Green synthesis of silver nano-particles (AgNPs) from flowers of *Rhododendron campanulatum* and its potential applications as photo-catalyst and antioxidant

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Abstract-The present work demonstrates the Green synthesis of silver nano-particles (AgNPs) using the *Rhododendron campanulatum* flower extract and their potential applications as photo catalyst and antioxidant. Several spectral approaches viz., UV-Vis, XRD, SEM-EDAX, BET isotherm and TEM were used to characterise the biosynthesized AgNPs in detail.The UV-Vis spectroscopy showed that the biosynthesized AgNPs have surface plasmon resonance (SPR) peak at 472 nm. The X-ray diffraction (XRD) studies confirmed the crystalline nature of biosynthesized AgNPs with mean crystalline size ~22.97 nm. According to the TEM examinations, the synthesized AgNPs were poly dispersed, smooth in morphology, irregularly shaped, and had an average grain size of 43.75 nm. The adsorption studies showed that the biosynthesized AgNPs have the single point surface area (at P/Po) was 0.1398 m^2/gm, BET surface area was 0.1057 m^2/gm, Langmuir surface area was 0.1465 m^2/gm, and the micro pore area was 0.7834 m^2/gm. The antioxidant efficacy of as synthesized AgNPs was determined using DPPH scavenging method, and the IC_{50} value was observed 38.68 µg/mL. Photocatalytic activity of synthesized AgNPs was determined by studying the adsorption/desorption equilibrium between the AgNPs and the solutions of MB, and RB dyes. The synthesized AgNPs showed the dye degradation efficacy of 73.88% for methylene blue (MB) dye, and 76.62% for Rose Bengal (RB) dye; however, in the absence of AgNPs, the degradation efficacy was observed as17.55% and 48.80% in 4 hours under photo-irradiation for MB and RB dye, respectively.

Keywords: *Rhododendron campanulatum* , Green Synthesis, Agnps, Antioxidant, Photo-Catalytic Degradation, Methylene Blue, Rose Bengal Dye.

Introduction

Nano-technology is one of the most fascinating and emerging area of research across all discipline of sciences. The controlled fabrication of materials at

different nano size scale is the major advantage of nanotechnology, which offers liberty to researchers to design the material of their need. These nano sized particles exhibit some unique characteristics compared to those of macro sized particles of bulk materials. If the nano-material synthesis is achieved via Greener synthetic approach it makes the process more acceptable due to strict environmental protocols associated with the uses of chemicals in synthesis. Therefore, in the synthesis of nano-material Greener approach in synthesizing the nano-material is presently an area of greater interest among the researchers due to its biocompatibility, less toxicity, easy adaptability and cost effectiveness. These nano-structured materials synthesized via green approach are applicable as a purifying agent, sensors, nano-medicines, optoelectronic devices, detoxifying agents, and many more[1-5]. Green mediated nano-material from plants, microorganisms, algae[6], and employing biological materials with enhanced properties, such as greater cost-effectiveness, low hazard, more eco friendly nature and well-defined shape and size[7]. Nanotechnology is a revolutionary technology for the modern society due to its enhanced applications in agricultural and food industries[8].The formation of toxic and non-degradable chemicals is grim point of discussion in present time, which pollute the major resources of our environment *viz.* air, water, plant growth, ecology and many more[9-10]. Therefore, the researchers are focusing on detoxification and recycling of waste-water by applying nano-material in the treatment of waste water[11].

The development of such nano-materials with outstanding physical, chemical, and biological properties, such as biosensing, optical, antioxidant, catalytic capabilities comprising antibacterial, antiviral, drug transport, and so forth, has been the main emphasis of the current effort[12-16].The novel aspect of this work is the creation of silver nano material using an extract of flowers from a *Rhododendron campanulatum* tree in the Garhwal Himalaya region of Uttarakhand, India. This was done for the first time using this plant material and is in accordance with the principles of green chemistry. The reason behind the selection of this plant was the richness of phenolic compounds in its extract, which is responsible for anti-oxidant activity[17]. Selected plant *R. campanulatum* has of medicinal properties like it is quite useful in cold, chronic fever, sciatica and hemicrania, while its bark is useful for digestive and respiratory disorders, and roots are useful to recover from many diseases like boils, headache, fever and so on[18]. Antioxidants play an important role in scavenging radicals and thus providing protection against infections and degenerative diseases[19-20]. By eliminating the free radical intermediates, antioxidants stop these chain reactions and stop additional oxidation processes. A potential source of bioactive substances with antioxidant effects is plants. Several medicinal plants include antioxidants, such as vitamin C (ascorbic acid), vitamin E, lycopene, etc., which aid in scavenging free radicals[21-23].For the breakdown of harmful dyes like methylene blue, methyl orange, alizarin red, acridine orange, malachite green, and many others, the majority of silver nano particles (AgNPs) have excellent photo-catalytic activity[24].

Material and methods

Chemicals- $AgNO_3$, methylene blue

(MB) and rose bengal (RB) dyes were purchased from Sigma Aldrich and plant material flowers of *Rhododendron campanulatum* voucher specimens GUH 0743[18] deposited at HNB Garhwal University were collected from Tungnath region of Garhwal Himalaya, Uttarakhand (India) and were utilized in this study.

Methodology

In the preparation of flowers extract of *Rhododendron campanulatum* (RCF), fresh flowers of *R.campanulatum* **(see** Figure**.1)** were collected and dried in the absence of sunlight at room temperature for 10-15 days for the preparation of non-volatile as well as stable compounds of nano particles, then the dried flowers were crushed in powder form. In a clean round bottom flask, 5 gm of crushed flowers was boiled in 100 mL of distilled water at 75−80°C for 30 min. The extract was cooled and filtered twice with Whattman filter paper no. 1. The freshly prepared extract was collected for analysis of targeted object[18-19].

Synthesis of silver nano particles (AgNPs) takes place via green route chemistry, in which flowers extract of *R. campanulatum* added into 5 milli molar aqueous solution of $AgNO_3$ in the ratio of 1:11, respectively. After three to four days the colourless solution of plant extract and silver nitrate was turned into dark brown red colour due to surface plasmon resonance (SPR) as well as presence of some phytochemical compounds present in the plant extract, which stabilize the formation of nano particles. After the reduction of solution, it was centrifuged at 5000 rpm at room temperature for 30 minutes, then residue as AgNPs collected and washed thrice by deionised water and kept at cool and dry place for further analysis. Different instrumental methods, such as the UV double beam spectrophotometer, XRD, SEM-EDAX, BET isotherm, and TEM, were used to characterise the synthesised nano materials[12,18-19].

Evaluation of Antioxidant activity

Antioxidant activity of synthesized silver nano particles (AgNPs) had been done by DPPH scavenging method. For this stock solution of sample was prepared by dissolving 100 mg of AgNPs in 100 mL of DMSO (Dimethyl sulphoxide), which was separated in several dilutions*i.e.*10µg/mL to 80 µg/mL for the test of antioxidant activity. Antioxidant activity was done against the ascorbic acid as a standard at similar dilution (10µg/mL to 80µg/mL) of stock solution. After 15 minutes, the DPPH sample's final absorbance at various concentrations was measured at 517 nm. Protocol for antioxidant activity has been already described in our previous work[20-24]. Percentage inhibitions of DPPH radical given by green synthesized AgNPs were determined by the following formula:

$$\% \textbf{ inhibition } = \frac{(\mathbf{Ac} - \mathbf{As})}{\mathbf{Ac}} \mathbf{x100}$$

A_C and A_S represents the absorbance of control and sample respectively.

Determination of Catalytic activity

To evaluate the photo catalytic activity, 10 mg of biosynthesized AgNPs, were separately added to 100 mL aqueous solution containing 5 mg of methylene blue (MB) and rose bengal (RB) dyes separately. To establish adsorption / desorption equilibrium between the AgNPs and the solutions of MB and RB dyes, the mixture was constantly agitated in the dark for 1 hour. The suspension was then exposed to photo-radiation. The

suspensions were removed from the reactor, centrifuged at 10min intervals, and their distinctive absorption spectra were recorded using de-ionized water as a reference on a UV-vis spectro-photometer[25-30,33]. The concentrations of the dyes were calculated using calibration curves. Methodology also discussed in previous paper Sati *et.al.*, 2021.

The following formula was used to determine how well synthesized nanomaterial (AgNPs) degraded dyes:

$$\textbf{Degradation}\ (\%) = \frac{(\mathbf{Co} - \mathbf{Ct})}{\mathbf{Co}} \mathbf{x100}$$

Where;

C_o and C_t are the concentration of dye sat t=0 and at time *t* after solar irradiation respectively.

Results and discussion

Characterization of silver nano-particles

Green synthesis of metal nano particles (AgNPs) was characterized by several instrumental techniques. These Results are as follows:

First of all, the formation of AgNPs was confirmed by bio-reduction of Ag^+ metal ion into AgNPs, which shows the UV absorption maxima peak (UV model 3375 Electronics India) for AgNPs at 472 nm for $5RCF_{1:11}$.2θ values of XRD (PAN analytical, X'PERT PRO) peaks were 37°, 43° and 63° correspond to the (111), (200), and (220) planes, respectively, for *fcc* crystals of synthesized AgNPs and the crystal size was calculated 22.97 nm (approx.). SEM images indicated the presence of agglomerated nano-material of AgNPs. To confirmation of AgNPs formation, EDAX was performed, in which a strong peak of elemental Ag (69.14 weight %) and other peaks of O (22.24 weight %) and C (8.62 weight %) elements were present. The synthesized powdered sample of nano material additionally used for TEM analysis in which it was observed that the green AgNPs were polydispersed, smooth morphology and irregular shaped grains having average size of 43.75nm[12,18-19,31-36]. BET isotherm plot **(see** Figure**.2)** of synthesized sample revealed that the synthesized AgNPs have single point surface area (SPSA at P/Po) was 0.1398 m^2/gm, BET surface area was 0.1057 m^2/gm, Langmuir surface area (LSA) was 0.1465m^2/gm, and the micro pore area was 0.7834 m^2/gm[28], on the basis of observed data due to which it may become a corrosion free material and may use as a good anti-corrosion material as well as protecting material.

Figure**ure1-** Flowers of *R.campanulatum* (RCF);

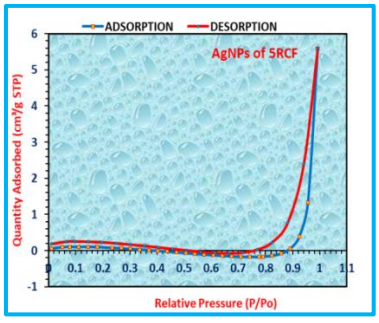


Figure **ure 2-** BET isotherm plot of AgNPs.

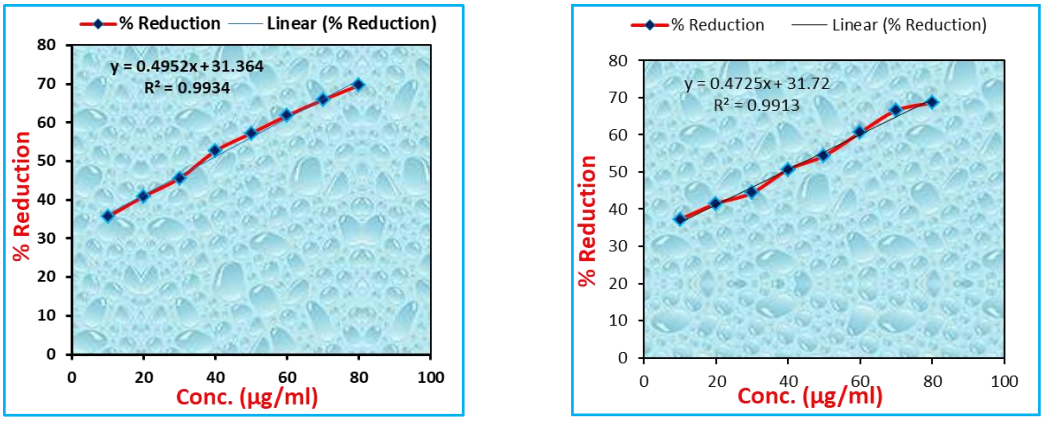
Evaluation of Antioxidant activity

Antioxidant activity of synthesized nano materials had been performed by DPPH method with respect to ascorbic acid as

standard and AgNPs as sample. In the determination of antioxidant activity, the following (Concentration v/s Percentage Reduction) graph and data shows that synthesized nano-material (AgNPs) were a good antioxidant material, for this IC_{50} value is 38.68µg/mL (**see Table-1;** Figure**ure-3**), which is just near to the IC_{50} value of the standard ascorbic acid (*i.e.* 37.63µg/mL)[20-24,32,34].

Table-1 Antioxidant activity data of Standard and sample (AgNPs)

S. No.	Conc. (µg/mL)	Absorbance		% Reduction		IC_{50} Value (µg/mL)
		Standard	AgNPs	Standard	AgNPs	
1.	10	0.300	0.310	35.60	37.28	**Standard 37.63**
2.	20	0.290	0.280	40.89	41.47	
3.	30	0.275	0.265	45.59	44.39	
4.	40	0.250	0.250	52.65	50.64	
5.	50	0.220	0.243	57.23	54.35	**AgNPs 38.68**
6.	60	0.187	0.230	61.78	60.58	
7.	70	0.164	0.218	65.86	66.54	
8.	80	0.147	0.210	69.60	68.60	

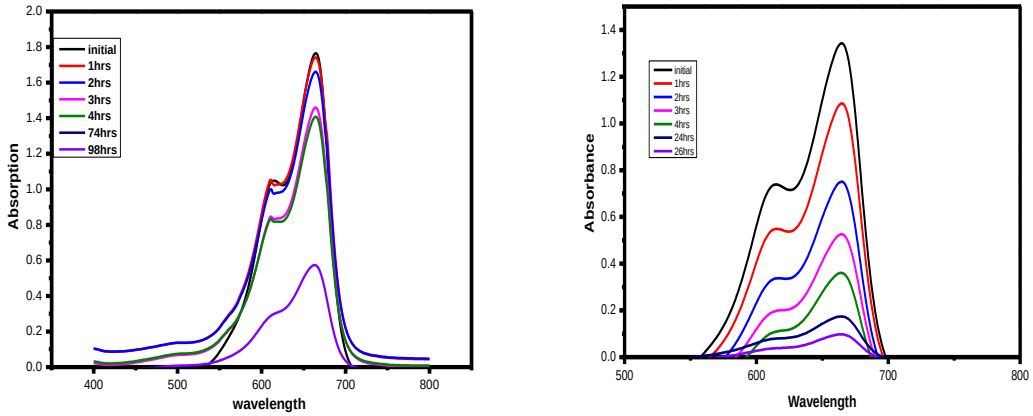


(a) **(b)**

Figure**ure 3-** Graph of Antioxidant activity for **(a)** Standard (Ascorbic acid); and **(b)** Sample (AgNPs).

Determination of Catalytic activity

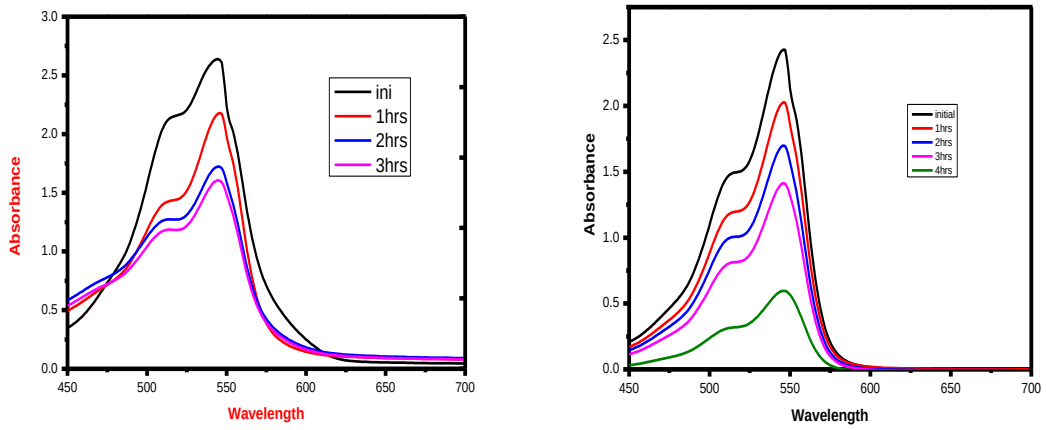
Catalytic degradation of methylene blue and rose bengaldye was analyzed by UV spectrometer, in which the absorption maxima peak of MB and RB dye was shifted towards lower absorption maxima value *i.e.* hypochromic shiftin characteristic peak at the time intervals of 1 hour. The hypochromic shift of characteristic peak of MB and RB dye was the indication of dye degradation process. The amount of dye degradation was calculated 73.88% in 4 hours **[**Figure**ure 4 (b)]** in the presence of AgNPs as a nano catalyst, while in the absence of nano catalyst (AgNPs) it shows 17.55% degradation of methylene blue dye **[**Figure**ure-4(a)]**. These findings show unequivocally that the MB dye decayed very quickly in the presence of produced AgNPs, but this degradation proceeded very slowly in the absence of AgNPs[23-27,29-30, 33].



(a) (b)

Figure**ure 4-** Degradation of MB dye (a)without AgNPs, and (b) with AgNPs as catalyst.

Similar Results were obtained in the photo-catalytic degradation of the dye rose bengal (RB), which was likewise facilitated by artificial AgNPs. The UV graph showed a reduction in the RB absorption peak within four hours. Initially (at t=0 hr), the value of absorption maxima peak of MB dye was 2.541 (at 543 nm) which was decreased very sharply upto 0.594 value of absorption maxima peak due to 76.62% degradation of dye on exposing to sunlight in the presence of synthesized NPs[see Figure.5(b)], It shows that the photo-catalytic degradation of the RB dye was completed to a 76.62% level within 4 hours, however in the absence of AgNPs, it was discovered that the nano catalyst only degraded the RB to a 48.80% level of its starting amount within 4 hours as seen by a UV-Visible spectrophoto-meter. These findings show unequivocally that green nano-catalyst caused MB and RB dyes to degrade quickly[23-28]. Therefore, AgNPs are good catalyst for toxic dye degradation.



(a) **(b)**

Figure**ure 5-** Degradation of RB dye (a) without AgNPs, and (b) with AgNPs as catalyst

Conclusion

In the present study, AgNPs were synthesized by using flowers extract of *R. campanulatum* via green chemistry synthesis. Due to richness of various phyto-chemical compounds in flowers extract which played an important role as a reducing as well as capping agent in the formation of AgNPs from $AgNO_3$ solution

of 5mM.In this environment friendly procedure, average size of synthesized NPs was 43.75 nm, and the BET isotherm plot revealed that the surface area of synthesized AgNPs was 0.1057 m^2/gm, which shows the significant presence of silver metal in synthesized sample. In the application part of nano material, synthesized silver nano material showed significant antioxidant property for which the IC_{50} value was 38.68µg/mL with respect to ascorbic acid as standard (IC_{50} value was 37.63µg/mL).The photo-catalytic degradation of the poisonous dyes methylene blue (MB) and rose bengal (RB) dyes demonstrated the remarkable catalytic properties of green produced nano materials. Within 4 hours of solar exposure, the MB dye was degraded by 73.88% in the presence of AgNPs, compared to 17.55% in the absence of the nano catalyst (AgNPs). Similar to this, after 4 hours of solar irradiation, the RB dye decomposed 76.62% in the presence of AgNPs and 48.80% in the absence of AgNPs. As increasing the time period of solar irradiation of the MB dye degraded 93.22% with AgNPs and without AgNPs degraded only 23% within 26 hours.These facts demonstrate the superior antioxidant and catalytic properties of the environment friendly nano-material (AgNPs) for the photo degradation of harmful colours. So, we can conclude that synthesized nano material may be applicable in the development of strong oxidizing agent, anti-corrosion material, water purifying agents as a removal of toxic contaminants as well as dye degrading agent in pure water conservation and in industries e.g. pharmaceutical, cosmetics, electronics and many more.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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