test organisms while nitrofuratoin had limited effect on test organisms.

The observed results for antibacterial activity of silver nanoparticles using *Azadirachta indica* and *Plectranthus purpuratus*against test organisms were shown in table,4 and 5.

Table: 3 Zone Of Inhibition for Antibiotics against Test Organisms

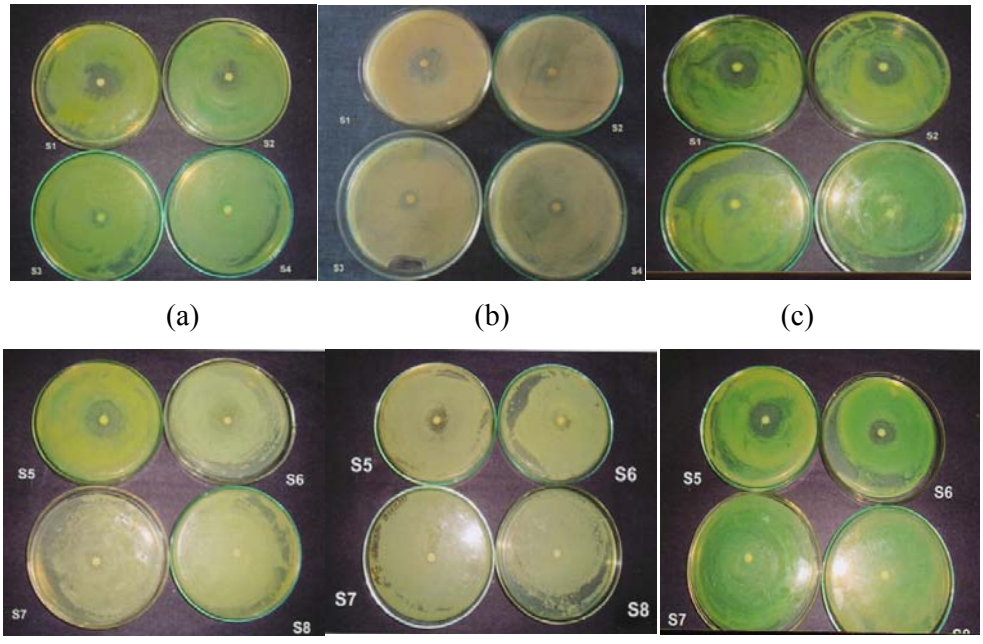
	Antibiotics	**Zone of inhibition (cm)**				
		Bacillus subtilis	***Escherichia coli***	***Pseudomonas aerugenosa***	***Staphylococcus aureus***	***Streptococcus anginosus***
1.	Amikacin	0.5	0.3	0.5	0.3	0.8
2.	Bacitracin	-	-	-	0.1	0.9
3.	Ciprofloxacin	0.3	0.3	0.3	0.8	0.7
4.	Nitrofuratoin	-	-	-	-	-

Table: 4 Antibacterial activity of silver nanoparticles using *Azadirachta indica* against test organisms

S. No	Samples	Zone of inhibition (cm)				
		Bacillus subtilis	***Escherichia coli***	***Pseudomonas aerugenosa***	***Staphylococcus aureus***	***Streptococcus anginosus***
1.	S1	1.1	1.3	1.1	1	0.6
2.	S2	0.8	0.5	1	0.1	-
3.	S3	-	0.4	0.5	0.1	-
4.	S4	-	0.2	-	-	-

Table: 5 Antibacterial activity of silver nanoparticles using *Plectranthus purpuratus*against test organisms

S. No	Samples	Zone of inhibition (cm)				
		Bacillus subtilis	***Escherichia coli***	***Pseudomonas aerugenosa***	***Staphylococcus aureus***	***Streptococcus anginosus***
1.	S5	1.2	0.5	1.3	0.7	0.5
2.	S6	-	-	1	0.1	0.3
3.	S7	-	-	-	-	0.3
4.	S8	-	-	-	-	0.1



(a) (b) (c)

(d) (e) (f)

(a) Antibacterial activity of silver nanaoparticles using *Azadirachta indica* against *Bacillus subtilis*

(b) Antibacterial activity of silver nanaoparticles using *Azadirachta indica* against *Escherichia coli*

(c) Antibacterial activity of silver nanaoparticles using *Azadirachta indica* against *Pseudomonas aerugenosa*

(d) Antibacterial activity of silver nanaoparticles using *Plectranthus purpuratus* against *Bacillus subtilis*

(e) Antibacterial activity of silver nanaoparticles using *Plectranthus purpuratus* against *Escherichia coli*

(f) Antibacterial activity of silver nanaoparticles using *Plectranthus purpuratus* against *Pseudomonas aerugenosa*

Antibacterial activity of silver nanoparticles using *Azadirachta indica* against test organisms was found to be highest at S1 & S2 concentration whereas S3 & S4 had minimal inhibitory effect. And also antibacterial activity of silver nanoparticles using *Plectranthus purpuratus*against test organisms were found to be highest at S5 concentration whereas S6 & S7 and S8 had intermediary effect on the organisms.

From this result, it is observed that high concentration of aqueous extract of plant is needed for the reduction of silver ions. More than that, the inhibitory activities of silver nanoparticles at S1 & S5 concentration of aqueous extract are comparable with standard antibiotics like Ciprofloxin, Amikacin and Bacitracin.

The silver nanoparticles synthesize by green route was found highly toxic against 5 clinically isolated bacterial species at a concentration of 30µ1 silver nanoparticles revealed higher antibacterial *E.coli, K.phenonia and P.Areuginosa.* The inhibitory activities in culture media of the silver nanoparticles reported in were comparable with standard antimicrobics, viz, Chloramphenicol.

The silver nanoparticles synthesized via green route were highly toxic to multidrug resistant bacteria hence has a great potential in biomedical applications.

CONCLUSION

The silver nanoparticles were green synthesized using leaf extract of *Azadirachta indica* and *Plectranthus purpuratus*at four different concentrations each. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such ecofriendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large scale synthesis of other inorganic materials (nanoparticles). Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

The nanoparticles were characterized by UV-Visible spectrophotometer analysis and FTIR spectra analysis. UV-Visible spectra show the characteristic Plasmon absorption peak for the

silver nanoparticles at 300nm. The FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration.

Further the Antibacterial activity of silver nanoparticles was investigated against Bacillus Subtilis, Escherichia Coli, Pseudomonas aerugenas, Staphylococcus aureus and Streptococcus anginosus. The study revealed to possess an effective antibacterial property. The inhibitory activities of all the silver nanoparticles are comparable with standard antibiotics. Thus the present study emphasizes the use of plant medicinal for the synthesis of silver nanoparticles with antibacterial effect.

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