Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/1362
DOI URL: http://dx.doi.org/10.21474/IJAR01/1362
ISSN NO. 2320-5407

RESEARCH ARTICLE

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES.

Gonsalves Chrislyn[1], Salanke Prachi[2], Shetty Sangeeta[4]* and Sundarrajan Priya[3].
Department of Life Science and Biochemistry, St. Xavier's College-Autonomous, Mumbai.

Manuscript Info

Manuscript History

Received: 12 June 2016
Final Accepted: 16 July 2016
Published: August 2016

Key words:-
Green synthesis, Silver nanoparticles, Antimicrobial activity, Disinfection of water.

Abstract

Nanoparticles have altered properties because of their size and result of which they have found enormous applications. Silver nanoparticles have been known to have antimicrobial properties. Biological synthesis of nanomaterials are not only ecofriendly but also the nanoparticles synthesized have superior properties than those synthesized using physical and chemical methods. In the present study, silver nanoparticles (AgNPs) were synthesized using various plant extracts like fern, jackfruit, and onion. The effect of varying pH of these extracts on the synthesis of AgNPs was studied, and alkaline medium was found to decrease the time taken for synthesis. The synthesized AgNPs were found to have absorption maxima close to 420 nm, and mean size less than 50 nm. The antimicrobial activity of AgNPs was checked against pathogens like, *E. coli, S. aureus, S. pyogenes, S. typhi, S. paratyphi A, S. paratyphi B, Shigella* and *Proteus.* The MIC of these AgNPs against *E. coli* and *S. aureus* was determined. It was found that these AgNPs when adsorbed on to activated carbon could be used to disinfect water contaminated with *E. coli.*

Introduction:-

Nanotechnology, is a modern technology in science that deals with the synthesis, design, and application of nanomaterials by controlling their shape and size. Nanoparticles are defined as discrete objects having all of their three Cartesian dimensions in the nano range i.e. 1-100 nm. The properties of these nanomaterials are different than those of their bulk materials due to their higher surface to volume ratio. Hence, the nanomaterials are used in various applications due to their improved properties. Nanotechnology finds its application in the fields of electronics, food industry, packaging industry, biomedical devices, drug delivery systems, medical diagnosis, biological tissue regeneration, space industry, optical devices, water purification, environment monitoring, and many more [Hirsch et al., 2013] [Koria et al., 2011] [Wang et al., 2011].

Significant amount of research is being done on the various ways to synthesize silver nanoparticles, like physical, chemical and biological/green methods. Green methods have gained more importance over the years as it is more eco-friendly than the other two methods. It does not make use of toxic reducing agents that are employed in chemical methods and the energy input is comparatively much less than that used during physical methods of nanoparticle synthesis [Iravani et al., 2014].

Corresponding Author:- Shetty Sangeeta
There has been equal contribution of 1 and 2 towards this paper.
Address:-Department of Life Science and Biochemistry, St. Xavier's College-Autonomous, Mumbai.

Since AgNPs have antimicrobial activity, they are used to coat various medico-surgical instruments like catheters, ventilators and surgical implants like artificial joints, pacemakers, etc. to prevent colonization of bacteria [Furno et al., 2004]. AgNPs have been impregnated on the dressings and bandages to prevent infections in the wounds. The antimicrobial property is also made use of in water disinfection [Hameed et al., 2013].

The aim of this study was to synthesize silver nanoparticles by the green synthesis method using Fern, Jackfruit and Onion extracts and to check for its antimicrobial activity against pathogenic microorganisms.

Materials and methods:-

Synthesis of Silver Nanoparticles:-

Fern (*Nephrolepis exaltata*), Jackfruit (*Artocarpus heterophyllus*), and Onion (*Alium cepa*) were obtained from local market. 1% (w/v) extract of Fern leaves and 10% extracts (w/v) of sundried Jackfruit pulp and onion bulb in deionised water (DIW) were prepared by boiling for two minutes. After cooling, the extracts were centrifuged at 8,000 rpm for 10 minutes to separate out particulate matter. To these extracts Silver Nitrate solution was added to make final concentration of 10 mM. The mixture was incubated in the presence of sunlight until a stable colour change was obtained.

AgNPs were also synthesized using chemical method to compare the two methods of nanoparticle synthesis, i.e. green method and chemical method. 50 ml of 1 mM $AgNO_3$ was boiled and 5 ml of 10% trisodium citrate was added drop wise. The mixture was constantly shaken till a stable colour was observed [Rashid et al., 2013].

Effect of pH on the synthesis of Silver Nanoparticle:-

The extracts prepared using the aforementioned green protocol were found to have pH of 4.5. The pH of the extracts was adjusted to 7.0 and 10.0 using 10N NaOH. To these extracts at varying pH (4.5, 7.0, and 10.0) silver nitrate solution was added, and the mixture was incubated in the presence of sunlight until a stable colour change was obtained. [Darroudi et al., 2010]

Characterization of synthesized Silver nanoparticles:-

Once a stable colour change was obtained, the solution was first centrifuged at 5000 rpm for 10 minutes to remove any plant preciptate. The supernatant obtained was further centrifuged at 12000 rpm for 30 minutes. The pellet obtained was washed thrice with DIW to remove residual plant extract. The final pellet obtained was resuspended in DIW and was used for characterization.

Spectrophotometric analysis:-

A spectrum scan from 300-800 nm was carried out using UV-Visible spectrophotometer to determine the absorption maxima of synthesized Silver nanoparticles. [Manivasagan et al., 2013]

Nanoparticle tracking system:-

The synthesized AgNPs were characterized using nanoparticle tracking system (Nanosight LM20) to determine the size of the nanoparticles. [Filipe et al., 2010]

Antimicrobial studies of the isolated AgNPs:-

Agar cup method:-

Antimicrobial activity of the synthesized AgNPs was tested against pathogens like, *E. coli, S. aureus, S. pyogenes, S. typhi, S. paratyphi A, S. paratyphi B, Shigella* and *Proteus* using agar cup method. Sterile DIW was used as a control. The plates were incubated overnight at 37^0C. [Elbeshehy et al., 2015]

Broth dilution method:-

The minimum inhibitory (MIC) of Ag-NPs was determined by two-fold broth dilution method using Nutrient Broth (NB). AgNPs were dried overnight at 60^0C to make the stock in sterile DIW from which a two-fold serial dilutions were made in NB. Overnight grown culture of approximately 10^6 cfu/ml *E. coli* and *S. aureus* were inoculated in the tubes containing NB alone (Positive control), NB containing AgNPs at a final concentration ranging from 1.5 to 200 µg/ml. Whereas, sterile uninoculated NB was used as a negative control. The tubes were incubated overnight at 37^0C. The MIC values corresponded to the AgNPs dose that inhibited bacterial growth (when compared to the positive control). [Elbeshehy et al., 2015]

Water disinfection:-
Activated carbon (AC) was incubated at 110˚C, for three hours. 1.6 g of this activated carbon and 10 ml of AgNPs (2mg/ml) (obtained from jackfruit and onion at pH 7.0) were mixed and kept on a magnetic stirrer overnight, to bring about adsorption of the nanoparticles onto the activated carbon under aseptic conditions [Karimi et al., 2012].

One ml of the activated charcoal adsorbed with nanoparticles (AC-AgNPs) was centrifuged at 7,000 rpm for 5 minutes to separate the non-adsorbed SNPs from the AC-AgNPs. The pellet obtained was washed twice with sterile deionized water to remove any loosely adsorbed AgNPs. The pellet obtained after washing was resuspended in sterile distilled water.

E. coli cells of density 10^6 CFU/ml suspended in sterile distilled water (19 ml), were exposed to 1 ml of the above AC-AgNPs. 10^6 CFU/ml of *E. coli* suspended in sterile distilled water was exposed to 1 ml of activated carbon, to serve as a charcoal control. Whereas, 10^6 CFU/ml of *E. coli* suspended in sterile distilled water was used as a culture control. At different time intervals (0, 2, 4, 8, and 12 minutes), aliquots were filtered using sterile Whattman filter paper. The filtrate was surface spread on sterile nutrient agar plates, in duplicates and the plates were incubated overnight at 37˚C.

Results and discussions:-

Synthesis of Silver Nanoparticles:-
The extracts of Fern (*Nephrolepis exaltata*), Jackfruit (*Artocarpus heterophyllus*), and Onion (*Alium cepa*) were used for synthesis of silver nanoparticles. On addition of silver nitrate a colour change to brown was observed for all the three extracts used. A change in colour of the mixture, indicates the reduction of silver ions (Ag^+) to atomic silver (Ag^0) which coalesce to form SNPs [Saxena et al., 2010], [Krishnaraj et al., 2010]. The time required for the colour change varied for each biological extract, ranging from 22, 56 and 57 minutes for Onion, Fern and Jackfruit extract respectively.

Effect of pH on the synthesis of Silver Nanoparticle:-
Effect of pH on the synthesis of SNPs was studied. It was found that at pH 10 the time taken for sythesis of the SNPs was the least for all three extracts (Table 1). This could be because alkaline conditions might enhance the efficiency of the reducing and stabilizing agents present in the extracts, thus resulting in faster synthesis [Mostafa et al., 2014].

Table 1:- Effect of varying pH of the extracts on the rate of synthesis of AgNPs

Extract	Fern			Jackfruit			Onion		
pH	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**
Time taken for synthesis (min)	56	36	29	57	57	31	22	11	4

Characterization of synthesized Silver nanoparticles:-

Spectrophotometric analysis:-
The absorption maxima of the AgNPs synthesized using green method with varying pH and chemical method were found to be in the range of 418 to 427 (Table 2). This is close to the characteristic absorption maxima of the AgNPs, i.e. 420 nm [Manivasagan et al., 2013].

Table 2:-Spectrophotometric analysis of the synthesized AgNPs

Extract	Fern			Jackfruit			Onion			Chemical synthesis
pH	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	
Absorption maxima (nm)	421	424	427	423	427	418	419	425	421	418

Nanoparticle tracking system::-
Nanoparticle tracking system (Nanosight LM20, Manuf*) was used to determine the size of the nanoparticles. The synthesized AgNPs were found to have mean sizes ranging from 22-44 nm (Table 3). The AgNPs showed a large standard deviation which proves that AgNPs are polydispersed. This is consistent with the size distribution of

AgNPs synthesized using green method without any external addition of capping or stabilizing agents [Raut et al., 2010].

Table 3: Representative **s**ize distribution obtained from the Nanoparticle Tracking System of the synthesized AgNPs at different pH values.

	Extract	Fern			Jackfruit			Onion			Chemical synthesis
	pH	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	
Size (nm)	**Mean**	28	31	33	44	36	26	37	40	36	22
	Mode	19	22	22	30	26	17	26	33	29	16
	Standard deviation	17	17	25	32	25	20	23	27	26	11

Antimicrobial studies:-

Agar cup method:-

The AgNPs synthesized using green method showed antimicrobial activity against various microorganisms which were tested in this study (Table 4). However, the chemically synthesized AgNPs showed no antimicrobial activity against any of the cultures tested. This could be attributed to the shape of the chemically synthesized AgNPs as compared to the green synthesis method [Pal et al., 2007].

Table 4:-Qualitative agar cup assay for determination of antimicrobial activity of synthesized AgNPs.

Culture	Fern (pH 4.5, 7.0, 10.0)	Jackfruit (pH 4.5, 7.0, 10.0)	Onion (pH 4.5, 7.0, 10.0)	Chemical synthesis
E. coli	+	+	+	-
S. typhi	-	-	+	-
***S. paratyphi* A**	-	-	-	-
***S. paratyphi* B**	-	-	+	-
Proteus	+	+	-	-
Shigella	+	+	+	-
S. aureus	+	+	+	-
S. pyogenes	+	+	+	-

Broth dilution method:-

The minimum inhibitory (MIC) of Ag-NPs was determined by two-fold broth dilution method using Nutrient Broth (NB). It was observed that the MIC of synthesized AgNPs, for *S. aureus* is higher than that for *E. coli* (Table 5). Since *S. aureus* is a Gram positive microorganism containing a thicker peptidoglycan layer in its cell wall than that in *E. coli*, probably AgNPs are not able to cross this barrier as efficiently as they can cross the thinner peptidoglycan layer in *E. coli*.

Table 5:-Minimum Inhibitory Concentrations of synthesized AgNPs

	Extract	Fern			Jackfruit			Onion		
	pH	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**	**4.5**	**7.0**	**10.0**
MIC (µg/ml)	***E. coli***	-	20	20	25	25	-	25	25	50
	S. aureus	-	20	40	100	100	-	50	50	-

Water disinfection:-

To determine the efficacy of AgNps for water disinfection, AgNPs were adsorbed on to Activated carbon under aseptic conditions. The AC control itself had a 10-fold reduction in cell count as AC is known to adsorb organic matter. However, AgNP-AC showed a further 1000-fold reduction in the cell count of the contaminated water (Table 6). Therefore, AgNPs adsorbed onto AC together showed a 10,000-fold reduction in the cell count. Zero cell count after zero-minute exposure could be attributed to the time lag during filtration of the aliquots during which AgNP-AC could have acted for extended time. Hence, this method can be used to disinfect water contaminated with sewage sample, having microbial load as high as 10^6 cfu/ml.

Table 6:-Results for water disinfection assay.

Time (min)	CFU/ml			
	Culture control	AC control	SNP-AC Jackfruit	SNP-AC Onion
0	8500	1115	0	0
2	28000	845	0	0
4	10000	2155	0	0
8	28200	2775	0	0
12	15000	1445	0	0

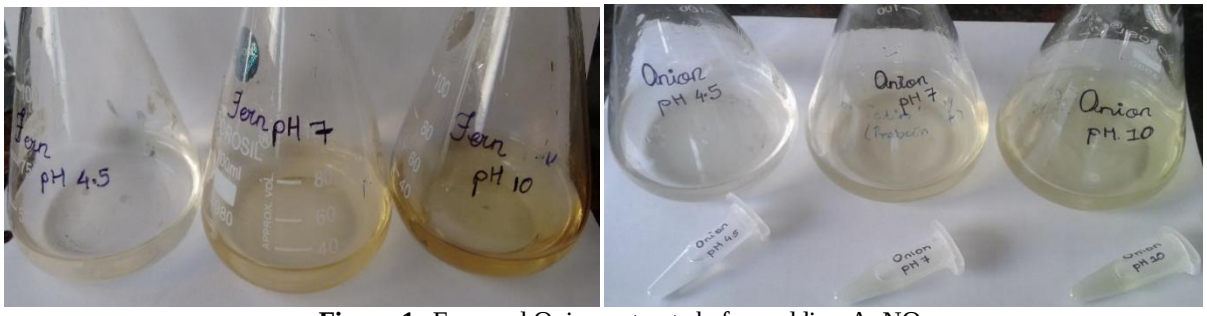

Figure 1:-Fern and Onion extracts before adding $AgNO_3$

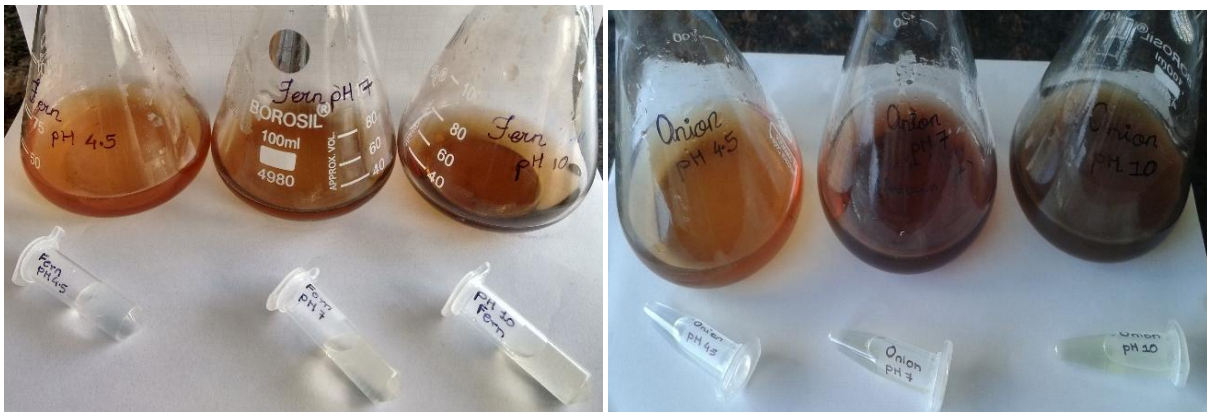

Figure 2:-Fern and Onion extracts after reduction of silver.

Conclusion:-

The green synthesis of silver nanoparticles was carried out by using Fern, Jackfruit and Onion extracts. The effect of pH on the synthesis of AgNPs was studied, and it was observed that in an alkaline medium the synthesis of AgNPs was faster. The size of the synthesized AgNPs was determined using a nanoparticle tracking system, and the synthesized AgNPs were found to have a mean size of 22-44 nm. Antimicrobial studies were carried out using the AgNPs synthesized by biological and chemical synthesis. Where the chemically synthesized AgNPs did not show antimicrobial activity against any of the eight cultures used, the biologically synthesized SNPs showed antimicrobial activity against some of the eight cultures used. The MIC of the synthesized AgNPs against *E. coli* and *S. aureus* was determined, using the broth dilution method. The antimicrobial property of these AgNPs was used to disinfect water containing a known number *E. coli* CFU/ml. We can hence conclude that AgNPs adsorbed to AC can be used effectivelyin water purifiers to reduce microbial load of contaminated water.

Acknowledgment:-

The authors are thankful to Dr. Rajesh Raut from the Institute of Science, Mumbai; for helping with the characterization of nanoparticles using the Nanoparticles Tracking System.

References:-

1. Darroudi, M., Ahmad, M. B., Abdullah, A. H., Ibrahim, N. A. and Shameli, K. (2010): Effect of Accelerator in Green Synthesis of Silver Nanoparticles. International Journal of Molecular Sciences, 11(10): 3898–3905.
2. Elbeshehy, E. K. F., Elazzazy, A. M. and Aggelis, G. (2015): Silver nanoparticles synthesis mediated by new isolates of *Bacillus* spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Front. Microbiol., 6: 453.
3. Furno, F., Morley, K., Wong, B., Sharp, B., Arnold, P., Howdle, S. , R., Paul D. Brown, P., Winship, P., and Reid, H. (2004). Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? J. Antimicrob. Chemother, 54 (6): 1019-1024.
4. Filipe, V., Hawe, A. and Jiskoot, W. (2010): Critical evaluation of nanoparticle tracking analysis (NTA) by Nanosight for the measurement of nanoparticles and protein aggregates. Pharmaceutical research, 27(5): 796-810.
5. Hameed, A., Moustafa, M., Amr, M., and Shawky, H. (2013). Using silver nanoparticles coated on activated carbon granules in columns for microbiological pollutants water disinfection in Abu Rawash area, Great Cairo, Egypt. Australian journal of basic and applied sciences,7(1):422-432.
6. Hirsch, L. R., Stafford, R. J., Bankson, J. A.,Sershen, S. R., Rivera, B.,Price, R. E., Hazle, J. D., Halas, N. J.,and West, J. L. (2003). Nanoshell-Mediated Near-Infrared Thermal Therapy of Tumors under Magnetic Resonance Guidance. Proceedings of the National Academy of Sciences of the United States of America, 100(23): 13549–54.
7. Iravani, S. (2014). Bacteria in Nanoparticle Synthesis: Current Status and Future Prospects. International Scholarly Research Notices, 2014.
8. Karimi, H., Mousavi, S. and Sadeghian, B. (2012): Silver nanoparticle loaded on activated carbon as efficient adsorbent for removal of methyl orange. Indian Journal of Science and Technology, 5(3): 2346-2353.
9. Koria, P., Yagi, H., Kitagawa,Y., Megeed, Z., Nahmias, Y., Sheridan, R., and Yarmush, M. (2011). Self-Assembling Elastin-like Peptides Growth Factor Chimeric Nanoparticles for the Treatment of Chronic Wounds. Proceedings of the National Academy of Sciences of the United States of America, 108(3): 1034–9.
10. Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T. and Mohan, N. (2010): Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces: Biointerfaces, 76: 50-56.
11. Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K. and Kim SK. (2013): Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel Nocardiopsis sp. MBRC-1. Biomed Res Int., 2013:287638.
12. Mostafa, M.H., Eman, H., Khaled, Z. and Doaa. (2014): Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arabian Journal of Chemistry, 7(6): 1131-1139.
13. Pal, S., Tak, Y. and Song, J. (2007): Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and environmental microbiology, 73(6): 1712-1720.
14. Rashid, M., Bhuiyan, K. and Quayum, M. (2013): Synthesis of silver nanoparticles and their uses for quantitative analysis of vitamin C tablets. Dhaka Univ. J. Pharma. Sci., 12(1): 29-33.
15. Raut, R., Kolekar, N., Lakkakula, J., Mendhulkar, V., and Kashid, S. (2010): Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamiapinnata* (L) pierre. Nano-Micro Letters, 2 (2):106-113.
16. Saxena, A., Tripathi, R.M. and Singh, R. P. (2010): Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity. Journal of Nanomaterials and Biostructures, 5(2): 427-432.
17. Wang, Y., Tong, M., Zhang, D., and Gao, Z. (2011). Improving the Performance of Catalytic Combustion Type Methane Gas Sensors Using Nanostructure Elements Doped with Rare Earth Cocatalysts. Sensors, 11(1): 19–31.