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Biological synthesis of silver nanoparticles by Neptunia oleraceae

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Abstract: **Introduction: Green synthesis of nanoparticles is a novel way to synthesize nanoparticles by using biological sources. It is gaining attention due to its cost effectiveness, eco-friendly nature and large scale production possibilities. Again silver nanoparticles are of special interest to researcher due to its evocative physical and chemical properties.**

Aim: The study was designed to synthesize silver nanoparticles using the aqueous solution of Neptunia oleraceae fresh leaf extract and AgNO3.

Materials and Methods: A fixed ratio of plant extract to metal ion was prepared and the colour change was observed that proved the formation of nanoparticles. The silver nanoparticles synthesized were confirmed by their change of colour to dark brown due to the phenomenon of surface plasmon resonance. The characterization was done by UV-VIS spectroscopy and X-RAY diffraction analysis. The size of the silver crystallites has been estimated using Debye Scherer formula. Stability of the nanoparticles under different conditions was studied and the phytochemicals responsible for the reduction of silver nanoparticles were also tested qualitatively. SEM was done to confirm the size and presence of the nanoparticles.

Results: The size of the silver nanoparticles was found to be 8.35 nm, which confirms the formation of nanocrystalline silver. Again the shape of the nanoparticles was spherical and appeared in cluster. The biological synthesis of silver nanoparticles was possible by using the plant extract Neptunia oleraceae.

Conclusion: The biological approach is the need of the hour in the field of nanotechnology and the advancement in India in this aspect is still in its infancy. Also, the antimicrobial activity of the silver nanoparticles synthesized by the current study needs to be carried out along with FTIR analysis to know the responsible phytochemicals.

Keywords: biosynthesis, silvernanoparticles, Neptunia oleraceae, UV-VIS Spectrophotometer, XRAY Diffraction, SEM.

1. INTRODUCTION:

Nanoparticles have been extensively used due to their immense application in diverse fields. Gold, carbon, iron, zinc, silver nanoparticles are of special interest as they have been found to show promising roles in different biological burning issues. In recent years, the concepts of nanotechnology are being applied in various fields' like biotechnology, physics, chemistry, material sciences, engineering, medicine etc. Nanoparticles can be synthesized by various physical and chemical methods, but such methods encounters many problems like expensive reagent, hazardous reaction condition, tedious and sophisticated techniques to isolate nanoparticles etc. Moreover, these methods are also time consuming. Hence, cost effective, cheap, rapid and eco-friendly protocols to synthesize nanoparticles are the need of the hour.

The impact of nanotechnology is growing all spheres of human life. The biological processes leading to the formation of nano scale inorganic material is strongly taken as an eco-friendly alternative to the other methods which suffers various drawbacks. Nano materials can be used as novel antimicrobial agents due to their unique properties which include attaching with the microbes and permeating their cells thereby destroying it completely. The biosynthesis of silver particles via green chemistry route and their effectiveness as antimicrobial agents has been immensely emphasized. Moreover, the biosynthetic method that uses plant extracts has attracted the scientists from different fields due to its simplicity, and environmentally-friendly nature as it does not involve the use of harsh, toxic and expensive chemicals. Biosynthesis would have greater commercial acceptance if the nanoparticles could be synthesized economically on a large scale in a faster rate. Presently, bacteria, fungi and algae are being used for the synthesis of nanoparticles but the green method which is using plant extract reduces the cost. Also it does not involve any special culture preparation and isolation techniques.

Nanoparticles are generally in the range of 1-100 nm which can be made from materials of different chemical nature, metals, polymers, biomolecules, organics, silicates etc. They can be of different morphologies like spheres, cylindrical, dumbbell shaped or platelets. Nanoparticles vary depending upon some factors like their size, morphologies, chemical nature, medium in which they are present etc. They have been grouped into two types namely, organic and inorganic nanoparticles. Nanoparticles can be synthesized in different ways like physical, chemical and biological approaches. Physical approaches include evaporation-condensation and laser ablation technique; chemical approaches include chemical reduction by organic and inorganic reagents; and biological approaches include reduction by microorganisms and plants.

Silver nanoparticles are being extensively used nowadays in antimicrobial applications, cosmetic products, biosensor materials, optical catalysts, electrometer, medical imaging , drug delivery, Nano composites, memory schemes, bio labeling nanowires ,animal husbandry, health industry , food storage, textile coating etc.

1.1 Synthesis of silver nanoparticles from plants

Various microorganisms such as bacteria, algae, fungi and yeasts are used for the biosynthesis of nanoparticles but recently a new trend has come to force i.e., the use of plants for the fabrication of nanoparticles because of its spontaneous, economical, eco-friendly protocol, suitable for large scale production and single step technique for the biosynthesis process (Huang et al., 2007). The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides (Prabhu et al., 2012).

Neptunia oleracea

Neptunia oleracea, commonly known as **water mimosa** is a pantropical nitrogen-fixing perennial legume, an aquatic plant that has sensitive leaves and white spongy tissues around its stems.

2. MATERIALS AND METHODS

2.1 Preparation of plant extracts:

Fresh leaves of the plants Neptunia oleraceae were purchased from the local markets of Guwahati, Assamand were weighed for 50 grams separately.They were then washed several times to remove the dust particles and then finally washed with distilled water.The leaves were then crushed with the help of juice extractorand then diluted to 100ml of distilled water in a conical flask which was then heated to 60 -80 degree celsius in the hot water bath for 10 to 15 minutes. The extract was filtered with whattman filter paper and then centrifuged at 5000 rpm for 20minutes. 0.05M of silver nitrate solution is prepared in distilled water and 1ml of the plant extract is added to 10ml of the 0.05M silver nitrate solution in a test tube and is heated in the hot water bath to 100 degree celsius for 15minutes. A change in colour of the solution from colourless to pale yellow to dark brown is the first confirmation test for the reduction of silver ions to silver nano particles. A part of the solution is given for analyzing the absorbance of the solution using UV-Vis Spectrophotometer in Guwahati Biotech Park, IIT GHY [INSTRUMENT NAME AND MODEL NUMBER] by diluting a part of the colored solution.

2.2 Stability of silver nanoparticles:

To check the stability of the silver nanoparticles, the solution of the silver nitrate after adding the plant extract is checked in three ways with and without applying physical stress- first by directly applying heat to 100 degree Celsius, second by keeping it at room temperature without heating and third by keeping it at dark room without heat. By analysing the above three ways of the reaction it was found that the desired colour change took place in case of applying direct heat and at room temperature without heating within 15 minutes of time. The colour change was not observed in case of dark room even after keeping it for a week which gives us the information that some amount of heat is needed for the reduction process.

2.3UV-VISIBLE Spectrophotometer analysis:

Absorbance spectroscopy is used to determine the optical properties of a solution. A part of the solution was analyzed for absorbance using UV-Vis Spectrophotometer in Guwahati Biotech Park, IIT GHY [INSTRUMENT NAME AND MODEL NUMBER] at Specord 210 plus spectrophotometer at a resolution of 2 nm (from 320 to 650 nm) in 2 ml quartz cuvette with the speed of 20 nm/sec.

2.4 X-RAY diffraction analysis:

X-ray crystallography is a tool used for identifying the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-raysto diffract into many specific directions. The XRD machine of IASST ,GHY [NAME AND MODEL NUMBER] was run on the sample with a voltage of40 KV,

current 40 mA, step size 0.04degree with the range from 10 to 55 degree angle.The coloured solution containing the silver nitrate and the plant extract was washed many times with distilled water to remove the impurities by centrifuging it with the help of table top refrigerated centrifuge [Hermle] in Guwahati Biotech Park and removing the supernatant and concentrating on the pellet.The pellet was then loaded on a square glass slide with the help of a micropipette and then kept in a lyophilizer [Labcanco] attached to a vacuum pump for about 2 hours. After the sample got dried it was taken out and sent for XRD analysis.

1.6 SEM and EDAX analysis:

The coloured sample was made powdered using Speed Vac –Eppendorf Concentrator plus. A very small portion of the sample was taken and thencoated with gold using Fine Coat Ion Sputter JFC-1100 and then given for SEM analysis which was done using JEOL JSM-6360 SEM as well as for EDAX analysis which was done using EDAX-OXFORD Instrument INCA Penta FET *3 in SAIF , NEHU Shillong.

2. RESULTS:

3.1 Stability of silver nanoparticles:

The colour change was not observed in case of dark room even after keeping it for a week which gives us the information that some amount of heat is needed for the reduction process. The change in colour at different time intervals and application of heat is reflected in the colour change.

3.2 UV-Visible spectrometry analysis:

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-VIS spectrum of the Specord 210 plus spectrophotometer at a resolution of 2 nm (from 320 to 650 nm) in 2 ml quartz corvette with the speed of 20 nm/sec. The UV absorption peak of silver nanoparticles ranges from 400 nm – 450 nm. Figure 4 shows the UV absorption peaks of N.oleraceae, UV-Vis spectra showed the peaks from 400 to 440 nm clearly indicating the formation of spherical AgNPs in the plants extract. The occurrence of the peak at this range is due to the phenomenon of surface plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field. The peaks gradually decreased which indicates no further formation of nanoparticles (Figure 1).

3.3 XRD- Analysis

From the XRD analysis, the FWHM was achieved, diffraction angle for a certain peak in the 2θ versus intensity graph and using the known value of the wavelength and shape factor the crystallite size of the deposited film was determined. The observed diffraction peak is at diffraction angle of 38.10 with d-spacing of 2.359 ^{0}A matched with JCPDS pattern number 00-004-0783 of FCC silver. Along with that, the presence of Silver Oxide (AgO) (JCPDS Pattern number 01-076-1489) at 2□□value of 32.08 with a d-spacing of 2.78 ^{0}A is also detected. AgO may be formed after formation of silver nanoparticles, which reacts with water (H_2O) in the solution since the nanoparticles are highly reactive due to their high surface to volume ratio. The broad hump at around 2□□value of 23.50 may be due to glass substrate. Further, two other diffraction peak with value of 27.66 and 46.15 were identified. The size of the silver crystallites has been estimated using Debye Scherrer formula,$t=k\lambda/(B\cos\theta_B)$. Taking $\lambda = 1.54056$Å (Cu K□), k = 0.9 the size has been found to be 8.35 nm, which confirms the formation of nanocrystalline silver [Figure 2].

3.4 SEM Analysis:

The SEM analysis of the powdered sample confirmed the presence of silver as nanoparticles. The images show the presence of the silver nanoparticles in clusters as well as in the form of individual particles [Figure 3]. It gives a brief idea about the morphology and size of the nano particles. The analysis confirms their size to be less than 10 nm and the shape to be spherical.

3.5 EDAX Analysis:

The EDAX analysis gives an account of the elements present in the sample. The elements present are shown in the form of different peaks which varies depending upon the amount of the particular element present. The graph also shows the peak of silver thereby confirming its presence. The weight percentage of the elements Cl K, Rh L, AgL, Au M has been found to be 10.80, 8.83, 70.93, and 9.44 respectively and the atomic percentage as 27.79, 7.83, 60.00 and 4.37 respectively [Figure 3].

3. DISCUSSION:

The present study reports the bio reduction of silver ions through fresh leaf extracts and testing for their phytochemicals qualitatively. The photosynthesis of silver nanoparticles was confirmed firstly by visual observation: the colorless sample turned brown after addition of AgNO3 0.05

M solution due to excitation of surface plasmon vibrations indicating the formation of silver nanostructures. The synthesis of silver nanoparticles using fresh leaves were detected also by UV-VIS absorption spectra showing a strong plasmon resonance which was centered between 410-450 nm, the peaks formed in that range gradually decreased which indicated completion of the formation of the nanoparticles after a period of time which was accordance of the findings in the "Biosynthesis of silver nano particles' by Bunghez et al where the absorption spectra of silver phyto-nanoparticles were recorded after 24 hours after their preparation and exhibited absorbance peaks at 415 to 455 nm range. The structure was confirmed by XRD analysis. The possible explanation for the nanoparticles formation can be due to the presence of different phytochemicals like tannin, glycosides, terpenoids, phenol, flavanoids etc. which were tested qualitatively and found positive in the study sample. The stability was also studied by observing the colour change in the presence of light, high temperature and also dark room, but the colour change was not observed in case of dark room even after many days which shows the importance of heat in the process of synthesis.

In the XRD analysis, it has been found that the observed diffraction peak at diffraction angle (2□) of 38.10 with d-spacing of 2.359 ^{0}A matches with JCPDS Pattern number 00-004-0783 of FCC silver. Along with that, the presence of Silver Oxide (AgO) (JCPDS Pattern number 01-076-1489) at 2□□value of 32.08 with a d-spacing of 2.78 ^{0}A is also detected. AgO may be formed after formation of silver nanoparticles, which reacts with water in the solution since the nanoparticles are highly reactive due to their high surface to volume ratio. Also, the broad hump at around 2□□value of 23.50 may be due to glass substrate. Further, two other diffraction peaks at 2□□value of 27.66 and 46.15 could be identified.

4. CONCLUSION:

The size of the silver crystallites was found to be 8.35 nm. Green synthesis of silver nanoparticles with the help of green plants is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale. Neptunia oleraceae showed great capability to synthesize AgNPs at optimum temperature conditions. For further studies, the nanoparticles can be visualized through SEM or TEM analysis, the exact phytochemicals responsible for the reduction of AgNP scan be found out and the synthesized nanoparticles can be applied to different microbial cultures and study their inhibition zones.FTIR analysis shall explain the exact phytochemicals or protein responsible for nanoparticles synthesis.

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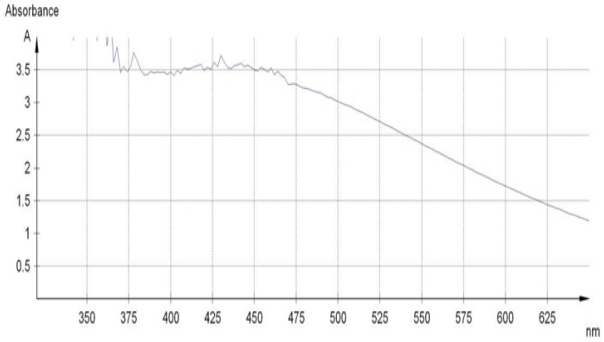

Figure 1: Graph showing results of UV-VIS analysis.

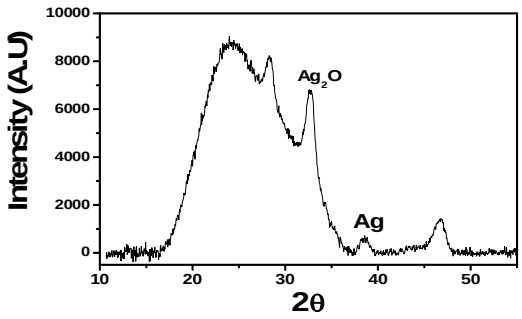

Fig 2: X-ray diffraction pattern of nanoparticles film

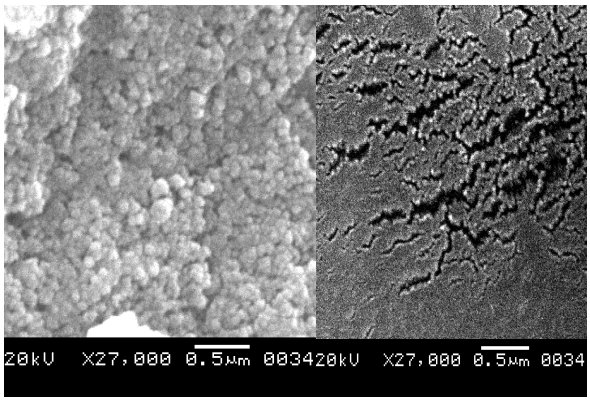

Figure 3: SEM photographs showing the cluster of spherical nanoparticles.

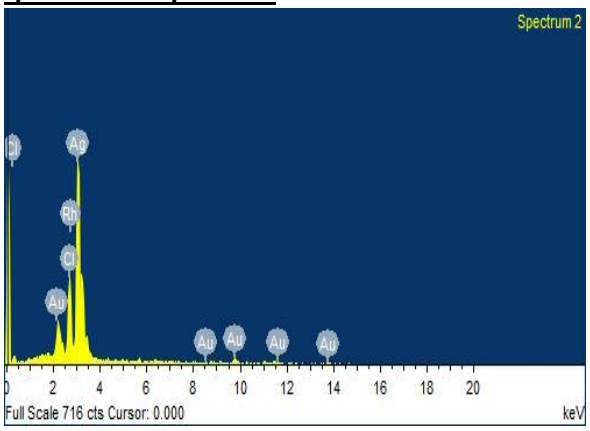

Figure 4: EDM peaks.