ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Antimicrobial Study of Green Synthesized Silver Nanoparticles (AgNPs) by Using *Ageratina adenophora* and its Characterization

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Article Information

Received: 24 March 2021
Revised version received: 13 June 2021
Accepted: 16 June 2021
Published: 28 June 2021

Cite this article as:
S.K. Gautam et al. (2021) Int. J. Appl. Sci. Biotechnol. Vol 9(2): 128-132. DOI: 10.3126/ijasbt.v9i2.37822

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Peer reviewed under authority of IJASBT

Abstract

Green chemistry refers to the design of chemical product and processes that reduce or eliminate the generation of hazardous substances. Silver nanoparticles (AgNPs) were synthesized successfully from $AgNO_3$ through a simple green synthetic route using *Ageratina adenophora* leaf extract which acts as both reducing and capping agents. As synthesized AgNPs were characterized with the help of X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. XRD study shows crystalline nature of silver nanoparticles and average particle size was calculated as 24 nm using Debye Scherrer equation. Functional group responsible for the reduction of silver ion was investigated using FTIR spectroscopy. Hydroxyl group, amine group, aliphatic amine group were detected from FTIR analysis. Further, the green synthesized nanoparticles were found to be highly toxic against bacteria: *Bacillus subtilis* and *Escherichia coli* showing zone of inhibition of 11 mm and 9 mm, respectively.

Keywords: AgNPs; Green synthesis; X-ray diffraction (XRD); Fourier Transform Infra-Red (FTIR) Spectroscopy; Antimicrobial activity

Introduction

Green chemistry deals with the creation and development of functional material that are environmentally friendly and non-toxic in nature. AgNps have attracted intensive research interest because of their advantageous application in biomedical, drug delivery, agriculture, textile industry, water treatment, catalysis and as antifungal and antimicrobial agent. Silver, a white soft lustrous metal of Group 11 (IB) and period 5 of the periodic table is valued for its decorative beauty (Nurani *et al.*, 2015).

Ageratina adenophora belong to the Asteraceae family which was introduced from around 1950s in Nepal (Balami and Thapa, 2017). It is a perennial herbaceous plant or small short-stemmed shrub that usually grows up to 1-2 m tall which is originally native to Mexico. Locally it is known as "kalo banmara" and common name as croften wood. In Nepal, *Ageratina adenophora* is found in Terai, Hills and low mountain regions of Central, Eastern and Western Regions (Shrestha, 2016).

There are different types of approaches for synthesizing nanoparticles but biological method is most preferable over other approaches due to non- toxic, low cost and environment friendly nature. Biological methods involve the synthesis of nanoparticles using plant extract and microorganisms such as bacteria and fungi. Phyto-nanotechnology has shown the new field for the synthesis of nanoparticles using different parts of plant like leaf, stem, root and fruit. Extract prepared from plant parts with universal solvent (Water), acts both as reducing and capping agents in the synthesis of nanoparticles. The reducing or capping agent used in chemical and physical methods are expensive, hazardous and highly toxic whereas in biological methods, the plant extract itself acts as capping agent (Ijaz *et al.*, 2020).

In this research work, we have demonstrated biosynthesis of AgNps by reduction of aqueous silver nitrate with aqueous leaf extract of *Ageratina adenophora*.

This article utilizes green chemistry principles in nanotechnology where there is reduction of use and generation of hazardous chemicals. This field of chemistry is environment friendly and sustainable and therefore, there is a growing demand of green chemistry methods.

Fig. 1: Leaves of *Ageratina adenophora* plant.

Materials and Methods

Synthesis of Silver Nanoparticles

In this work, the green synthesis of AgNPs was carried out using $AgNO_3$ solution and leaves extract of *Ageratina adenophora*. 1.69 g of $AgNO_3$ in 250 mL of distilled water was stirred at room temperature for about 5 mins. Then, 50 mL of leaves extract solution was poured dropwise from the burette. The $AgNO_3$ solution was placed above the magnetic stirrer for homogenous mixing of the components and at the same time titration was carried out using leaves extract at the room temperature. Initially, the solution was transparent but after the completion of titration the colour was converted into brown. Then the stirred solution was kept in dry place for eighteen hours and was filtered out twice using Whatmann filter paper no. 41. Greenish black coloured substance was obtained which was dried in an oven. It was then powdered using a glass rod was stored in the preservative vials. The reaction involved during this process is given as

$$AgNO_3 \leftrightarrow Ag^+ + NO_3^-$$

$$Ag^+ + e^- \rightarrow Ag$$

Characterization Techniques

Synthesized AgNPs were characterized using X-ray diffraction (XRD) analysis and Fourier transform infrared (FTIR) spectroscopy to determine the average particles size and to identify the functional group responsible for the reduction of silver ion.

The crystalline structure and average size of the powdered sample were determined by the XRD patterns obtained employing CuKα radiation (λ= 0.15406 nm) for 2θ values ranging from 20^0 to 80^0 in X-ray diffractometer (Rigaku ultima IV model).

The average particle size, D can be estimated from the peak broadening of XRD pattern using Debye Scherrer's equation (Gautam *et al*., 2008; Regmi *et al*., 2019).

$$\text{Crystal size, } D = \frac{\kappa\lambda}{\beta Cos\theta}$$

Where, κ = Scherrer's constant (0.94)

λ = wavelength of X- rays (0.15406 nm)

β = full width at half maximum intensity (FWHM)

θ = diffraction angle of each peak.

The functional groups present in the sample were detected/analyzed using FTIR (IRTracer-100, SHIMADZU) spectroscopy in a range of 4500-500 cm^{-1}.

Antimicrobial Activity of AgNPs

Bacillus subtilis (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) are used to investigate the antimicrobial activity of powdered samples. The investigation was carried out in the lab of Polytechnical Research Institute Nepal (PORIN), Kathmandu.

38 g of nutrient agar (Muller Hinton Agar) was dissolved in 1 litre of water and was autoclaved at 125°C for 5 minutes. It was then transferred and cooled down to about 37°C. After lowering the temperature, the media was transferred to Petridis at the amount of 25 mL. The prepared media plate was then stored at refrigerator until its use.

At the beginning, the bacterial strains of ATCC 9637 *Escherichia coli* (Gram negative) and *Bacillus subtilis* ATCC 6051 (Gram positive) were cultured in the liquid nutrient media for 24 hrs. Then 100 uL culture broth of each strain was plated on the nutrient agar plate for 15 min at 37°C. After incubation, 10 uL samples was piped and dispensed on filter paper disc kept on the nutrient agar plate and incubated overnight at 37°C.

Results and Discussion

i. X-ray Diffraction (XRD) Analysis

The peaks obtained were analysed and their 2θ values were noted for indexing the peaks by using origin software. There are peaks appearing at 2θ values of 38.6°, 44.6°, 64.7° and 77.4° corresponding to the hkl planes (111), (200), (220) and (311), respectively which is shown in Fig. 2. Besides, no extra peaks of impurity are detected, indicating the synthesized AgNPs are pure and crystalline in nature. The structure obtained from XRD peaks of the powdered sample was found to be face centred cubic phase (JCPDS file no. 04-0783) (Mehta *et al.*, 2017). Lorentzian profile was fitted to calculate the particle size of silver nanoparticles as displayed in Fig. 3. The average crystalline size of AgNPs was calculated from the intense peak using Debye Scherrer equation and was found to be 24 nm.

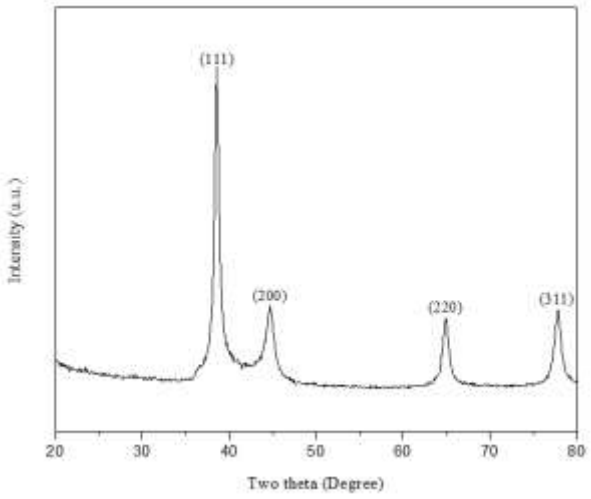

Fig. 2: XRD pattern of AgNPs synthesized using *Ageratina adenophora* leaf extract.

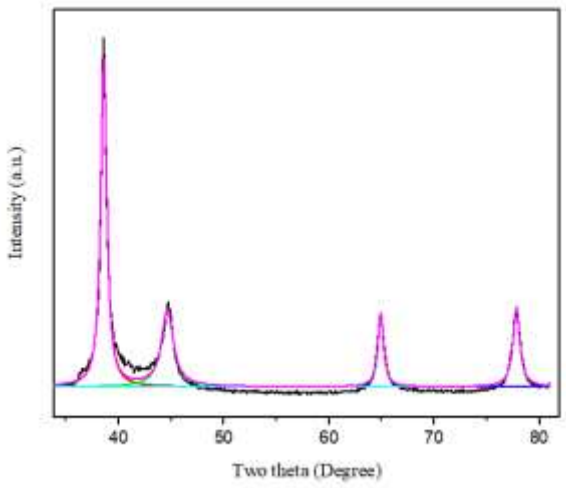

Fig. 3: Lorentzian fitting of XRD pattern of AgNPs synthesized using *Ageratina adenophora* leaf extract.

ii. Fourier Transmission Infra-Red (FTIR) Spectroscopy Analysis

FTIR spectroscopy explained about the interaction of AgNPs from the leaf biomolecules *of A. adhenophora* and to identify organic impurities present in the sample. The FTIR spectroscopy of AgNPs was done within the range of 500-4500 cm^{-1} wavelength which is shown in Fig. 4. AgNPs sample shows characteristics peak at different wavelength range. The peak at about of 3270 cm^{-1} corresponds to the stretching vibration of O-H bond group of alcohol and phenols (Awwad and Salem, 2012). The peak at about of 1596 cm^{-1} corresponds to the N-H bond, (Bhujel *et al.*, 2012), primary amines (Redriguez-Luis *et al.*, 2020). The peak at about of 1246 cm^{-1} corresponds to the stretching vibration of C-N bond, aliphatic amine (Balashanmugam and Kalaichelvan, 2015; Dhungana, 2016).

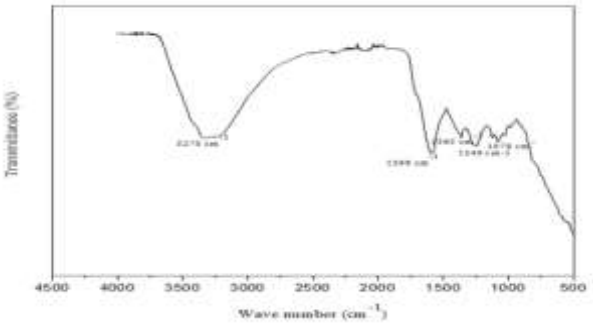

Fig. 4: FTIR spectra of AgNPs synthesized using *Agaratina adenophora* leaf extract.

iii. Antimicrobial activity of AgNPs

Antimicrobial activity of silver is well known that has been used for treatment of several diseases since ancient time. The antimicrobial screening of AgNPs were tested against Gram positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli*. The result showed the zone of inhibition of *B. subtilis and E. coli* found to be 11 mm and 9 mm, respectively which is shown in Fig. 5. The nanoparticles showed significant antimicrobial activity in the *B. subtilis* and *E. coli*. which is slightly more effective in case of Gram-negative bacteria. The difference in sensitivity of Gram positive and Gram-negative bacteria against AgNPs may result from the variation in the thickness and molecular composition of the membranes. Gram positive bacteria cell wall composed of peptidoglycan is comparatively thicker than that of Gram-negative bacteria. Therefore, it showed more effective in Gram positive bacteria compared to the Gram negative (Prakash *et al.*, 1017; Balami *et al.*, 2017).

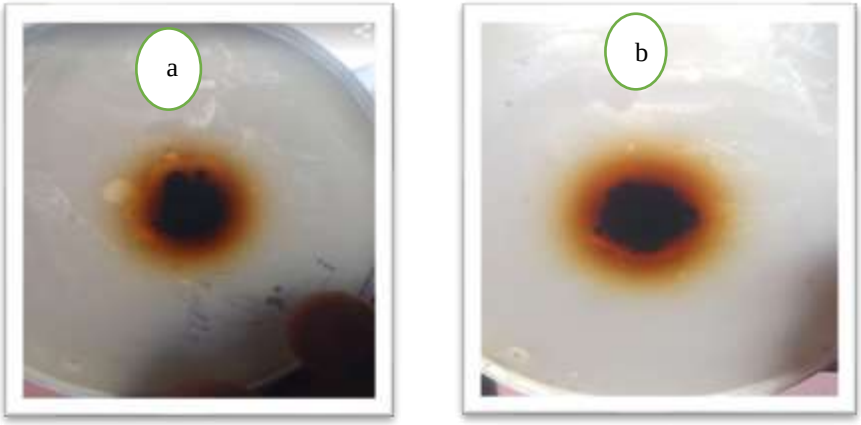

Fig. 5: Antimicrobial activity of silver nanoparticle against bacteria

(a) *Bacillus subtilis*, (b) *Escherichia coli.*

Conclusion

Silver nanoparticles were successfully synthesized via green synthetic route using leaf extract of *Ageratina adenophora*. As synthesized $AgNP_s$ were characterized using X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. XRD study reveals the pure crystalline nature of $AgNP_s$ having face centred cubic gemeotry with average particle size of 24 nm. FTIR analysis shows the presence of hydroxyl (O-H) group, amine (N-H), and aliphatic amine (C-N) group. $AgNO_3$ is proven significant against bacteria *Bacillus subtilis* and *Escherichia coli* showing zone of inhibition of 11 mm and 9 mm, respectivley.

Authors' Contribution

S.K. Gautam, T.R. Binadi & B. Regmi designed the research plan; Y. Baid & P. Thapa Magar, performed experimental works & collected the required data. All authors collectively analysed the data; S.K. Gautam, Y. Baid & P. Thapa Magar prepared the manuscript. All authors critically revised, finalized & approved the final form of the manuscript.

Conflict of Interest

This is the authors' declaration that guarantes objectives and fair research. The research results are not influenced by external factors or misconduct & there is no conflict of interest with present publication.

Acknowledgements

Authors are thankful to Department of Chemistry, Tri-Chandra Multiple Campus, for providing laboratory facitlities. We are greatly beholden to Nepal Academy of Science and Technology (NAST) for XRD analysis and Central Department of Chemistry, Tribhuvan University for FTIR analysis. Our sincere gratitude towards Polytechnic Research Institute of Nepal (PORIN) for antimicrobial test.

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