

ORIGINAL RESEARCH PAPER

Plant-mediated biosynthesis of Silver nanoparticles from *Gymnema sylvestre* and their use in photodegradation of Methyl orange dye

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ABSTRACT

The present study reports one step green synthesis of silver nanoparticles using *Gymnema sylvestre* aqueous extract at room temperature and their usage in the photodegradation of methyl orange dye. The silver nanoparticles are synthesized using an aqueous extract of stem and root of *Gymnema sylvestre*. UV-Visible spectral analysis showed absorbance peak at 430 nm with special reference to the excitation of surfaces plasmon vibration by silver nanoparticles. FT-IR analysis of nanoparticles reveals the presence of molecular functional groups such as amides, phenolic compounds, and carboxylic acid. These phytochemicals act capping and stabilizing agents for silver nanoparticles. EDAX elemental analysis shows the presence of silver as the main element in synthesized nanoparticles. The average crystalline size of silver nanoparticles was found to be 25.3 nm and 9.97 nm for Stem-AgNPs and Root-AgNPs respectively by Scherer formula. XRD patterns also suggest the occurrence of crystalline silver ions. Further, photocatalytic degradation of methyl orange was measured spectrophotometrically by using silver nanoparticles as nanocatalyst under solar light effect. The results revealed that biosynthesized silver nanoparticles using *G. sylvestre* was found to be notable in degrading methyl orange dye under the influence of sunlight.

Keywords: FTIR; *Gymnema sylvestre*; Photodegradation; SEM; Silver nanoparticles; Stem and Root extract; XRD

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INTRODUCTION

The thumb challenge in nanotechnology is for the improvement of efficient and green chemistry involved experimental procedures for the synthesis of nanomaterials for a required size, shape, and dispersivity [1]. In the current scenario of developing clean and green technologies for the nanomaterials synthesis, these aspects assume significant importance [2]. Using different plant extracts and reducible bio-excretory in the synthesis of novel metal nanoparticles is an attractive opportunity.

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As an alternative to the physical method, green synthesis method employing plant extracts are proved to be more feasible and simple. Nanoparticle synthesis can do using different microbial strains [3], enzymes, metabolites, biodegradable products [4], fungi, mushrooms [5] and plant extracts [6]. The current method of nanoparticle synthesis with plant extract is advantageous because it is simple, highly reproducible, nontoxicity and can be processed at the room temperature. Recent reports show the green synthesis of metallic nanoparticles

like using Au and Ag salts with part of the plant like *Cinnamomum camphora* [6], geranium leaf broth [7], and lemongrass extract [8], tamarind leaf extract [9] and *aloe vera* plant extracts [10]. Biosynthesis of nanoparticles using *G. sylvestre* leaf extract has been previously reported to be having anticancer properties [11].

Plant extract-mediated bioreduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. The selection of metal salt depends upon which metal nanoparticle is going to be synthesized. Modification of reduction reaction conditions like temperature, pH, amount of plant extract and concentration of metal salt may be affecting the reaction rate. The biosynthesis can be performed in ambient conditions and by the standard prescribed in green chemistry. The problems with the current physical and chemical methods are poor ability, short time stability, the involvement of toxic chemicals and safety problem in the usage of these particles and such problems can be satisfactorily solved by biosynthetic processes. In this study, aqueous extract of *G. sylvestre* is used for the synthesis of silver nanoparticles. The synthesis of *G. sylvestre* silver nanoparticles occurs at the room temperature in various periods.

G. sylvestre plant has medicinal importance, although synthesized nanoparticles (NPs) were used as nanocatalyst in the photodegradation of methyl orange dye. Photodegradation using nanoparticles is an effective and rapid technique in the removal of pollutants from wastewater [12]. In this study, we discuss the photocatalytic efficiency of one step synthesized silver nanoparticles for the degradation of methyl orange dye.

Gymnema sylvestre R.Br. (Family: Asclepiadaceae), commonly known as 'Gurmar', is a well-known medicinal plant. It is valuable medicinal plant used in folk medicine to treat diabetes, obesity, asthma etc. in India for antiquity. The plant is specially used in the treatment of diabetes and many other ailments. A recent review on *G. sylvestre* describes the antimicrobial [13, 14] hepatoprotective, antihypercholesterolemic, anti-inflammatory [15] activities of leaves of this plant. The plant *G. sylvestre* was used to control diabetes, obesity, atherosclerosis etc., by traditional medicinal practitioners of India [16, 17, 18]. It is well known that a group of more than twenty saponin glycosides of olenane-type including a mixture of gymnemic acids I-XVIII (antisweet compounds) and gymnema saponins are the active

constituents of these leaves [15]. The extract of *G. sylvestre* leaves (stem and root) is rich in reducing sugars, flavanoids, terpenoids, saponins phenols, which acts as a good antioxidant and an excellent antimicrobial agent [13]. These phytochemical are important natural resources and play an aggressive role in both stabilization and reduction of nanoparticles. The attention of the present work is to apply the accurate principles of green chemistry for the synthesis of silver nanoparticles by using stem and root *G. sylvestre* extracts as reducing and capping agent.

MATERIALS AND METHODS

Plant material

The plant of *G. sylvestre* (2 Kg) was collected from 'Pune' Maharashtra, India. The plant was authenticated by Botanical Survey of India, Pune (BSI). The material has been deposited at AHMA herbarium at BSI (Voucher No.SVS-1/783).

Chemicals: Analytical grade AgNO_3 procured from Hi Media Lab and used for the experiment. Tripally Ionized Water is for preparation of solutions.

Abbreviations: SWE: Stem Water Extract, RWE: Root water extract, NPs: Nanoparticles, AgNPs: silver nanoparticles, GS: *Gymnema sylvestre*, GS-SAgNPs: *Gymnema sylvestre* stem silver nanoparticles, GS-RAgNPs: *Gymnema sylvestre* root silver nanoparticles, SPR: Surface plasma resonance.

Preparation of extracts from G. sylvestre

The Plant material was cleaned with distilled water and shade dried for 15 days. The plant parts stem and root were separated. The air-dried Stem and Root of *G. sylvestre* (10g) were cut into the size of 0.25 cm X 0.25 cm were taken in a wide neck Borosil flask. Then 100 mL of tripally distilled water is poured into the flask and subjected to heating for 1 hr. The solution is then filtered in hot condition using Whattman filter paper No.44 to remove the solid fibrous residues. The clear filtrate (aqueous extract) of stem and root were labeled as SWE and RWE. The water extracts of *G. sylvestre* are stored in a freezer at 4°C and used for the silver nanoparticles synthesis.

Biosynthesis of Silver nanoparticles from G. Sylvestre

Analytical grade AgNO_3 procured from Hi Media Labs is prepared in 3mM is used for the experiment. 3.0 mL of Stem water extract (SWE) is added to 50

mL of AgNO₃ (3mM) aqueous solution. Similarly 3.0 mL of Root water extract (RWE) is added to 50 mL of AgNO₃ (3mM) aqueous solution. Then the flasks are placed on a magnetic stirrer at room temperature for the process of NPs synthesis for about 48 hrs. The appearance of Yellow-brown color indicates the formation of Silver Nanoparticles. A control solution (3 mM AgNO₃) was also kept under the same condition. The reaction medium containing AgNPs was centrifuged at 10 000 rpm for 30 min. The obtained precipitation was freeze-dried and used for further studies.

Characterization of Biosynthesized silver nanoparticles

The different techniques used for the characterization of biosynthesized nanoparticles are as follows, Visual Inspection and Intensity Characterization, Spectroscopy techniques like UV-vis to understand the formation of metallic nanoparticles and Fourier transform infrared spectroscopy (FTIR): to understand the probable pathway of biosynthesis. FESEM analysis provides information on chemical composition near the surface. EDAX and XRD data are used in getting the confirmation on the chemical composition and crystalline nature of nanoparticles.

The GSAgNPs were characterized using a UV-Vis 3000+ double-beam spectrophotometer (Lab India, Maharashtra, India). The spectrometric range was 200–800 nm, and scanning interval was 0.5 nm. The functional characterization of molecules present in the GSAgNPs from the stem extract and Root extract of *G. sylvestre* were done by FTIR spectrometry (RX1; Perkin-Elmer, Waltham, MA, USA). The surface morphology of the biofunctionalized AgNPs was characterized by SEM analysis (JSM-5600LV; JEOL, Tokyo, Japan) and the elemental compositions were determined by EDAX analysis (S-3400N; Hitachi, Tokyo, Japan). The crystal size of the synthesized AgNPs was determined by XRD measurements using an XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan). The crystallite domain size was calculated from the width of the XRD peaks by assuming that they were free from non-uniform strains using the following Scherer formula [19].

Photocatalytic degradation of Methyl Orange Dye

The photocatalytic degradation of methyl orange was studied by using biosynthesized GS-SAgNPs and GS-RAgNPs nanoparticles using reported method [20]. All the experiments were

performed outdoor with the sun as the main source of light. 50 ml of methyl orange solution (5 mg/l) was prepared and used in this degradation reaction. The two different test solutions of methyl orange containing GS-SAgNPs and GS-RAgNPs (3 mg/ml) were prepared. 50 ml of methyl orange (5mg/ml, without GS-AgNPs) was used as a control to monitor the degradation. Later, the mixtures were allowed to stir constantly for about 30 min in darkness to ensure constant equilibrium of GS-AgNPs in the organic solution. Then test solutions and control were kept under sunlight within a Pyrex glass beaker and stirred continuously on a magnetic stirrer. The mean temperature was found to be 30°C. The absorption spectrum of the reaction mixture was measured periodically using a UV-visible spectrophotometer (Shimadzu, UV-2450, Japan) after centrifugation to ensure the degradation of methyl orange solution. The % Degradation of Methyl orange dye by GS-SAgNPs and GS-RAgNPs were determined by the following equation:

$$\% \text{ Degradation} = \frac{[A_{C(0)} - A_{C(t)}] \times 100}{A_{C(0)}}$$

Where, $A_{C(0)}$ is the absorbance of Methyl orange dye at 0 min (Control), $A_{C(t)}$ is the absorbance of methyl orange dye after addition of GS-SAgNPs and GS-RAgNPs at 30,60,90 and 120 min. respectively.

RESULTS AND DISCUSSION

Visual Inspection

After the addition of aqueous plant extract of *G. sylvestre* to AgNO₃ (3mM) aqueous solution, a light yellowish color was observed which changed to dark brown color on subjecting the reaction mixture to room temperature. This change of color with respect to time indicates that the formation of silver NPs has taken place [21]. The reaction mixture is kept for 48 hr. After 48 hr, it was observed that the intensity of the brown color of solution increases. The intensity of the color is recorded as absorbance with respect to time which clears that the GS-AgNPs were found to be time-dependent. The color intensity (Absorbance intensity) of the reaction mixture increases exponentially with time as shown in Fig. 1(a) for GS-SAgNPs and Fig.1 (b) GS-RAgNPs respectively.

The reaction process takes place rapidly and after complete reaction process, it is observed that more than 90% of silver ions will be reduced to GS-AgNP in 48hr. The AgNPs formation is confirmed by an increase in the color of the reaction mixture which

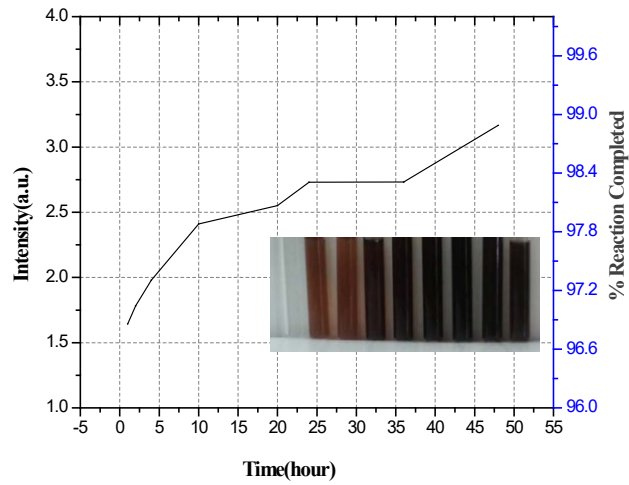


Fig.1 (a): SAgNPs: Intensity graph of reaction mixture containing aqueous 3mM AgNO₃ solution treated with Stem Water extract (SWE) versus time (hour) showing that the 90% of SNPs biosynthesis completed within 2 hr. Inset: increase in the color intensity of the reaction mixture with time.

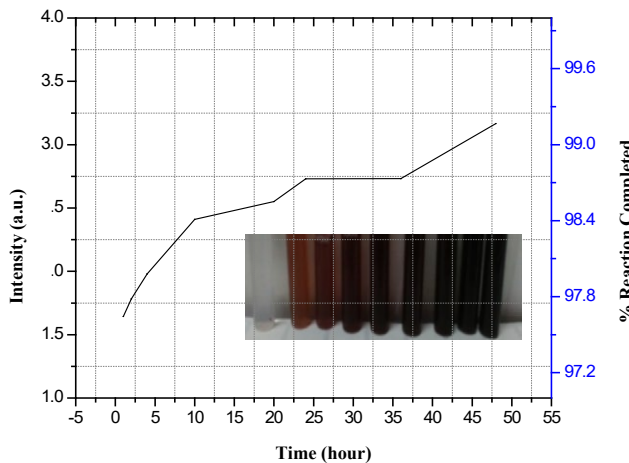


Fig.1. (b): RAgNPs: Intensity graph of reaction mixture containing aqueous 3mM AgNO₃ solution treated with Root Water extract (RWE) versus time (hour) showing that the 90% of NPs biosynthesis completed within 2 hr. Inset: increase in the color intensity of the reaction mixture with time.

is observed with Surface Plasma resonance (SPR) in the visible range of the spectra. AgNP exhibits brownish color in water owing to excitation in the Vibrations of SPR. The characteristic color change obtained may perhaps be due to the excitation of surface plasma resonance (SPR) and reduction of AgNO₃ [12]. The control AgNO₃ solution remained as such without any change in color as shown in Figs. 1 (a) and (b). This suggests that the color intensity of the nanoparticles solution is directly proportional to the incubation time.

UV-vis spectroscopy

UV-visible spectroscopy is a preliminary and convenient tool for measuring the reduction of

metal ions based on optical properties called SPR. The reaction mixture after 48 hr has an absorption maximum at 430 nm suggesting the formation of Ag nanoparticles [5] as shown in Figs. 2(a) and (b).

The shape of the absorption band was found to be symmetrical, which indicates the presence of spherical-polydispersed nanoparticles and further confirmed by FE-SEM studies. The incubation time plays an important role in the formation of nanoparticles by reducing the silver ions at room temperature. Our results showed that there are no further changes in UV-vis spectrum signifying the reaction is completed. Thus, it is evident from this experiment the formation of nanoparticles in solution is time dependent as shown in Figs. 3(a) and (b).

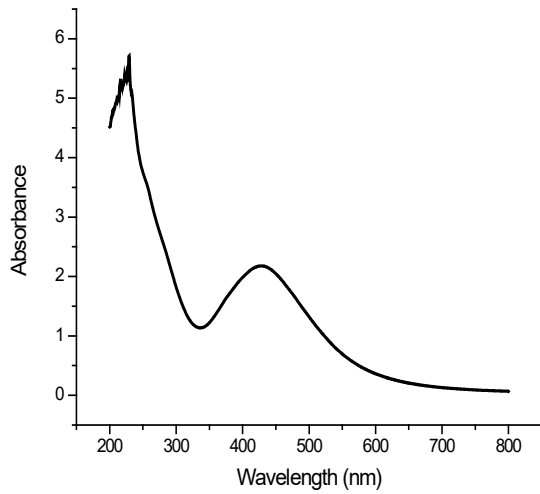


Fig. 2(a): UV-VIS Spectra of GS-SAgNPS at 3mM AgNO₃

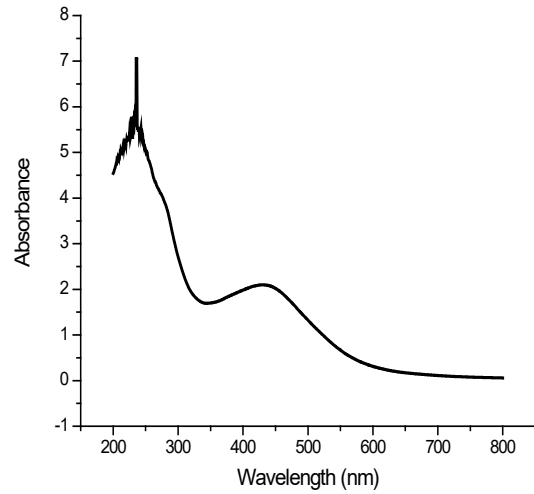


Fig. 2(b): UV-VIS Spectra of GS-RAgNPS at 3mM AgNO₃

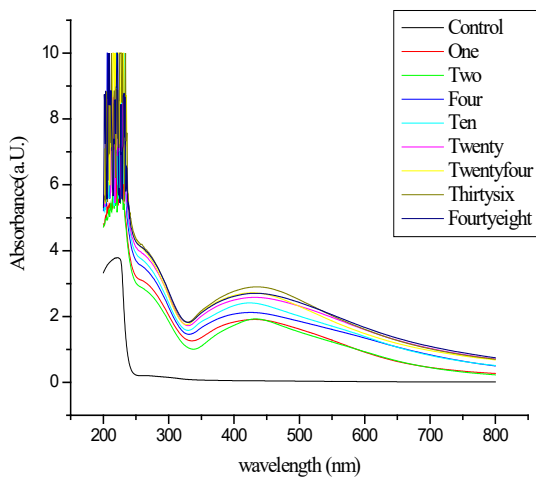


Fig. 3 (a): Time dependent Biosynthesis of GS-SAgNPs

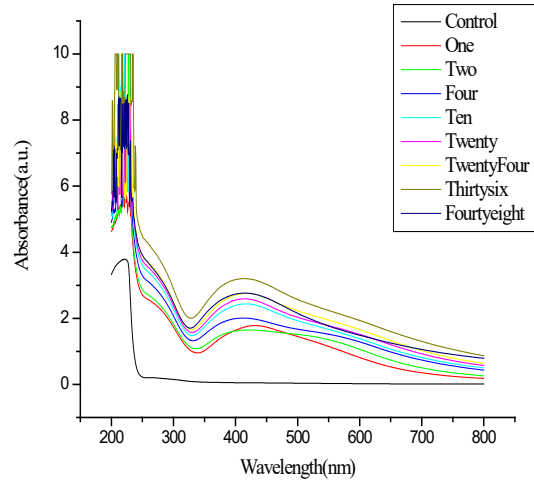


Fig. 3 (b): Time dependent Biosynthesis of GS-RAgNPs

IR- spectroscopy

The plant extract acts as reducing agent, capping agent and stabilizing agent for biosynthesized Silver NPs. The FTIR spectra for stem water extract and corresponding GS-SAgNPs are shown in Fig. 4(a). Similarly, FTIR spectra for Root water extract and corresponding obtained GS-RAgNPs are shown in Fig. 4 (b).

FT-IR analysis showed that the presence of molecular functional groups that are responsible for the reduction of silver ions as shown Fig. 3(a) and Fig. 3(b). The FTIR analysis of GS-SNPs showed three sharp absorption peaks at 3456 Cm⁻¹, 1635 Cm⁻¹, and 705.0Cm⁻¹. The peak at 3456 Cm⁻¹ indicating the possibility of the presence of O-H stretching. The absorption peak at 1640 cm⁻¹ could be due to the amide bond from the carbonyl group

of a protein. The sharp peak at 705 Cm⁻¹ is due to C-Cl stretching vibrations. The detailed peak descriptions in FTIR analysis are given in Tables 1 and 2 These results suggested that the biological molecules possibly perform the dual function of synthesis and stabilization of silver nanoparticles [12, 22].

SEM images of AgNP are shown at resolution of 0.5 μm in Figs. 5(a) and (b). Biosynthesized Ag-NPs are spherical shaped and well distributed with aggregation. This image gives information about the organic moieties adsorbed on the surface of nanoparticles which serve as a reducing and also as a capping agent.

The quantitative analysis of synthesized nanoparticles was carried out using EDAX. The EDAX showed the high silver content of 41%.

Table 1: IR Frequency Band for GS-SAgNPs

Sr. No.	IR Frequency for GS-SAgNPs	Possible Functional Group
1	3434 Cm^{-1}	O-H stretching-bonded
2	1635 Cm^{-1}	Amide Stretching
3	2792 Cm^{-1}	-C-H and C-H (Methoxy compounds) stretching vibrations
4	2330 Cm^{-1}	Primary amine group of protein
5	1332 Cm^{-1}	C-C and C-N stretching
6	705 Cm^{-1}	C-Cl stretching

Table 2: IR Frequency Band for GS-RAgNPs

Sr. No.	IR Frequency for GS-RAgNPs	Possible Functional Group
1	3456 Cm^{-1}	O-H stretching
2	1645 Cm^{-1}	Amide C-O stretching
3	2758 Cm^{-1}	-C-H and C-H (Methoxy compounds) Stretching Vibrations
4	1350 Cm^{-1}	C-C and C-N Stretching
5	705 Cm^{-1}	C-Cl Stretching

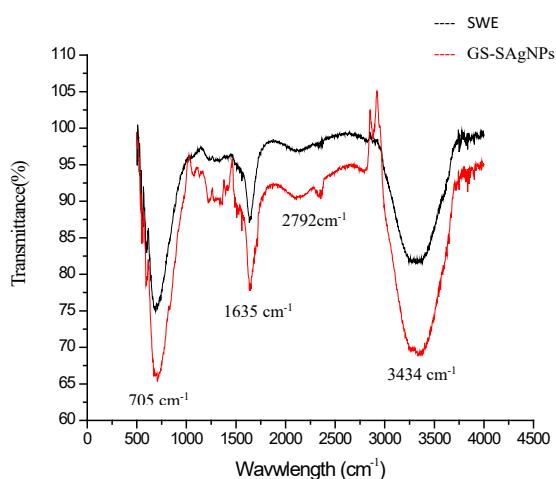


Fig. 4(a): IR Spectra of SWE and GS-SAgNPs

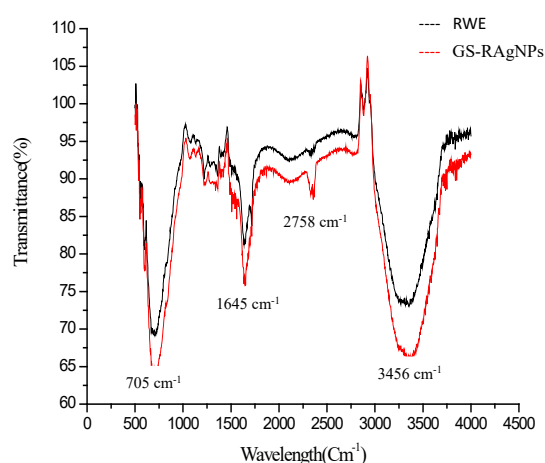


Fig. 4 (b): IR Spectra of RWE and GS-RAgNPs

The EDAX spectrum also showed the presence of Oxygen and Carbon of 46.11% and 7.11%, respectively.

X-ray diffraction patterns of synthesized silver nanoparticles were shown in Figs. 6(a) and (b). The XRD pattern showed diffraction peaks of the range of 2θ ($20-80^\circ$) which were corresponding to (111) (200) (220) and (311) planes [23]. A peak at $2\theta = 32.2^\circ$ represents the formation of pure silver (Ag) at the start of the reaction. The average crystalline size of the Ag nanoparticles was determined by Scherrer's formula and found to 25.3 nm and 9.97 nm for SAgNPs and RAgNPs respectively.

Photocatalytic degradation of methyl orange dye was investigated using biometrically synthesized GS-SAgNPs and GS-RAgNPs by solar irradiation technique at different time intervals as shown in Figs. 7(a) and (b). The characteristic absorption peak of methyl orange solution was found to be 460 nm (λ_{max}). Degradation of methyl orange was visualized by a decrease in peak intensity within 2 hr of incubation time in sunlight. There is no considerable shift in peak position for methyl orange solution without Ag nanoparticles. As compared to other irradiation techniques, the solar light was found to be faster in decolorizing methyl orange in the presence of

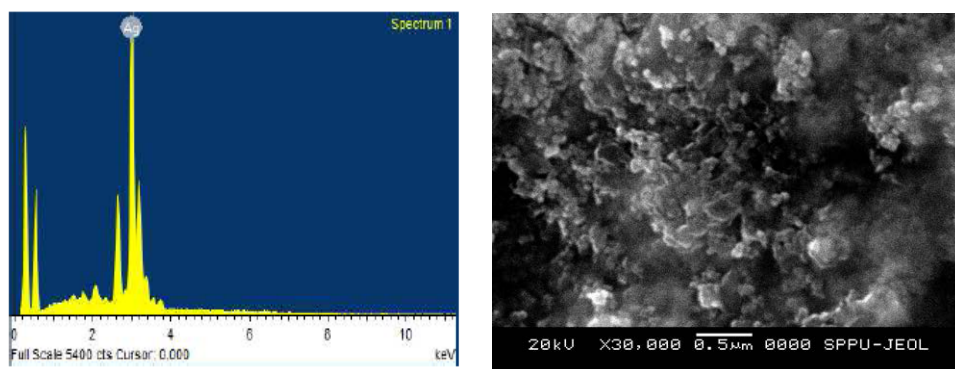


Fig. 5(a): EDAX and SEM of GS-SAgNPs

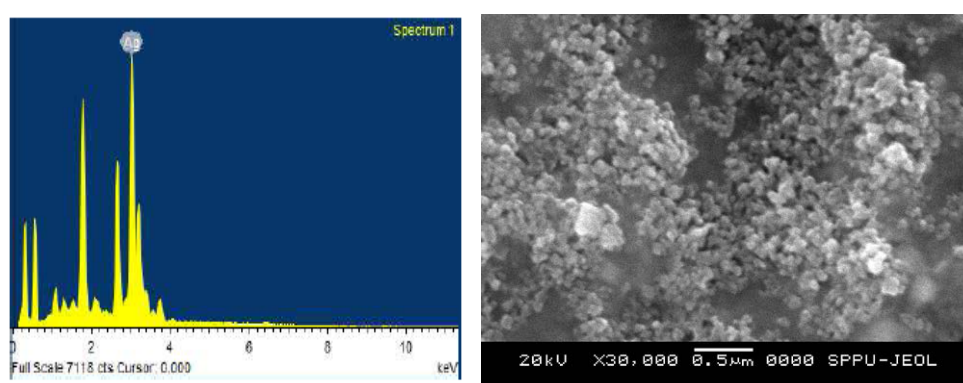


Fig. 5(b): EDAX and SEM of GS-RAgNPs

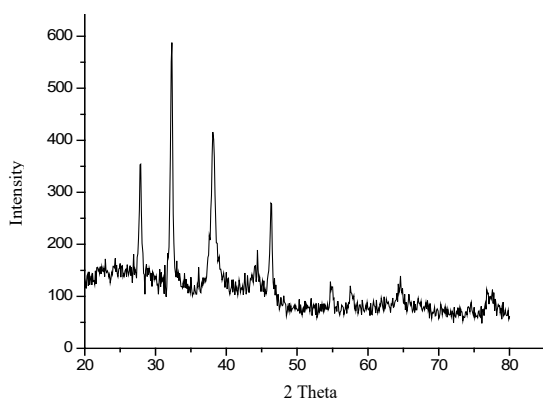


Fig. 6(a): XRD Pattern of GS-SAgNPs

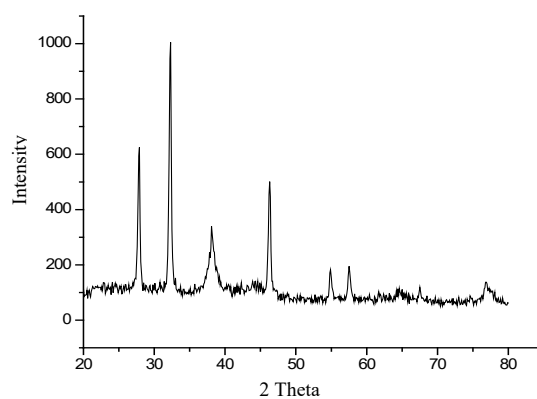


Fig. 6(b): XRD Pattern of GS-RAgNPs

metal catalyst [24].

The rate of degradation of Methyl orange dye by GS-SAgNPs was found to be faster as compared with GS-RAgNPs. Initially, % degradation of methyl orange was low (30 min) for GS-SAgNPs is 22.81% and for GS-RAgNPs is 2.68%. The notable photo catalytic activity of biosynthesized GS-SAgNPs was mainly because of the presence of protein moiety that surrounded the Ag-NP [FTIR

band at 2330 cm^{-1}]. The % degradation of Methyl orange dye is as shown in Fig. 8. It is observed that degradation of methyl orange by GS-SAgNPs is 57.04% after 2 hr and by GS-RAgNPs is 47.65%. The results show that 50 % dye degradation can be done by silver nanoparticles synthesized from *G. sylvestre*. *G. sylvestre* AgNPs can be used as efficient nanocatalyst for degradation of methyl orange dye.

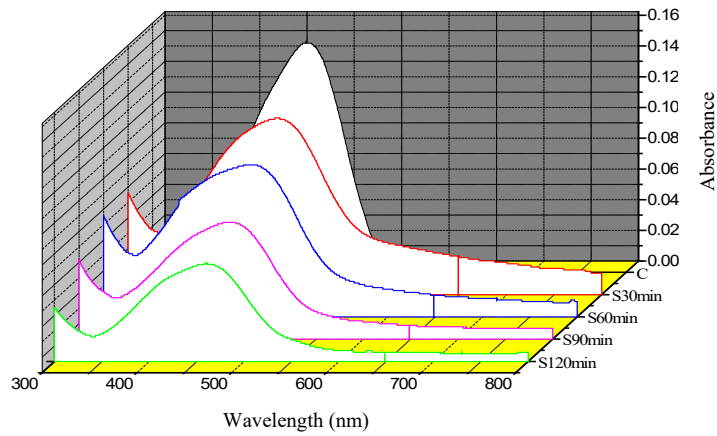


Fig. 7(a): Degradation of Methyl orange dye using GS-SAgNPs

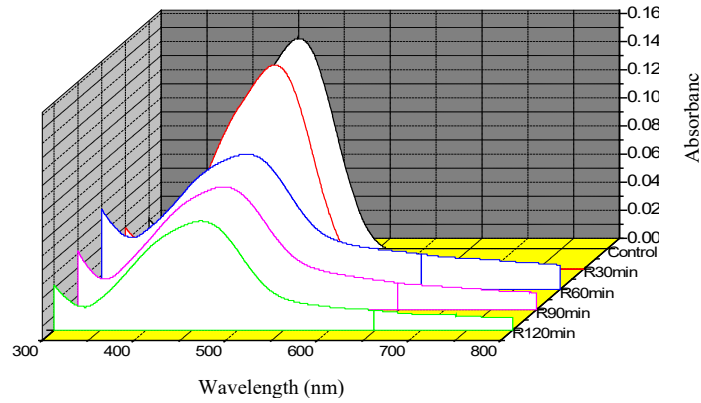


Fig. 7(b): Degradation of Methyl orange dye using GS-RAgNPs

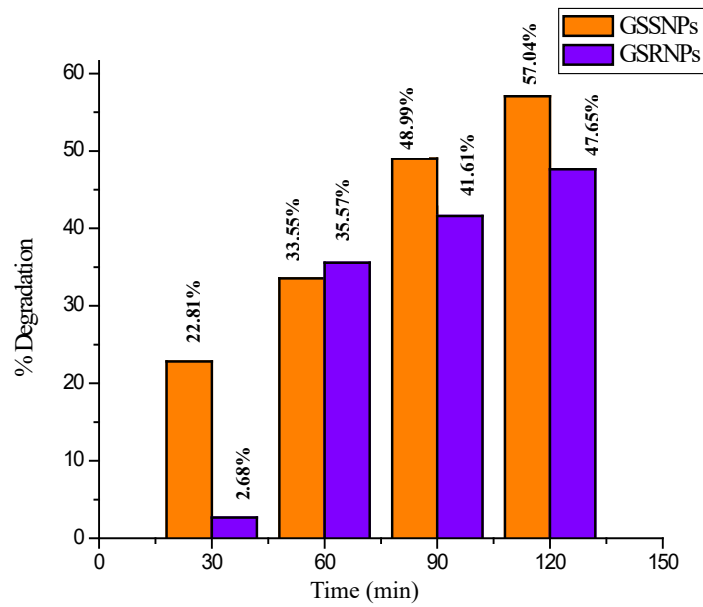


Fig. 8: % Degradation of Methyl orange dye using GS-SAgNPs and RAgNPs

CONCLUSIONS

To conclude, in this study, the nanoparticles synthesized from aqueous extract *G. sylvestre* (stem and root) were labeled as GS-SAgNPs and GS-RAgNPs respectively. These green-synthesized SNPs (GS-SAgNPs and GS-RAgNPs) of *G. sylvestre* were examined by techniques Ultraviolet-visible (UV-vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray analysis (EDAX), and X-ray diffraction (XRD) analysis for studying their size and morphology. silver nanoparticles from *G. sylvestre* were synthesized biometrically at room temperature within 48 hr of incubation time. The formation of silver nanoparticles was confirmed by UV spectra, FT-IR, SEM, and XRD studies. The nanoparticles were found to be active in degrading methyl orange solution with solar light illumination. These findings suggest that silver nanoparticles synthesized by a facile method from *G. sylvestre* can able to degrade dyes in the presence of sun light. This current work provides information about the application of *G. sylvestre* for biosynthesis of metal nanoparticle and their use in Photocatalytic degradation of contaminants present in water

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

- Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials*. 2009;8(7):543-57.
- Sharma NC, Sahi SV, Nath S, Parsons JG, Gardea-Torresde JL, Pal T. Synthesis of Plant-Mediated Gold Nanoparticles and Catalytic Role of Biomatrix-Embedded Nanomaterials. *Environmental Science & Technology*. 2007;41(14):5137-42.
- Hamed M, Givianrad M, Moradi A. Biosynthesis of Silver Nanoparticles Using Marine Sponge. *Oriental Journal of Chemistry*. 2015;31(4):1961-7.
- Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*. 2010;75(1):1-18.
- Owaid MN, Ibraheem IJ. Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *European Journal of Nanomedicine*. 2017;9(1).
- Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2009;339(1-3):134-9.
- Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*. 2007;18(10):105104.
- Shankar SS, Ahmad A, Pasricha R, Sastry M. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. *Journal of Materials Chemistry*. 2003;13(7):1822.
- Rai A, Singh A, Ahmad A, Sastry M. Role of Halide Ions and Temperature on the Morphology of Biologically Synthesized Gold Nanotriangles. *Langmuir*. 2006;22(2):736-41.
- Ankamwar B, Chaudhary M, Sastry M. Gold Nanotriangles Biologically Synthesized using Tamarind Leaf Extract and Potential Application in Vapor Sensing. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*. 2005;35(1):19-26.
- Arunachalam K, Arun LB, Annamalai SK, Arunachalam AM. Potential anticancer properties of bioactive compounds of *Gymnema sylvestre* and its biofunctionalized silver nanoparticles. *International Journal of Nanomedicine*. 2014:31.
- Muthukrishnan S BS, Muthukumar M SM, Rao Mv SKT. Catalytic Degradation of Organic Dyes using Synthesized Silver Nanoparticles: A Green Approach. *Journal of Bioremediation & Biodegradation*. 2015;06(05).
- Rupnar S V, S.S. Pingale, C.N. Dandge and D. Kshirsagar, 2016, Phytochemical Screening and In vitro evaluation of antioxidant & antimicrobial activity of *Gymnema sylvestre*, *International Journal of Current Research*, 8:11.
- Gopiesh khanna V, Kannabiran K. Antimicrobial activity of saponin fractions of the leaves of *Gymnema sylvestre* and *Eclipta prostrata*. *World Journal of Microbiology and Biotechnology*. 2008;24(11):2737-40.
- Kanetkar P, R. Singha and M. Kamat, 2007, *Gymnema sylvestre*: A Memoir. *Journal of Clinical and Biochemistry Nutrition*, 41:77-81
- Agarwal S K, S.S. Singh, S. Verma, V. Lakshmi and A. Sharma, 2000, Chemistry and medicinal uses of *Gymnema sylvestre* (Gur-mar) leaves: A review. *Indian Drugs*, 37 :354-360.
- Subramaniyan V and P. Srinivasan, 2014, *Gymnema sylvestre*: A Key for Diabetes Management A Review, *Pharmacology & Toxicology Research*, 1 :1-10.
- Kumar M S, N. Astalakshmi, P. T. Arshida, K. Deepthi, N.M. Devassia, P.M .Shafna and G. Babu, 2014, A Concise Review on gurmara *Gymnema sylvestre* R.Br., *World Journal of pharmacy and Pharmaceutical Science*. 4:430-448.
- Muthukrishnan S, Bhakya S, Senthil Kumar T, Rao MV.

- Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii* – An endemic species. *Industrial Crops and Products*. 2015;63:119-24.
20. Amutha M, Lalitha P, Firdhouse MJ. Biosynthesis of Silver Nanoparticles Using *Kedrostis foetidissima* (Jacq.) Cogn. *Journal of Nanotechnology*. 2014;2014:1-5.
 21. Owaid MN, Raman J, Lakshmanan H, Al-Saeedi SSS, Sabaratnam V, Abed IA. Mycosynthesis of silver nanoparticles by *Pleurotus cornucopiae* var. *citrinopileatus* and its inhibitory effects against *Candida* sp. *Materials Letters*. 2015;153:186-90.
 22. Kumar P, Govindaraju M, Senthamilselvi S, Premkumar K. Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. *Colloids and Surfaces B: Biointerfaces*. 2013;103:658-61.
 23. Patil RS, Kokate MR, Kolekar SS. Bioinspired synthesis of highly stabilized silver nanoparticles using *Ocimum tenuiflorum* leaf extract and their antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2012;91:234-8.
 24. Venkatesan J, Kim S-K, Shim M. Antimicrobial, Antioxidant, and Anticancer Activities of Biosynthesized Silver Nanoparticles Using Marine Algae *Ecklonia cava*. *Nanomaterials*. 2016;6(12):235.