ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Phyllanthus reticulatus mediated synthesis and characterization of silver nanoparticles and its antibacterial activity against gram positive and gram negative pathogens

Gomathi M[1], Prakasam A[*1], Rajkumar P.V[2], Rajeshkumar S[3], Chandrasekaran R[4], Kannan S[5]

[1]Research Department of Physics, Thiruvalluvar Government Arts College, Rasipuram-637401, Tamil Nadu, India
[2]Research Department of Physics, AVVM Sri Pushpam College (Autonomous), Poondi, Thanjavur-613 503, Tamil Nadu, India
[3]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-600077, Tamil Nadu, India
[4]Department of Zoology, Mizoram Central University, Aizawl, India
[5]Department of Electronics & Communication, Selvam Arts and Science College, Namakkal, Tamil Nadu, India

Article History:

Received on: 09.04.2019
Revised on: 15.07.2019
Accepted on: 20.07.2019

Keywords:

green synthesis,
optical,
structural,
antibacterial studies

ABSTRACT

In this study, silver nanoparticles were successfully synthesized by using *Phyllanthus reticulatus* leaf extract at different concentration (5, 10, 15 mL). The prepared samples were analyzed their optical, structural, surface morphological properties, and along with these properties, the antibacterial properties were analyzed. The UV-vis absorption spectra show SPR band around 450 nm, shift to lower wavelength due to increasing extract concentration. This shift can be ascribing to a decrease in particle size. The crystallinity nature of the prepared samples is observed by the XRD profile the calculated particles size by Scherrer's formula displays a decrease in size with respect to leaf extract volume. From the SEM image, it can be seen that irregular and large size particles prepared at 5-10 mL and the smooth spherical shape and tiny particles were observed in the sample prepared at 15 mL. The small and spherical shape nanoparticles show good antibacterial activity against gram positive and negative bacterial pathogens. The activity of large and irregular shape particles may be due to the release of Ag+ ions and generation of ROS. Because of cell wall difference in bacteria, the better activity is observed against gram negative bacteria.

*Corresponding Author

Name: Prakasam A
Phone: +919944501455
Email: aptgac@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i4.1603

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

In recent times, silver nanoparticles (Ag NPs) have been extensively studied by their distinct physicochemical properties (Sunar *et al.*, 2019; Aafreen *et al.*, 2019; Vignesh *et al.*, 2019; Das *et al.*, 2019) and are widely used in different fields such as biosensors (Anjum *et al.*, 2019), optical devices (Pranati *et al.*, 2019), catalytic and antibacterial applications (Chellakannu *et al.*, 2019; Santhoshkumar *et al.*, 2019). Verities of physical and chemical methods such as laser-ablation (Kumar *et al.*, 2018), chemical-reduction method (Agarwal *et al.*, 2018a; Shanmugam, 2017), electrochemical (Rajeshkumar and Bharath, 2017) and thermal decomposition (Rajeshkumar and Naik, 2018) have been employed for the synthesis of AgNPs. However, most of these methods are expensive and use toxic chemicals during synthesis, which are significantly hazardous to the environment. Viet et al. reported that the chemical methods use different kinds of chemical for the synthesis of AgNPs and face difficulties to control the size and the morphology of the particles (Agarwal *et al.*, 2018b).

In this sense, the biological method (green synthesis) is developed for the synthesis of metal nanoparticles and is a successful alternative to the physical and chemical methods. It is simple, low-cost, no toxic chemicals and eco-friendly method. This method offers several advantages over the other methods for the synthesis of AgNPs. Many works already reported that leaf extracts were used for the synthesis of Ag NPs such as *Azadirachta indica* (Ahmed *et al.*, 2016), *Justicia glauca* (Emmanuel *et al.*, 2015), *Sesbania grandiflora* (Das *et al.*, 2013) , *M. oleifera* (Nayak *et al.*, 2015) , *Acalypha indica* (Krishnaraj *et al.*, 2010) .

In the present study, silver nanoparticles are successfully synthesized using *Phyllanthus reticulatus* leaf extract as a reducing agent. The impact of extract concentration on structural, optical, and surface morphological properties of AgNPs are studied and reported. In addition, the antibacterial potential of synthesized AgNPs is examined against (*S. aureu)* and gram negative strains (*E. coli*) using the disc diffusion method. The synthesized AgNPs can be applied for antibacterial applications.

MATERIALS AND METHODS

Preparation of leaf extract

Phyllanthus reticulates leaves were collected from Ananmalai University, South India. Initially, the collected fresh leaves were washed carefully with tape water for several times, and then, the leaves further cleaned through de-ionized water to remove the dust and to avoid the contamination if any present on the surface of the leaf. The perfectly cleaned leaves were sliced as tiny pieces. The 5 gm of sliced leaves was boiled for 10 min in 100 mL deionized water and then filtered using Whatman No.1 filter paper. The filtered extract was keeping at 4 ºC by the refrigerator. This leaf extract plays an important role as a reducing agent in the green synthesis for the preparation of AgNPs.

Green Synthesis of Ag Ps

The 0.1 M of AgNO3 was dissolved homogenously in 40 mL deionized water. The leaf extract was introduced drop by drop into the AgNO3 solution and at the same time, the reaction mixture was stirred at 60 ºC for 5 min. The reaction mixture colour is turned rapidly to dark brown, which indicates the reduction of Ag and the reduction process is saturated within 10 min. The brown colloidal suspension was filtered and then dried at 200 ºC in a muffle furnace.

Characterization of Synthesized of Ag Ps

The structural, optical, surface morphological properties and FTIR analysis of synthesized Ag NPs were studied.

Antibacterial activity of Synthesized of Ag N s

The antibacterial activity of synthesized Ag NPs was studied against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative bacteria) using the disc diffusion method. For the evaluation of antibacterial activity, terilized petriplates were filled with fresh Muller Hinton agar medium and then allowed to solidify the agar medium using a laminar airflow chamber. After that, the bacterial culture was spread over the plate, and five 6 mm discs were placed over the agar medium. Two discs were assigned for control and streptomycin (standard). Three discs were silver nanoparticles loaded with 200 μL stack solution. Finally, the petriplates were incubated at 36 ºC for 24 hrs. The zone of inhibition around the disc was measured; the inhibition zone around the disc indicates the antibacterial activity of synthesized AgNPs. The Streptomycin and solution absence of Ag NPs stack solution were used as a standard antibiotic as well as control.

RESULTS AND DISCUSSION

Optical studies

UV-visible absorption spectra of AgNPs, synthesized from *Phyllanthus reticulatus* leaf extract of various concentrations (5, 10 and 15 mL), are shown in Figure 1. The optical analysis is the simplest way to discover the development of Ag NPs from an aqueous mixture of AgNO3 and leaf extract. The colorless aqueous AgNO3 was turned into dark brown when *Phyllanthus reticulatus* leaf extract was added into the solution mixture. The change in mixture color indicates that the formation of AgNPs. The absorption spectra show peak around 450 nm, indicating Surface Plasmon Resonance (SPR) between electron clouds from AgNPs surface and incident light photons. The absorption peak occurs at 451 nm when the addition of 5 mL leaf extract, while for 10 mL leaf extract, the absorption peak shifts to 450 nm and a further increase in the leaf extract to 15 mL, it shows a significant change in the occurrence of absorption

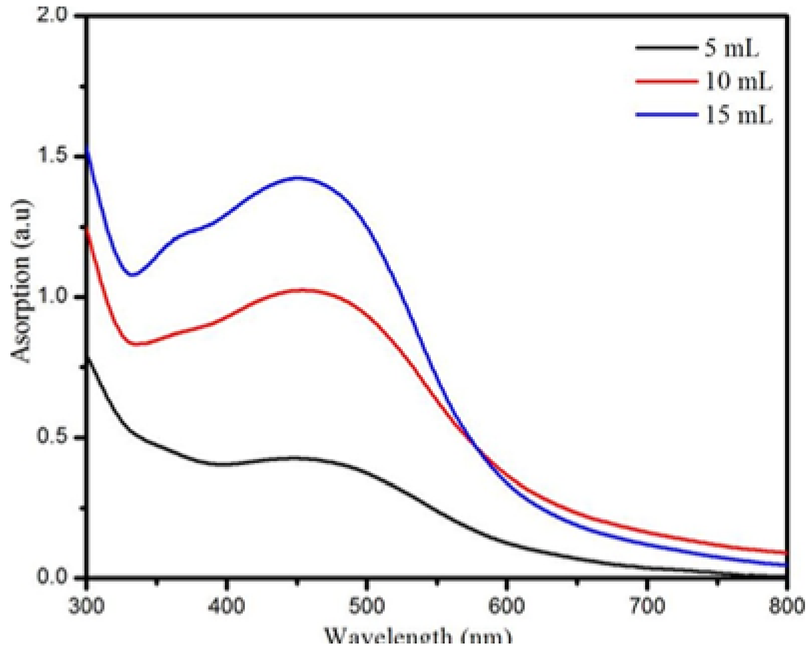

Figure 1: UV- absorption spectra of Ag

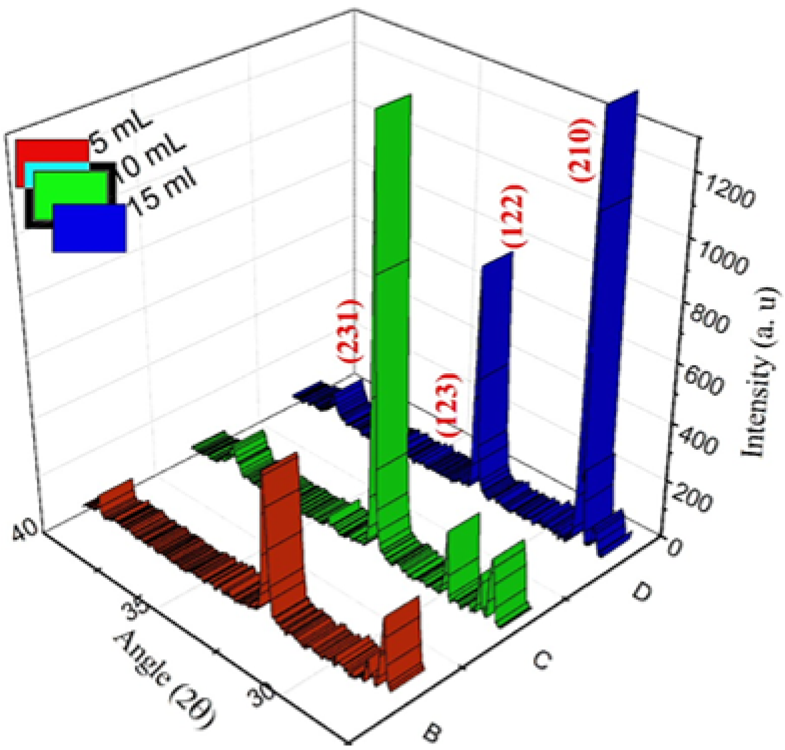

Figure 2: XRD pattern of Ag NPs

peak (at 448 nm) in the spectra.

The similar pattern is also observed for various researchers in different leaf extract of different concentration since the obtained results are well agreed with previous reports (Kathiravan, 2018; Gomathi *et al.*, 2018). The variation in the occurrence of absorption peak plays a vital role in the size of Ag NPs. In this work, the absorption peak shifts towards shorter wavelength while increasing the extract concentration, due to the reduction of particle size in accordance with the concentration of possible biological reducing agents in the *Phyllanthus reticulatus* leaf extract. Moreover, along with the peak shift, the intensities of the peak increase with the increase in extract concentration which can be attributed to the scale of production of Ag NPs increasing.

Structural studies

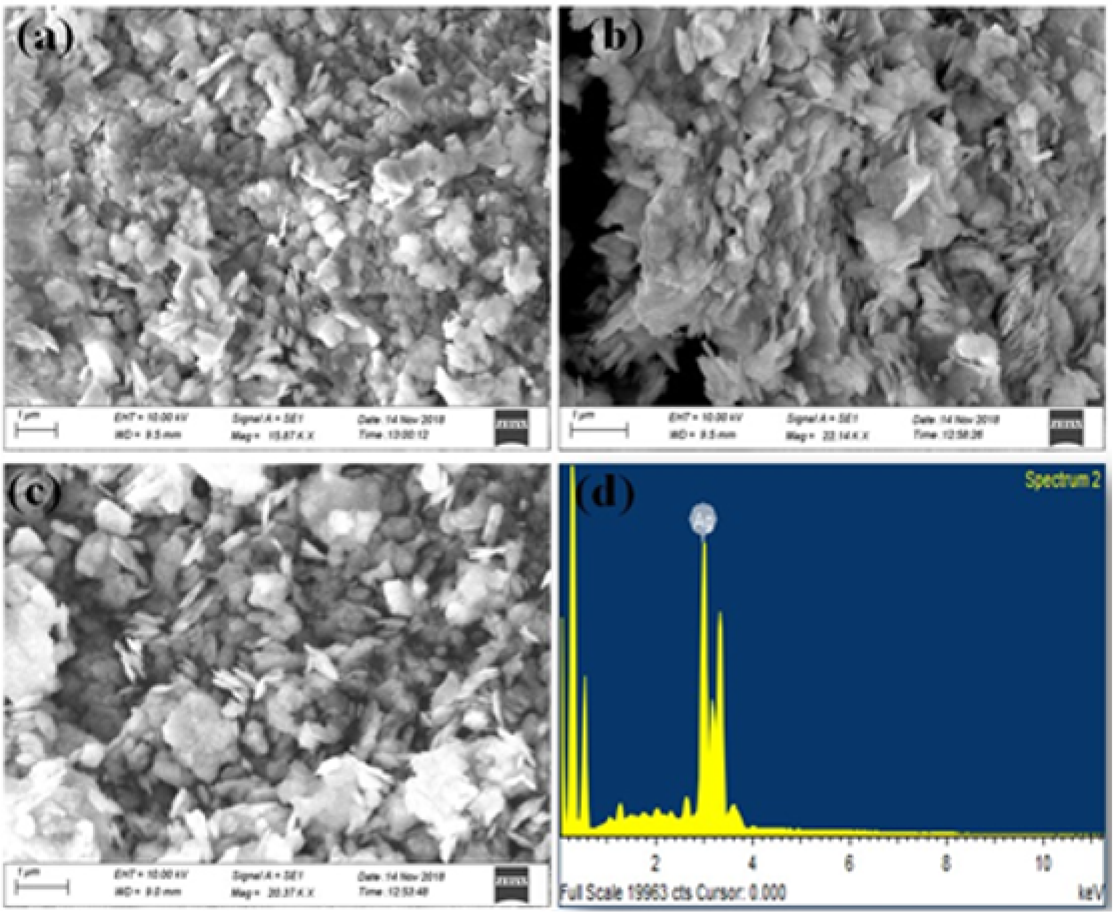

Figure 3: (a-c) SEM images of Ag NPs, (d) EDX spectrum of Ag NPs

Figure 2 shows the XRD pattern of green synthesized Ag NPs, *Phyllanthus reticulatus* leaf extract used as a reducing agent. From the spectra, it can be clearly observed that the diffraction.

peaks at 27.72 °, 32.17 °, 39. 22 ° and 46.17 ° are related to (210), (122), (123) and (231) planes of the silver crystal. It is strongly suggested that the synthesized NPs are mixed phases (cubic, face-centred cubic) of Ag NPs. The absence of peaks related to other Ag compound was observed, indicating the phase purity of the synthesis Ag NPs. Similarly, the same XRD patterns were observed for green synthesized Ag NPs using *Ocimum canum* leaf extract by (Jayaseelan and Rahuman, 2012). Suman et al. reported that the biphasic nature Ag NPs was obtained when the *Morinda citrifolia* root extract introduced as reducing agent (Dipankar and Murugan, 2012). Dipankar et al. reported that the diffraction angle (2θ) at 27.72 ° is belonged to the face-centred cubic structure of AgNPs (Suman *et al.*, 2013).

At 5 mL leaf extract, the Ag NPs are predominantly grown through (1 2 2) plane and also grown along (2 1 0), (1 2 3) and (2 3 1) planes. The similar grown pattern is also observed for 10 mL leaf extract, but the full-width half maximum (FWHM) of (1 2 2) is slightly decreased than the previous case. While increase leaf extract to 15 mL, the crystallites turn its growth predominantly to (2 1 0) planes. In addition, FWHM of (210) plane considerably reduced and other regular planes retained its growth even though the change in a preferred orientation. The reduction in FWHM of dominant peak obviously represents a decrease in particle size, which can powerfully support the results (decrease in particle size) observed in the optical studies.

The average particle size of AgNPs is calculated using Scherrer's formula from FWHM of predominant peaks.

D = 0.94λ/β cosθ

Where λ is the wavelength of XRD (1.5406 Å) used in this study, β is FWHM of predominant peak and θ is Bragg's diffraction angle. The average particle size is 31 nm for 5 mL leaf extract.

The value of particle size decreased to 27 nm for 10 mL of leaf extract. The introduction of leaf extract into the solution mixture further increased to 15 mL, as the result particle size drastically decreased to 22 nm. This decrease in particle size can be

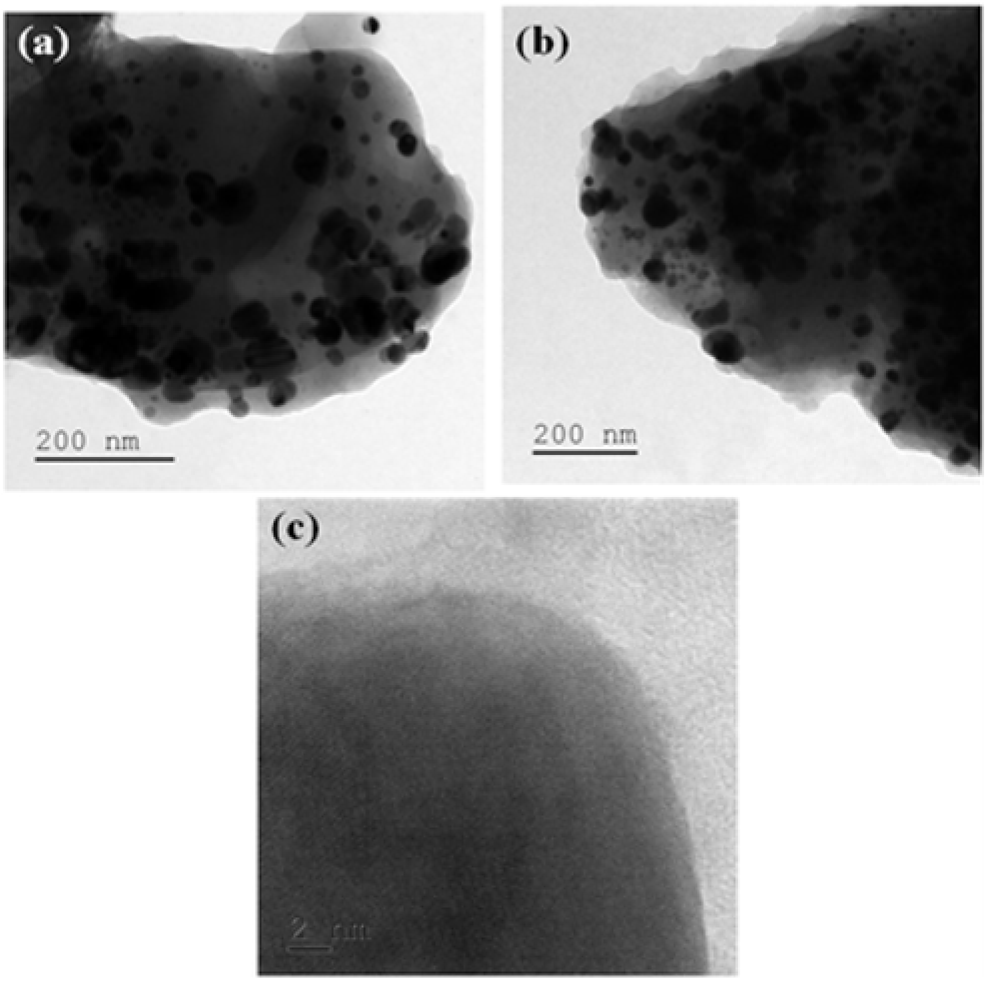

Figure 4: TEM images of Ag NPs

ascribed to Zener pinning effect; the rapid growth of particle having large defects gives the way to stop the periodic extension of crystallite growth as large size (Rajkumar *et al.*, 2015) .

Surface morphological studies

SEM images of 5, 10 and 15 mL leaf extract synthesized AgNPs are shown in Figure 3 a, Figure 3 b and Figure 3 c, respectively. The surface morphology of AgNPs is obviously illustrated from the SEM images. In the case of 5 mL extract a synthesized sample, most of the particles are undefined shape, because of the combination of number particles and only a few of them is roughly spherical shape AgNPs. There is no significant changes observed in throughout SEM image of 10 mL extract synthesized sample. But, entirely different morphology is observed by the sample synthesized from 15 mL leaf extract and the image contains smooth and spherical shape Ag NPs. Majority of the image are having small size Ag NPs. However, we can see large size particle which can be attributed to aggregation of a large number of smallest once. The EDX image was recorded to affirm the formation of Ag NPs. The EDX image is given in (Figure 3 d). From the image, it is noticed that the strong energy peaks are emerged and associated to the anticipated element Ag.

Figure 4 shows TEM images of green synthesized AgNPs. TEM analysis clearly illustrates the size and shape of AgNPs. It exposes that Ag NPs are well segregated and mainly spherical in shape. However, a few of them is found to be irregular in shape. From the TEM image, it can conclude that the AgNPs are in nano-size, roughly spherical in shape with an average diameter of 30-20 nm.

FT-IR analysis

Figure 5 shows the FT-IR spectrum of synthesized AgNPs. The spectrum was recorded in the range of 400- 4000 cm-1 to find the possible bio-molecules dependable for the reduction and stabilization of AgNPs from *hyllanthus reticulatus* leaf extract. The bands at 1111 cm-1, 1046 cm-1, 1626 cm-1, 2923 cm-1, 2848 cm-1, 3430 cm-1 were observed from the spectrum. The spectrum exhibits a strong band at 3430 cm-1, which can be due to –OH stretching vibrations representing the presence of

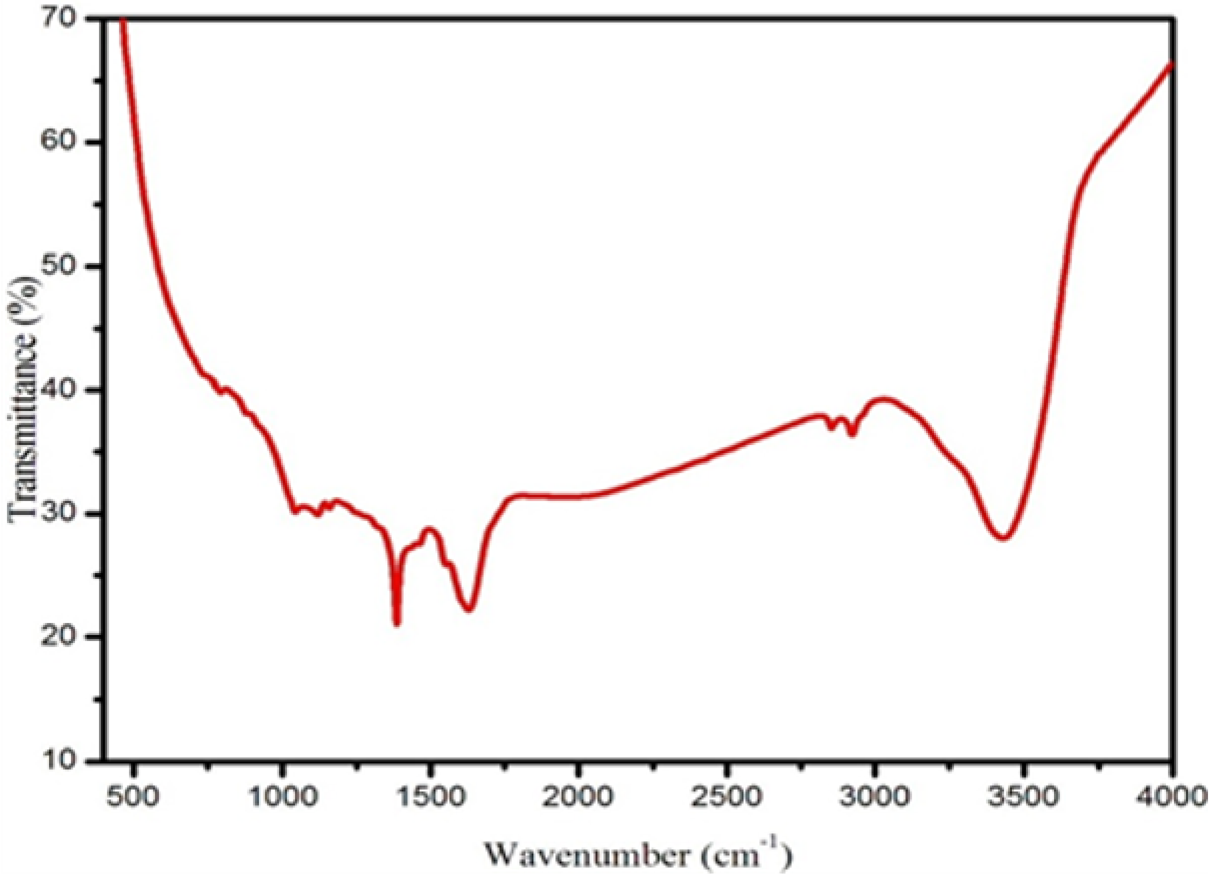

Figure 5: FTIR spectrum of Ag NPs

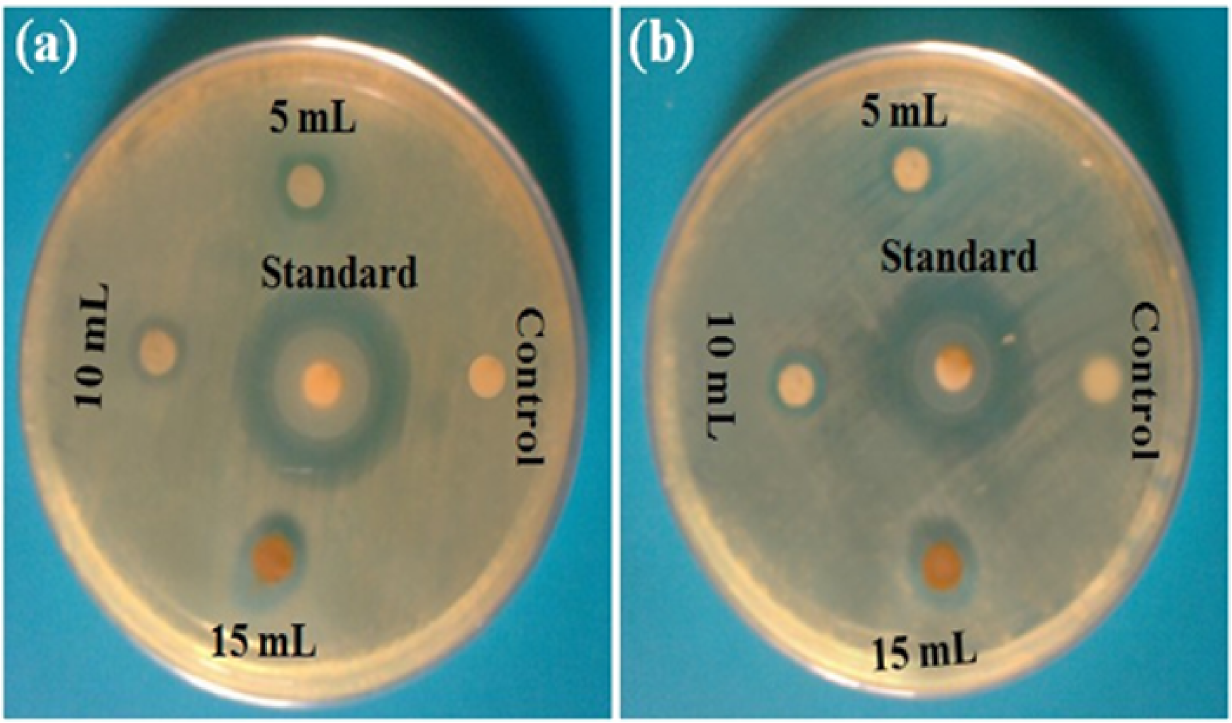

Figure 6: Antibacterial activity of synthesized Ag NPs

hydroxyl groups as phenols and H-bonded alcohols. The strong band at 1626 cm−1 is mainly attributed to the secondary amine, N-H stretching vibrations of proteins. The stretching peak located at 1389 cm-1 is ascribed to stretching vibration of C–H rock alkenes. The small bands at 1111 cm-1 and 1046 cm-1 specify the presence of carboxylic acids, C–O stretching alcohols and esters. The small intense peaks at 2923 cm−1and 2848 cm−1 can be attributed to the presence of C–H stretching vibrations and secondary amines. Hence, the FT-IR spectrum of AgNPs exhibits the absorption peaks related to the presence of amine, hydroxyl group and proteins. These compounds work as a reducing agent to the formation of Ag NPs in green synthesis.

Antibacterial studies

The antibacterial efficacy of *Phyllanthus reticulatus* leaf extract synthesized AgNPs were examined against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative bacteria) using the disc diffusion technique. The photographic image of the zone of inhibition is shown in Figure 6. The control disc doesn't show any inhibition zone around the disc in all cases. The synthesized AgNPs resists the growth of the bacteria around the disc, which clearly shows synthesized AgNPs possess antibacterial activity against both gram positive and gram negative bacteria.

Ag NPs synthesized at a higher extract concentration of leaf extract holds better inhibition zone than the others, since the antibacterial activity strongly depends on the size and shape of the particles. At lower extract concentrations (5-10 mL), asymmetry in shape and large size particles are grown, which have less surface area. The spherically symmetry and small size particles are got the highly interacting surface, which makes contact with more bacteria. Naturally, nanoscale particle quickly attached to the cell membrane and significantly distort its regular task such as permeability and respiration.

Ag NPs are the source of slow-release of Ag+ ions, which also interact with phosphorus-containing compounds like DNA as well as sulfur-containing protein in the cell wall membrane. These are the main factors for the antibacterial activity of asymmetry shape and large size NPs. Better, antibacterial activity is observed on gram negative bacteria, the thick cell wall membrane of gram positive bacteria made up of peptidoglycans resists the enter of NPs into the bacteria. While gram-negative bacterial are got thin cell wall membrane, made up of peptidoglycans and lipopolysaccharides, which includes sulfur-containing protein. This protein interacts with Ag NPs and directly affects the respiration system.

Addition to this, the generation of reactive oxygen species (ROS) such as superoxide anions (O -), hydroxyl radicals (OH) and hydrogen peroxide (H O) inhibit the growth of bacteria. These ROS can easily penetrate into the cell wall, damaging bacteria severely and eventually died.

CONCLUSION

In this work, silver nanoparticles have been successfully synthesized from *Phyllanthus reticulatus* leaf extract at different concentration (5, 10, 15 mL). From the absorption spectra, it. Can see that the SPR band shifts to lower wavelength when the extract concentration increases, which can be a result of a decrease in particle size. The XRD studies show the good crystalline nature of synthesized Ag NPs. SEM image exhibits small and spherical shape Ag NPs at 15 mL leaf extract. EDX spectrum confirms that the synthesized sample is Ag NPs. The synthesized samples are having good antibacterial action against disease, causing bacterial pathogens.

REFERENCES

Aafreen, M. M., Anitha, R., Preethi, R. C., Rajeshkumar, S., Lakshmi, T. 2019. Anti-Inflammatory Activity of Silver Nanoparticles Prepared from Ginger Oil-An Invitro Approach. *Indian Journal of Public Health Research & Development*, 10(7):145–145.

Agarwal, H., Kumar, S. V., Rajeshkumar, S. 2018a. Antidiabetic effect of silver nanoparticles synthesized using lemongrass (cymbopogon citratus) through conventional heating and microwave irradiation approach. *Journal of Microbiology Biotechnology and Food Sciences*, 7(3):371–376.

Agarwal, H., Menon, S., Kumar, S. V., Rajeshkumar, S. 2018b. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. *Chemico-Biological Interactions*, 286:60–70.

Ahmed, S., Saifullah, S. A., Swami, M., Ikram, B. L. 2016. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of Radiation Research and Applied Sciences*, 9(1):1–7.

Anjum, A. S., Anitha, R., Rajeshkumar, S., Lakshmi, T. 2019. Synthesis of White Pepper Oleoresin Mediated Silver Nanoparticles and its Antioxidant Effect Biomedicine. 31(1):165–169.

Chellakannu, M., Panneerselvam, T., Rajeshkumar, S. 2019. Kinetic study on the herbal synthesis of silver nanoparticles and its antioxidant and antibacterial effect against gastrointestinal pathogens. *International Journal of Research in Pharmaceutical Sciences*, 10(1):407–414.

Das, A., Roy, A., Rajeshkumar, S., Lakshmi, T. 2019. Anti-inflammatory Activity of Turmeric Oil Mediated Silver Nanoparticles. *Research Journal of Pharmacy and Technology*, 12(7):3507–3510.

Das, J., Das, M. P., Velusamy, P. 2013. Sesbania grandiflora leaf extract mediated green synthesis of antibacterial silver nanoparticles against selected human pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 104:265–270.

Dipankar, C., Murugan, S. 2012. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. *Colloids and Surfaces B: Biointerfaces*, 98:112–119.

Emmanuel, R., Palanisamy, S., Chen, S. M., Chelladurai, K., Padmavathy, S., Saravanan, M., Al-Hemaid, F. M. A. 2015. Antimicrobial efficacy of green synthesized drug blended silver nanoparticles against dental caries and periodontal disease causing microorganisms. *Materials Science and Engineering: C*, 56:374–379.

Gomathi, M., Rajkumar, P. V., Prakasam, A. 2018. Study of dislocation density (defects such as Ag vacancies and interstitials) of silver nanoparticles, green-synthesized using Barleria cristata leaf extract and the impact of defects on the antibacterial activity. *Results in Physics*, 10:858–864.

Jayaseelan, C., Rahuman, A. A. 2012. Acaricidal efficacy of synthesized silver nanoparticles using aqueous leaf extract of Ocimum canum against Hyalomma anatolicum anatolicum and Hyalomma marginatum isaaci (Acari: Ixodidae). *Parasitology Research*, 111(3):1369–1378.

Kathiravan, V. 2018. Green synthesis of silver nanoparticles using different volumes of Trichodesma indicum leaf extract and their antibacterial and photocatalytic activities. *Research on Chemical Intermediates*, 44(9):4999–5012.

Krishnaraj, C., Jagan, E. G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. T., Mohan, N. 2010. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B: Biointerfaces*, 76(1):50–56.

Kumar, S. V., Karpagambigai, S., Rosy, P. J., Rajeshkumar, S. 2018. Controlling of disease causing pathogens using silver nanoparticles synthesized by one step green procedure. *Journal of Applied Pharmaceutical Science*, 8(1):142–146.

Nayak, D., Pradhan, S., Ashe, S., Rauta, P. R., Nayak, B. 2015. Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. *Journal of Colloid and Interface Science*, 457:329–338.

Pranati, T., Anitha, R., Rajeshkumar, S., Lakshmi, T. 2019. Preparation of Silver nanoparticles using Nutmeg oleoresin and its Antimicrobial activity against Oral pathogens. *Research Journal of Pharmacy and Technology*, 12(6):2799–2803.

Rajeshkumar, S., Bharath, L. V. 2017. Mechanism of plant-mediated synthesis of silver nanoparticles - A review on biomolecules involved, characterisation and antibacterial activity. *Chemico-Biological Interactions*, 273:219–227.

Rajeshkumar, S., Naik, P. 2018. Synthesis and biomedical applications of Cerium oxide nanoparticles - A Review. *Biotechnology Reports*, 17:1–5.

Rajkumar, P. V., Ravichandran, K., Baneto, M., Ravidhas, C., Sakthivel, B., Dineshbabu, N. 2015. Enhancement of optical and electrical properties of SILAR deposited ZnO thin films through fluorine doping and vacuum annealing for photovoltaic applications. *Materials Science in Semiconductor Processing*, 35:189–196.

Santhoshkumar, J., Sowmya, B., Kumar, S. V., Rajeshkumar, S. 2019. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of Passiflora caerulea. *South African Journal of Chemical Engineering*, 29:17–23.

Shanmugam, R. 2017. Antioxidant activity of characterized silver nanoparticles synthesized using flower extracts of Chrysanthemum indicum. *Research Journal of Biotechnology*, 12(8):38–43.

Suman, T. Y., Rajasree, S. R. R., Kanchana, A., Elizabeth, S. B. 2013. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using Morinda citrifolia root extract. *Colloids and Surfaces B: Biointerfaces*, 106:74–78.

Sunar, S., Rajeshkumar, S., Roy, A., Lakshmi, T. 2019. Preparation of herbal formulation and it's application on nanoparticles synthesis and antibacterial activity. *International Journal of Research in Pharmaceutical Sciences*, 10(3):2177–2180.

Vignesh, S., Roy, A., Rajeshkumar, S., Lakshmi, T. 2019. Evaluation of the Antimicrobial activity of Cumin oil mediated silver nanoparticles on Oral microbes Research. *J. Pharm. and Tech*, 12(8):3709–3712.

