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Green Synthesis of Silver Nanoparticles Using *Azadirachta indica* Bark Extract for Characterization and Biological Application

Anurag Bharti*, Dr. Yogendra Singh, Saurabh Jain

Shri Ramnath Singh Mahavidhyalaya College Of Pharmacy, Gormi, Bhind (M.P.) – 477660 India.

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ABSTRACT

In recent science Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, ecofriendly and less time consuming. The Green synthesis was done by using the aqueous solution of Azadirachta indica leaf extract and $AgNO_3$. Silver was of a particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, DLS, Zeta Analysis, and SEM. The nanoparticles were found have the size ranges from 160-180 nm.

KEYWORDS: Neem, Azadirachta indica, Silver nanoparticles, green synthesis

INTRODUCTION

The 'green' environment friendly processes in chemistry and chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns (1). Silver is the one of the most commercialised nano-material with five hundred tons of silver nanoparticles production per year (2) and is estimated to increase in next few years. Including its profound role in field of high sensitivity biomolecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities (3).

A number of techniques are available for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc. (4, 5,6); unfortunately many of the nanoparticle syntheses methods involve the use of hazardous chemicals or high energy requirements, which are rather difficult and including wasteful purifications (7). Thus; a scenario is that whatever the method followed, will always leading to the chemical contaminations during their syntheses procedures or in later applications with associated limitations. Yet; one cannot deny their ever growing applications in daily life. For instances; "The Noble Silver Nanoparticles" are striving towards the edge-level utilities in every aspect of science and technology including the medical fields; thus cannot be neglected just because of their source of generation.

Hence, it is becoming a responsibility to emphasise on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green syntheses are rendering themselves as key procedure and proving their potential at the top. The techniques for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, etc.), plant extracts, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology (8). Greener syntheses of nanoparticles also provides advancement over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials (9). Microorganisms can also be utilized to produce nanoparticles but the rate of syntheses are slow compared to routes involving plants mediated synthesis.

Considering the vast potentiality of plants as sources this work aims to apply a biological green technique for the synthesis of silver nanoparticles as an alternative to conventional methods. In this regard, bark extract of Azadirachta indica (commonly known as neem) a species of family Meliaceae was used for bioconversion of silver ions to nanoparticles. This plant is commonly available in India and each part of this tree has been used as a household remedy against various human ailments from antiquity and for treatment against viral, bacterial and fungal infections (10). Silver nanoparticles can be produced at low concentration of bark extract without using any additional harmful chemical/physical methods. The effect of concentration of metal ions and concentration of bark extract quantity were also evaluated to optimize route to synthesise silver nanoparticle for antimicrobial activity. The method applied here is simple, cost effective, easy to perform and sustainable.

MATERIALS AND METHODS

Sample Collection and Authentication: Azadirachta indica bark was collected from Rohilkhand Region of Bareilly district, Uttar Pradesh, India. Then it was cleaned properly with water. The plant specimen was identified and authenticated by Dr. Alok Shrivastava, Department of Plant Science, MJP Rohilkhand University, Bareilly Uttar Pradesh, India. The plant Voucher Specimen No. was RU/PS/2024/06.

Preparation of Plant Extract: 20g of dried powdered neem bark was extracted sequentially by hot continuous percolation method in Soxhlet apparatus using 100 ml aqueous extract. The solution was cooled at room temperature and filtered by Whatman filter paper No.1 to remove fibrous impurities. The aqueous extracts were collected and stored in 250 ml Erlenmeyer flask at 4°C in refrigerator. The stored aqueous extract was used as reducing agent and stabilizing agent within 1 week for the biosynthesis of silver nanoparticle from silver nitrate. All glassware and equipment used in the study were properly washed with distilled water and dried in oven. (11)

Synthesis of Silver nanoparticles:

i) Preparation of 1mM Silver nitrate:17mg silver nitrate was accurately weighed and transferred into 100 ml standard flasks to get 1mM Silver nitrate solution.

ii) Microwave Irradiation Technique: 100 ml solution of 1 Mm silver nitrate was added to the various concentration of aqueous extract solution and kept on magnetic stirrer for 15 minutes to achieve uniform mixing. Beaker containing solution was transferred into microwave oven. The microwave power was kept constant at 840Watts and the colour change was observed. Less time required for reduction of silver nitrate in extract solution to obtain silver nanoparticles was optimized. Reddish brown colour confirms the formation of silver nanoparticles and analyzed by UV Visible spectrophotometer. Four concentration ratios of plant and metal ions were prepared (30:1, 60:1,120:1 & 240:1) by increasing the concentration of plant extract concentration in the solution. (12)

iii) Colour changes during reduction of silver nanoparticles: During reduction of silver nitrate colour change was noted. Initially it was yellowish orange colour and turns to reddish brown colour which shows the formation of AgNPS.

iv) Separation of Silver Pellets: The centrifugal force was used to separate silver pellets. The centrifugal force was used at 5000 rpm for 1 hour. The supernatant was discarded, the AgNPs was redispersed in distilled water. To obtain sample powders, AgNPs sols were dried. (13)

Characterization Of Silver Nanoparticles:

Visual Examination: The primary confirmation of the synthesized Azadirachta indica silver nanoparticles is done by visual basis. The colour change of Azadirachta indica extract and silver nitrate solution with respect to time was observed.

UV-Vis Analysis: The optical property of Ag-NPs was determined by UV-Vis spectrophotometer (Perkin-Elmer). After the addition of $AgNO_3$ to the plant extract, the spectra were taken in different time intervals up to 24 hrs between 200 nm to 500 nm. Then the spectra was taken after 24hrs of $AgNO_3$ addition. (14, 15)

FTIR Analysis: The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75 °C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method. (16)

SEM Analysis: The morphological features of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of

the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV. (17, 18)

Dynamic light scattering (DLS) & Zeta-Potential Analysis: Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionised water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min at 25 °C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs). (19, 20)

In Vitro Antimicrobial Activity

Antibacterial bioassay: The different concentration of prepared silver naoparticles were subjected to antibacterial evaluation against four bacterial strains Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi), Staphylococcus aureus (S. aureus) as described by Usman et al. Muller Hinton Agar (Oxoid UK) was prepared in conical flask in accordance to the directions provided by the manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121 °C and 15 psi pressure. The media was poured into Petri dishes under aseptic condition. The stock solutions of corresponding fractions were prepared in dimethyl sulfoxide (DMSO). (21) The modified method of Perez et al., (1990) was followed. All of the four bacterial strains were obtained from Department of Microbiology, Rohilkhand Medical College, Bareilly, Uttar Pradesh, India. Bacterial culture was inoculated on MHA (corresponding to 10^6 CFU/ml). Bacterial strains were spread on the solidified agar media, then 7 mm wells were punched in the agar media by using sterile metallic borer. Stock solutions of crude extract and fractions in DMSO at concentration of 20 mg/ml were prepared and 200 µl from each stock solution was added into respective wells. The petri dishes were incubated at 37 °C for 24 hours and control wells containing antibiotic (Levofloxacin), which is a positive control, was also run side by side. After 24 hours antibacterial activities were measured as diameter of the zones of inhibition and compared with the zone of inhibition of control (Levofloxacin). (22, 23)

Antifungal bioassay: The antifungal bioassay was determined by agar tube dilution method (24). Four fungal strains i.e. Aspergillus flavus (A. flavus), Fusariun solani (F. solani), Aspergillus fumigatus (A. fumigatus) and Aspergillus niger (A. niger) were used for antifungal activities. To refresh fungal strains, 13g/L nutrient broth in distilled water was prepared. Sterilized in autoclave and four flasks of 250ml were filled from broth, to each flask fungal colonies were inoculated separately. These flasks were then placed in incubator at 30°C for 3 days for refreshing fungal strains. SDA was used for growth of fungal strains. The flask was sterilized in autoclave at 121°C for 15 minutes at 1.5 pounds pressure. (25)

Antibiotic, Clotrimazole (Canesten) an antifungal drug was taken as a positive control and dissolved in distilled water (30µg/ 6µL) while DMSO was incorporated as negative control. About 7ml of medium was added to clean, dry and sterilized test tubes. Solutions of crude extracts and sub fractions were prepared each of 2µg/µL. One ml of sample (2µg/µL) was also added to each test tube, the test tube was kept in inclined position to make a slant. The same process was repeated for all of test tubes. (26) After cooling and solidifying, the fungi inoculums suspension was spread over the SDA medium uniformly using sterile cotton swabs. After that the test tubes were kept in incubator for 3 days at 30°C. After 3 days the fungal growth was observed in each test tube by the absence and presence of fungal strains. (27, 28, 29)

RESULTS AND DISCUSSION

Visual Examination: Initial colour of Azadirachta indica bark extract was yellow and it turns to dark brown colour after the addition of silver nitrate solution. Beyond 90 minutes there is no significant change in colour indicating the completion of the reduction reaction. Azadirachta indica bark extract is shown in figure 1. The change in colour of the reaction mixture after 2 hours is presented in Figure 1 which indicated the formation of AgNPs. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range.

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Figure 1: Azadirachta indica extract and Silver nanoparticles

UV-Vis Spectrophotometer Analysis: Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The sharp bands of silver nanoparticles were observed around 421 nm in case of Azadirachta indica. So we confirmed that Azadirachta indica leaf extract has more potential to reduce Ag ions into Ag nanoparticles, which lead us for further research on synthesis of silver nanoparticles from Azadirachta indica leaf extracts. The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles the insets to Figure: 2, 3, 4 and 5 represent the plots of absorbance at λ_{max} (i.e., at 420 nm) versus time of reaction.

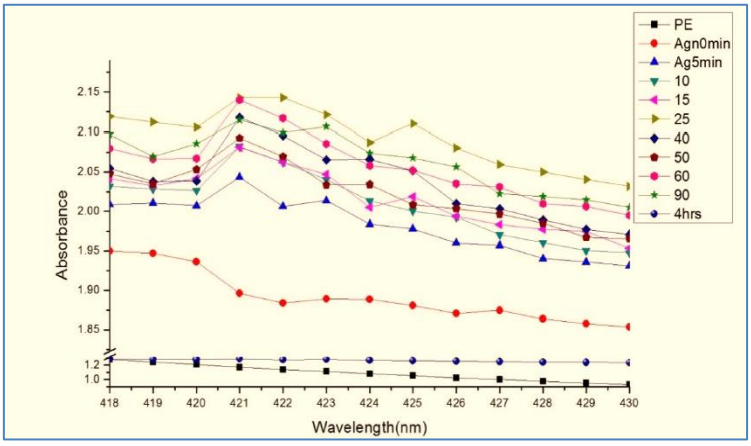

Figure 2: UV-vis spectra of Azadirachta indica 60:1 ratio at different time interval

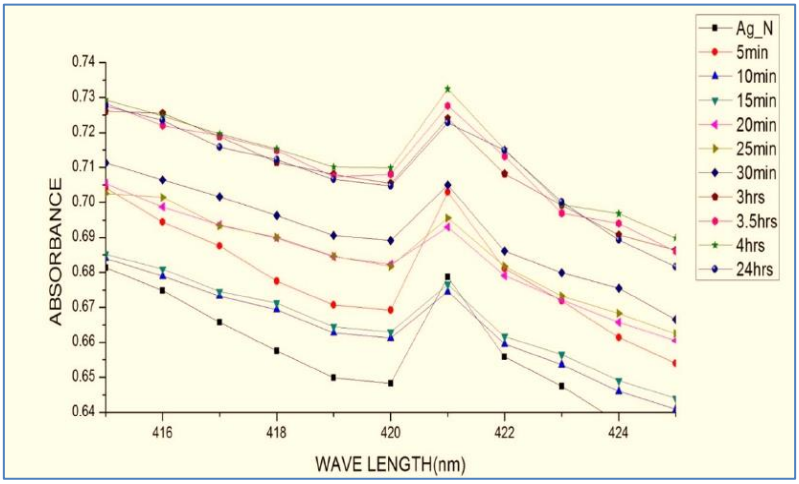

Figure 3: UV-vis spectra of Azadirachta indica 60:1 ratio at different time interval

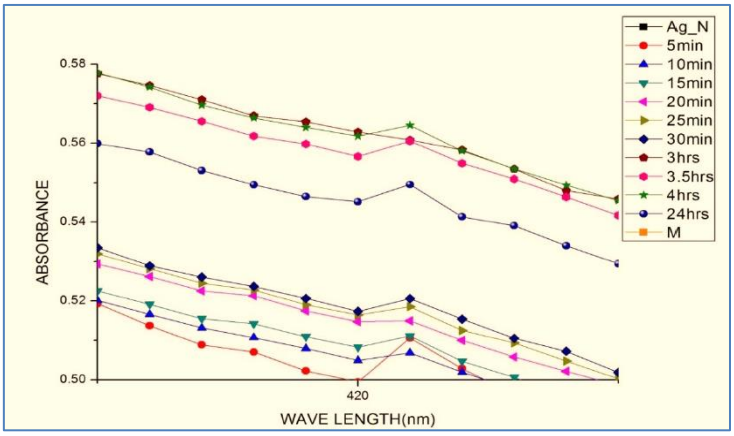

Figure 4: UV-vis spectra of A. indica 120:1 ratio at different time interval

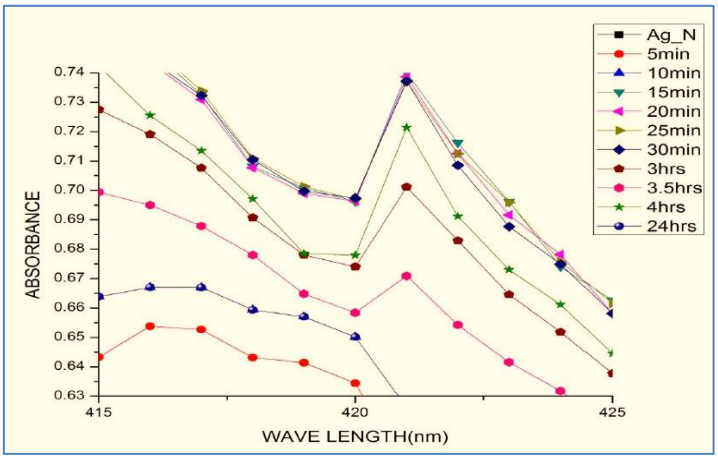

Figure 5: UV-vis spectra of A. indica 240:1 ratio at different time interval

FTIR Analysis: FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (Figure 6 and 7) in case both of 60:1 and 120:1 ratios showed the band between 3490-3500 cm-1 corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm^{-1} showed a stretch for C-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N-H. Where as the stretch for Ag-NPs were found around 500-550 cm^{-1}.

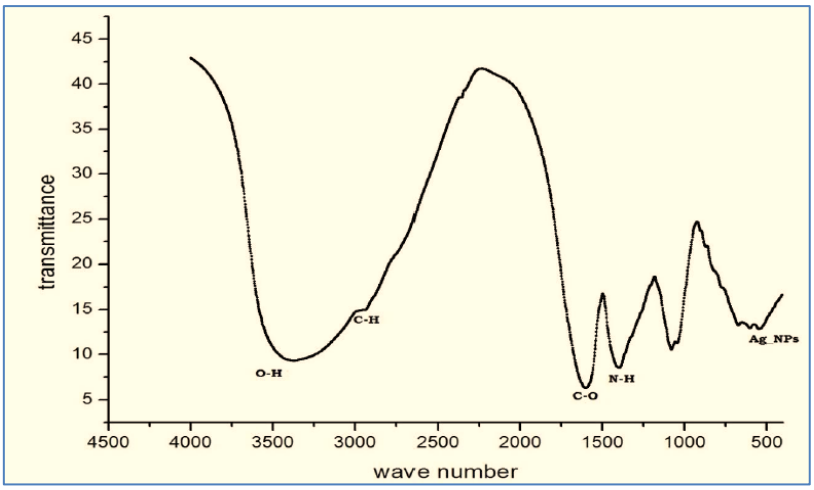

Figure 6 FTIR result for 60:1 ratio silver nanoparticles

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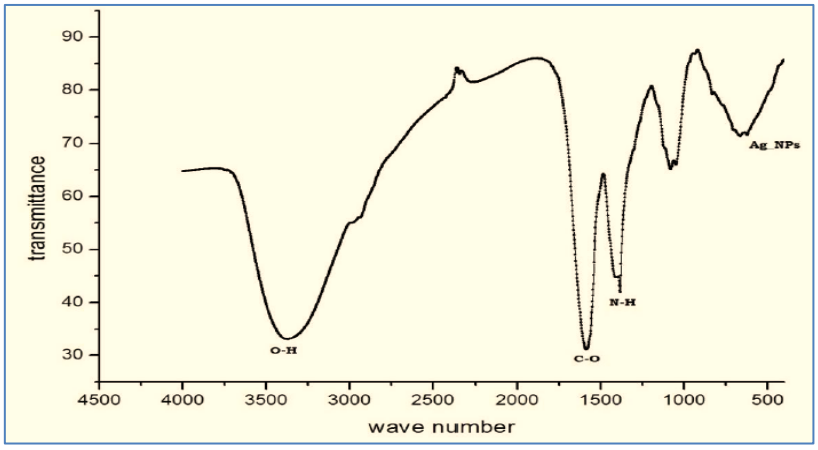

Figure 7: FTIR result for 120:1 ratio silver nanoparticles

SEM Analysis: SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 120:1 ratios where as in 60:1 ratio the size is of several nm. (Figure 8 and to 9).

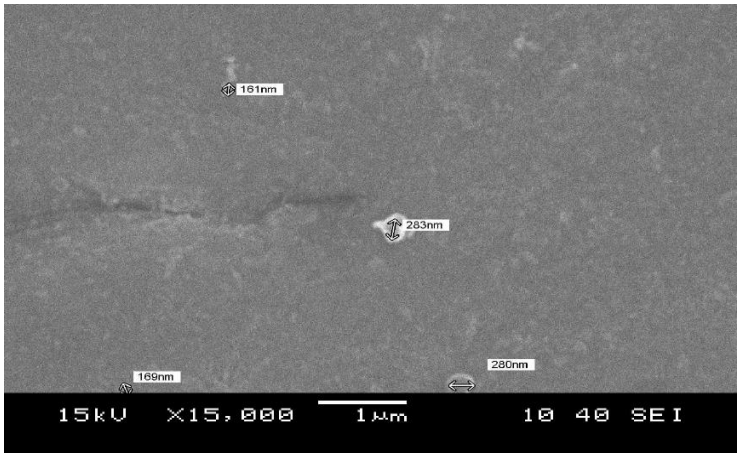

Figure 8: SEM image for 60:1 ratio silver nanoparticles

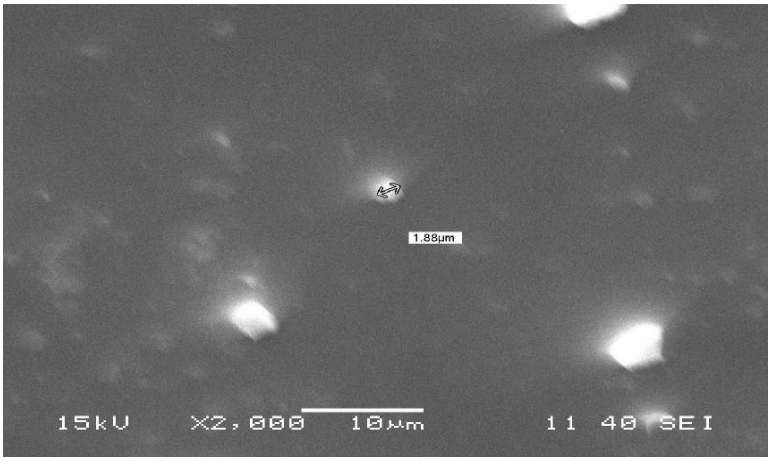

Figure 9: SEM image for 120:1 ratio silver nanoparticles

The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of 60:1, and 120:1 ratios.

DLS Analysis: The particle size distribution (PSD) of synthesized silver nanoparticles of different ratios like 30:1, 60:1, 120:1, and 240:1 are shown in the figures. (Figure 10 and 11). According to the figure 10 the colloidal solution of silver nanoparticles of ratio 60:1, the solution contains particles of uniform sizes ranging from 68 nm to 396 nm. The average size of nanoparticles is 160 nm. The particle size in case of 120:1 ratio ranges from 78 nm to 255 nm with mean particle size of 169 nm.

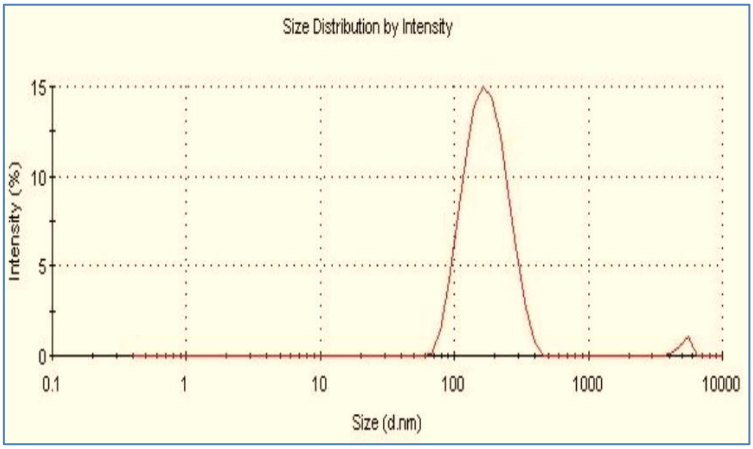

Figure 10: DLS result for 60:1 ratio silver nanoparticles

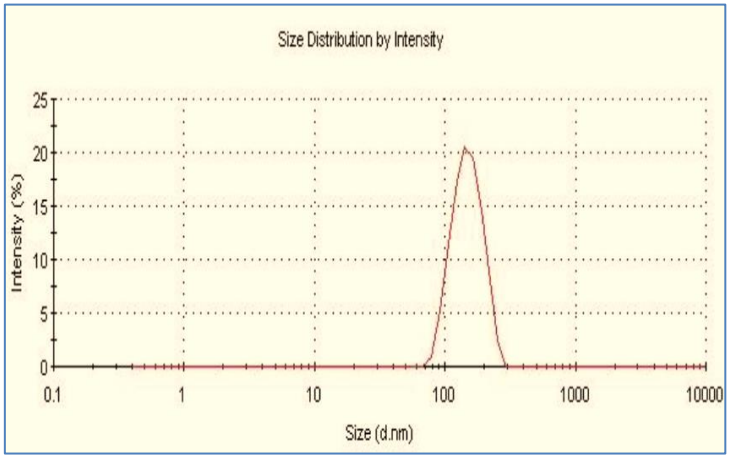

Figure 11: DLS result for 120:1 ratio silver nanoparticles

Zeta Potential Analysis: The Zeta potential measurements of silver nanoparticles synthesized with different ratios like 60:1, and 120:1, are 1.92 mV, and 6.12 mV respectively. From the analysis the order of stability of nanoparticles synthesized from different ratios is 120:1 > 60:1. Nanoparticles are very small in size for which they are energetically very unstable. Therefore, the particles undergo agglomeration/aggregation to stabilize themselves. So, there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis.

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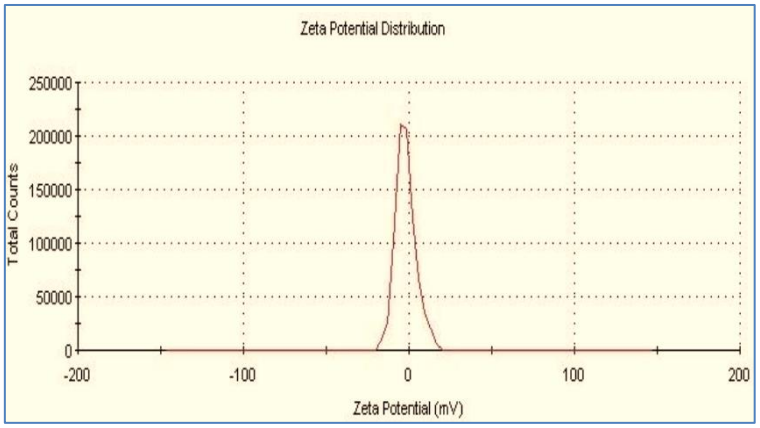

Figure 12: Zeta Analysis result for 60:1 ratio silver nanoparticles

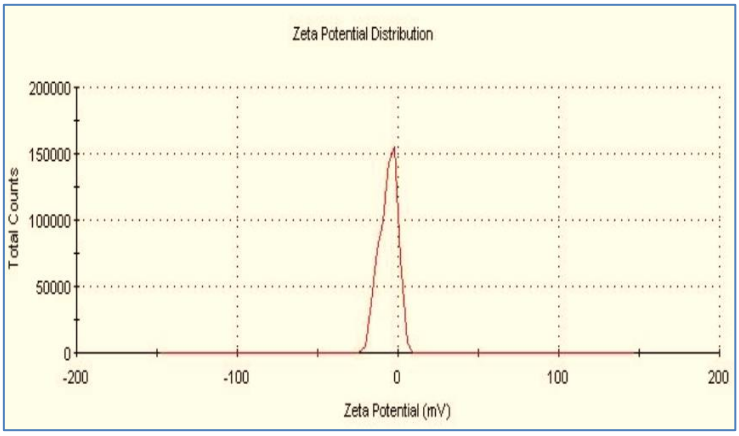

Figure 13: Zeta Analysis result for 120:1 ratio silver nanoparticles

ANTIMICROBIAL ACTIVITIES

Antibacterial Activity: The extract of A. indica was evaluated for in vitro antibacterial activity against, E. coli, S. typhi, P. aeruginosa and S. Aureus indicating different zones of inhibition (Table 1). Our results revealed that chloroform, butanol, ethyl acetate extract of A. indica showed significantly higher inhibitory activity (between 15-19 mm) against E. coli, P. aeruginosa and S. aureus. All extracts of A. indica showed activity (12-14 mm) against S. typhi (Table 1). The present investigation has shown that the different concentration of prepared silver nanoparticles of A. indica have active phytochemical, which can inhibit the growth of pathogenic bacteria and fungi.

Antifungal activities: The ethyl acetate and butanol extracts of A. indica showed activity against all studied fungal strains. However, 60:1 and 120:1 fractions did not show any activity against A. flavus and F. solani, respectively (Table 2). Similarly, the n-hexane and chloroform extracts of M. falcata showed activity against all studied fungal strains.

Table 1: Zone of inhibition (mm) against four bacterial strains.

Silver Nanoparticles	**Concentration**	**EC**	**PA**	**ST**	**SA**	**ANOVA**
	30:1	18.66±1.15	15.66±0.577	13.33±1.15	18.66±1.15	$p < 0.01$
	60:1	17±1	17.33±1.15	14.33±1.15	15.33±0.57	$p < 0.05$
	120:1	17.33±1.15	17±1	13±1	15.33±1.15	$p < 0.01$
	240:1	15±1	17.33±0.57	13.66±0.57	19±1	$p < 0.01$
DMSO		0	0	0	0	
Standard drug (Levofloxacin)		22	21	22	23	

EC Escherichia coli; PA-Pseudomonas aeruginosa; ST-Salmonella typhi; SA-Staphylococcus aureus

Table 2: Zone of inhibition (mm) against four fungal strains.

Silver Nanoparticles	Concentration	AF	AN	AF	FS
	30:1	-	+	-	+
	60:1	+	-	+	-
	120:1	+	+	+	+
	240:1	+	+	-	+
Standard drug Clotrimazole (Canesten)		+	+	+	+

AF-Aspergillus flavus; AN-Aspergillus niger; AF-Aspergillus fumigatusl FS-Fusarium solani

+ Presence of antifungal activity

-– Absence of antifungal activity

CONCLUSION

A simple one-pot green synthesis of stable silver nanoparticles using A. indica bark extract at room temperature was reported in this study. Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized nanoparticles which exclude external stabilizers/reducing agents. It proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The synthesised silver nanoparticles showed efficient antimicrobial activities against both E. coli and S. aureus. Benefits of using plant extract for synthesis is that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer products. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

REFERENCES

1. Crabtree JH, B.R., Siddiqi Ra, Huen IT, Handott LL, Fishman A. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. Perit Dial Int, 2003. 23(4): p. 368-374.
2. Larue, C., Castillo-Michel, H., Sobanska, S., Cecillon, L., Bureau, S., Barthes, V. Foliar exposure of the crop Lactuca sativa to silver nanoparticles: evidence for internalization and changes in Ag speciation. Journal of Hazardous Materials, 2014; 264, 98e106.
3. El-Chaghaby G. A., & Ahmad, A. F. Biosynthesis of silver nanoparticles using pistacia lentiscus leaves extract and investigation of their antimicrobial effect. Oriental Journal of Chemistry, 2011; 27, 929e936.
4. Garima S.R.B., Kunal K., Sharma A.R., Singh R.P. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J Nanopart Res, 2011; 13: p. 2981-2988.
5. Mukherjee P.A.A., Mandal D.S., Senapati S., Sainkar R., Khan M.I., Parishcha R., Ajaykumar P.V., Alam M., Kumar R., Sastry M., "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis.". Nano Lett, 2001; 1: 515-519.
6. Spring H. S. K. Diversity of magnetotactic bacteria. Syst Appl Microbiol, 1995. 18(2): p. 147-153.
7. Nelson D.P.D., Oswaldo L.A., Gabriel I.H.D.S., Elisa E. Mechanical aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. Journal of Nanobiotechnology, 2005; 3: p. 8.
8. Hemanth N.K.S., Karthik L., Bhaskara R.K.V. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus Penicillium sp. Arch Appl Sci Res, 2010; 2(6): 161-167.
9. Natarajan K.S.S., Ramchandra M. Microbial production of silver nanoparticles. Dig J Nanomat Bios, 2010. 5(1): p. 135-140.
10. Ibrahim, H. M. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. Journal of Radiation Research and Applied Sciences. http://dx.doi.org/10.1016/j.jrras.2015.01.007.
11. Khalil, M. M. H. (2013). Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arabian Journal of Chemistry, 1131e1139.
12. Khalil, M. M. H., Ismail, E. H., & El-Magdoub, F. (2012). Biosynthesis of Au nanoparticles using olive leaf extract. 1st nano updates. Arabian Journal of Chemistry, 5, 431e437.
13. Kharissova, O. V., Dias, H. V. R., Kharisov, B. I., Perez, B. O., & Perez, V. M. J. (2013). The greener synthesis of nanoparticles. Trends in Biotechnology, 31, 240e248.
14. 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, 50e56.

15. Sinha, S. N., Paul, D., Halder, N., Sengupta, D., & Patra, S. K. (2014). Green synthesis of silver nanoparticles using fresh water green alga Pithophora oedogonia (Mont.) Wittrock and evaluation of their antibacterial activity. Applied Nanoscience. http://dx.doi.org/10.1007/s13204-014-0366-6.
16. Sre, P. R. R., Reka, M., Poovazhagi, R., Kumar, M. A., & Murugesan, K. (2015). Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 135, 1137e1144.
17. Thuesombat, P., Hannongbua, S., Akasit, S., & Chadchawan, S. (2014). Ecotoxicology and environmental safety effect of silver nanoparticles on rice (Oryza sativa L. cv. KDML 105) seed germination and seedling growth. Ecotoxicology and Environmental Safety, 104, 302e309.
18. Obaid, A. Y., Al-Thabaiti, S. A., Al-Harbi, L. M., & Khan, Z. (2015). Extracellular bio-synthesis of silver nanoparticles. Global Advanced Research Journal of Microbiology, 3(8), 119e126.
19. Omoja, V. U., Anaga, A. O., Obidike, I. R., Ihedioha, T. E., Umeakuana, P. U., Mhomga, L. I., et al. (2011). The effects of combination of methanolic leaf extract of Azadirachta indica and diminazene diaceturate in the treatment of experimental Trypanosoma brucei brucei infection in rats. Asian Pacific Journal of Tropical Medicine, 4, 337e341.
20. Padalia, H., Moteriya, P., & Chanda, S. (2014). Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. Arabian Journal of Chemistry. http://dx.doi.org/10.1016/j.arabjc.2014.11.015.
21. Prathna, T. C., Chandrasekaran, N., Raichur, A. M., & Mukherjee, A. (2011). Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. Colloids and Surfaces B: Biointerfaces, 82, 152e159.
22. Sadeghi, B., & Gholamhoseinpoor, F. (2015). A study on the stability and green synthesis of silver nanoparticles usingZiziphora tenuior (Zt) extract at room temperature. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134, 310e315.
23. Salari, Z., Danafar, F., Dabaghi, S., & Ataei, S. A. (2014). Sustainable synthesis of silver nanoparticles using macroalgae Spirogyra varians and analysis of their antibacterial activity. Journal of Saudi Chemical Society. http:// dx.doi.org/10.1016/j.jscs.2014.10.004.
24. Sasikala, A., Linga Rao, M., Savithramma, N., & Prasad, T. N. V. K. V. (2014). Synthesis of silver nanoparticles from stem bark of Cochlospermum religiosum (L.) Alston: an important medicinal plant and evaluation of their antimicrobial efficacy. Applied Nanoscience. http://dx.doi.org/10.1007/s13204-014-0380-8.
25. Tripathy, A., Raichur, A. M., Chandrasekaran, N., Prathna, T. C., & Mukherjee, A. (2010). Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachta indica (Neem) leaves. Journal of Nanoparticle Research, 12, 237e246.
26. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., et al. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society, 15, 113e120.
27. Elumalai E.K., Hemachandran J., Vivivan Therasa S., Thirumalai T., David E. Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities. J Phram Sci, 2010; 2(9): p. 549-554.
28. Ankamwar B.D.C., Ahmad A., Sastry M. Biosynthesis of gold and silver nanoparticles using Emblics Officinalis Fruit extract and their Phase Transfer and Transmetallation in an Organic Solution. J nanosci nanotechnol, 2005; 5(10): 1665-1671.
29. Kasthuri J.K.K., Rajendran N. Phyllanthin assisted biosynthesis of silver and gold nanoarticles a novel biological approach. J Nanopart Res, 2009; 11: 1075-1085.

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