ANTIMICROBIAL ACTIVITY OF TRACHYSPERMUM AMMI LEAVES MEDIATED SILVER NANOPARTICLES : GREEN APPROACH

Sonam Sahu[1], Deepti Rawat[2], Darshan Singh[3*]

[1] *Student, Amity Institute of Applied Science, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India*
[2] *Assistant Professor, Department of Chemistry, Miranda House, University of Delhi, Delhi, India*
[3] *Assistant Professor, Department of Chemistry, Daulat Ram Collage, University of Delhi, Delhi, India*
***Corresponding Author. Tel.no. +91 9873428781, Email: darshnachem2004@yahoo.co.in (Darshan Singh)**

Abstract

This paper describes a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 3mM $AgNO_3$ solution through the extract of Trachyspermum ammi leaf using domestic microwave irradiation method. On mixing leaf extract with silver salt solution in ratio 1:4 the color changed from colourless to yellowish brown which partially confirmed the degradation of silver ions to silver nanoparticles (SNPs). The synthesized nanoparticles were characterized by using UV–visible spectroscopic techniques, Energy dispersive X-ray spectroscopy (EDAX), Scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR). The shape of the synthesized nanoparticles was found to be cubical. The antimicrobial activities of the synthesized silver nanoparticles against two human pathogens were evaluated by using disc diffusion and broth dilution method.

KEYWORDS: *Trachyspermum ammi Leaves, SNPs, MIC, SEM-EDAX, Green Synthesis*

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1. INTRODUCTION

Nanotechnology is arising as a new interdisciplinary field that connects the knowledge of biology, chemistry, physics, material science, engineering, pharmacology and medical [1]. The term nanotechnology can be defined as the creation, utilization and exploitation of materials at a nanoscale [2]. Nanoparticles exhibit completely new or improved properties than bulk material such as size, distribution and morphology. As the specific surface area of nanoparticles increases, the biological effectiveness also increases on the account of a rise in surface energy. These small size nanoparticles can modify the physiochemical properties of the materials, which can lead to adverse biological effect on living cells [3]. There are many types of NPs as shown in figure 1, out of which Inorganic Nanoparticles are easily available, rich in functionality, compatible, capable of targeted drug delivery and controlled release of drugs. These features make them capable of being used for cellular delivery [4]. Among all, Silver nanoparticles (SNPs) have received great attention due to their applications in wide areas such as biotechnology, packaging, electronics, medicine and coatings. Their strong antibacterial properties as well as low toxicity towards human cells has led to their application in various areas such as wound dressing, medicinal imaging, protective clothing, therapeutic compounds, antibacterial surfaces, water treatment, preservation of food and cosmetics to kill living bacterial and disinfecting agents [4]. The biomedical efficacy of silver nanoparticles (SNPs) depends only on the sensitivity of pathogen and size of nanoparticles. Smaller the size of the nanoparticle, greater will be the surface area and the better will be the antibacterial efficacy [5,6]. Literature survey has revealed that Silver nanoparticles can be used against many infectious organisms such as E.coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphilis typhus & Staphylococcus [3].

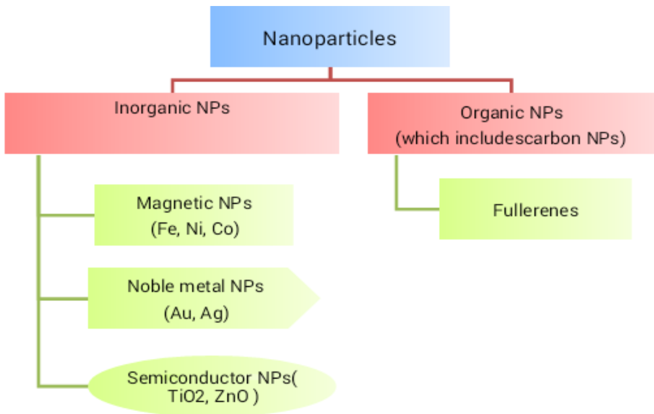

Figure 1: Types of Nanoparticles

There are various physical, chemical and biological methods to synthesize metal nanoparticles [7]. But "Green" chemistry methods have been successively used for sustainable production and development of NPs because these methods are eco-friendly, economical, can easily be utilized for mass-scale synthesis. Moreover, these methods do not require high temperature, high pressure, high energy and toxic chemicals unlike chemical methods. Also, the results obtained are relatively reproducible [8]. Plants, algae, fungi, bacteria and viruses have been utilized for the bio production of NPs in the last few years [9-17]. But the maintenance of cell culture is not easy. Therefore, production of nanoparticles from microbes has been replaced by plant extract because plant extract acts as both reducing and capping agents during the synthesis. They reduce metal ions faster than fungi or bacteria and there is a no need to maintain a cell culture [18]. Almost all parts of a plant are composed of antioxidants or sugars which are water soluble, and therefore can be utilized in the synthesis of NPs [19]. The rate of production of NPs, their yield and various other characteristics are controlled by the nature of the plant, concentration of the metal salt as well as plant extract, pH, temperature and contact time [20].

Trachyspermum ammi (L.) commonly called Ajwain, is an herbaceous herb belonging to the family Apiaceae. Its seeds are known to possess antiseptic, stimulant, diuretic, anesthetic, antimicrobial, antiviral, nematicidal, antiulcer, antihypertensive properties etc. Trachyspermum ammi leaves contains phytochemicals such as carbohydrates (24.6%), tannins, glycosides, moisture (8.9%), protein (17.1%), saponins, flavones and various other components (7.1%) involving metals which are responsible for reduction and stabilization of NPs [21].

The present paper reports the microwave assisted green synthesis of silver nanoparticles using Trachyspermum ammi leaf extract. It is a one pot synthesis of metallic NPs in solution which is more economical, consumes less energy and reaction time and gives better product yield [22]. Therefore, the study assumed that microwave assisted preparation of silver NPs using T.ammi would be useful than other methods which requires excessive heating, longer reaction time for studying antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Materials

Silver nitrate was procured from Merck, India and used as received. NaCl, NaOH, yeast and Tryptone was purchased from Spectrochem. All other reagents were of analytical grade. Domestic microwave was purchased from LG, India. Fresh leaves of Trachyspermum ammi were collected from Botanical garden of Daulat Ram College, India.

2.2. Preparation of Leaf Extract

Fresh Trachyspermum ammi leaves were collected from the botanical garden of Daulat Ram College, Delhi, India. The leaves were washed under running tap water for several times and later with distilled water. They were grinded in mortar with the help of a pestle and then the extract was made from the grinded paste by heating on a water bath for 15 minutes. It was then cooled and was filtered on a whattmann filter paper 44.

2.3. Biosynthesis of Silver Nanoparticles

A standard solution of 3mM silver nitrate was prepared. 5 ml of prepared Trachyspermum ammi leaves extract was added to the 20 ml standard solution of silver nitrate. The mixture was then microwaved for some time. A colour change was observed from colourless to yellowish brown solution. Initial formation of silver nanoparticles was determined by its colour change and then by recording the UV-Visible spectrum of each solution after a fixed interval of time.

2.4 Characterization

UV-Vis spectrophotometer (UV-2600 series Shimadzu spectrophotometer) was used to get the absorption peaks to confirm the synthesis of silver nanoparticles. FT-IR spectroscopy was used to recognize the functional groups that bound on the silver surface and involved in the formation of silver nanoparticles. Model-Perkin Elmer spectrophotometer FT-IR spectrum in the range 4000-400 cm^{-1} at a resolution of 4.0 cm^{-1} was used. Thin sample disc was prepared by pressing with the disc preparing machine. However the size and shape of particles were obtained by SEM (model-JEOL-5800-LV 16) with an accelerating voltage of 15 KW and a sample current of 41μA.

2.5 Antimicrobial Analysis

The synthesized silver nanoparticles from Trachyspermum ammi leaf extract was tested for their antimicrobial activity against pathogenic organisms like Escherichia coli and bacillus subtilis by using Broth dilution and disc diffusion method.

2.5.1 Broth Dilution Analysis

1000 µL of LB medium was taken in 6 test tubes. In the first test tube 950 µL of LB medium was added and then 50 µL of bacteria culture (i.e., Escherichia coli and bacillus subtilis), was mixed properly and 100 µL of a solution from 1st test tube was discarded. To the 1st test tube 100 µL of synthesized Ag NPs was added and again mixed it. After this, serial dilution was carried out to the last test tube.

2.5.2. Disc Diffusion Analysis

The silver nanoparticles synthesized using Trachyspermum ammi leave extract was tested for antimicrobial activity by disc diffusion method against Escherichia coli and bacillus substilis. Approximately 20ml of molten and cooled media (Lauria Bertini) was poured into sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth. 0.1ml from 10^{-8} dilution of different pathogenic bacteria suspension was spread on LB agar plates. Filter discs of 5 mm in diameter were impregnated with Trachyspermum ammi mediated silver particles and placed on the plates. The plates were then incubated at 37°C for 24 hrs and the zones of inhibition of silver nanoparticles and silver nitrate were measured.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of Silver Nanoparticles

Figure 2 shows the colour intensity of mixture of silver nitrate and Trachyspermum ammi leaf extract before and after microwave heating for 3 min. When standard silver nitrate solution was mixed with Trachyspermum ammi leaf extract in a particular ratio then no noticeable change in the color was observed. After microwave heating, the colorless mixture was changed to yellowish brown that indicated the formation of silver nanoparticles from bio-reduction of silver ions.

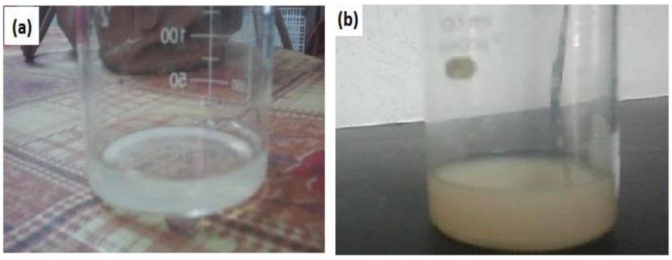

Figure 2: Mixture of Silver Salt and Trachyspermum ammi Leaf Extract (a) before heating. (b) after microwave Heating.

3.2. UV–Visible Spectrophotometer Analysis:

The formation and stability of the Trachyspermum ammi mediated silver particles was monitored by UV–Vis spectrophotometer analysis. Figure 3 shows the UV-Visible spectrum of synthesized silver nanoparticles which showed the peaks at 380 & 430 nm corresponding to the surface plasmon resonance (SPR) of silver nanoparticles.

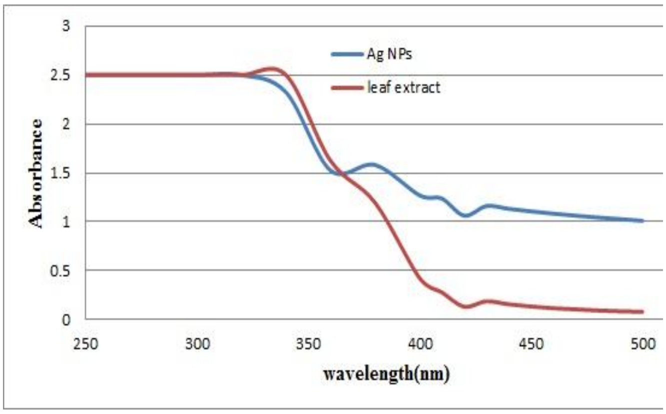

Figure 3: UV-Visible Spectrum of Synthesized Silver Nanoparticles

3.3. SEM-EDAX Analysis:

The formation of Trachyspermum ammi mediated silver nanoparticles as well as their morphological dimensions was studied by SEM analysis. It was found that the average size of synthesized nanoparticles are in the range of 45-100 nm and are cubical in shape as shown in Figure 4. EDAX of synthesized silver nanoparticles showed characteristic peak of silver as well as some constituents of plants extract as shown in Figure 5.

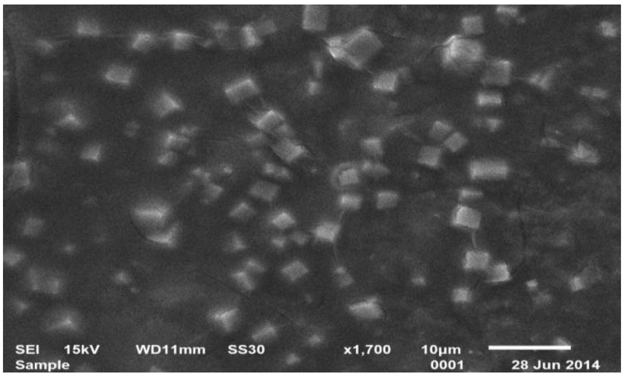

Figure 4: SEM Image of Synthesized Silver Nanoparticles

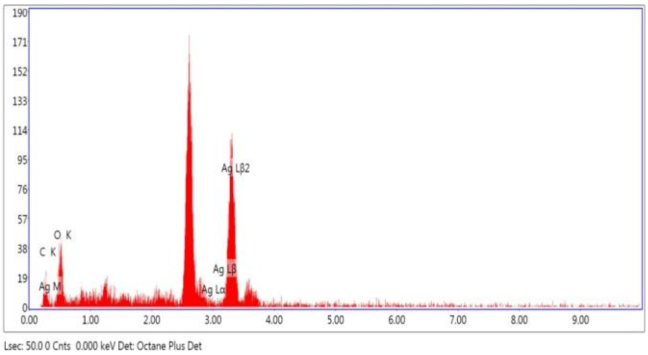

Figure 5: EDAX of Synthesized Silver Nanoparticles

3.4. FT-IR Analysis:

FT-IR was done to determine the functional groups responsible for reduction and molecular interaction(s) between the stabilizers present in the Trachyspermum ammi leaves and the synthesized NPs. Figure 6 shows the characteristic peaks at 3393.86 cm^{-1} due to the stretching of -OH group, at 2987.84 cm^{-1} due to –C-H stretching. The peak at 1401.75 cm^{-1} indicated the N-H stretching which confirmed the presence of amine group. The intensity of peaks decreased from the unreacted mixture of silver salt and leaf extract than the reacted mixture but the number of peaks are same which confirmed the reaction is taking place between the silver salt and leaf extract.

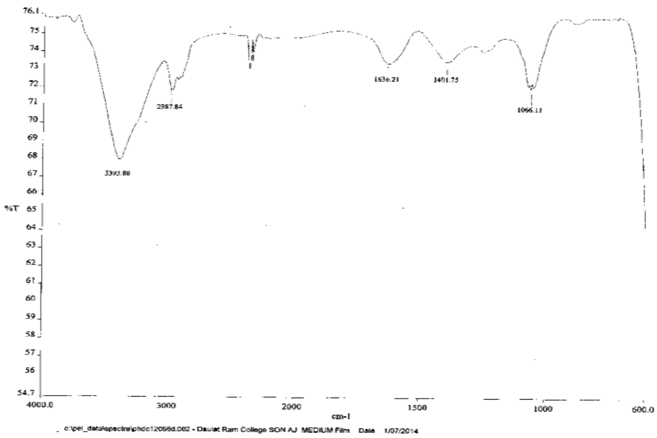

Figure 6: FT-IR Spectrum of Silver Nanoparticles synthesized from Trachyspermum ammi

3.5. Antimicrobial Study:

In this study, the application of silver nanoparticles as an antibacterial agent was investigated against both gram negative and gram positive bacteria. In disk diffusion method, Zones of inhibition for bacillus substilis and E.Coli were found to be 18 mm and 10 mm. Also from broth dilution method, minimum inhibitory concentration (MIC) was observed at 6.25µL/ml for bacillus substilis and at 100µL/ml for E. Coli. These results shows that silver nanoparticles synthesized using Trachyspermum ammi leaves showed maximum antibacterial activity against bacillus subtilis as compared to Escherichia coli bacteria. This indicates that E. coli is more resistive to NPs than B. subtilis was [23].

4. CONCLUSION

In this experiment, an attempt was made to synthesize bio inspired silver nanoparticles (SNPs) using leaf extract of Trachyspermum ammi leaves which acts as a reducing as well as capping agent. The rate of reduction for the synthesis of nanoparticles by this method was rapid, simple and without the use of any hazardous chemicals, etc. The synthesized SNPs were confirmed by colour transformation and UV-Visible Spectrophometric analysis. FT-IR spectra showed that the proteins existing in the plant extract are the biomolecules which are accountable for the capping and stabilization of the synthesized nanoparticles. SEM study revealed that the synthesized SNPs are cubical in shape with variation in their sizes. A peak of Ag in the EDAX data also confirmed the Ag NPs formation. Due to the non-uniformity in size, maximum antibacterial activity was found against bacillus subtilis as compared to Escherichia coli bacteria this indicates that E. coli is more resistive to NPs than B. subtilis was. So in conclusion, the SNPs manufactured using a plant extracts are much more proficient antibacterial agents as compared to chemically synthesized SNPs.

5. ACKNOWLEDGMENT

The authors are grateful to the Daulat Ram College, University of Delhi for carrying out experimental work. We express our sincere thanks to the director, USIC, University of Delhi for providing instrumentation techniques which is necessary for carrying out this research work.

REFERENCES

[1] Michał Moritz, Małgorzata Geszke-Moritz.The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. Chemical Engineering Journal 228 (2013) 596–613.

[2] K.S. Kavitha, Baker Syed, et al. Review Paper on Plants as Green Source towards Synthesis of Nanoparticles. Int. Res. J. Biological Sci. Vol. 2(6), June 2013, pp. 66-76.

[3] Nel A, Xia T, Madler L and Li N. Toxic potentials of materials at the nanolevel. *Science (2006)*311, 622-627.

[4] Xu Z P, Zeng Q P, Lu G Q and Yu A B (2006), "Inorganic Nanoparticles As Carriers For Efficient Cellular Delivery", Chemical Engineering Science, Vol. 61, pp. 1027-1040.

[5] Steven J.Oldenburg. Silver nanoparticles: properties and applications/ Material Science/ Nanomaterials (online). http://www.sigmaaldrich.com

[6] Miguel Jose Yacaman. Synthesis, Characterisation and Properties of Nanostructured Materials/Yacaman Research Group (online). http://www.cenava.cl/downloads/noticias/noticia6/charlas/full/Yacaman%202.pdf, 2009.

[7] Thomas Nesakumar Jebakumar Immanuel Edisonand Mathur Gopalakrishnan Sethuraman. Electrocatalytic Reduction of BenzylChloride by Green Synthesized silver Nanoparticles Using Pod Extract of Acacia nilotica. ACS Sustainable Chem. Eng. 1(2013), 1326-1332

[8]. Dhillon GS, Brar SK, Kaur S, Verma M. Green approach for nanoparticle biosynthesis by fungi: current trends and applications. Crit Rev Biotechnol (32) 2012, PP.49–73.

[9] Edison, T.J.L; Sethuraman, M. G. Instant green synthesis of silver nanoparticles using Terminalia chebula fruit extract and evalution of their catalytic activity on reduction of methylene blue. Process. Biochem. 47(2012) 1351-1357.

[10] Edison, T.J.L; Sethuraman, M. G. Biogenic robust synthesis of silver nanoparticles using Punica granatum peel and its application as a green catalyst for the reduction of an anthropogenic pollutant 4-nitrophenol. Spectrochim. Acta, Part A,104(1), 2013, 262−264.

[11] Merin,D.D.;Prakash,S.;Bhimba, B.V. Antibacterial screening of silver nanoparticles synthesized by marine micro algae. Asian. Pac. J. Trop.Med. 3(10), 2010, 797-799.

[12] Gajbhiye, M.; Kesharwani,J.; Ingle,A.; Gade, A.; Rai,M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed.: Nanotechnol .Bio. Med. 5(4) (2009), 382−386.

[13] Jaidev,L.R.; Narasimha,G. Fungal mediated biosynthesis of silver nanoparticles ,characterization and antimicrobial activity. Colloids and Surface.,B 81(2) 2010,430−433.

[14] Sadowski,Z.;Maliszewska,I.H.;Grochowalska,B.;Polowczyk ,I ;Kozlecki,T. Synthesis of silver nanoparticles using microorganisms.Mater.Sci−Pol.2008,26(2),419.

[15] Nadagouda,M.N.;Castle,A.B.;Murdock,R.C.;Hussain,S.M.; Varma,R.S. In vitro biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols. Green Chem.2010,12,114−122.

[16] Nadagouda,M.N.; Varma,R.S. Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chem.2008,10,859−862.

[17] Sankar Narayan Sinha and Dipak Paul. Eco-friendly Green Synthesis and Spectrophotometric Characterization of Silver Nanoparticles Synthesized using Some Common Indian Spices. IJGHC 3(2),2014, 401-408.

[18] Nameirakpam Nirjanta Devi, P.Dheeban shankar, et al. Antimicrobial efficacy of green synthesized silver nanopafrom the medicinal plant plectranthus amboinicus. International Journal of Pharmaceutical Sciences Review and Research. 12 (2012) 164-168.

[19] Mayur Valodkar, N. Ravirajsinh Jadeja et al. Biocompatible synthesis of peptide capped Cu NPs and their biological effect on tumor cell.Material Chem. and Physics 128(1),2011, 83-89.
[20] Dwivedi A D,Gopal K. Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract. Colloids Surf A 2010;(369)27–33.
[21] Mohammad M.Zarshenas et al. An overview on Ajwain (Trachyspermum ammi) Pharmacological effects; modern and Traditional. J. Natural Remedies 14(1) 2014, 98-105.
[22] Nadagouda,M.N.; Peth et al. Microwave assisted green thesis of silver structure.Acc.Chem Res 44(2011) 469-478.
[23] Ki-Young Yoon, Jeong Hoon Byeon, Jae-Hong Park, Jungho Hwang. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. Science of the Total Environment 373 (2007) 572–575.