ECO-FRIENDLY PHYTO-SYNTHESIS OF SILVER NANOPARTICLES USING COLCHICUM AUTUMNALE AND ITS CHARACTERIZATION.

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Abstract

Metal nanoparticles have been produced chemically and physically for a long time; however, their biological production has only been investigated very recently. Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology. In the present study, we report biogenic fabrication of silver nanoparticles by a simple procedure using leaf broth of Colchicum autumnale without extra surfactant, capping agent, and/or template at ambient conditions. The AgNPs were characterized by UV-visible spectrophotometer, X-ray diffraction (XRD), particle size analyzer (DLS), scanning electron microscopy (SEM). The size of synthesized nanoparticles are in the range of 70-250nm which was confirmed by DLS and SEM micrographs. The size of nanoparticles decrease with increase in broth concentration. Morphology of the silver nanoparticles wasspherical and its distribution, monodisperse. AgNPs are crystallized in face centered cubic symmetry.

Introduction:

In recent times, a hotspot of interest in the green nanotechnology area has been the work on the expansion of a modest, ecofriendly, and safe approach for synthesizing metallic nanoparticles (MNPs). Biomaterials have been considered as an attractive resource for green synthesis due to their diversity, regenerative ability, and safety [1-4]. The remarkable success in this field has opened up avenues to develop “greener” methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials. Conventionally, the chemical and physical methods used to synthesize silver nanoparticles are expensive and often raise questions of environmental risk because of the involvement of toxic and hazardous chemicals [5].

Silver nanoparticles (AgNPs) have been recognized to possess immense importance and thus, have been extensively studied. Silver is well-known since ancient time for to its medicinal value and preservative properties. It is efficient antimicrobial agent compared to other salts due to their extremely large surface area, which provides better contact with microorganisms [6]. Several microorganisms, such as bacteria, fungi and yeasts, have come up as nanofactories, synthesizing metal nanoparticles. Biological approaches using microorganisms and plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods for its rapid, economical and eco-friendly protocol. Studies have shown that Alfalfa roots can absorb Ag (0) from agar medium and are able to transport it to the plant shoot in the same state of oxidation [7]. Existing literature also reports successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the latex obtained from Jatropha curcas [8]. Ag NPs were also obtained using Aloe vera [9], Acalypha indica [10], Garcinia mangostana [11] leaf extracts, Crataegus douglasii fruit extract [12] as well as various other plant extracts [13] as reducing agent.

Nanotechnology is important in developing sustainable technologies for the future, for humanity and the environment [14, 15]. New applications of nanoparticles are emerging rapidly. Colloidal silver is of particular interest.
because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds.

The reason for selecting plant for biosynthesis is because they contain important reducing agents like Citric acid, Ascorbic acids, flavonoids, terpenoids, reductases and dehydrogenases that may play an important role in biosynthesis of metal nanoparticles. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world’s population. Colchicum autumnale or autumn crocus, is recorded in early herbal guides as a treatment for inflammation. Autumn crocus is valued for its chemotherapeutic properties. The potent chemical Colchicine present in this plant have medicinal assets, including anti-cancer effects. It is widely used in plant breeding for its property as a mitotic toxin. Colchicine is present in all parts of the plant, the highest concentration of toxins is found in the seeds and the bulb (corm) (Cooper & Johnson, 1984; Frohne & Pfander, 1983). Colchicine is present in the flowers (0.1 to 0.8% in fresh flowers; up to 1.8% in dried flowers), in the seeds (0.2 to 0.8%) in the bulb (corm) (0.4 to 0.6%). Leaves contain very low amounts of colchicine (Gessner & Orzechowski, 1972). The additional toxins present, which are closely related to colchicine, include: desacetylmethylcolchicine (CHNO5), desacetylthiocolchicine (CH23NO4S), colchicoside, demethyl desacetylcolchicine, colchicine amide.

Here in, we report the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate (AgNO3) by Colchicum autumnale leaf broth. Further, these biologically synthesized nanoparticles were characterized and the morphology was studied.

Experimental:-
Colchicum autumnale leaves were taken from the plant and leaf broth solution was prepared by taking 10 g of finely cut leaves in a 500 mL Erlenmeyer flask with 100 mL of deionized water and then boiling the mixture for 20 minutes at 60°C before finally decanting it. This leaf broth was centrifuged, filtered and stored at room temperature for further experiments and used within 5 days.

For the synthesis of Ag nanoparticles, silver nitrate (AgNO3) [Sigma Aldrich] was used. Ag nanoparticles synthesis was carried out by taking 0.4mL, 0.8mL and 1.2mL of leaf broth and adding 19.6mL, 19.2mL and 18.8mL of 1×10⁻⁴ M aqueous AgNO3. The reaction mixture was allowed to stay stagnant at room temperature to observe the color change (Table-1).

Characterization of Silver nanoparticles:-
UV-vis spectrophotometer:-
To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken over the range of 200-800nm using a UV–vis spectrophotometer (Shimadzu-2450 UV-vis spectrophotometer). The leaf broth was used as blank.

Zeta potential and Size distribution studies:-
Mean particle diameter of silver nanoparticles and zeta potential was examined using the Zetasizer Nano ZS (Malvern instruments, UK) with a 663nm red laser and was capable of both particle size analysis (using DLS-Dynamic Light Scattering) and Zeta Potential measurement (using Doppler Electrophoresis). For the analysis, the nanoparticle sample of desired concentration was flushed through a disposable capillary cell (DTS 1060).

Field Emission-Scanning Electron Microscope (FE-SEM):-
Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4800. Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry.

XRD analysis:-
Powder XRD patterns were recorded using a powder X-ray diffractometer (Model- D8 Advance, made in BRUKER Germany). XRD is a rapid analytical technique primarily used for phase identification of crystalline material and can provide information on unit cell dimensions. By scanning the sample through a range of 20 angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material.
Results and discussion:-
The appearance of brown color following mixing of the plant extract with AgNO₃ indicated the formation of silver nanoparticles (AgNPs). This color change was due to the reaction between various biomolecules present in the leaf broth and silver ions. (Figure 2)

UV-visible and kinetic studies of AgNPs:-
The appearance of brown color was due to the excitation of surface plasmon vibrations and it provides a convenient spectroscopic signature to indicate the formation of silver nanoparticles. Noble metals are known to exhibit unique optical properties due to the property of Surface Plasmon Resonance (SPR) which is the collective oscillation of the conduction electrons in resonance with the wavelength of irradiated light. The size and shape of metal nanoparticles determine the spectral position of plasmon band absorption as well as its width. Silver nanoparticles have shown to exhibit size dependent optical properties [19]. They are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm due to this property [20].

The amount of leaf broth determines the size of the AgNPs. Figure 3-5 shows UV-vis absorption spectra of the AgNPs (Sample A, B, and C) over different periods of time. The SPR band becomes narrower and fairly sharp as the volume of leaf broth increases in the solution. (Figure 6). Concentration variation study of Colchicum autumnale leaf broth was carried out with 1x10⁻⁴ M Silver nitrate (AgNO₃) showing that under UV-vis spectrophotometry, the absorbance lies between 535-545 nm (Table 1).

Table 1:- Comparative study – Three concentrations.

| Sample | Concentration (%) | Volume of AgNO₃ solution (mL) | Volume of leaf broth (mL) | λ-max = SPR band (nm) | Absorbance |
|--------|-------------------|-----------------------------|--------------------------|-----------------------|------------|
| A      | 2%                | 19.6                        | 0.4                      | 430.00                | 0.662      |
| B      | 4%                | 19.2                        | 0.8                      | 439.50                | 1.327      |
| C      | 6%                | 18.8                        | 1.2                      | 439.50                | 1.806      |

Particle size distribution and zeta potential study:-
Nanoparticle size was characterized using the Zetasizer Nano ZS (Malvern instruments, UK), a Dynamic Light Scattering (DLS) instrument operating in the backward (a scattering angle of 173°) and forward (13°) scattering modes. Particle size of Sample1-3 shows various sizes of particles ranging from 70-250 nm. The Z-average of the samples being 116.7 nm, 103.4 nm and 96.89 nm for sample A, B, and C respectively. The Z-average for each sample increases with time over 24 hours (Figure 7-9). However, the average particle size decreases with the increase in leaf broth volume (Table 2, Graph 1). This might happen when the solvent molecules contribute to the particle size distribution in the scarcity of substrate molecules (leaf broth), yielding erroneous results. Whereas, when the volume of leaf broth increases the particle size decreases because of abundance of substrate molecules where solvent molecules bind. Also, since the particles are in various phases of nucleation they lead to varying size of particles in the solution.

Table 2:- Comparative study – Z-average versus Polydispersity index (PDI)

| Sample | Z-average (d.nm) | PDI  |
|--------|------------------|------|
| A      | 116.7            | 0.233|
| B      | 103.4            | 0.249|
| C      | 96.89            | 0.257|

The zeta potential of a sample determines whether the particles within a liquid tend to flocculate or not. Nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. The zeta potential values are -10.0 mV, -8.45 mV and -8.19 mV for samples A, B and C respectively (Figure 10-12). The results depict that the synthesized AgNPs might flocculate or form agglomerates.

Polydispersity is a measure of the heterogeneity of size of molecules or particles in a mixture. The maximum PDI value is arbitrarily limited to 1. Increase in the PDI is attributed to the formation of large number of particles formed as a result of high concentration of broth reducing the silver ions. The PDI increases with decrease in the particle size but, the difference in PDI of each solution is too low and hence, the synthesized AgNPs are monodisperse (Table 2, Graph 2).
FE-SEM:-
Field Emission-SEM micrographs were obtained using Hitachi FE-SEM, Model S-4800. The surface morphology and topography of synthesized AgNPs were examined. Well defined spherical and monodisperse AgNPs were evidenced. The particle size range from 70-250 nm. The size of nanoparticles conform to the results obtained by Zetasizer Nano ZS.

XRD:-
The structure of biologically synthesized Ag nanoparticles were analyzed by XRD measurements. A typical XRD pattern of the Ag synthesized by biobased process was found to possess face centered cubic (fcc) structure. The Bragg’s reflections at 2θ=38.20, 44.39, 64.59 can be indexed to the (111), (200) and (220) orientations, respectively, confirmed the presence of silver nanoparticles (Figure-13). In addition, the unassigned peaks suggested the crystallization of bioorganic phase occurs on the surface of the nanoparticles. T.C. Pratha et al. found the similar outcomes in their investigation on silver nanoparticles [21]. Bragg reflections of face centered cubic structure of metallic silver (Joint Committee on Powder Diffraction Standards No.-04-0784), reveals that the synthesized are composed of pure crystalline silver.

Figures:-

Figure 1:- Schematic diagram of nanoparticle synthesis and characterization.

Figure 2:- Color change of solutions depicting synthesis of silver nanoparticles for various concentrations of leaf broth.
Figure 3:- UV-vis spectra results of sample A showing peak for silver nanoparticle.
Figure 4: UV-vis spectra results of sample B showing peak for silver nanoparticle
Figure 5: UV-vis spectra results of sample C showing peak for silver nanoparticle.
Figure 6: UV-vis spectra results of three different concentrations showing peaks for silver nanoparticle.

Figure 7: Size distribution results for sample A over time.
Figure 8: Size distribution results for sample B over time.

Figure 9: Size distribution results for sample C over time.

Figure 9: Zeta Potential distribution result of sample A.
Figure 10: Zeta Potential distribution result of sample B.

Figure 11: Zeta Potential distribution result of sample C.

Figure 12: SEM images of silver nanoparticles
Figure 13:- XRD pattern of synthesized AgNPs.

Graph 1:- Comparative study of particle size for different concentrations after 24 hours.
Graph 2:- Comparative study of polydispersity for different concentrations.

Conclusion:-
The rapid reduction of Ag ions into spherical-shaped and monodisperse particles using Colchicum autumnale leaf broth, provides several advantages in the direction of biogenic process and also denotes the superiority over the chemical synthesis in providing green, environmentally safer method of nanoparticle production. Silver nanoparticles were synthesized in ambient conditions and characterization of synthesized nanoparticles was carried out by UV–Vis spectrophotometer, DLS, FE-SEM and XRD. It is believed that phytochemicals present in the leaf broth of Colchicum autumnale have reduced the silver nanoparticles. The synthesized AgNPs were spherical shaped, monodisperse and 90-250nm in diameter. DLS results show a typical trend wherein the particle size reduces with increase in the concentration of leaf broth. XRD spectra indicate the face centered cubic symmetry of synthesized AgNPs.

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References:-
1. Kong Y, Chen J, Gao F, et al. A multifunctional ribonuclease-A conjugated CdTe quantum dot cluster nanosystem for synchronous cancer imaging and therapy. Small.; 6(21):2367–2373(2010).
2. Zhang Y, Yang D, Kong Y, Wang X, Pandoli O, Gao G. Synergetic antibacterial effects of silver nanoparticles on aloe vera prepared via a green method. Nano Biomed Eng.;2(4):252–257(2010).
3. Yang DP, Chen S, Huang P, et al. Bacteria-template synthesized silver microspheres with hollow and porous structures as excellent SERS substrate. Green Chem.;12:2038–2042(2010).
4. Shukla R, Nune SK, Chanda N, et al. Soybeans as a phytochemical reservoir for the production and stabilization of biocompatible gold nanoparticles. Small.;4(9):1425–1436(2008).
5. Tripathy, A.; Raichur, A. M.; Chandrasekaran, N.; Prathna, T.C.; Mukherjee, A.;Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachta indica (Neem) leaves J. Nanopart. Res. 12, 237(2010).
6. David E, Elumalai EK, Prasad TNVKV, Venkata Kambala, Nagajyothisi PC. Green synthesis of silver nanoparticle using Euphorbia hirta L and their antifungal activities. Archives of Applied Science Research, 2:76-81(2010).
7. Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19:1357–1361, (2003).
8. Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids Surf A Physicochem Eng Asp 339:134–139, (2009).
9. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol Prog 22:577–583, (2006).
10. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B: Biointerfaces, 76:50–56, (2010).
11. Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N, Dhanaraj SA Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. J Saudi Chem Soc 15:113–120, (2010).
12. Ghaffari-Moghaddam M, Hadi-Dabanlou R Plant mediated green synthesis and antibacterial activity of silver nanoparticles using Crataegus douglasii fruit extract. J Indus Eng Chem 20:739–744, (2014).
13. Ghaffari-Moghaddam M, Hadi-Dabanlou R, Khajeh M, Rakhshanipour M, Shameli K Green synthesis of silver nanoparticles using plant extracts. Korean J Chem Eng 31:548–557, (2014).
14. G. Oberdörster, E. Oberdörster and J. Oberdörster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ Health Perspect., 113, 823–839 (2005).
15. Anastas, P. T., Meeting the challenges to sustainability through green chemistry. Green Chemistry, 5 (2), G29-G34, (2003).
16. Frattini A, Pellegrin N, Nicastro D, De Sanctis O. Effect of amine groups in the synthesis of Ag nanoparticles using amino silanes Mater Chem Phys, 94, pp.148, (2005).
17. M. Ip, S. L. Lui, V. K. M. Poon, I. Lung, A. Burd Antimicrobial activities of silver dressings: an in vitro comparison, J. Medical Microbiol. 55, 59–63, (2006).
18. Pandey S, Oza G, Mewada A, Sharon M. Green Synthesis of Highly Stable Gold Nanoparticles using, Momordica charantia as Nanofabricator. Archives of Applied Science Research., 4: 1135-1141, (2012).
19. Y. Zhao, Y. Jiang, Y. Fang. Spectroscopy property of Ag nanoparticles, Spectochim. Acta A. 65, 1003, (2006).
20. M. Sastry, K.S. Mayya, K. Bandyopadhyay, pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles, Colloids Surf. A, 127 221, (1997).
21. T. C. Pratha, Lazar Mathew, N. Chandrasekaran, Ashok M. Raiuchur and Amitava Mukherjee, Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size, Colloids Surf B: Biointerfaces 82, 152–159, (2011).