Biosynthesis, Characterization and Antimicrobial activity of Silver Nanoparticles using Justicia Adhatoda.

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***Abstract*: Biosynthesis is a simple, non-toxic, economical and eco-friendly approach for the synthesis of nanoparticles. In the present work, nanoparticles of silver were synthesized by using aqueous solution of *Justicia Adhatoda* leaf extract as a reducing agent. These synthesized Ag-NPs were further evaluated for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis.* The characteristics of silver nanoparticles were studied using UV-Visible spectroscopy and Fourier Transform Infra-red spectroscopy (FTIR). The potential applications of bio-synthesized nanoparticles showed antimicrobial efficacy against *Escherichia Coli* (12 mm) and *Bacillus subtilis* (11 mm) respectively.**

***Keywords*: Biosynthesis; *Justicia Adhatoda* extract; Silver nanoparticles; Antimicrobial activity.**

1. INTRODUCTION

Nanotechnology is an important field of modern research mainly concern with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use of human benefits. Biosynthesis provides advancement over chemical and physical method as it has no need to use high pressure, energy, temperature and toxic chemicals ¤1¥. Nanomaterials are seen as a solution to many technological and environmental challenges in the field of solar energy conversion, catalysis, medicine, and water treatment. Research on the synthesis of Nano sized material is of great interest because of their unique properties like optoelectronic, magnetic and mechanical which differs from bulk.

The biosynthesis of Silver oxide nanoparticles of different sizes and shapes has been reported using bacteria, fungi and plant extract ¤2¥. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Physical and chemical methods uses harmful chemicals such as sodium borohydride, sodium citrate dehydrate which are possibly hazardous and very costly to the natural settings and organic functions. Bio inspired synthesis of these particles proves to be cost effective environmental alternative to chemical and physical methods in preparing of nanoparticles, ecofriendly and helps to reduce harmful effects on environment ¤3,4¥.

In the present study Silver nanoparticles were synthesized through bio inspired synthetic approach. The applications of Silver nanoparticles (AgNPs) are a very promising, efficient and cost effective method for remediating this environmental health concern. Plant extracts have shown prospects in AgNPS synthesis which is a green chemistry synthetic approach ¤5¥. Plant extract mediated synthesis of nanoparticle is one of the stable and suitable alternatives in comparision with other conventional methods. *Justicia Adhatoda* known as Vasaka or Malabar nut belongs to *Acanthaceae* family. It has many medicinal properties such as antibacterial, antifungal, anti-inflammatory. Biologically synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antibacterial and antimicrobial properties ¤6, 7¥.The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, FTIR spectroscopy and tested for their antimicrobial activity.

2. MATERIALS AND METHODS

2.1 Preparation of *Justicia Adhatoda* Leaf Extract

Fresh leaves of *Justicia Adhatoda* were collected from A.S.C College campus, Indapur. The collected leaves were thoroughly washed several times with distilled water and shade dried for a day. 10 g leaves was added in 100 mL of distilled water and heated at 80 °C for 15-20 minutes. Allowed to cool at room temperature and filtered through Whatman No. 42. The filtrate was centrifuged at 1500 rpm for 10 minutes. The supernatant filtrate was used as leaf extract for the synthesis of nanoparticles, stored for further use.

2.2 Preparation of 0.01M $AgNO_3$ solutions

0.169 gm. A.R grade $AgNO_3$ dissolved in 100 mL distilled water (DW) and stored in amber colored bottle in cool and dry place.

2.3 Preparation of Silver Nanoparticles

50 mL 0.01 M Silver nitrate were taken in beaker and stirred using a magnetic stirrer for 5 minutes. After 5 minutes, 25 mL leaf extract was added slowly into this beaker for uniform precipitation of silver nanoparticles. The solution was left undisturbed and allowed to room temperature. The yellowish brown colored precipitate get deposited at the bottom, indicates the formation of nanoparticles. The deposited nanoparticles were washed with sterile distilled water and was subjected to centrifugation at 10,000 rpm for 20 minutes. The nanoparticles was dried in hot air oven for 1 hour at 80°C and subjected for characterization. Reduction of silver ions to silver nanoparticles was confirmed by color changes from colorless to yellowish brown (figure 2).

(a)

(b)

Fig. 1: (a) Solution of silver nitrate and plant extract (b) Silver Nanoparticles

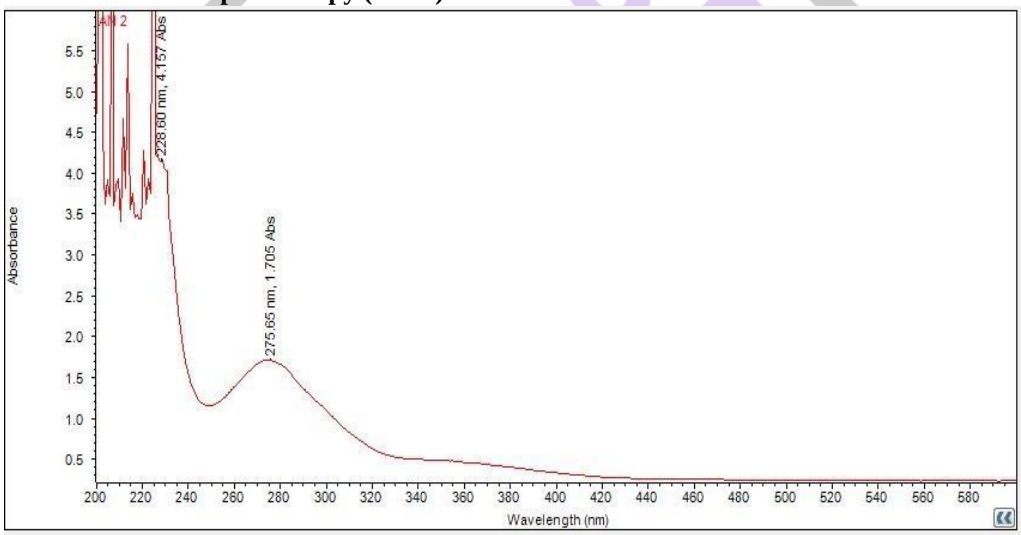
3. RESULTS AND DISCUSSION

3.1 UV-Visible Spectroscopy

It is well known that silver nanoparticles exhibit a brown color in aqueous solution due to excitation of surface Plasmon vibration in silver nanoparticles ¤8,9¥. The absorption spectra of synthesized silver nanoparticles after the completion of reaction shown in figure 1. The absorption spectrum of the synthesized nanoparticles was observed in the UV region at 275 nm. This observation indicates formation of silver nanoparticles ¤10, 11¥.

Fig.2: UV-Visible absorption spectrum of obtained Silver Nanoparticle

3.2 Fourier Transform Infrared Spectroscopy (FTIR)



The FTIR band intensities in various regions of the synthesized AgNPS spectrum (figure 3) were analyzed to reveal the compound responsible for nanoparticle synthesis. Band at 1033.28 cm^{-1} corresponds to OH bending, frequency 1318.30 cm^{-1} attributed to C = O of ketonic carboxylic acid. The peak at 1456.89 cm^{-1} attributed to primary amines and alkaline peak observed at 2917.14 cm^{-1} may be due to aldehydes stretching of alkane respectively.

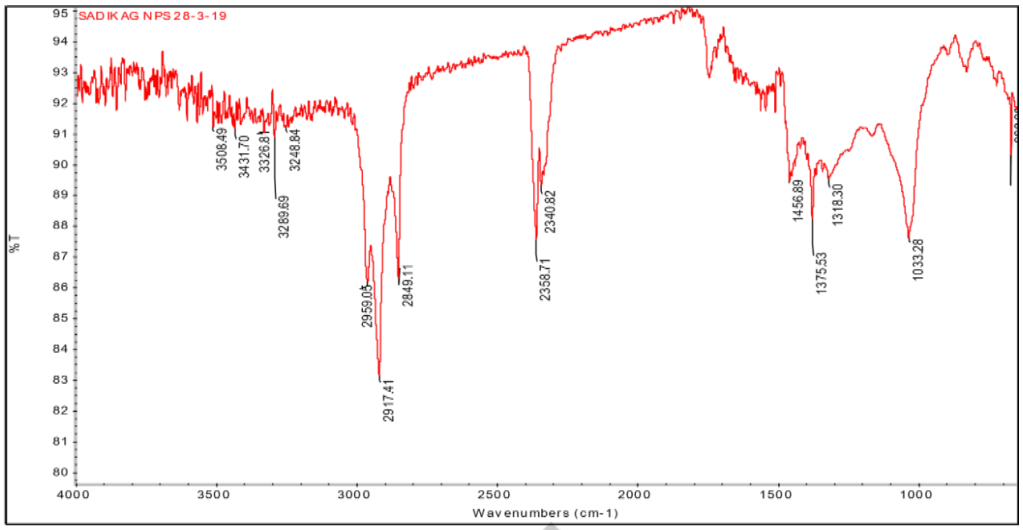


Fig. 3: FTIR spectrum of synthesized Silver Nanoparticles

3.3 Antimicrobial efficacy

Synthesized silver nanoparticles were tested against bacterial strains such as *Escherichia Coli*, *Bacillus Subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by disc diffusion method for their zone of inhibition. Amongst these gram positive and gram negative strains, *Escherichia Coli and Bacillus Subtilis* were found to be sensitive to silver nanoparticles showing zone of inhibition (12 mm) and (11 mm) respectively (figure 4 and Table 1) and no activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*¤12, 13¥.

Table 1: Antimicrobial Activity of silver nanoparticles

Plants extract/drug	Micro-organisms (zone of inhibition in mm.)			
	Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Amoxicillin (100 µg/mL)	–	–	–	–
Ciprofloxacin (100 µg/mL)	35 mm	22 mm	20mm	20mm
AgNPs (50µg/mL)	12 mm	11 mm	–	–

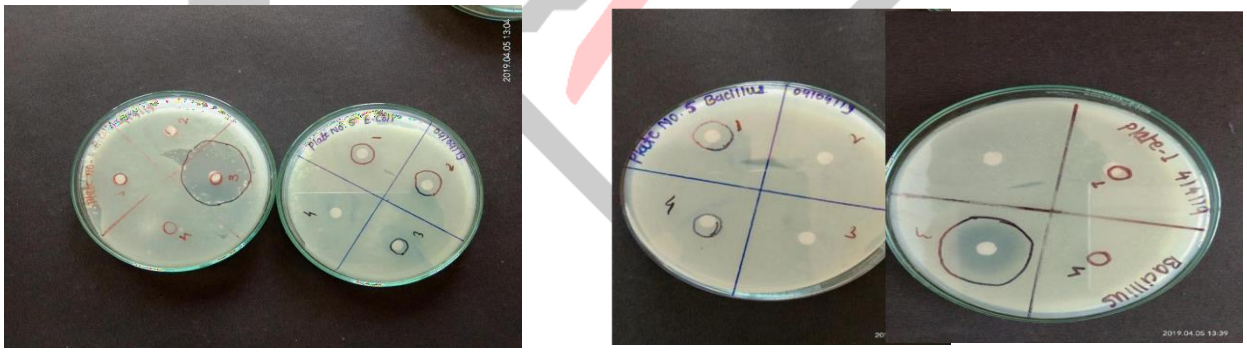


Fig. 4: Antimicrobial activity of synthesized Silver nanoparticles against pathogens

CONCLUSION

Biosynthesis is reliable and eco-friendly processes for synthesis of metallic nanoparticles method using *Justicia adhatoda*. Here, we have reported a simple biological and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bio reduction method. The functional groups like amide, ketone, and aliphatic amines played as reducing and stabilizing agents. Green synthesis provide faster synthesis comparable to other synthesis and has many applications. The studied Silver nanoparticles were completely inactive towards *Staphylococcus aureus* and *Pseudomonas aeruginosa*. But inhibited the growth of residual gram positive rods *Eschericha coil* and gram positive rods *Bacillus Subtilis*.

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REFERENCES

[1] D. Bharathi, S. Ramalakshmi, P. T. Kalaichelven, A. kalaichelvan, "Biological synthesis of silver nanoparticle by using leaf extract of Justicia adhatoda," Scholar Research Library Der Pharmacia Letter, vol. 7(7), pp. 391-395 (2015).
[2] I.V. Nagati, R. Koyyati, P. Rudra, "Green synthesis of plant mediated silver nanoparticles using Withania somnifera leaf extract and evaluation of their antimicrobial activity," I.J.A.R., vol. (9), pp 307-313 (2013).ISSN: 2320-5407.
[3] P.A. Rashmi, R. John, L. Mathew, "Isolation and Characterization of Vasicine from in vitro cultures of *Justicia adhatoda,"* Int. Journal of Pharm and Bio Sciences, vol. 3, pp.58-64 (2012).
[4] S. R. Kuchekar, M. P. Patil, H. A. Aher and V. B. Gaikwad, "*Azadirachta Indica* Assisted Green Synthesis of Iron Oxide Nanoparticles," Science, Technology and Development, vol. 10(1), pp. 241-246 (2021)
[5] M. Pattanayak and P. L. Nayak,"Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of *Azadirachta Indica* (Neem)," World Journal of Nano Science and Technology, vol. 2(1), pp. 06-09 (2013).
[6] S. Dhankhar, R. Kaur, S. Ruhil, M. Balhara, A.K. Chhillar, "A Review on *Justicia adhatoda*: A potential source of natural medicine. African Journal of Plant Sciences," vol. 5(11), pp. 620-627 (2011).
[7] B. Pragati, R. Khilari1, V. Karale, D. Ingole, "Isolation and Characterization of *Endophytes* from *Justicia Adhatoda* as a Source of Novel Antibiotics," International Advanced Research Journal in Science, Engineering and Technology, vol. 7(2), pp. 18-22 (2020).
[8] S. R. Kuchekar, M. P. Patil, V. B. Gaikwad and Sung. H. Han, "Synthesis and Characterization of Silver Nanoparticles Using *Azadirchta Indica* (Neem) leaf extract," Int. J. of Engineering and Science Invention, vol. 6(9), pp. 66-70 (2017).
[9] M. Forough, K. Farhadi., "Biological and green synthesis of silver nanoparticle," Turkish Journal of Engineering and Environmental Sciences, vol. 34, pp. 281-287 (2010).
[10] R. P. Visweswara, TNVKV Prasad, A.S.Rayees, K.B.Satheesh, N. Ganapathi, S.R. Cirandur, "Biological synthesis of silver nanoparticles by using leaf extract of *Justicia adhatoda*," International Journal of Nanomedicine, vol.8, pp. 335-3364 (2013).
[11] G. Sivaperumal, K. Kulanthai, G. Sadhasivam, P. Pachiappan, S. Rajamani and D. Paramasivam, "Extraction, isolation, characterization, semi-synthesis and antiplasmodial activity of *Justicia adathoda* leaves," Bangladesh Journal of Pharmacology, vol. 11, pp. 878-885 (2016).
[12] G. Bhumi, M. Linga rao , N. Savithramma ,"Green synthesis of silver nanoparticles from the leaf extract of *adhatoda vasicanees* and assessment of its antibacterial activity," vol. 8(3), pp. 517-522, ISSN - 0974-1244 (2015).
[13] A. Karthikeyan, V. Shanthi, A. Nagasathya, "Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* (L)," Int. Journal of Green Pharm. vol. 3, pp. 78-80 (2009).