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Silver Nanoparticles Green Synthesised Using Catharanthus Roseus and Its Anti-Bacterial Efficacy

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Abstract

Nanotechnology involves the design and development of highly ordered nanostructures. Among various methods for preparation of nanomaterials, green synthesis is put forward as an economic and eco-friendly replacement to chemical or physical approach which are toxic and stressful to the environment. This work presents a modest and feasible fabrication of silver nanoparticles (AgNPs) through bio-reduction using aqueous extract of Catharanthus roseus (Vinca Rosea) flowers. Characterisation such as XRD, SEM, UV-Visible Spectroscopy and FTIR are done to examine its surface morphology and physiochemical properties. Anti-bacterial activity against Enterococcus Sp, E.coli, P. aeroginosa and K. pneumonia were studied via agar well Diffusion Assay and the results are discussed.

AgNPs synthesized from C. roseus flowers show high crystallinity with diffracted angles in XRD analysis, corresponding to face centred cubic Ag lattice with small crystallite size. SEM analysis reveal predominantly spherical and uniformly shaped nanomaterial (NM). FT-IR spectrum shows characteristic peaks confirming that phytochemicals in Catharanthus roseus stands as potential capping and stabilizing agent through the presence of biomolecules like alkaloids (Vinblastine, Vincristine, etc) and flavonoids. Zone of inhibition formed against Gram-negative Pseudomonas aeruginosa bacteria is stronger when compared to the other species of interest. Thus, the green synthesised AgNPs from C. roseus flowers can be potentially used as antifouling agent against Pseudomonas aeruginosa bacteria in bio-medical systems and also in the pharmaceutical industry as strong antibacterial agent while administrating drug delivery.

Key Words: Green synthesis; Catharanthus roseus; Silver nanoparticles; X-Ray Diffraction analysis; Scanning Electron Microscopy; Antibacterial activity.

1.Introduction

Nanotechnology has emerged as a widely developed research field in nano-dimensional materials in various application like energy storage / harvesting, sensing / bio-sensing, water treatment, bio-medical, etc. In this 21st century, nanoscience has become ground for rich technological innovations. Nanoparticles exhibit extremely improved properties as compared with their massive counterparts derived due to their major characteristics such as morphology, reduced size and distribution [1]. Most important and unique property is that they express large surface area to volume ratio. Synthesis of nanomaterials (NM) is done by numerous physical and chemical methods but biological methods are simple and eco-friendly [2].

Conventional fabrication of metal nanoparticles (NPs) have huge price tag and hazardous to the environment. Hence "Green Technology" is preferred. Recently, preparation of nanoparticles using plant materials like flower or leaf extract as reducing agent has received more attention in the field of nanofabrication [3]. Several medicinal plants contain bioactive compounds like alkaloids and flavonoids that are effective to cure diseases and used in pharmaceuticals. Vinca rosea (Catharanthus roseus (L.) G.Don) is a perennial herb grown in Australia, Africa India and South Europe for its medical use. It contains more than 70 alkaloids, mostly of indole type [11]. Alkaloids like Ajamalicine, Reserpine and Serpentine are renowned for their antispasmodic and hypotensive properties. The root consists of alkaloid, which has Alstonine, has the ability to reduce blood pressure. Vinblastine and Vincristine are the Alkaloids which acts as anticancer drugs in the treatment of different type of cancers like Lymphomas, Hodgkin disease, Breast cancer, acute lymphocytic leukaemia, soft tissue sarcomas. Multiple myeloma and Neuro blastoma [12–16]. Presence of phytochemicals as bioreducing agents in medicinal plants like Catharanthus roseus have an advantage to develop plant based bio-nanomaterials [4]. AgNPs synthesized from aqueous extract of C.roseus [1] shows new horizon for targeted drug delivery.

AgNPs are used extensively as anti-bacterial agents in the health industry for disinfecting medical devices, also in water treatment, food storage, textile coatings and a number of environmental applications. Sheshadri et al reported synthesis of AgNPs from aqueous extract of C. roseus leaf and elucidated the effectiveness of melatonin, a natural hormone found in C. roseus as growth promoter and stress alleviator [5]. Identical work has been reported all over the world on the biogenic synthesis and biomedical applications of AgNPs [6]. Dillenia indica fruit extract mediated AgNPs with antioxidant potency and its biochemical composition was conferred [7]. Green synthesized AgNPs were formed by the usage of leaf extract of Mimusops elengi. Due to presence of various phyto-organic compounds like alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides and benzenoids the AgNPs show good antimicrobial efficacy against multi drug resistant clinical isolates [8]. A correlative study of morphology, reactivity and stability of AgNPs using Gram positive bacteria Bacillus subtitles and Catharanthus roseus has also been reported [9]. Antioxidant potential and indole alkaloid profile variations with water deficit stress from root, stem, leaf, flower and pods of Catharanthus roseus (Pink flowered) and white -flowered alba has been confirmed [10].

This paper exhibits modest and bio friendly fabrication method of silver (Ag) nanoparticles (NPs) through bio-reduction with dried flower aqueous extract of Catharanthus roseus (L.) G.Don (Vinca Rosea). The NM is crystalline in nature which was confirmed by Scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) and X-ray diffraction pattern (XRD). Due to Surface Plasmon Resonance the UV Visible spectrum show strong absorbance at 422nm. FTIR peaks corresponding to the various functional groups, indicate the action of phytochemicals from the plant extract in the formation and stability of the bio-reduced AgNPs. Results from Agar Well Diffusion Assay reveal the NM to have potent antibacterial effect against all bacterial species with high inhibition capability against Gram-negative Pseudomonas aeruginosa bacteria. This proves that the NM can be incorporated in bio-medical systems to best tackle Pseudomonas aeruginosa bacterial population.

2. Material and Methods

2.1. Preparation of Bio-Extract

Fresh flowers of C. roseus plant were collected and thoroughly washed, before drying under sunshade and powdered by using mechanical grinder. 10g powdered flowers were added to 100 ml of distilled water. The mixture is allowed to boil for an hour at 60°C in water bath for the complete extraction of phytochemicals and later cooled down to room temperature. As prepared bio-solution is filtered and stored at 4°C for future work.

2.2. Synthesis of Silver nano particles (AgNPs)

100mM aqueous solution of $AgNO_3$ was prepared in a conical flask. 50ml of flower extract was mixed with 50ml of silver nitrate solution placed under a magnetic stirrer. Within a particular period of time straw yellow colour solution changes to coffee brown indicating the formation of AgNPs. The mixture is kept in room temperature under continuous stirring at 100rpm for an appropriate period of time until complete bio-reduction happens. Similar colour changes were observed in other plant mediated synthesis of AgNPs [17–22] and hence reaction was completed by the confirmation of silver nitrate reduced to form AgNPs, through plant extracts. Once the solution colour turns to almost black, the obtained nano-solution is centrifuged and the settled nano-compound is collected. The nano-compound is put in a crucible in a furnace to completely dry it from its moisture content. The dried nano-compound is taken in a motor and pestle and grinded manually by hand to form Ag nano-powder into AgNPs, which is then stored for futher characterisations and applications.

2.3. Characterization

The prepared C. roseus flower mediated AgNPs was monitored by spectral analysis. Bio reduction of pure Ag^+ ions done with dried flower samples of C. roseus was observed by optical absorbance spectra with nanosample suspended in distilled water and noted on UV-Vis spectrophotometer (Shimadzu 2400 double beamed UV-Vis) in 200-700nm wavelength range. FTIR analysis was done by using Perkin- Elmer LS-55 spectrometer to identify the presence of biomolecules responsible for the bio reduction. Powder XRD analysis was carried out using Shimadzu XRD -6000/6100 MODEL with 30Kv, 30mA with CuKα radians at 2Θ range of 20^{o}-80^{o} and then inspected using XRDA software. The crystallite size of the AgNPs was derived by Debye Scherer's equation, $D=0.94\ \lambda/\beta\cos\theta$. Scanning electron microscopic (SEM) investigation was carried out using Hitachi S-4500 SEM instrument equipped with a thermo EDX attachments. As prepared powdered AgNPs were spread on a carbon coated copper grid and observed the size and morphological features.

2.4. Antibacterial assay

Antibacterial activity of the C. roseus mediated AgNPs were examined against both gram positive Enterococcus species and gram negative Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa bacteria via Agar Well Diffusion Assay. 1ml of selected suspension cultures were spread over 15ml of moulded Muller Hinton Agar plate. 5mm of wells were formed by cork-borer and 5mg/ml of samples were loaded on the wells respectively. Sterile water used as a negative control and the 10µg of streptomycin used as a positive control. The inoculated plates were incubated 24hrs at 37°C. The diameter of the zone of inhibition (ZOI) was observed and measured in millimetre around wells.

3. Results and discussion

3.1. UV-Vis Spectrophotometric analysis.

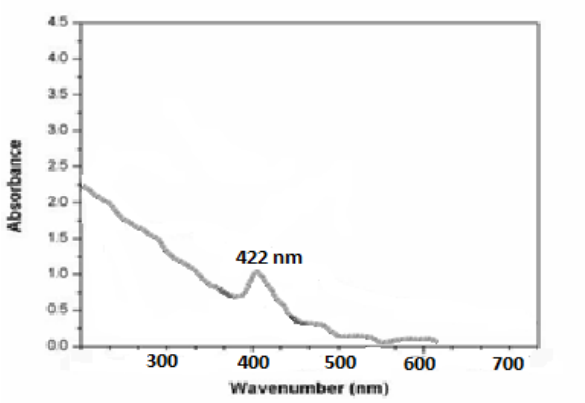

Figure 1. UV-Visible absorption spectra of C. roseus flower mediated AgNPs

The C. roseus mediated AgNPs show Surface Plasmon Resonance (SPR) absorption at 422nm depicted in Figure 1, owing to collective oscillation of electrons [23] at that particular wavelength of light. Sharp absorption edge clearly depicts smaller particle size and spherical shape of the metal NPs. In other studies reported, UV-Visible spectrum peaks ranging from 413-424nm at various time intervals (10,30,60,120 and 180mins) has been revealed in the same plant mediated AgNPs [24] and also 448.5nm of C. roseus leaf mediated AgNPs is reported [25].

3.2. FT IR analysis.

FT-IR spectrum of C. roseus flower mediated AgNPs displayed in Figure 2, shows characteristic peaks at 3408, 2931, 1730, 1620, 1327, 1188, 1073, 825, 763 and 607 cm^{-1} confirming that phytochemicals in C. roseus stands as potential capping and stabilization agent in the bio-reduction of AgNPs. These characteristic peaks are assigned to functional groups O-H bond among alcohols and phenols, C-H stretch denotes alkane, C=C stretch alkanes, Amide-I bands arising out of C=O stretch, C-H stretch due to methyl group, C-N stretch amines, C-N stretch aliphatic amines, respectively and peaks at 825, 763, 607cm^{-1} correlate to =C-H bending as tabulated in Table 1. This confirms phytochemicals present in biomolecules like alkaloids and flavonoids have successfully been deposited onto the surface of the NPs, capping and stabilising them. In previous studies some of the stretching bands were responsible for respective functional groups [26] which was reported as heterocyclic compounds due to the presence of alkaloids and flavonoids in plant sources [27,28].

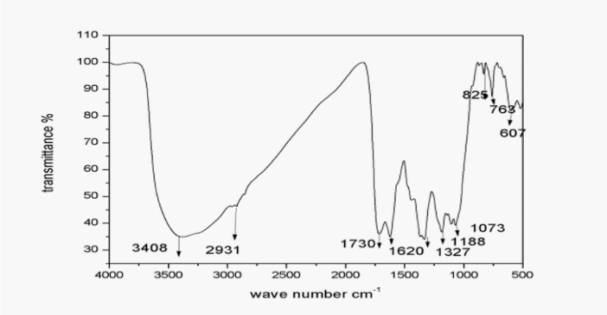

Figure 2. FTIR analysis of Catharanthus roseus flower mediated AgNPs

Wave number cm^{-1}	Peak assignment
3408	O-H bond among alcohols and phenols
2931	C-H stretch denotes alkane
1730	C=C stretch alkanes
1620	Amide -I bands arising out of C=O stretch
1327	C-H stretch due to methyl group
1188	C-N stretch amines
1073	C-N stretch aliphatic amines
607,763,825	=C-H bend

Table 1: Characteristic peak assignment for C. roseus flower AgNPs.

3.3. Powder X-ray diffraction analysis.

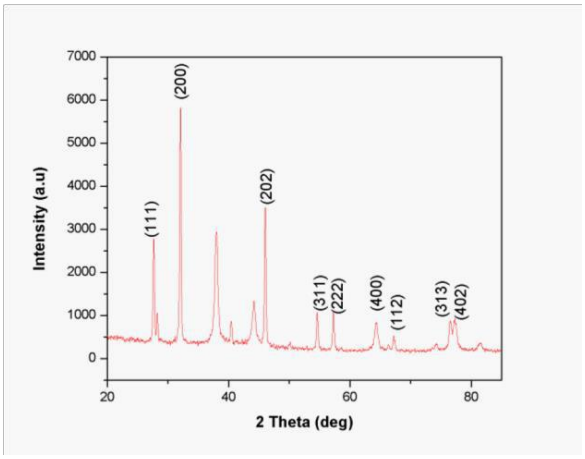

Figure 3. XRD pattern of C. roseus flower mediated AgNPs

AgNPs synthesized with C.roseus flowers show high crystallinity with prominent diffracted angles at 27.66^{O}, 32.07^{O}, 46.02^{O} and 54.6^{O} in Figure 3, which correspond to face centred cubic (fcc) Ag lines with JCPDS NO 04.0784 indexed at (111), (200),(202) and (311) hkl planes. Average crystallite size is calculated to be 11nm using Debye Scherrer equation. The lattice constant value of the synthesised Ag fcc nanocrystals are a=b=c=5.577(A^{O}).

3.4. Scanning electron microscopy (SEM) and Energy dispersive X-ray (EDX) Analysis.

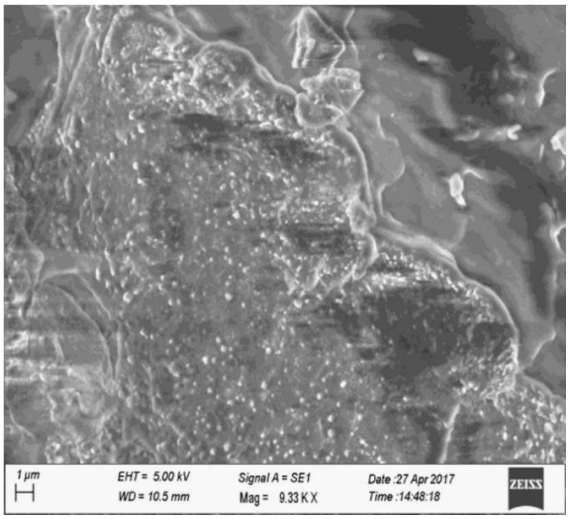

Figure 4. SEM image of C. roseus flower mediated AgNPs.

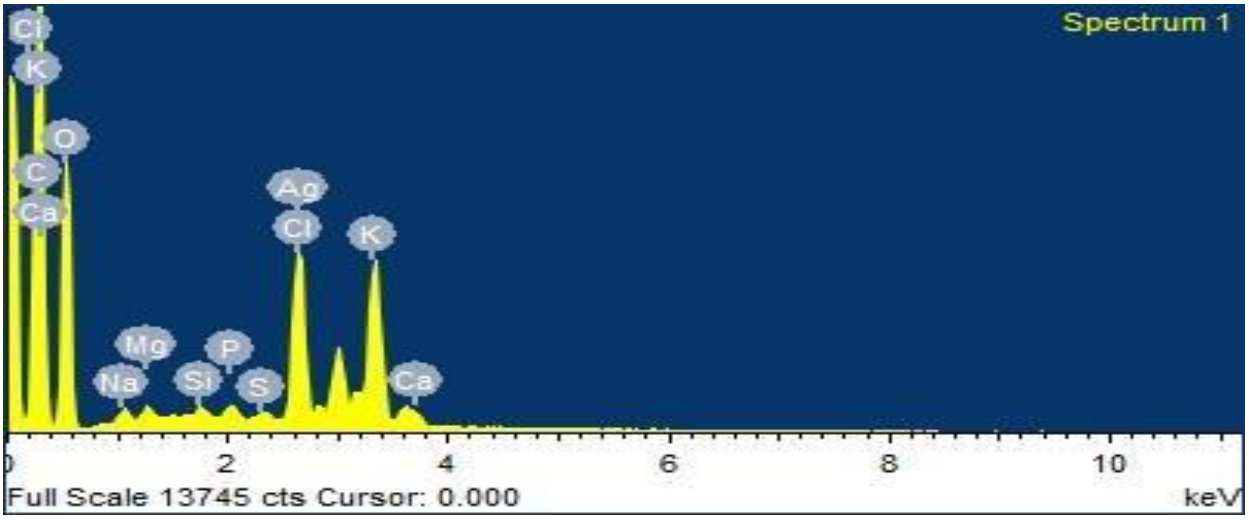

Figure 5. EDX analysis C. roseus flower mediated AgNPs.

C. roseus mediated AgNPs formed are predominantly spherical with uniform shape and diameter ranging between 6 – 11nm (Figure 4). The NM exhibit predominantly quasi-spherical morphology, rough surface texture and moderate agglomeration, which are characteristic features of plant-mediated NM. The observed morphology indicates efficient bio-reduction and stabilization by the phytochemicals present in the flower extract, making the synthesized Ag nanoparticles suitable for applications in antimicrobial activity and biomedical fields. Similar occurrence was described by Chandran et al [30] in Aloevera plant mediated AgNPs [31] and in Cassia auriculata leaf mediated AgNPs. EDX mapping (Figure 5) confirm the presence of elemental Ag. The reported identification lines for the major emission energies at 2.6kev confirm bio reduction of Ag ions to AgNPs. From EDX mapping, C. roseus flower bio reduced AgNPs show weight percentage as 3.73% and 17.44% respectively.

3.5. Antibacterial analysis.

Antibacterial activity of C. roseus flower mediated AgNPs was determined against one Gram-positive bacterium in the Enterococcus species and three Gram-negative bacterium Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa at 5mg/ml concentration via Agar Well Diffusion Assay. While the antibacterial activity of AgNPs was quite dormant when compared to the streptomycin positive control, good Zone of Inhibitions (ZOI) was observed. The growth of Enterococcus faecalis was inhibited upto 8.93mm, Escherichia coli had an inhibition zone of 9.23mm, Pseudomonas aeruginosa upto 9.88mm and Klebsiella pneumonia revealed a ZOI of 7.28mm by the green synthesised C. roseus flower mediated AgNPs. From the results obtained, it can be concluded that among all bacterial strains, the inhibition activity of Gram-negative Pseudomonas aeruginosa bacteria is stronger when compared to the other species of interest revealing the action of phytochemicals while the bio-reduction of AgNPs. Raut Rajesh et al explored the anti bacterial activities of C. roseus flower and leaf mediated AgNPs against S.aureas, E.coli, P.aeroginosa and K. pneumonia [32]. Similarly antibacterial activities of AgNPs against S.aureas and E.coli was reported by Kim et al [33]. Thus it can be concluded that the synthesised nanomaterial has great potential to be incorporated as antibacterial coating in Pseudomonas aeruginosa prone medical systems.

Conclusion

This study proves that Catharanthus roseus dried flower extract is found suitable for bio reduction of AgNPs by green synthesis, with the reaction time and concentration ratio of plant extract to metal salt being decisive for in aqueous synthesis. XRD analysis and spectroscopic characterization from UV-Vis, FTIR confirm the formation and stability of the bio synthesized AgNPs, while SEM reveals its morphology. Average crystallite size calculated from Scherrer equation is found to be 11nm, proving the spherical AgNPs to have great bio-medical activity against bacterial strains. Antibacterial activity was discussed for four bacterial species at a concentration of 5mg/ml AgNPs and concluded that though the nanomaterial has robust inhibition agaist all the bacterial strains, the inhibition is maximum for Gram-negative Pseudomonas aeruginosa bacteria. This simple, eco-friendly, less expensive, free of chemical contaminants, green synthesis method of AgNPs by Vinca rosea may be used as anti-bio-fouling agent and also in technological applications in pharmaceutical industries to combat bacterial infections.

Author Contribution: Francis Tisha – conceptualised the research idea, collected literatures and data, conducted the experimental work and analysis and drafted the manuscript. Christma Eunice Sherina – Proof read and revised the manuscript. N.S. Nirmala Jothi – Supervised the work and reviewed the manuscript. All authors read and approved the final manuscript.

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Data Availability: All data and supporting materials applied in the study will be available from the corresponding author(s) only on academic requests.

Authorship

The corresponding author(s) confirm they have read the journal policies and is submitting their manuscript in accordance to it.

Declarations

Ethics approval and consent to publish, participate: We confirm that this manuscript has not been published elsewhere before and would not be considered to be published in other journals.

Competing Interests: The authors declare no conflict of interest.

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