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Polygonum glabrum Willd. leaf extract mediated green synthesis of silver nanoparticles and their assessment of antimicrobial activity

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Abstract: *Green nanotechnology is gaining importance due to the elimination of harmful reagents and provides effective synthesis of expected products in an economically manner. In the present investigation aqueous leaf extract of Polygonum glabrum Willd. was used for the synthesis of silver nanoparticles. The colour of reaction mixture was changes from colouless to brown colour indicates the formation of silver nanoparticles, further confirmed by characteristic UV-Vis absorption peak at 429 nm. Synthesized nanoparticles were characterized by FTIR, Fluorescence Spectroscopy and SEM. Fluorescence Spectroscopy is a powerful tool to study the tertiary structure of proteins. FTIR data revels that biomolecules involved in the reduction and capping of silver nanoparticles. The SEM result shows the size of nanoparticles ranges from 10 to 35 nm and are spherical in shape. XRD data showed that crystalline nature of nanosilver. The biogenic silver nanoparticles showed excellent antimicrobial activity against S. typhi, E. coli, P. aeruginosa and S.aureus.*
Keywords*: Silver nanoparticles, Polygonum glabrum Willd, FTIR, Antimicrobial activity*

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I. Introduction

Nanotechnology is referred to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macroscale products, also now referred to as molecular nanotechnology [1]. Dimensions between approximately 1 and 100 nanometers are known as the nanoscale. The synthesis of nanoparticles and their characterization is an emerging field of nanotechnology since past two decades due to their wide applications in the fields of physics, chemistry, biology and medicine. Unusual physical, chemical and biological properties can emerge in materials at the nanoscale. These properties may differ in important ways from the properties of bulk materials and single atoms or molecules. Although metal is a poor catalyst in bulk form, nanosized particles can exhibit excellent catalytic activity due to their relative high surface area-to-volume ratio and their interface-dominated properties, which significantly differ from those of the bulk material [2]. Controlled size and composition of nanoparticles are of fundamental interest since they provide solutions to environmental and technological challenges in the areas of catalysis, solar energy conversion and waste water treatment. More recently, nanoscale materials has been looked at with interest for synthesis of advanced materials, energy storage devices, electronic and optical displays, chemical and biosensors, drug delivery, optical spectroscopy including surface-enhanced raman scattering [3] , detection and diagonostic, antimicrobial, therapeutics [4,5,6,7], biomedicine, waste water treatment [8] , food industry [9] , Antidiabetic [10, 11], Antiviral [12] , anticancer[13], Anti-inflammatory [14] and antiplasmodial [15] .

Nanoparticles are synthesized by physical and chemical methods but these methods have certain disadvantages due to involvement of toxic chemicals. A large amount of toxic chemicals are produced during the synthesis of nanomaterials and these chemicals pose a serious threat to environment. Thus, there is a need for safe, clean, nontoxic and environment-friendly method for the synthesis of nanoparticles. Researchers in the field of nanoparticles have laid emphasis on biological method for synthesis of nanoparticles by biomimetic approach. Bio-inspired synthesis of metal nanoparticles can be considered as an emerging branch of green chemistry in which plant extract, bacteria, fungi and algae have been used for the reduction of Ag^{+} to Ag^{0}. Recently, number of plants and plant parts such as leaf, fruit, flower, tuber and rhizome extract were used for the synthesis of silver and gold nanoparticles, further testing the efficacy of nanoparticles for antimicrobial activity Viz. Garcinia mangostana [16] , Dioscorea bulbifera [17] , Alternanthera sessilis [18] , Terminalia arjuna [19] , Phyllanthus maderaspatensis [20], Cansjera rheedii J. F. [21], Hemidesmus indicus [22] , Linum usitatissimum L. [23], Dimocarpus longan [24], Convolvulus pluricaulis [25] , Lavandula intermedia [26] , Allophylus serratus [27] , Origanum Vulgare L. [28] , Bauhinia acuminate and Biophytum sensitivum [29]. Polygonum glabrum Willd. is an annual or perennial herb, which belongs to the family Polygonaceae (Figure 1). The leaf extract of P. glabrum contains

flavonoids, terpenoids, quinones, proteins, enzymes, amino acids etc. The present investigation was undertaken to study phytosynthesis of silver nanoparticles by using leaf extract of P. glabrum and testing the antibacterial activity of synthesized silver nanoparticles.

II. Materials and methods:

Silver nitrate was obtained from Sigma-Aldrich chemicals. All glassware's were washed with distilled water and dried in an oven. Fresh leaves of P. glabrum were collected from botanical garden of Karnatak University campus, Dharwad.

Preparation of leaf extract: The one gram of P. glabrum leaf material was incisor into small pieces and macerated by adding 10 ml sterile distilled water with the help of pestle and mortar. During the extract preparation, 20 ml of sterile distilled water was used for centrifugation at rotation speed of 1200 rpm for 20 min. The supernatant was decanted and pellet was re-macerated in 10 ml distilled water and again followed by centrifugation. The supernatant was used as extract for conducting experiments.

Figure 1: Shows the Habit of Polygonum glabrum Willd.

Synthesis of silver nanoparticles: The 5 ml P. glabrum leaf extract was added to 250 ml Erlenmeyer conical flask containing 100 ml of 1mM silver nitrate solution. The tightly capped flasks were kept in a shaker at a rotation speed of 200 rpm at 27^{o}C and pH of the solution was maintained at 9 pH. The change in the colour of solution to dark brown was noted after reaction period which indicates the formation of silver nanoparticles further confirmed by characteristic absorption peak of UV-Vis spectrometer.

Characterization of silver nanoparticles: The UV–VIS spectra of the sample was measured on a UV-2450 (Shimadzu) spectrophotometer operated at a resolution of 1 nm. The bio-reduction of silver ions in aqueous solution was monitored by UV–Vis spectrum between the ranges from 400 to 800 nm. Detection of tryptophan / tyrosine residues in proteins present in the reaction mixture or extract was analyzed spectrophotometrically by the measurement of absorbance in the range between 200-300 nm wavelength regions by using U-3010 spectrophotometer.

Fluorescence Spectroscopy is a powerful tool to study the tertiary structure of proteins. The Fluorescence of the solution of silver nanoparticles was studied by using F-7000FL spectrophotometer. The detection of tryptophan / tyrosine residues in protein was made by excitation at 250 nm for the confirmation of their presence. FTIR characterization of reaction mixture using FTIR (F-7000FL) spectrophotometer for identification of biomolecules involved in bioreduction and capping of silver nanoparticles. In order to remove any free biomass residue, the residual solution after reaction was centrifuged at 4000 rpm for 20 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing processes were repeated for three times. Finally, the dried samples were palletized with KBr and analyzed using FT-IR.

After bioreduction, solution containing silver nanoparticles was dried at 60^{o}C for 2-3 days in an oven. Dried sample was collected for the determination crystalline structure of Ag nanoparticles by X' Pert pro X-ray diffractometer operated at an voltage of 40 kv and a current of 30mA with Cu Kα radiation. The bioreduced solution was dried at 60^{o}C in an oven. After complete drying, fine powdered material was separated and collected. This material was used for SEM (scanning electron microscopy) observation. The material was mounted on clear aluminum stub using double sided adhesive cellotape. The sample was gold plated in a vacuum evaporator. SEM image was taken on JEOL, JSL 35 C model operated at an accelerating voltage of 20 kv at a magnification 27,000 X.

Antimicrobial activity: Antimicrobial activity of biogenic silver nanoparticles was assessed by well diffusion method against gram negative (Escherichia coli MTCC 723, 1554, Pseudomonas aeruginosa MTCC 736 and Salmonella typhi MTCC3216) and gram positive (Staphylococcus aureus MTCC3160) microorganisms.

Initially, the stock cultures of bacteria were revived by inoculating broth media and grown at 37ºC for 18 hrs. The agar plates were poured by muller hinton media and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with desired quantity (20, 40, 60, 80 and 100 µl) of nanoparticles solution. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone was noted.

III. Results and discussion

When leaf extract of P.glabrum was added to aqueous 1mM silver nitrate solution which leads to the appearance of dark brown colour within 10 min. The colour change was arising due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles [30]. UV-Visible absorption spectroscopy is widely used to study the optical properties and electronic structure of nanoparticles. The process of bio reduction of silver ions was gradually monitored in UV-Vis spectroscopy Figure 2 depicts a series of absorption spectra recorded from the solution of silver nanoparticles at different time intervals. The absorption spectra showed an intense peak at 429 nm due to the surface Plasmon resonance (SPR) band of silver nanoparticles. Noble metals exhibit unique optical properties due to the property of surface plasmon resonance (SPR) which is the collective oscillation of the conduction electrons induced by the interacting electromagnetic field. The size and shape of metal nanoparticles determine the spectral position of plasmon band absorption as well as its width [31] .The appearance of single prominent peak shows that the particles are spherical and uniform in size [32] which is further confirmed by SEM photograph.

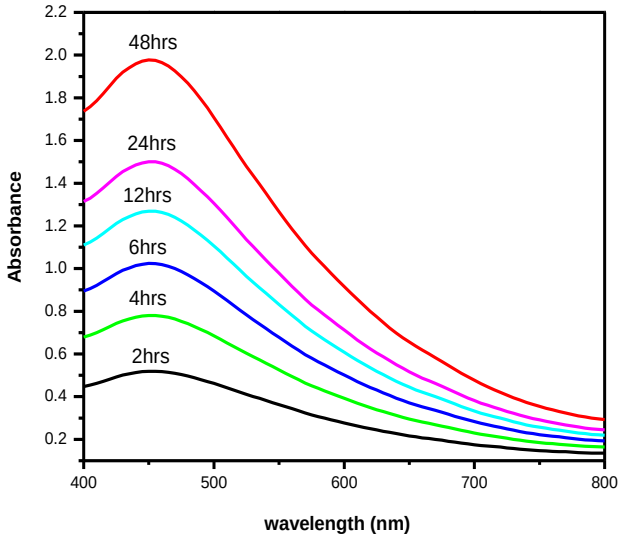

Figure 2: UV-Vis spectra recorded as a function of reaction time of silver nanoparticles

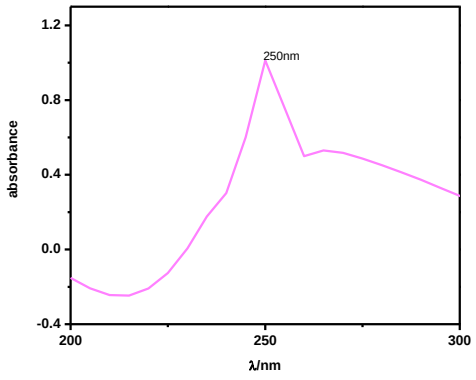

Figure 3: UV-Vis spectrum recorded at lower wavelength of silver nanoparticles

Further in lower wavelength region of UV-Vis spectrum recorded from the reaction medium at 72 hrs (Figure 3) showed an absorption band at 250 nm is clearly indicates that presence of aromatic amino acid viz. tryptophan and tyrosine in the protein [33] . The presence of this amino acid which may be involved in reduction

and capping of silver nanoparticles. Figure 4 shows the fluorescence spectra recorded from the silver nanoparticles solution. An emission band observed at 330 nm due to proteins which are present in the solution are in their native form no change in the tertiary confirmation of proteins while the reduction process [33].

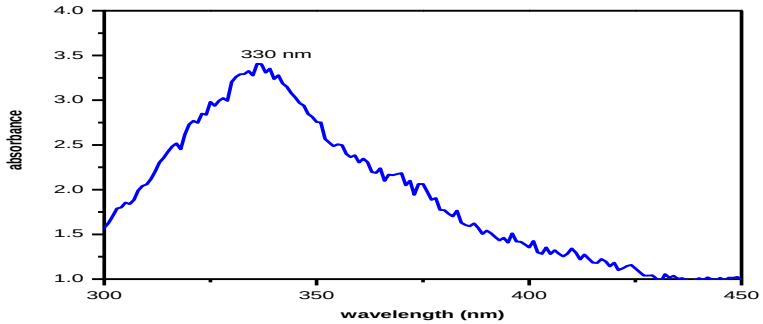

Figure 4: Fluorescence emission spectrum recorded from the silver nanoparticles solution

FTIR measurements were carried out to identify the possible reducing biomolecules in the leaf extract responsible for the formation of silver nanoparticles and to identify the chemical change of the functional groups involved in bioreduction. Figure 5 and Table 1 shows that the FTIR spectrum of silver nanoparticles synthesized by leaf extract of P.glabrum. The absorption band at ~1640 cm^{-1} was arised due to amide-I and was assigned to stretch mode of carbonyl group (C=O) coupled to amide linkage. The spectrum exhibit an intense band at ~2100 cm^{-1} was arised due to S-H stretching vibration in amino acid residues. The band at ~1250 cm^{-1} was arised due to stretching vibration of N-H group which is a characteristic of amide band-III in the amide linkage. The spectrum of P. glabrum showed a broad intense band at ~3300 cm^{-1} which arised due to O-H group present in phenols. The band at ~1730cm^{-1} is a characteristic of carbonyl stretch vibration in carboxylic acids and phenols. The amide linkage between amino acid residues in polypeptide and protein gave rise to well-known signatures in the infrared region of the electromagnetic spectrum. The band at ~1640 cm^{-1} is close to that reported for native proteins, which suggests that proteins are interacting with biosynthesized silver nanoparticles and also their secondary structure was not affected during reaction with Ag^{+} ions or after binding with Ag^{0} nanoparticles. The similar results were observed in the carob leaf extract [34]. The carbonyl group of proteins which have a strong ability to bind silver and formation of layer around the silver nanoparticles act as a capping agent to prevent agglomeration and provide stability.

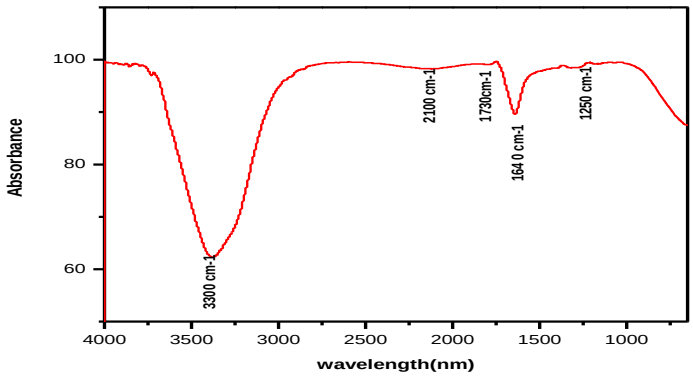

Figure 5: FTIR spectrum of leaf extract of P. glabrum after bioreduction of 1mM silver nitrate solution.

Table 1. FTIR absorption peaks and their functional groups of silver nanoparticles synthesized by P. glabrum.

S. No.	Absorption peak(cm^{-1})	Functional groups
1	3300	Stretch frequency of the O-H band
2	2100	Stretching vibration in S-H
3	1730	Stretching vibration in carbonyl groups
4	1640	Amide-I band (Stretch mode of carbonyl group)
5	1250	Amide-III band

X-ray diffraction is a very powerful and versatile quantitative technique to elucidate the complete three-dimensional structure of matter at molecular and atomic levels. Elucidation of detailed structural features at atomic levels is possible if the specimen is in crystalline state. The XRD patterns of silver nanoparticles

synthesized by leaf extract of P. glabrum shows (Figure 6) braggs peaks at 2θ 38.4 °, 44.6°, 64.4 ° and 77.6 corresponds to (111), (200), (220) and (311) planes of face centred cubic (fcc) of elemental silver. It is confirm that silver nanoparticles are spherical shape and crystalline in nature. The lattice constant calculated from this pattern was a=4.088Å, 'a' value which is in agreement with the value reported in literature for silver (a=4.088 Å JCPDS-04-0783). Further, the sharpness of the peak (111) clearly specifies the nano formulations that were confirmed by using the Debye-Scherrer formula [35]

$D=k\lambda/\beta\cos\theta$

Where 'D' is the mean crystalline size of the particle, K is the shape factor whose value is 1-0.9, λ is the wavelength of the X-ray radiation source that is 0.154 nm, β is $(\pi/180)^{o}$ full width at half maximum (FWHM) and 'θ' is the bragg angle respectively. The sizes of silver nanoparticles of leaf extract of P.glabrum 21.59 nm.

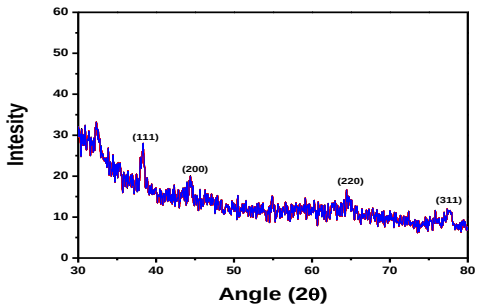

Figure 6: XRD pattern of silver nanoparticles

A representative SEM microphotograph of the silver nanoparticles formed by P. glabrum are shown in Figure 7. This photograph shows spherical shaped nanoparticles as well as some aggregates. Observation of such images in an optical microscope, reveals that assembles were found to be aggregates of silver nanoparticles in the size ranges 10 to 35 nm. The nanoparticles were not in direct contact even within the aggregates indicating stabilization of the nanoparticles by capping agent. The separation between silver nanoparticles seen in the SEM images could be due to capping by proteins and would explain the UV-Vis spectroscopy measurement which is characteristic of well dispersed silver nanoparticles. The above findings corroborates with the results of previous observation made by [36] in their study on biosynthesis of silver nanoparticles in Aeromonas species SH10.

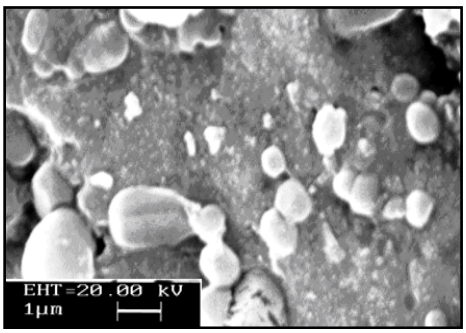

Figure 7: SEM images of silver nanoparticles synthesized by leaf extract P. glabrum

Antimicrobial activity

Silver nanoparticles shows good antimicrobial activity against both gram positive and gram negative bacteria. Gram negative bacteria were more sensitive to silver nanoparticles than the gram positive bacteria. Silver nanoparticles at different concentration (20, 40, 60, 80 and 100µl) showed different zone of inhibition (ZOI) with respect to different microorganisms. The gram negative bacteria S. typhi, P. aeruginosa and E. coli showed maximum zone of inhibition at 12, 8 and 8 mm respectively but, gram positive bacterium S. aurues showed 6 mm (Figure 8 and table 2). Similar results were found in the fruit extract of Leea indica [37]. In the present investigation S. typhi shows more sensitive towards silver nanoparticles and produces 12 mm ZOI and least activity was observed in S. aurues. The differential sensitivity of gram negative and gram positive bacteria towards silver nanoparticles is possibly depends on cell wall structure. The cell wall of gram positive bacteria composed of a thick peptidoglycan layer, which consisting of linear polysaccharide chains cross linked by short peptides thus forming more rigid structure leading to difficult penetration of the silver nanoparticles compared to the gram negative bacteria where the cell wall possesses thinner peptidoglycan layer [38] .The silver

nanoparticles attached to the negatively charged cell wall cause accumulation of protein precursor which alters membrane permeability results in dissipation of the proton motive force [39]. Silver has a greater affinity towards phosphrous and sulphur containing biomolecules in the cells. Sulphur containing proteins in the cell membrane and phosphrous containing DNA elements are preferential sites for the silver nanoparticles binding [40]affecting the replication machinery.

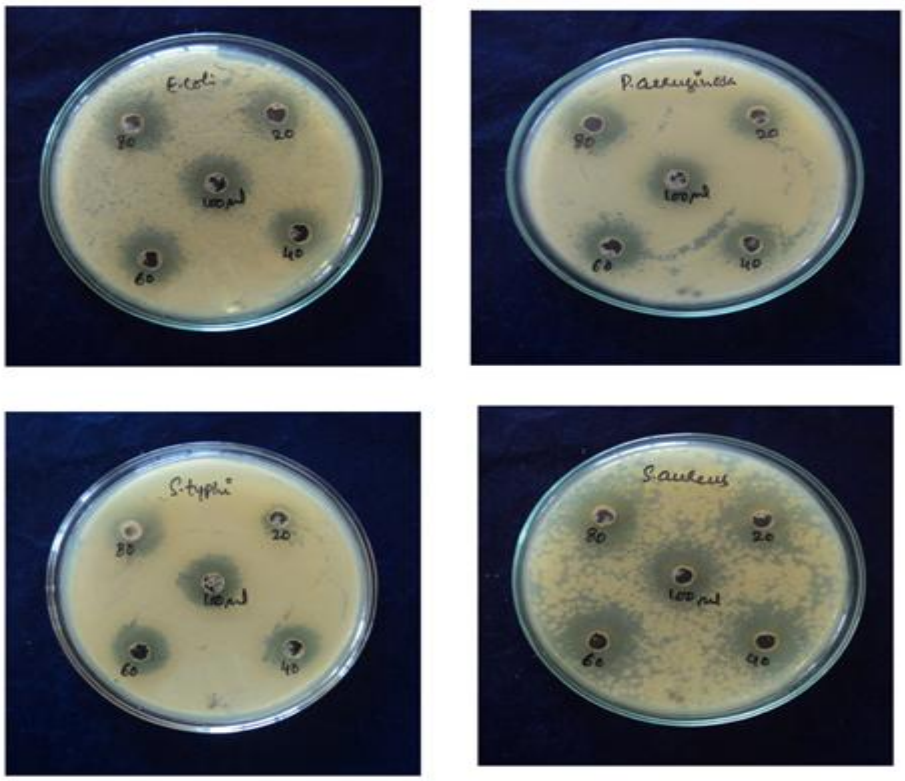

Figure 8: Antimicrobial activity of concentrations of silver nanoparticles (20, 40, 60, 80 and 100 µl)

Table 2. Zone of inhibition (mm) of different concentration of silver nanoparticles on the test pathogens by well diffusion method

AgNPs (µl/ well)	Zone of inhibition (mm)			
	E.coli	S.typhi	P.aeruginosa	S.auerus
20	5	6	5	2
40	6	9	7	4
60	6	11	8	5
80	7	12	8	6
100	8	12	8	6

IV. Conclusion

In the present investigation leaf extract of P, glabrum was used to synthesize biogenic silver nanoparticles. The XRD and SEM results reveal that size of nanoparticles ranges between 10 to 35 nm and are spherical in shape. Plant mediated biosynthesis offers a rapid, cheap, clean, safe and eco-friendly approach. The biogenic silver nanoparticles showed excellent antimicrobial activity against gram negative (S. typhi, E. coli and P.aeruginosa) and gram positive (S. auerus) bacteria. The S. typhi are more sensitive to silver nanoparticles synthesized by leaf extract of P. glabrum The gram negative bacteria shows highest antimicrobial activity than gram positive bacteria.

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