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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CITRUS SINESIS FRUIT EXTRACT AND ITS ANTIBACTERIAL ACTIVITIES

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ABSTRACT- The synthesis of metal nanoparticles is a growing area for research due to its potentiality in the application and development of advanced technologies. In general, nanoparticles are synthesized by using chemical methods which are not eco-friendly. Here, we have used a fast, convenient and environment-friendly method for the synthesis of silver nanoparticles by reducing silver nitrate with fruit extract of Orange (Citrus Sinesis). Characterization of the metallic nanoparticles was done by XRD analysis, UV Visible spectroscopy, FTIR, Scanning electron microscopy and Energy Dispersive X-ray Spectroscopy (EDAX), Antibacterial activity.

Keywords- Orange fruit extract, silver Nano particle, UV, FTIR, XRD, SEM, EDAX, Antibacterial activity

1. INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly [1, 2, 3]. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics [4], antimicrobials and therapeutics [5,6], Catalysis[7] and micro-

electronics[8]. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions[9], chemical and photochemical reactions in reverse micelles[10], thermal decomposition of silver compounds[11], radiation assisted[12], electrochemical[13], sonochemical [14], microwave assisted process[15] and recently via green chemistry route[16,17,18].

The use of environmentally benign materials like plant leaf extract [19], bacteria [20], fungi [21] and enzymes [22] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [23, 24]. 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.

Here in, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of orange fruit. Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens.

2. PREPARATION OF FRUIT EXTRACT

Fresh orange was washed and later smashed inside a grinder. The smashed fruit was then filtered to remove the debris. The filtered juice was centrifuged at 10000 rpm for 10 minutes to obtain the liquid fruit extract at last the clear solution was collected and stored 4°C for further experiment

3. SYNTHESIS OF SILVER NANOPARTICLES

10ml of 0.01M Silver nitrate solution (10ml) and orange extract (10ml) was mixed in the ratio 1:1 and the mixture was constantly stirred. The mixture was kept in microwave oven at 60°C for 3 minutes, and cooled to room temperature. After 1 hours change in the color from white color to black color and the silver Nanoparticle was presented. That solution was kept in microwave oven at 150°C for 3 hours and cooled to room temperature. The silver Nanoparticle was presented the beaker.

4. RESULT AND DISCUSSION

4. 1. Optical Studies

The bio reduction of Ag ions in aqueous solution is monitored by periodic sampling of aliquots of the mixture and subsequently measuring UV-V is spectra. The UV-V spectral analysis was done by using spectrophotometer at the range of 421nm and observed the absorption peak for Citrus Sinesis due to excitation of surface Plasmon vibrations in the silver nanoparticle, which are identical to the characteristic UV-Visible spectrum of metallic silver and it was recorded. In the synthesis of silver nanoparticles, the color changes occurred when orange fruit extract is added with AgNO₃ solution. This color change was due to the reduction of Ag⁺ into Ag⁰ which leads to the formation of silver nanoparticles.

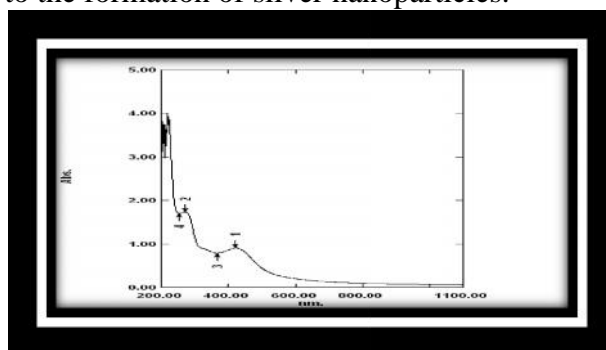


Figure 1- UV-Visible spectra of AgNPS for Citrus sinesis

5. STRUCTURAL STUDIES

The application of Ag NPs was highly dependent on the chemical composition, shape, size, and mono disparity of particles. For the crystalline nature of the AgNPs, intense XRD peaks were observed corresponding to the (111), (200), (220), (311) planes at 2 angles of 38.28°, 44.38°, 64.54°, and 77.64°, respectively. This was in good agreement with the unit cell of the face centered cubic (fcc) structure (JCPDS card no #893722) with a lattice parameter of $a = 4.085 \text{ \AA}$. Because of the biomass residue, other crystallographic impurities were also observed in the XRD profile. The size of AgNPs according to the XRD was about 24 nm.

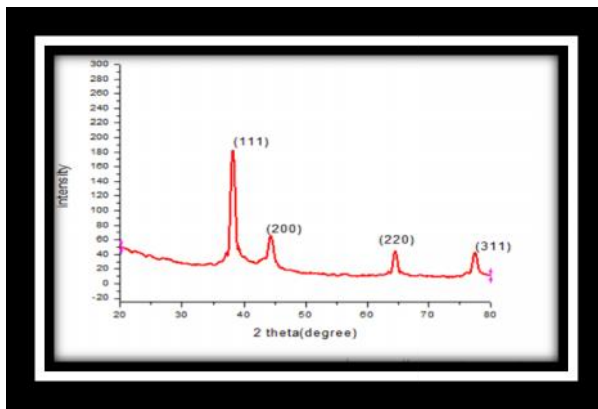


Figure 2- The result of XRD spectrum.

6. FTIR STUDIES

FTIR was used to analyze the functional group present in the synthesized silver nanoparticles. FTIR spectrum of synthesized silver nanoparticles was represented in figure FTIR measurements were carried out to identify the possible biomolecules in the orange fruit extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic Nanoparticle solution.

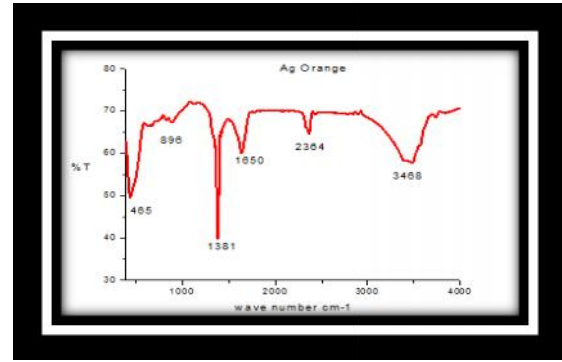


Figure 3- The result of FTIR analysis

WAVENUMBER (cm ⁻¹)	MOLECULAR MOTION	FUNCTIONAL GROUP
896	PH bend	Phosphines
1381	-NO ₂ (aliphatic)	Nitro groups
1650	C=O stretch	Amides
2364	O-H stretch	Carboxylic acids
3468	N-Hstretch(1 per N-H bond)	Amines

7. ENERGY DISPERSIVE X-RAY SPECTROSCOPY

The presence of metal elemental Ag was determined. The samples were dried at room temperature and then analyzed for the samples composition of a synthesized Nanoparticles. The presence of the elemental silver can be seen in 3kev in the graph presented by the EDAX analysis, which indicate the reduction of silver ions. It has been reported that Nanoparticle synthesized using fruit extract are surrounded by a thin layer of some capping organic materials from the fruit that remains stable in the solution even after synthesis.

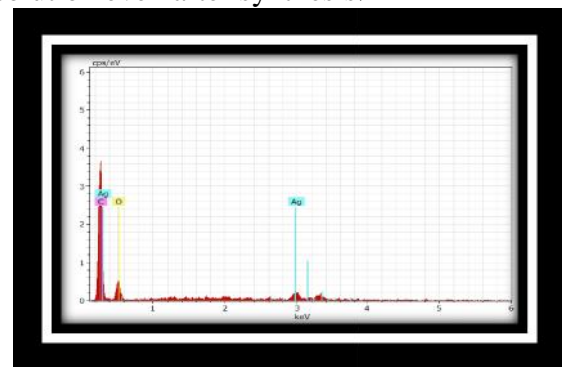


Figure 4- The result of Energy Dispersive X- Ray Spectroscopy for Ag Nano Particles

8. SCANNING ELECTRON MICROSCOPY

The size of synthesized nanoparticles was determined by SEM. SEM images provided information about the morphology and size of the biosynthesized silver nanoparticles. The below are the obtained SEM images for the synthesized of the fruit extract. The SEM images show Rock like structure.

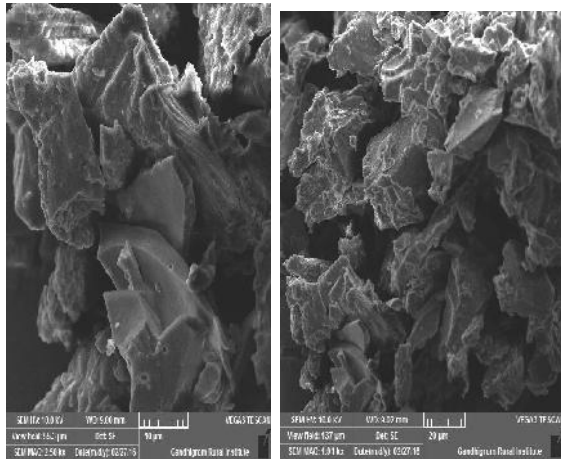


Figure 5- The result of SEM image for Ag Nano Particles

9. ANTIBACTERIAL ACTIVITY

The antibacterial activity done on E.coli and B.Cereus and the zone of inhibition is 11cm and 1 2cm respectively.

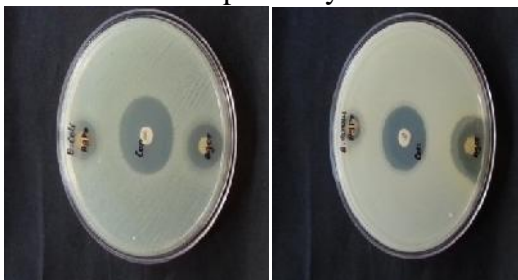


Figure 6- The result of Antibacterial activity image for Ag Nano Particles

The antimicrobial activity of prepared silver nanoparticles was performed against pathogenic bacteria. Pathogens subjected in the present study were E.coli and B.Cereus. Bacterial growth inhibition around the well is due to the release of diffusible inhibitory

compounds from silver nanoparticles. The present study clearly indicates that the synthesized silver nanoparticles have good antimicrobial action against E.coli and B.Cereus. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles.

CONCLUSION

The present study reported on the synthesis of silver nanoparticles by citrus sinesis fruit extract without using any harmful reducing or capping agents. The bio-reduction of aqueous Ag⁺ ions by the fruit extract has been demonstrated. It is found that these fruit can be a good source for the synthesis of silver nanoparticles. The green synthesis of silver nanoparticle using fruit extract were performed by adopting standard procedure were characterized by XRD,UV,FTIR,EDX and SEM studies. Due to surface Plasmon Resonance during the reaction with the ingredients present in the fruit extracts color changes which result in the formation of silver nanoparticles. The typical XRD pattern revealed that the average size of silver nanoparticles was found to be 24 nm.The spectroscopic analysis using UV-visible spectroscopy which gave a peak 421nm proved the formation of AgNPs. The FTIR analysis of the fruit extract mediated AgNPs was performed and indicate the presence of amines, amides, and nitro group's .The SEM image which we obtained is rock like structure, and EDAX indicate the presence of silver in the fruit extract. Application of such eco-friendly AgNPs in the different fields of science like medicine, catalysis, drug delivery system etc., makes this bio reduction process a highly suitable way for large scale synthesis. Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and it has the capability of killing different type of diseases causing microorganisms.

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