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Green synthesis of silver nanoparticles using *Indigofera cordifolia* leaf extract and their pharmacological potential

P. K. Siva¹, M. Sathish¹, T. Parvathi¹, M. Kamaraj² R. Bhuvaneswari³,
M. Arumugam^{1*}

¹PG and Research Department of Botany, J.J College of Arts and Science (Autonomous), Pudukkottai, Tamil Nadu – 622 422, India, ²PG and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli-620 020, Tamil Nadu, India, ³PG and Research Department of Physics, Government Arts College for Women (Autonomous), Pudukkottai-622001, Tamil Nadu, India (^{1,2,3}Affiliated to Bharathidasan University, Thiruchirappalli, Tamil Nadu-620024-India)

ABSTRACT

Biologically the silver nanoparticles were synthesized from *Indigofera cordifolia* leaves extract. The absorbance of the silver nanoparticles centered at four hundred and twenty nm, with respect to the surface plasmon resonance of silver nanoparticles wavelength. XRD method proves, biosynthesized NPs would retain the face centered cubic (fcc) structure. In TEM image analysis, silver NPs morphology was spherical in shape. Composition of the silver nanoparticles was obtained by EDAX analysis method. FTIR analysis concluded that biosynthesis Ag NPs was observed at 1384 cm⁻¹, with respect to -NO₂ stretching arises from AgNO₃. Ten types of bands are present in the broad emission because of organic matrix bound to silver nanoparticles, which reveals as the result of photoluminescence measurements. The silver NPs possess more antibacterial activity as compared to the standard drug, Amoxicillin.

KEYWORDS: *Indigofera cordifolia*, Silver nanoparticle, Green synthesis, Plasmon resonance, X-Ray Diffraction, Antibacterial activity

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*Corresponding Author:
M. Arumugam,
E-mail: aarubot@gmail.com

INTRODUCTION

The green synthesis methods which are eco friendly methods in the field of science and technologies are very much popular in the future and these methods are essential to minimize the issues in agriculture (Thuesombat *et al.*, 2014). The biological production of silver nanoparticles consist of three major stages, the process must studied on the basis of green chemistry perspective, they are selection of solvent medium, reducing agent that are environmentally non invading tumors, and nontoxic substances for the stability of Ag NPs.

Indigofera tinctoria L. belongs to the family Fabaceae, its synonym is *Indigofera sumatrana* Gaertn. Leaves of the plant are medicinally related. The plant is a shrub, flowering occurs in August to December. The plant is empirical in tainted forest areas and scrub jungles, also in the plains. The plant is spread in Paleotropics, widely cultivated in every districts of Kerala. Local names are Amari, Neelayamari. It is a subshrub, originate in moist deciduous forests and also in plains, the plant is widely cultivated (Sasidharan, 2004). *Indigofera tinctoria* is used in constipation, liver disease, heart palpitation and gout

(Amrithpal, 2006). The roots, stems and leaves are used for treating chronic bronchitis, asthma, ulcers, skin diseases and is useful for promoting growth of hair. The juice extracted from the leaves is useful in the treatment of hydrophobia. An extract of the plant is high-quality for epilepsy and neuropathy. The plant possesses anti-toxic property (Warriner *et al.*, 2007). The flavonoid fraction of *Indigofera tinctoria* had chemopreventive effect against benzo pyrene induced lung cancer (Ravichandran & Ravichandran, 2008). The tangential analgesic possessions of *Indigofera tinctoria* was reported Saravanakumar, 2009. The methanolic extract of the entire plant possessed antihelmintic activity against *Pheretima posthuma* (Gunasekaran, *et al.*, 2009). The ethanolic leaf extract *Indigofera tinctoria* have the ability to inhibit the growth of gram positive bacteria namely *Bacillus pumilus*, *Staphylococcus aureus* and *Streptococcus pyrogens*. Strong antioxidant activity was experiential both qualitatively and quantitatively. The cytotoxic effect of *Indigofera tinctoria* leaf extract on lung cancer cell line NCIH69 was studied. The proportion cell viability of cells was found to decrease at increasing attentiveness (Renukadevi & Sultana, 2011). present work, AgNPs are synthesized *Indigofera cordifolia* leaf extract using a green approach. We identified the morphological

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properties, optical properties and antimicrobial activities of NPs of Ag. This is the primary study in the NPs of Ag on green production from *Indigofera cordifolia* leaves extract. Subjected studies like UV-Visible, X-Ray diffraction, XPS, FESEM, EDAX, Fourier transform IR spectroscopy, photoluminescence and antimicrobial property studies.

MATERIALS AND METHODS

Green Synthesis of Silver Nanoparticles

Indigofera cordifolia leaves were collected from Musiri (10.9549° N, 78.4439° E) Tiruchirappalli (Tamil Nadu, India) and used for the preparation of the aqueous extract and it was subjected to extraction. The exact preparations consisted of different steps. The primary step was cleaning the plant leaves with fresh water, again washed with demineralized water and then cut into tiny parts. 10g of perfectly crushed plant leaves were added to hundred milliliters of double demineralized water. Then it vaporized for ten minutes at 80°C. The final product was extracted by using filter paper (Whatman no.1). The filtrate was collected and kept in the 250 mL Erlenmeyer flask. It was kept at room temperature and used for further analysis. 50 milliliter of the prepared extraction was mixed with 50 milliliters of 1 millimolar Silver nitrate solution at room temperature. Then the Silver nanoparticles were evidently identified after twenty minutes.

Characterization Techniques

The silver nanoparticles were characterized by XRD, XPS and FESEM analysis Angles between 30°-80° for and the monochromatic wavelength of 1.54 Å were applied for the nanoparticles. The Fourier-transform infrared spectroscopy would be measured on a range of four hundred to four thousand cm^{-1} in the Perkin-Elmer spectrometer. The electromagnetic spectrum of silver nanoparticles was identified in wavelength between 200nm to 1100 nm by Lambda 35 spectrometer. The photoluminescence spectra were identified by the Perkin Elmer-LS 14 spectrometer.

Antibacterial Assay

The antimicrobial activity of the AgNPs were determined by the well diffusion assay on MHA towards *Klebsiella pneumonia*, *Streptococcus aureus*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus pneumonia* and *Pseudomonas vulgaris*. For the antibacterial assay, Molten Nutrient Agar (MHA) plates were prepared and it streaked with bacterial inoculum for two to three times. During streaking, the MHA plates were rotated at a sixty degree angle the uniform distribution of the bacterial inoculum on the plates. After the inoculation of the microorganism on the plate disc with different concentrations are placed on the inoculated agar plate. The test sample concentrations include 40 $\mu\text{l/ml}$ (loaded for 1.5 mg) and 50 $\mu\text{l/ml}$ (loaded for 1.75 mg), which are loaded in the wells on the inoculated MHA plate. The MHA plated were subjected to incubation, the plates were placed on the incubator for twenty

four hours at thirty seven °C. After the incubation zone will be formed around the disc and that is called the zone of inhibition, it measure and also record. Amoxicillin (Hi-Media) antibiotic disc were used as positive control for *Klebsiella pneumoniae*, *Streptococcus aureus*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus pneumonia* and *Pseudomonas vulgaris* corresponding to as compared the capacity of the particular test sample. Dimethyl sulfoxide used as the result with negative action. It was found that dimethyl sulfoxide does not show biochemical possessions.

Cell Culture

Dulbecco's modified Eagle's medium, DMEM having the pH of 7.2 to which 10% FBS was supplemented were introduced for the cultivation of breast cancer MCF-7 cells. The culture media also treated by using gentamicin (100 U/mL) to reduce the contamination. An incubator with high moisture content having 5% carbon dioxide was proposed for the incubation of breast cancer cell. From the cell culture the 80–90% cells were collected by the use of trypsin. Then it was washed with phosphate buffered saline.

Cell Viability Assays

The viability of the cells was identified by using the slightly altered protocol of MTT cytotoxicity assay (Raveendran *et al.*, 2003). The cultured breast cancer cells would be developed to density of 2×10^4 cells/wells for 24 hrs. Introduced to various test concentrations of silver nanoparticles for 24 hrs. After the treatment phosphate buffered substrate with added 5.0 mg/mL MTT were introduced at the range of 10 microliter into each well and incubated for another 4 hrs. The transfer of MTT leads to the development of formazan crystals inside the hepatic cells. Dimethyl sulfoxide (100 μL) was treated with the formazan crystals which dissolved. The measurements were taken at 570 nm. ELISA readers (BioTek) were used to taking readings.

RESULTS AND DISCUSSION

UV-Vis Spectroscopic Studies

The *Indigofera cordifolia* leaf extract was used in the reduction of AgNO_3 into Ag^0 , and the reduction was initially confirmed by the color changes from colorless to yellowish brown color is shown in Figure 1a. On the basis of the arrangement of the size of the NPs, the produced Ag NPs manifest the absorbance between 400 nm to 450 nm. Then the absorbance of the nanoparticle will be centralized at 420nm peak.

The relationship of α and $h\nu$ can be expressed as,

$$\alpha h\nu = A(h\nu - E_g)^n$$

Where, absorption coefficient is α , $h\nu$, incident photon energy, E_g , optical band gap, A, constant, n is the exponent.

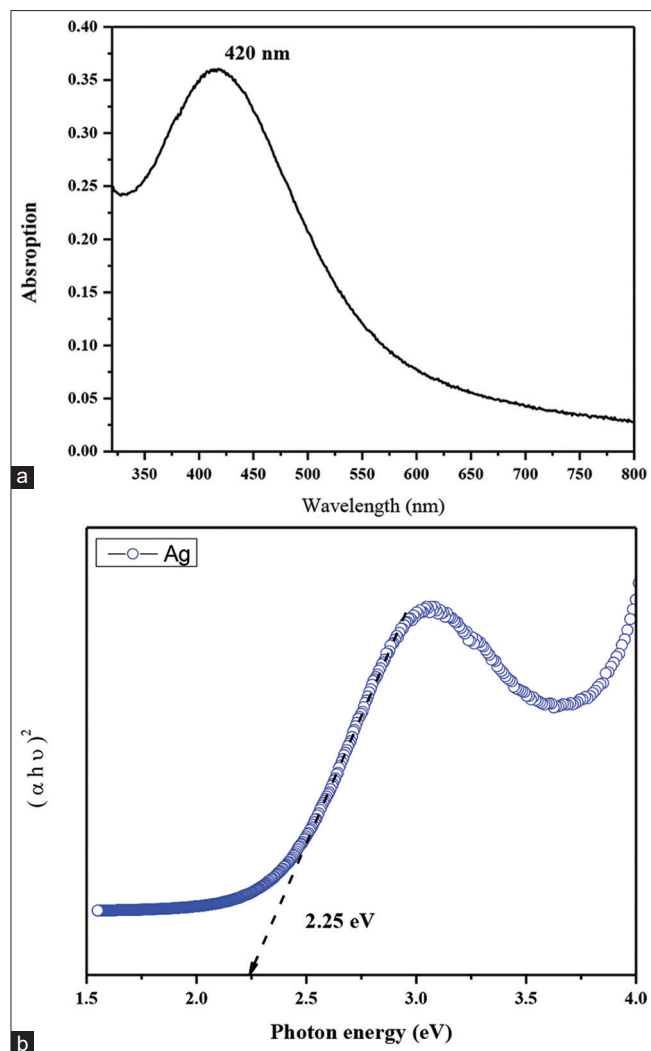


Figure 1: (a) UV-Vis spectrum of Ag NPs synthesized by *Indigofera cordifolia* leaf extract. (b) Photon energy level of the phyto-synthesized silver NPs

Allowed direct transition occurs when the value of n becomes half. A plot is drawn between $(\alpha h\nu)^2$ vs. photon energy, $h\nu$ for determining the direct and indirect transition and to find the intercept with energy axis, a linear portion of the edge is extrapolated (Figure 1b) At 2.25 eV, Ag NPs direct band gap is observed.

X-ray Diffraction Studies

The XRD pattern of Ag NPs are synthesis from *Indigofera cordifolia* leaf extraction is presented in Figure 2. The X-Ray diffraction patterns are shows at (2θ) of 38.02, 43.99, 64.48 and 77.31 with respect to various plane (111,200,220 and 311) of the Ag nanoparticles. The standard peaks on the graph represent the face-centered cubic of Ag Nanoparticles. XRD represents the Ag NPs generated due to the Ag^+ ion reduced by the leaf extract of *Indigofera cordifolia*

The lattice constant 'a' of silver found from this equation,

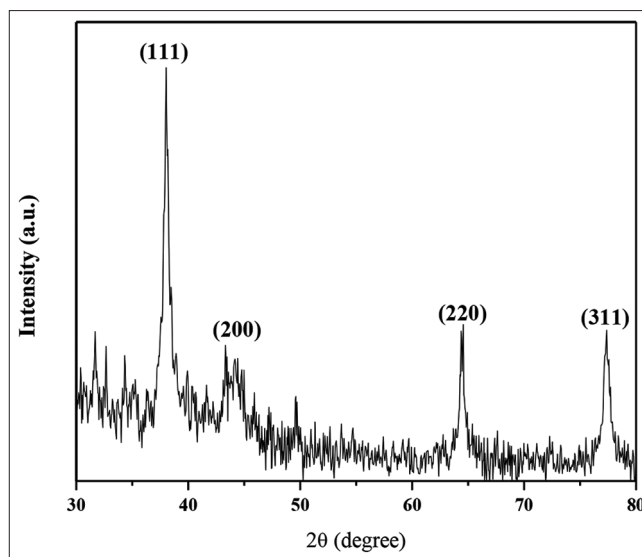


Figure 2: Diffraction patterns (X-ray) of synthesized silver nanoparticles

$$\frac{1}{d^2} = \left(\frac{h^2 + k^2 + l^2}{a^2} \right)$$

'a' is the lattice constant obtained from the formula, $a = \sqrt{d^2 (h^2 + k^2 + l^2)}$. Estimated value of 'a' is 4.0993 Å for silver nanoparticles. The unit cell volume calculated from the above mentioned relation is $V = a^3$. V is identified as 68.8857 Å³ for silver nanoparticles.

Debye-Scherrer's formula used to find D,

Where D is Average crystallite size of a nanoparticle.

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

Here, X-ray wavelength is represented as λ , the angular peak width at half maximum in radians is indicated as beta and Bragg's diffraction angle is θ . For Ag NPs the value (D) obtained is 28.46 nm.

X-ray Photoelectron Spectroscopy Analysis

The X-ray photoelectron spectra gives the knowledge of the oxidation state of C1s, O1s and silver 3d state for silver nanoparticles synthesized by *Indigofera cordifolia* leaf extract is represented in Figure 3(a-c). Figure 3a shows the C1s signal split into three symmetric peaks observed at (284.30 eV, 286.59 eV and 294.06 eV). The carbon C1s peck center at 284.30 eV associated with C-C state, in contrast to carbon atoms of Tyr, Trp or Phe phenyl rings. The carbonyl carbons C-O peak is observed at 286.59 eV for Ag NPs and were attributes to transfer of electron from carbons in carbonyl groups and Finally O-C=O peak center at 294.06 eV, which was most likely from carbons a to the carboxylic groups

for silver NPs synthesized by using *Indigofera cordifolia* leaf extract (Li *et al.*, 2007). Figure 3b indicates oxygen (1s) non symmetrical signal differentiated into two identical signal are obtained at 527.98 eV and 530.12 eV for Ag NPs, which is described to the carboxylate groups (Li *et al.*, 2007). Figure 3c represents the strong spin orbit coupling where the Ag (3d) signal splits into two identical peaks Ag 3d_{5/2} and Ag 3d_{3/2}. The silver nanoparticles, which peak in the graph, were situated at 367.57 eV and 373.63 eV. The spin-orbit split value was 6.06 eV which collaborated with the binding energy of metallic Ag (Kaushik, 1991). At last the ions of silver get changed to its metallic form (Ag). The bio Ag nanoparticles were capped by the extracts of *Indigofera cordifolia*.

The Morphology and Elemental Composition

The surface structure of the silver nanoparticles are produced from *Indigoferacordifolia* leaf extract is represented in Figure 4(a-b). The complete Field Emission Scanning Electron Microscopic image, the silver NPs is formed spherical shape. The elemental composition of the nanoparticle obtained by Energy Dispersive X-ray analysis spectra of the silver nanoparticles are represented in Figure 5. These spectra displayed the presence of C, O, and Ag in silver NPs using *Indigofera cordifolia* leaf extract.

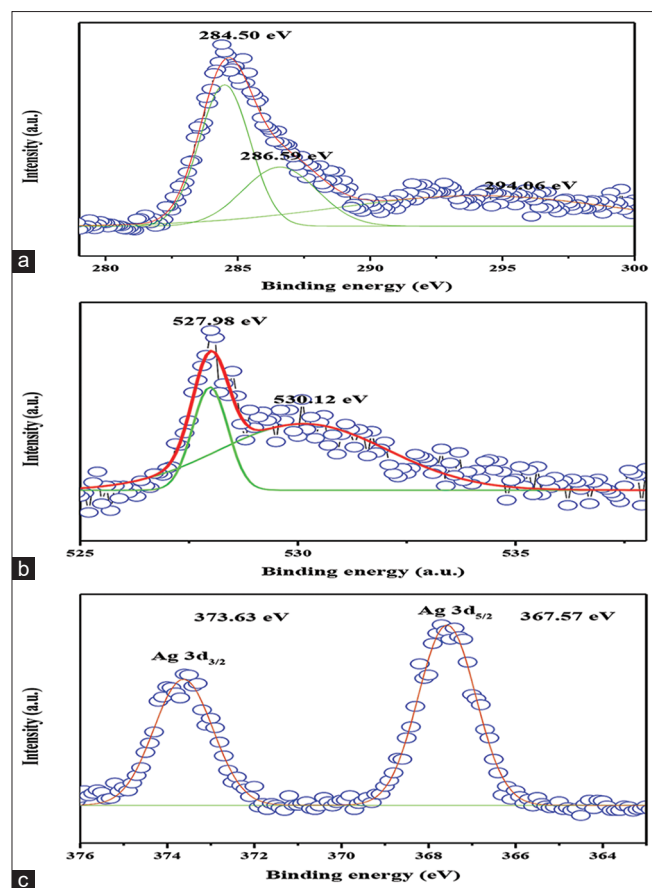


Figure 3: (a) XPS spectra of C (1s) for the silver NPs. (c) XPS spectra of Ag (3d) for the silver NPs, 3b: XPS spectra of O (1s) for the silver NPs

High Resolution Transmission Electron Microscopic Studies

Figure 6 represents that the HRTEM image of Ag Nanoparticles is produced from *Indigofera cordifolia* leaf extract. Silver NPs possessed a spherical structure, in a size range of 10-30 nm. SAED patterns can be assigned to the reflections (111, 200, 220 and 311) for Si and Ag NPs respectively.

FTIR Spectroscopic Studies

The extract was prepared from the plant which has two major functions such as capping and reducing properties. The availability of functional groups were discovered by Fourier transform infrared analysis of Ag NPs. Figure 7(a-b) shows the FTIR spectra for Ag nanoparticles were synthesized by *Indigofera cordifolia* leaf extract. The broad absorption band is observed at 3464 cm⁻¹ in the silver sample and this vibration frequency is bounded hydroxyl (-OH) or amine (-NH) groups present in the bio-macromolecules on leaf extract (Shah *et al.*, 2016) [5]. In literature C-H stretching of methyl groups vibration were observed at 2962 and 2873 cm⁻¹ for silver NPs. In our result, C-H stretching of methyl group's vibration is observed at 2972 and 2867 cm⁻¹ for silver NPs. From the Fourier transform infrared spectra of green synthesis silver nanoparticle vibration frequency are identified at 1384 cm⁻¹ with respect to -NO₃ stretching which comes from silver nitrate (Coates and Meyers, 2000; Edison *et al.*, 2016). The C-H bending vibration identified at 839 cm⁻¹ for silver nanoparticles. The metallic silver graph's peak obtained at

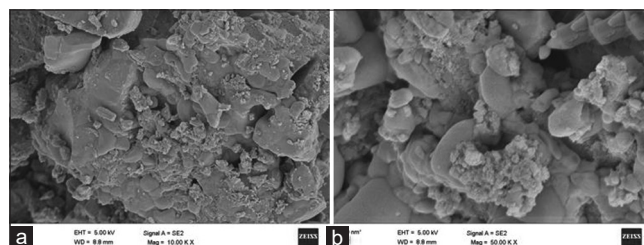


Figure 4: (a-b) Field Emission Scanning Electron Microscopy image of high and low magnification for silver NPs.

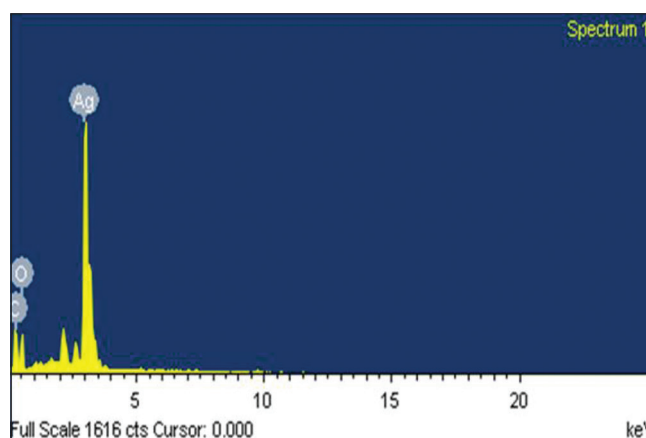


Figure 5: EDAX spectra of the silver NPs

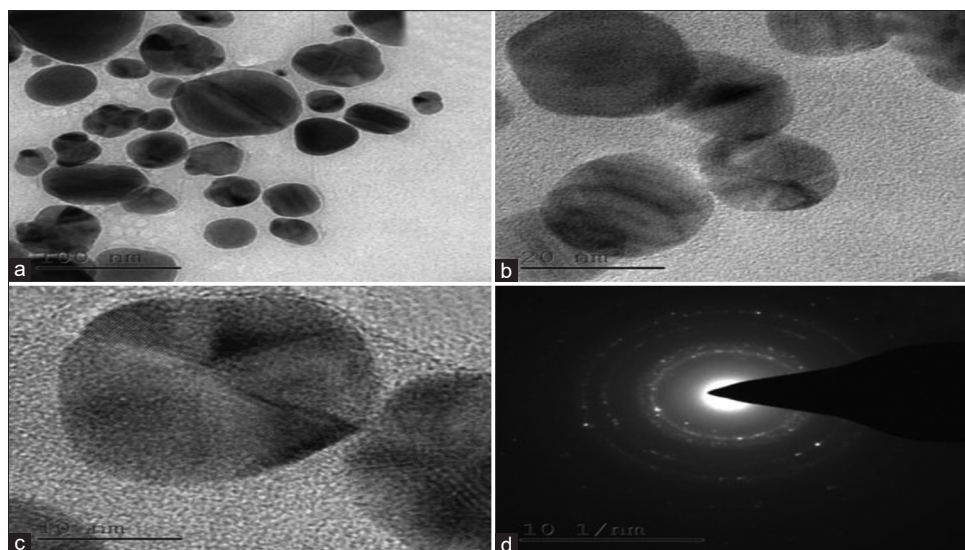


Figure 6: (a-c) High and low magnifications of silver NPs and (d) SAED pattern of silver NPs

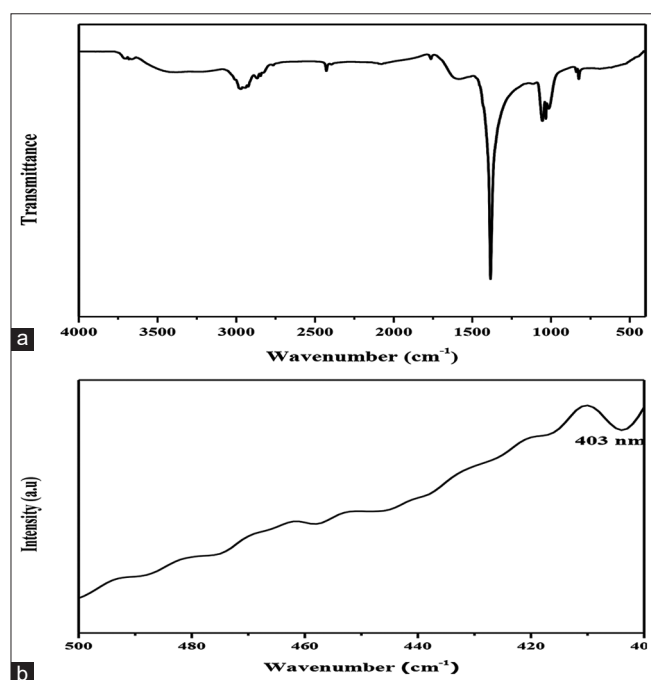


Figure 7: (a) FTIR spectra of silver NPs. (b) Extended 500-400 cm^{-1} region of FTIR spectra for silver NPs

403 cm^{-1} for Ag nanoparticles are synthesis from *Indigofera cordifolia* leaves extract (Figure 7b.).

Photoluminescence spectroscopic studies

Photoluminescence (PL) spectra are one of the effective tools to identify the optical activity of Ag NPs as photonic materials. Synthesized silver NPs using *Indigofera cordifolia* leaf extract is present in Figure 8. The excitation wavelength of silver nanoparticles is identified at 420 nm. The emission spectrum of the silver nano particle with six peaks is observed at 448 nm, 480 nanometer, 491 nanometer, 501 nanometer, 541 nanometer and

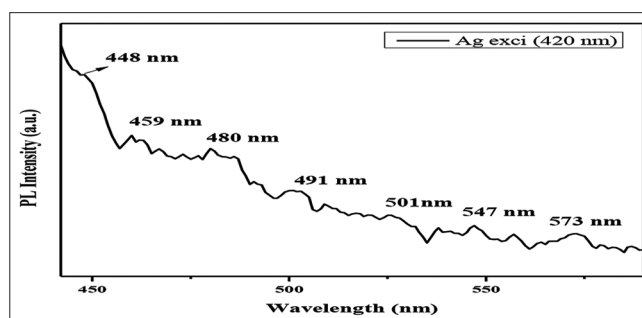


Figure 8: Photoluminescence spectra of silver NPs

573 nanometer. The two blue emission, blue-green emission, two green emission and yellow emission for silver NPs. The luminescence emission peaks at 440 and 600 nanometer shows because of the availability of antioxidant in *Indigofera cordifolia* plant extract. The green emission and yellow peaks at 501 nm, 541 nm and 573 nm of the organic matrix bound to silver nanoparticles.

Antibacterial Studies

In present work, silver NPs synthesized by *Indigofera cordifolia* leaf extract is treated with *S. aureus* and *S. pneumoniae* and *E. coli*, *Pseudomonas aeruginosa*, *P. vulgaris*, *K. pneumoniae* and *S. dysenteriae* analyzed well diffusion assay represented in Figure 9. The antimicrobial activity examined around the all-well concentration (20, 25, 30 and 35 $\mu\text{g/ml}$) of silver nanoparticles treated with test samples (Table 1). The zone inhibition of silver NPs possesses more antibacterial activity as compared to standard Amoxicillin (10 μg disc). Increasing silver nanoparticles concentrations, zone inhibition also increased. The nanoparticles synthesized from plant extract that act against the microorganisms by the production of secondary metabolites which is responsible for the inhibition of the microorganisms. The Ag NPs having large surface area which is the largest capacity of the particular nanoparticle against

the microbial cells. The NPs which bound with the plasma membrane of the bacterial cell and moves into the cytoplasm of the microorganism (Wang *et al.*, 2017; Sirelkhatim *et al.*, 2015; Mohd Yusof *et al.*, 2019; Liao *et al.*, 2019; Sánchez-López *et al.*, 2020). The microorganisms surface that bound with the S-containing membrane proteins. The AgNPs were act with the membrane proteins and also act with the Phosphorus containing Deoxyribonucleic acid.

The nanoparticles major advantage is its size, the nanoparticles are tiny particles which has high surface area which can actively react with the microorganisms. The nanoparticles have high efficiency than larger particles (Rai *et al.*, 2009). In our result, silver nanoparticles average size less than 10-30 nm that will

increase the activity of NPs by acting with the microorganism and there by producing the electronic effect (Rai *et al.*, 2009). From the photoluminescence spectra of the silver NPs, the wavelengths of the green and yellow lights are 501 nm, 547 nm and 573 nm for the Ag nanoparticles only because of the availability of O vacancy. So that the silver NPs have more antimicrobial activity.

MTT cell viability assay were used to find the cytotoxic property of AgNPs in MCF-7 cells (Table 2). Different concentrations of MCF-7 cells were prepared and the inhibition percentage was identified with the help of cells without mixed with the nanoparticles. It is clear that the concentration needed to inhibit 50% MCF-7 cells are low for AgNPs than the *Indigofera cordifolia*

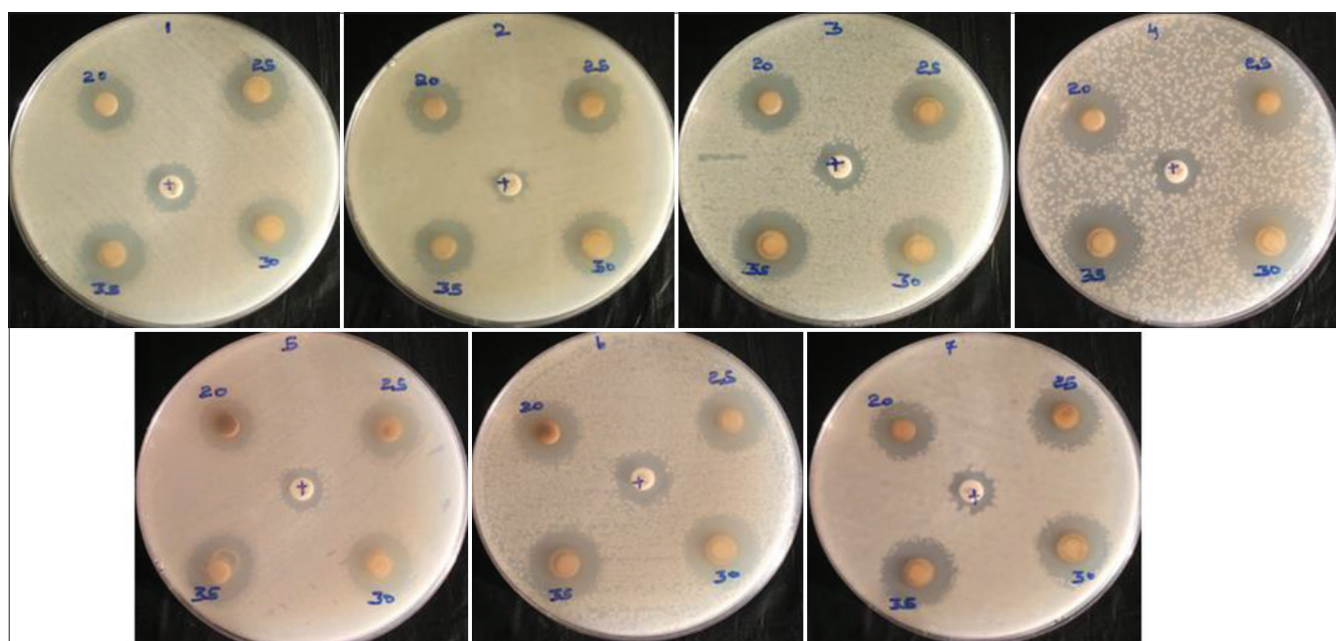


Figure 9: The antibacterial activity of silver NPs using *Indigofera cordifolia* leaf extract against *Streptococcus aureus* (2) *Staphylococcus pneumonia* (6) includes *Escherichia coli* (4), *Pseudomonas aeruginosa* (5), *Pseudomonas vulgaris* (7), *Klebsiella pneumonia* (1) *Shigella dysenteriae* (3)

Table 1. Antibacterial activity *Indigofera cordifolia* mediated AgNPS

S. No	Name of the bacterial strains	20μm/ml	25μm/ml	30μm/ml	35 m/ml	Amoxicillin
1.	<i>Klebsiella pneumonia</i>	22.32±1.21	23.40±0.91	25.22±0.65	25.19±1.12	18.22±0.39
2.	<i>Streptococcus aureus</i>	21.41±0.41	22.39±1.13	23.33±0.33	22.12±1.03	13.33±0.20
3.	<i>Shigella dysenteriae</i>	23.42±0.38	24.32±1.02	26.36±1.03	27.46±0.82	12.42±1.38
4.	<i>Escherichia coli</i>	22.38±0.59	24.29±0.36	24.11±0.26	26.31±0.69	13.23±0.31
5.	<i>Pseudomonas aeruginosa</i>	19.24±1.02	21.31±0.52	23.26±0.46	24.36±1.22	16.37±0.49
6.	<i>Staphylococcus pneumonia</i>	21.37±0.81	22.39±0.43	24.12±1.21	26.40±0.35	12.41±0.56
7.	<i>Pseudomonas vulgaris</i>	21.22±0.45	22.36±0.13	23.39±0.62	6.23±0.41	13.26±0.29

Mean Zone of inhibition ±SD (in mm)

Table 2: MCF-7 Cells MTT assay was done following the incubation period of the plant extract, silver nitrate and two concentrations of silver nanoparticles of *indigofera cordifolia*

Treatment	12hour		24hour		36hour	
	Viable (%)	Inhibition (%)	Viable (%)	Dead Inhibition (%)	Viable (%)	Dead Inhibition (%)
MCF-7 Cell+Plant Crude extract	68.38±0.12	31.00±0.10	62.00±0.41	36.00±0.03	58.00±0.91	45.12±0.02
MCF-7 Cell+AgNO ₃	74.41±1.21	26.01±0.91	72.00±0.38	23.02±0.12	70.32±0.45	24.21±1.57
MCF-7 Cell+50 mg/mL AgNPs	62.32±0.81	37.10±1.02	55.21±1.23	45.21±0.59	34.02±0.52	63.21±0.3
MCF-7 Cell+100 mg/mL AgNPs	54.23±0.64	46.01±1.21	28.00±1.41	72.20±1.15	12.00±0.43	88.00±1.06

leaves extract (Jacob *et al.*, 2012; Selvi *et al.*, 2016; Ciorîță *et al.*, 2020; Vijayarathna & Sasidharan, 2012; Venugopal *et al.*, 2017).

CONCLUSIONS

Ag NPs were produced from *Indigofera cordifolia* leaf extract using the Green method. The absorbance was centered at 420 nanometer, in contrast to the surface plasmon resonance of silver nanoparticles wavelength. XRD analysis proves that synthesized NPs retained the FCC structure and the crystalline size 28.46 nm for Ag NPs. The X-ray photoelectron spectrum provided the knowledge of the oxidation state of Cls, Ols and silver 3d state for silver nanoparticles. From the FESEM image, the silver nanoparticles would be seen as spherical shapes. The element composition was discovered by Energy Dispersive X-ray analysis spectra. In the TEM result, the surface structure of silver nanoparticles was a spherical structure with a particle size of 10-30 nm. In the Fourier Transform Infrared spectrum of biosynthesized silver nanoparticles frequency was obtained at 1384 cm⁻¹, this was due to nitrate stretching, which arises from silver nitrate. The green and yellow peaks were identified at 501 nm, 541 nm and 573 nm of the organic matrix bound to silver nanoparticles. The silver NPs have high microbicidal properties as they differentiate with the standard drug amoxicillin. The AgNPs have a bactericidal activity which can act against the bacterial cells. The produced secondary metabolites, that causes cell death.

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