

BIOGENIC POTENTIAL OF STABILIZED SILVER NANOPARTICLES USING *G. SYLVESTRAE* AND THEIR BIOLOGICAL ASSAYS

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ABSTRACT

Objective: The idea of green chemistry has gained immense fame due to replace chemical products and improves technologies to eradicate substances that are harmful to the environment. In this paper, a rapid cost-efficient method was employed using herbal extract *Gymnema sylvestrae* because of their biological constituents present in the sample.

Methods: Phytosynthesis of AgNPs were optimized under different reaction conditions using pH, temperature, incubated at various concentrations. Analyses of particles were revealed using UV-Vis, FTIR spectrum, morphology was observed in scanning electron microscope, particle analysis was done using Diffraction Light Scattering and bioactive constituents present in plant sample was analysed by High-performance liquid chromatography. Bioefficacy of synthesised AgNPs was assessed by means of microbicidal assay against various bacteria and fungi.

Results: UV and FTIR analysis reveals the presence of plant extract responsible for stabilization and efficient reduction. Peptides to proteins, polyphenols, and many other secondary metabolites involved in the bioreduction were identified. SEM micrograph reveals the nature, size and distribution of the sample. HPLC chromatogram indicated the presence of gymnemagenin responsible for their biological assays. Broad spectrum of microbicidal activity have been reported in 400 µl of biosynthesised AgNPs against *Bacillus sp.* (24.5 mm), and *S. epidermis* (22.3 mm).

Conclusion: Therefore *G. sylvestrae* synthesised silver nanoparticles were stable and acts as a reducing and capping agent detecting the presence of biomolecules. Biosynthesised AgNPs showing excellent antimicrobial activity and future prospects of this study indicates that these nanoparticles can be applied in drug delivery.

Keywords: Bioreduction, Biofunctionalized, Optimization, Gymnemagenin

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INTRODUCTION

Bio functionalized materials in nanotechnology are employed for the synthesis of their required size, shape, and dispersivity properties [1]. In the present scenario the development of hygienic and green technologies for the nano synthesis, plays a vital role [2]. As a substitute to the physical method, green synthesis method employing plant extracts are proved to be more viable and simple. Synthesis of novel metal nanoparticles is a striking opportunity by using reducible bio-excretory and different plant extracts. Nanoparticles (NPs) can be developed using different plant extracts, microbial strains [3], enzymes, metabolites, biodegradable products [4], fungi, mushrooms and AgNPs are being investigated for application in biomedicine and to be used as bactericide agent or for cancer treatment [5]. Plant materials, including leaf [6], bark [7], fruit [8], peel [9], seed [10], and root [11] extracts work so well in the green synthesis of AgNPs under mild experimental conditions and replacing hazardous chemicals by polyphenols, flavonoids, proteins, saponins or sugar as reducing agents as well as capping agents. Metal nanoparticles have potential applicability in a variety of areas such as electronics, textile, catalysis, energy and medicine [12], antimicrobial [13, 14], antitumor effect [15], sensitivity to detect the presence of various pollutants such as metals and dyes [16, 17], antibiotics. Therefore, the progress of environmentally conscious, energy-efficient, facile, and rapid green synthesis that evade toxic chemicals has concerned significant attention [18].

Gymnema sylvestre R. Br. belongs to the family Asclepiadaceae, is used as an efficient anti-diabetic medicinal herb. Leaves are rich in biological compounds like alkaloids, betaine choline, tartaric acid, triterpene saponins, gymnemic acid, anthraquinone derivatives and trimethylamine [19]. Anti sweet constituent of the leaves has been developed by the mixture of triterpene saponins. Chewing of leaves reduces sensitivity to sweet substances [20]. Glycosides isolated from plant have anaesthetic and topical effect resulting from the competition of the receptor sites between glycosides and the sweet substances [21].

Bioactive constituents of the plant include the gymnemic acids, saponins, stigmasterol, quercitol, and the amino acid derivatives betaine, choline, and trimethylamine. The saponin gymnemic acid, constituent of the leaves, was shown to suppress sweet taste sensation and to inhibit glucose absorption in the small intestine [22].

The aim of the present work was to apply the accurate principles of green methods for the development of biologically synthesised metal nanoparticles by using leaf extracts of *G. sylvestrae*. This method of synthesis with plant extract is advantageous because it is simple, highly reproducible, nontoxicity and can be processed at the room temperature. Microbicidal assessment of this phytosynthesised nanoparticles against pathogens indicate that this nanoparticle can be further used for the applications of drug delivery.

MATERIALS AND METHODS

Experimental section

Chemicals and reagents used

Silver Nitrate (AgNO_3) and solvents like acetone, chloroform, petroleum ether, ethanol, methanol, nutrient agar and potato dextrose agar were purchased from Sigma Aldrich, USA. All other reagents, chemicals and solutions used were of analytical grade.

Preparation of plant material

Gymnema sylvestrae was collected from local surroundings of Madurai of 9.9252 ° N latitude and 78.1198 ° E longitude region. Fresh leaves were used for the extraction of active components were shade dried at room temperature and powdered using electrical blender. These powdered samples were stored in an airtight container.

Qualitative phytochemical examination

The dried plant material was successively extracted with acetone, chloroform, petroleum ether, ethanol, methanol and kept in shaker for 2 d. The solvent was evaporated using rotary evaporator under

reduced pressure at 37 °C. The plant extract was subjected to phytochemical analysis by the method described by Harborne [23]. The extract was screened for the presence of bioactive compounds like alkaloid, flavonoid, glycosides, phenol, saponin, steroid, tannin and terpenoids.

Biosynthesis of silver nanoparticles

Aqueous solution (1 mmol) of silver nitrate (AgNO_3) was prepared and used for the synthesis of AgNPs. 5 ml of *G. sylvestrae* along with 95 ml of aqueous solution of 1 mmol AgNO_3 was mixed for reduction into Ag^+ ions. In a typical synthesis of silver (Ag) nanoparticles the leaf extract (1.5 ml) was added to 30 ml of 10^{-3} M AgNO_3 aqueous solution in a 250-ml Erlenmeyer flask and heated on water bath at 75 °C for 60 min. Bioreduction of Ag ions was observed by means of brownish colour further experimented using spectrophotometric determination. The product was redispersed in deionized water and the centrifugation was continued constantly for two to three times to remove the absorbed substances on the surface of nanoparticles [24].

Microorganisms

Bacteria such as *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Klebsiella terrigena*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Fungal organisms such as *Fusarium oxysporum*, *Penicillium* and *Aspergillus niger* were obtained from GH hospital, Madurai. The bacterial cultures were maintained on nutrient agar slants at 4 °C and the fungal cultures were maintained on potato dextrose broth at 25 °C.

Preparation of inoculum

The bacterial cultures were inoculated into the nutrient broth and incubated for 24 h at 37 °C. The growth was compared with 0.5 McFarland; the turbidity of the medium indicates the growth of organisms, while the fungal cultures were inoculated into potato dextrose broth and allowed to incubate at 25 °C for 48 h [25].

Antimicrobial assay of silver nanoparticles

The AgNPs synthesized from *Gymnema sylvestrae* were tested for antimicrobial activity by well-diffusion method against pathogenic microbes. Standard agar well diffusion method was employed to detect the assay of AgNPs against the microbial isolate according to Cheesbrough [26]. For microbicidal assay of the compounds, wells were made in plates containing nutrient agar medium seeded with 100 μl of 24 h of each microbial isolate. The wells were loaded with *G. sylvestrae*, AgNO_3 and plant-based synthesized silver nanoparticle was loaded using a micropipette. From each solution, that contains both Ag and *G. sylvestrae* extracts, as well as the control, synthesized

silver nitrate was placed in separate wells. AgNO_3 (200 μl) solution was used as a standard to determine their inhibition potential. The plates were left in the refrigerator for 2 h then incubated at 37 °C for 24 h. The diameter of the inhibition zones was calculated and tabulated by comparing with standard.

UV-Vis spectroscopy

The synthesized AgNP were observed visually for any colour change and one ml of the reaction mixture were withdrawn consecutively at various time levels by diluting a small aliquot (100 μl) of the sample 10-fold in deionized water for analysis of surface plasmon resonance of silver nanoparticles. The reduction of pure Ag^+ ions was monitored by measuring using a UV-Vis spectrophotometer (Shimadzu 1601 model, Japan) at the resolution of 1 nm in the range of 200–800 nm.

FT-IR analysis

The functional groups of the nanoparticles were qualitatively confirmed by using FTIR spectroscopy, with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. Approximately 3 mg of lyophilized sample along with 300 mg of dried KBr, was mixed and crushed well in mortar and pestle to prepare thin pellet for analysis. Scans per sample were performed in range of 400–4000 cm^{-1} [27].

Scanning electron microscopy (SEM)

The structure and composition of freeze-dried purified silver particles were analyzed by using a 10-kV ultra-high resolution scanning electron microscope. A drop of aqueous solution containing purified silver nanomaterials obtained after repetitive centrifugation was sputter coated on carbon-coated copper grids and the images of nanoparticles were studied using FEI QUANTA-200 SEM.

Particle size analysis and Zeta potential

Particle size analysis of silver nanoparticles was analysed on particle size analyzer system (Zeta sizer, Malvern Instruments Ltd., USA).

RESULTS AND DISCUSSION

Authentication of plant material

Fresh leaves of *G. sylvestrae* were authenticated by Dr. S. John Britto, The Director, The Rapinat herbarium and center for molecular systematic, St. Joseph's College, Tiruchirappalli, Tamilnadu. Voucher specimens of the dried drugs were deposited in the herbarium of the centre for molecular systematic, St. Joseph College, the obtained voucher number is AR 001/21/12/18.

Table 1: Phytoconstituents present in *G. sylvestrae*

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Alkaloid	-	+	+	+	+
Flavanoid	-	-	+	+	+
Saponin	-	-	-	+	+
Tannin	+	-	-	+	-
Phenol	+	+	+	+	-
Glycosides	-	-	+	-	+
Terpenoid	+	+	-	+	+
Steroid	-	+	-	+	+

+: Present, -: absent. The results are replicates of samples.

Phytochemical analysis of *G. sylvestrae*

Investigations on the phytochemical screening of *G. sylvestrae* revealed the presence of biological compounds were represented in table 1. Methanol extract possess majority of the active constituents except glycosides, whereas in aqueous extract tannins and phenols were absent. The presence of phytoconstituents such as alkaloids, flavonoids, tannins, and phenolic compounds was responsible for several medicinal properties such as antioxidant, antimicrobial, anti-inflammatory and anticancer activities etc. [28]. Ethyl acetate extract displayed positive result towards alkaloid, flavanoid, phenol

and glycosides. In hexane and chloroform extract flavanoid, saponin and glycosides were absent, whereas indicates the presence of phenol and terpenoid. Whereas metabolites like plants containing different proteins and secondary metabolites such as alkaloids, quinines, flavonoids, terpenoids and saponins [29, 30] are involved in the synthesis and stabilization of AgNPs.

Phytosynthesis of AgNPs

AgNPs are synthesized by means of plant extract mediated process, AgNO_3 along with *G. sylvestrae* is incubated for several hours

indicates the brownish colour. This can be attributed to the large amount of reductants (electron-rich phytomolecules) in the reaction medium, which cause the rapid reduction of Ag ions. The fast reduction of Ag ions usually facilitates that plant extract contain a variety of naturally occurring phytomolecules, such as water-soluble flavonoids, alkaloids, and several other phenolic compounds, which are broadly classified as polyphenols [31, 32]. These polyphenol-based phytomolecules acquire strong reducing properties and have a great tendency to adsorb on the surface of NPs [33].

UV-Vis analysis of synthesized AgNP using *G. sylvestrae*

Optimization parameters indicates the results of NPs synthesized were stable at 75 °C temperature within 2 h, at acidic (4) pH and basic (8.5) pH, in the concentration of 2 mmol is indicated in fig. 1–5. UV-visible spectroscopy is used for measuring the reduction of metal ions based on optical properties. Biological synthesis of AgNPs

involves mixing the aqueous extract with an aqueous solution of AgNO₃. The various biomolecules present in the plant extract such as enzymes, proteins, flavonoids, terpenoids and cofactors act as both reducing and capping agents [34]. Previous studies indicate that neutral pH is responsible for silver nanoparticle synthesis [35]. The biosynthesis can be performed in ambient conditions and by the standards prescribed in green chemistry. Reaction temperature and the dosage of the tea extract showed an effect on the production efficiency and formation rate of nanoparticles [36]. Chemical and physical methods are inconvenient as they are unstable and toxic, these conditions can be satisfactorily solved by biosynthetic processes. In this study, the aqueous extract of *G. sylvestrae* is used for the synthesis of Agnp. The synthesis of silver nanoparticles occurs at the room temperature in various periods and incubation time plays an important role in the formation of nanoparticles by reducing the silver ions at room temperature.

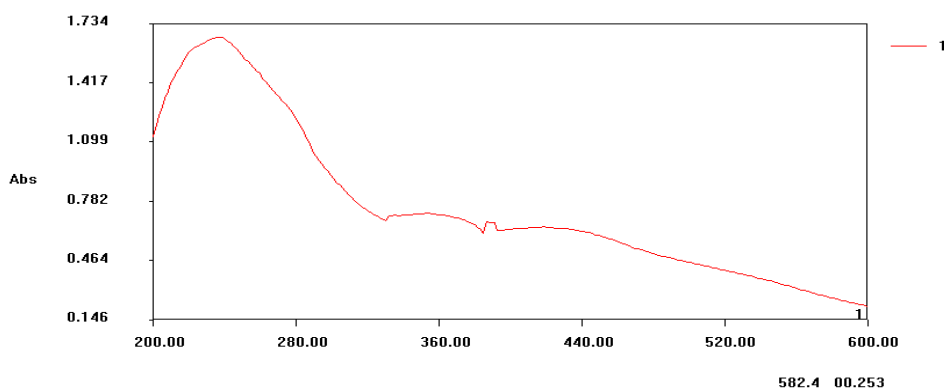


Fig. 1: UV-Vis analysis of synthesized AgNPs at 75 °C

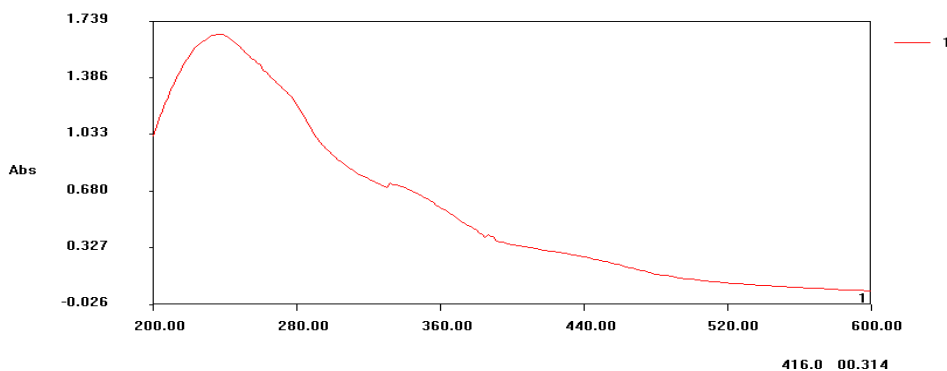


Fig. 2: UV-Vis analysis of synthesized AgNPs at 2 h

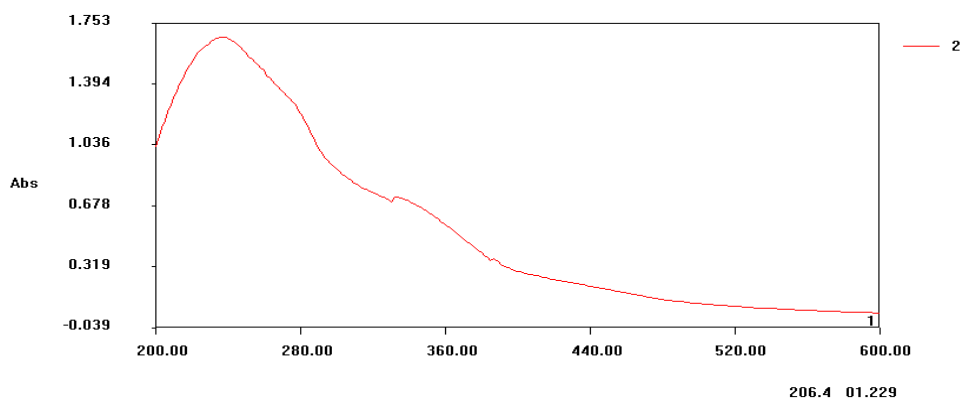


Fig. 3: UV-Vis analysis of synthesized AgNPs at pH 4.5

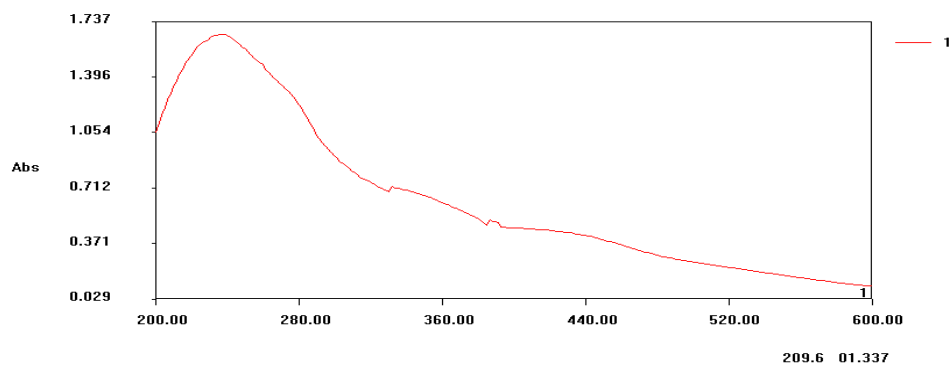


Fig. 4: UV-Vis analysis of synthesized AgNPs at pH 8.5

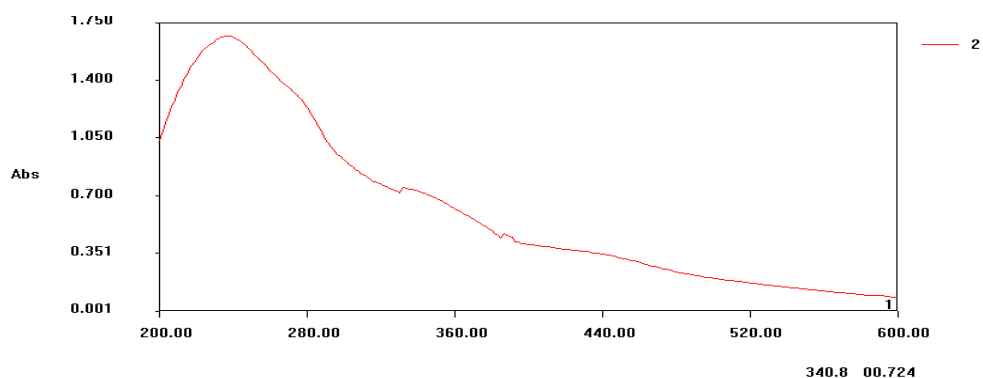


Fig. 5: UV-Vis analysis of synthesized AgNPs at 2 mmol

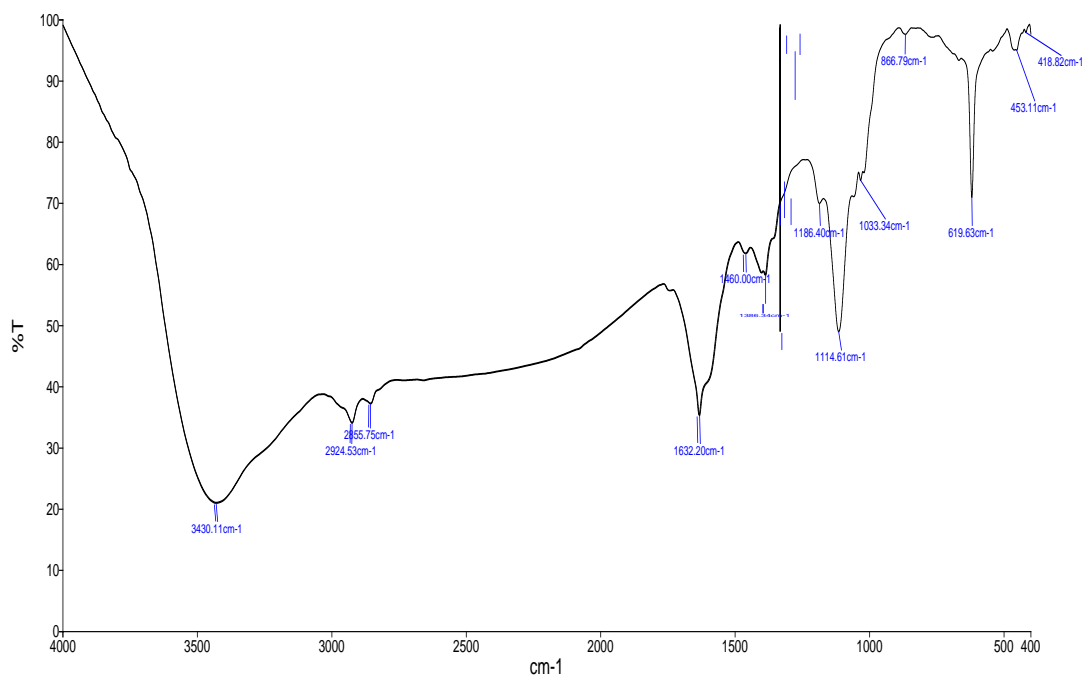


Fig. 6: FTIR spectrum of synthesized AgNP using *G. sylvestrae*

FTIR analysis of biosynthesized AgNPs

FTIR spectrum of phytosynthesized AgNPs using *G. sylvestrae* were depicted in fig. 6. The wavenumber at 3430 cm^{-1} corresponds to N-H stretch of amino compound, followed by 2924 cm^{-1} , 2855 cm^{-1} belongs to methylene C-H asymmetric/symmetric stretching of

alkane group. The spectral results revealed that most of the bands were representative of flavonoids and terpenoids and vibrational bands corresponding to bonds such as C=C, C-O-C, C=O, C-O and C-N were derived from the plant metabolites like thiamine, flavonoids and terpenoids present in *G. sylvestrae*. This result suggests that plants are responsible for proficient stabilization of

AgNPs [37, 38]. The functional group at 1632.20 cm^{-1} corresponds to primary amide NH_2 bending, followed by 1460 cm^{-1} of C=C stretching of aromatic compounds. *Ziziphora tenuiora* leaves functionalized with biomolecules that have primary amine group, carbonyl group, hydroxyl groups and other stabilizing functional groups as shown by FTIR spectroscopic technique [39]. The wavenumber at 1186.40 cm^{-1} , 1114.61 cm^{-1} , 1033.34 cm^{-1} belongs to C-O stretching of alcohol and phenol group, followed by 866.79 cm^{-1} belongs to out of plane C-H bending of aromatic compounds, 619 cm^{-1} of C-H bend of alkyne group. This complex

molecule becomes unstable and hydroxyl group reduces the silver ion (Ag^+) to silver (Ag^0), thus producing stable AgNPs [40]. Previous study indicates that the carboxyl ($\text{C}=\text{O}$), amine ($\text{N}-\text{H}$), and hydroxyl ($\text{O}-\text{H}$) groups in carob leaf extract are chiefly involved in the reduction of silver ions. Previous results reveal that the protein present in leaf extract is in charge of stabilizing the AgNPs and thereby prevents agglomeration. Aminoacids along with carbonyl group signify the formation of a covering layer on AgNPs and thus acting as a capping agent to avoid agglomeration in the aqueous medium [41].

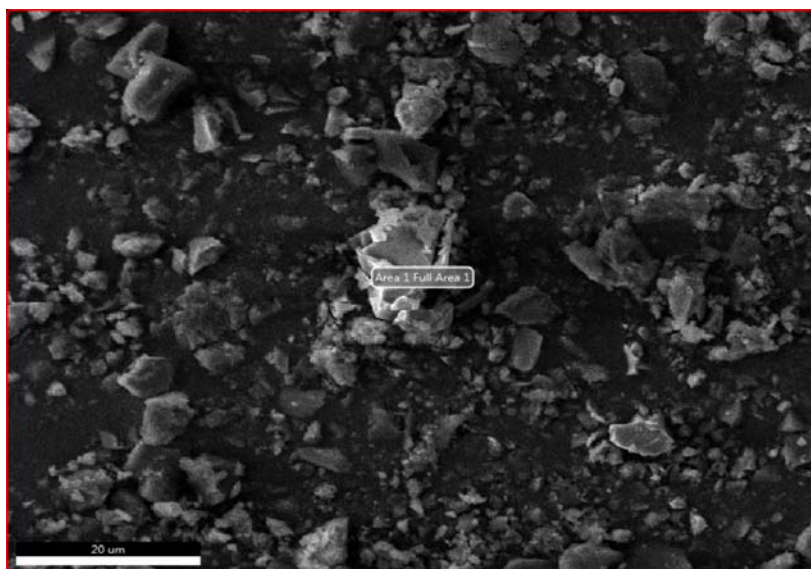


Fig. 7: Scanning electron micrograph of synthesized AgNPs using *G. sylvestrae*

SEM result of biosynthesized AgNPs

SEM images of AgNPs are depicted in fig. 7. Morphology and structure of biosynthesized AgNPs can be viewed by SEM. The

results indicate that particles were sphere shaped and well distributed with aggregation. Organic molecules observed on surface particles can be viewed which serve as a reducing and also as a capping agent.

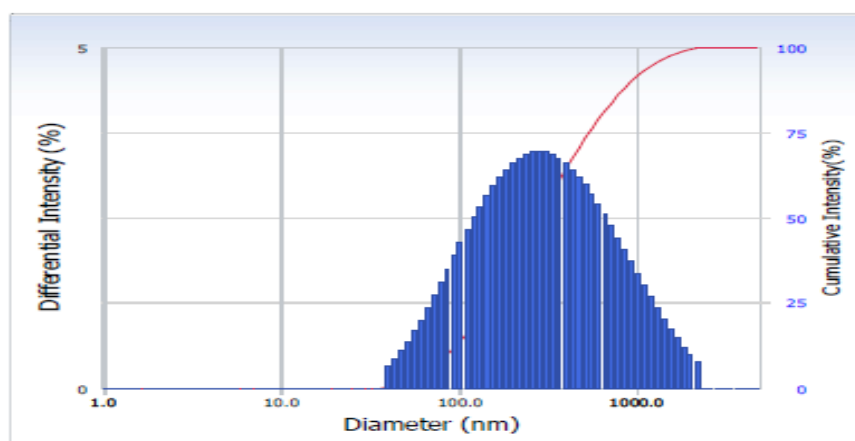


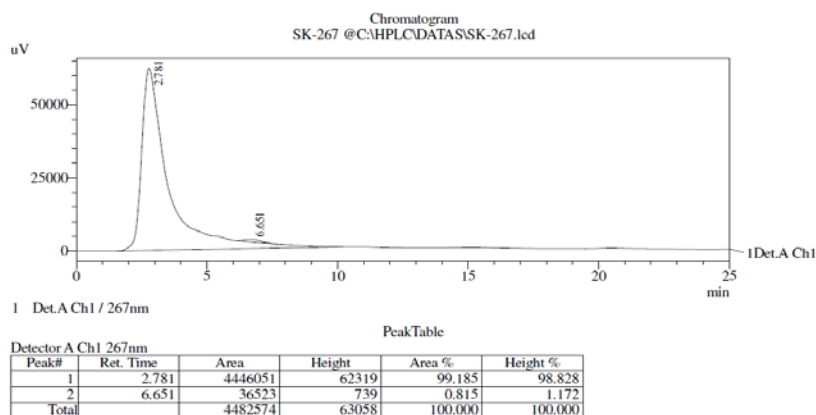
Fig. 8: DLS analysis of synthesized AgNP using *G. sylvestrae*

DLS analysis of *G. sylvestrae*

DLS analysis of plant-based AgNPs using *G. sylvestrae* were represented in fig. 8. The particle size distribution spectra for the silver nanoparticles were recorded as diameter (nm) on X axis versus frequency (%/nm) on Y axis. The results indicate that the nanoparticles were found to be well dispersed. Dynamic light

scattering technique has been used to measure hydrodynamic diameter of the hydrosol (particle suspension). The size obtained by the DLS is different because it gives the average size of the particles. *Gymnema sylvestre* AgNPs was found to be 100 nm.

The particle size obtained from DLS is different, due to the variation in principles used for measurement [42].

Fig. 9: HPLC analysis of *Gymnema sylvestrae***HPLC analysis of *G. sylvestrae***

HPLC chromatogram of *G. sylvestrae* was represented in fig. 9. As HPLC is most widely used tool for quantification of analytes with good reproducibility an attempt has been made to develop a dependable and reproducible validated method for gymnemagenin

estimation using HPLC. The retention time at 6.6 indicates the presence of gymnemagenin of 0.8%. Gymnemagenin is a common genin of gymnemic acids which can be produced only after acidic and basic hydrolysis. The method reported in the literature [43] reports properly validated HPLC method for gymnemagenin estimation.

Table 2: Antimicrobial activity of *G. sylvestrae*

S. No.	Microorganism	<i>Gymnema sylvestrae</i>				
		Acetone	Chloroform	Ethanol	Ethyl acetate	Petroleum ether
1.	<i>Bacillus cereus</i>	21.5±0	19.3±0.2	24±0.5	22±0	17.6±0.2
2.	<i>Klebsiella pneumonia</i>	16.3±0.2	15.5±0	19.5±0	17±0	14.6±0.2
3.	<i>Pseudomonas aeruginosa</i>	18±0	17.3±0.2	20.4±0.1	18.4±0.2	15±0
4.	<i>Staphylococcus aureus</i>	20±0	18.1±0.2	22±0	21.6±0.1	16.5±0
5.	<i>Escherichia coli</i>	19.3±0.1	18±0	21.3±0.2	19.5±0	15.3±0.2
6.	<i>Mycobacterium mucilaginosus</i>	14.3±0.2	14.3±0.2	18.3±0.1	15.1±0.2	13.3±0.2
7.	<i>Klebsiella terrigena</i>	15.5±0	15±0	19.1±0.1	16.5±0	14±0
8.	<i>Fusarium oxysporum</i>	14.1±0.2	12±0	17.1±0.2	16.5±0	11.5±0
9.	<i>Penicillium</i>	16.5±0	15.2±0.1	19±0	18.6±0.1	14.5±0
10.	<i>Aspergillus niger</i>	15.3±0.1	14.5±0	18.3±0.2	15.1±0.2	13.3±0.2

*values are mean of ±SD, n=3

Microbicidal assay of *G. sylvestrae*

Bactericidal and fungicidal assay of *G. sylvestrae* were depicted in table 2. Maximum inhibitory effect was observed in ethanol extract against *B. cereus* (24 mm), ethylacetate (22 mm) and acetone extract (21.5 mm), followed by *S. aureus* in ethanol (22 mm), ethyl acetate (21.6 mm) and acetone (20 mm). A glycoprotein isolated from *G. sylvestrae* exhibits good antibacterial activity against methicillin-resistant *Staphylococci* and multi-resistant *Enterococci* 44. Moderate inhibitory effect was observed in *P. aeruginosa* in ethanol (20.4 mm) and ethyl acetate (18.4 mm) followed by *K. pneumonia* (19.5 mm, 17.5 mm), whereas least inhibition was observed in *M. mucilaginosus* in chloroform (14.3 mm) and petroleum ether (13.3 mm). Stronger extraction capacity of ethanol has yielded a number of active constituents responsible for antimicrobial effect

[45]. The results suggest that chloroform and petroleum ether extract remained sensitive towards the organism. Results corroborated with former reports suggests that the ethanolic extract of *G. sylvestrae* leaves possess superior antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* [46]. The experimented activity may be due to the occurrence of potent phytoconstituents in the extracts, the results correlate with previous reports [47]. The chief compounds of *G. sylvestre* includes gymnemic acid, saponins and oleanane-type of triterpenoid. Maximum fungicidal activity was reported in ethanol (19 mm) and ethyl acetate (18.6 mm), followed by *A. niger* (18.3 mm, 15.1 mm), *F. oxysporum* (17.1 mm, 16.5 mm). The active principle is gymnemic acid also possesses antimicrobial and sweet suppressing activities. Chloroform (12 mm) and petroleum ether (11.5 mm) extract remained sensitive towards *F. oxysporum* possessing least inhibition.

Table 3: Antimicrobial activity of synthesized silver nanoparticle

Microorganism	Plant samples used in the study zone of inhibition in mm <i>Glycyrhiza glabra</i>				
	100 µl	200 µl	300 µl	400 µl	Agno ₃ solution 200 µl
<i>Bacillus sp.</i>	20.6±0.1	21±0	22.6±0.1	24.5±0	20±0
<i>Escherichia coli</i>	18.5±0	19.2±0.2	20.2±0.2	21.3±0.1	19.1±0.2
<i>Mycobacterium mucilaginosus</i>	14±0	15.5±0	16.4±0.3	17±0	14.5±0
<i>Klebsiella terrigena</i>	16.3±0.2	17±0	17.5±0	18.7±0.2	16.5±0
<i>Pseudomonas aeruginosa</i>	17.6±0.1	18.2±0	19.6±0.2	20.5±0	17.3±0.3
<i>Shigella</i>	16.5±0	17.7±0.1	18±0	19.4±0.2	16±0
<i>Staphylococcus epidermis</i>	19±0	20.6±0.1	21.5±0	22.3±0.3	19.1±0.2
<i>Fusarium oxysporum</i>	14±0	17.3±0.3	18.5±0	19.6±0.1	13.5±0
<i>Penicillium</i>	17.6±0.1	18±0	19.1±0.2	21.5±0	17±0
<i>Aspergillus niger</i>	16.5±0	17.5±0	19±0	21±0	16.3±0.2

*values are mean of ±SD, n=3

Microbicidal assay of synthesized AgNPs using *G. sylvestriae*

Microbicidal efficacy of phytosynthesized AgNPs using *G. sylvestriae* were represented in table 3. Among various metal nanoparticles, silver nanoparticles (AgNPs) in particular have been the focus of increasing interest due to their peculiar properties which can be tailored for a specific application by controlling the shape, size and morphology of the nanoparticles [48, 49, 50, 51, 52, 53]. 400 µl of AgNPs remained resistant against *Bacillus sp.* (24.5 mm), *S. epidermis* (22.3 mm) and *E. coli* (21.3 mm) followed by 300 µl of AgNPs (22.6 mm, 21.5 mm, 20.2 mm) possessing higher inhibition. *Abutilon indicum* synthesized nanoparticles in previous results revealed that high antimicrobial activities against *S. typhi*, *E. coli*, *S. aureus* and *B. subtilis* microorganisms [54]. The effective antimicrobial activity is due to a spherical shape and small size of AgNPs. *Bacillus sp.* (21 mm), *S. epidermis* (20.6 mm) and *E. coli* (19.2 mm) possess moderate zone of inhibition in 200 µl of AgNPs. The irregular release of insufficient concentrations of silver ions from AgNO₃ may be the reason for its restricted efficacy as antimicrobial agents, which can be enhanced using AgNPs because their large surface area makes them highly reactive [55]. Nps increases the contact area between AgNPs and microbe which in turn increases the inhibitory activity. The strain susceptible to Al₂O₃ nanoparticles exhibited a superior zone of inhibition (*E. coli*), whereas resistant strains exhibit a smaller zone of inhibition (*Proteus vulgaris*) [56]. Minimum inhibitory effect was observed in 100 µl against *M. mucilaginosus* (14 mm). Tamboli and Lee (2013) demonstrated that the antimicrobial effect of SNPs was due to the breakage of double-stranded DNA molecules present in the bacteria [57]. One such size-dependent property has led to the development of dressing materials incorporated with nano-sized silver to enhance the wound healing property of the dressing materials [58].

Fungicidal activity of photosynthesis AgNPs indicate that maximum inhibition was observed in 400 µl of AgNPs against *Penicillium* (21.5 mm), *A. niger* (21 mm) and *F. oxysporum* (19.6 mm). In addition, nanoparticles possess extremely large surface area that provides better contact and interaction with bacterial cells [59]. The biosynthesized AgNPs have been applied widely to prevent biomedical devices associated infections, food preservation, water purification, clothing, cosmetics, and other numerous pharmaceutical products [60, 61, 62, 63]. Zone of inhibition was found to be moderate in 200 µl of AgNPs (18.5 mm, 17.5 mm, 17.3 mm). Minimum inhibitory effect was observed in 100 µl against *Penicillium* (17.6 mm), *A. niger* (16.5 mm) and *F. oxysporum* (14 mm). In this perspective nanoscale materials have emerged up as novel antimicrobial agents due to its unique chemical and physical properties [64]. The effective antimicrobial activity of the biosynthesized AgNPs in this study provides the capability of AgNPs as potential topical ointments for various infections without any side effects. This environmentally friendly approach is more biocompatible and cost-efficient and includes the capability of supporting larger synthesis [65, 66].

CONCLUSION

Naturally, plants have a broad array of phyto-biomolecules which act as reducing as well as stabilizing agents, characteristically responsible for increasing the biomedical applications of nanoparticles synthesized by green based principle. Secondary metabolites present in plants play an important role in synthesis. AgNPs inhibited the bacterial and fungal growth corroborating their biological properties.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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