

Potential anticancer properties of bioactive compounds of *Gymnema sylvestre* and its biofunctionalized silver nanoparticles

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Background: *Gymnema sylvestre* is an ethno-pharmacologically important medicinal plant used in many polyherbal formulations for its potential health benefits. Silver nanoparticles (SNPs) were biofunctionalized using aqueous leaf extracts of *G. sylvestre*. The anticancer properties of the bioactive compounds and the biofunctionalized SNPs were compared using the HT29 human adenoma colon cancer cell line.

Methods: The preliminary phytochemical screening for bioactive compounds from aqueous extracts revealed the presence of alkaloids, triterpenes, flavonoids, steroids, and saponins. Biofunctionalized SNPs were synthesized using silver nitrate and characterized by ultraviolet–visible spectroscopy, scanning electron microscopy, energy-dispersive X-ray analysis, Fourier transform infrared spectroscopy, and X-ray diffraction for size and shape. The characterized biofunctionalized *G. sylvestre* were tested for its in vitro anticancer activity against HT29 human colon adenocarcinoma cells.

Results: The biofunctionalized *G. sylvestre* SNPs showed the surface plasmon resonance band at 430 nm. The scanning electron microscopy images showed the presence of spherical nanoparticles of various sizes, which were further determined using the Scherrer equation. In vitro cytotoxic activity of the biofunctionalized green-synthesized SNPs (GSNPs) indicated that the sensitivity of HT29 human colon adenocarcinoma cells for cytotoxic drugs is higher than that of Vero cell line for the same cytotoxic agents and also higher than the bioactive compound of the aqueous extract.

Conclusion: Our results show that the anticancer properties of the bioactive compounds of *G. sylvestre* can be enhanced through biofunctionalizing the SNPs using the bioactive compounds present in the plant extract without compromising their medicinal properties.

Keywords: *Gymnema sylvestre*, gymnemic acid, biofunctionalized silver nanoparticles, anticancer activity, HT29 cell line

Introduction

For treatment of various diseases, bioactive components from medicinal plants that are similar to chemical compounds are used.¹ In recent years, the use of ethno-botanical information in medicinal plant research has gained considerable attention in some segments of the scientific community.² In one of the ethno-botanical surveys of medicinal plants commonly used by the Kani tribals in Tirunelveli hills of the Western Ghats in Tamil Nadu, India, it was revealed that *Gymnema sylvestre* is the most important species based on its use.² The use of plant parts and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice for many decades.³

G. sylvestre R. Br, commonly known as “Meshashringi”, is distributed over most of India and has a reputation in traditional medicine as a stomachic, diuretic, and a remedy

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to control diabetes mellitus. *G. sylvestre* R. Br⁴ is a woody, climbing plant that grows in the tropical forests of Central and Southern India and in parts of Asia.⁵ It is a pubescent shrub with young stems and branches, and has a distichous phyllotactic opposite arrangement pattern of leaves which are 2.5–6 cm long and are usually ovate or elliptical. The flowers are small, yellow, and in umbellate cymes, and the follicles are terete, lanceolate, and up to 3 inches in length.⁶

In homeopathy, as well as in folk and ayurvedic medicine, *G. sylvestre* has been used for diabetes treatment.⁷ *G. sylvestre* has bioactive components that can cure asthma, eye ailments, snakebite, piles, chronic cough, breathing troubles, colic pain, cardiopathy, constipation, dyspepsia, hemorrhoids, and hepatosplenomegaly, as well as assist in family planning.⁸ In addition, it also possesses antimicrobial,⁹ antitumor,⁵ anti-obesity,¹⁰ anti-inflammatory,¹¹ anti-hyperglycemic,¹² antiulcer, anti-stress, and antiallergic activity.¹³

The presence of flavonoids, saponins, anthraquinones, quercitol, and other alkaloid have been reported in the flowers, leaves, and fruits of *G. sylvestre*.¹⁴ The presence of other therapeutic agents, such as gymnemagenin, gymnemic acids, gymnemanol, and β -amyrin-related glycosides, which play a key role in therapeutic applications, have also been reported. The focus of the present work is to assess the potential therapeutic medicinal value of this herb and to understand/enhance the mechanistic action of their bioactive components.¹⁴

G. sylvestre contains triterpenes, saponins, and gymnemic acids belonging to the oleanane and dammarene classes.^{15,16} The plant extract has also tested positive for alkaloids, acidic glycosides, and anthraquinone derivatives. Oleanane saponins are gymnemic acids and gymnema saponins, while dammarene saponins are gymnemasides.

As reported by Thakur et al¹⁴ the aqueous extracts of the *G. sylvestre* leaves showed the presence of gymnemic acids I–VI, while the saponin fraction of the leaves tested positive for the presence of gymnemic acids XV–XVIII. The gymnemic acid derivative of gymnemagenin was elucidated from the fraction VIII–XII, which is responsible for the antidiabetic activity, and the fraction VIII stimulates the pancreas for insulin secretion. The novel D-glucoside structure with anti-sweet principle is present in the I–V saponin fraction. The presence of pentatriacontane, α - and β -chlorophylls, phytin, resins, D-quercitol, tartaric acid, formic acid, butyric acid, lupeol, and stigmasterol has been reported as other plant constituents of *G. sylvestre*,¹⁴ while the extract has also been tested positive for alkaloids.^{13,17}

Sharma et al have reported the antioxidant activity of oleanane saponins from *G. sylvestre* plant extract and

determined the IC₅₀ values for 2,2-diphenylpicrylhydrazyl (DPPH) scavenging, superoxide radical scavenging, inhibition of in vitro lipid peroxidation, and protein carbonyl formation as 238 μ g/mL, 140 μ g/mL, 99 μ g/mL, and 28 μ g/mL, respectively, which may be due to the presence of flavonoids, phenols, tannins, and triterpenoids.¹⁸ The enhanced radiation (8 Gy)-induced augmentation of lipid peroxidation and depletion of glutathione and protein in mouse brain were reported by Sharma et al¹⁸ using multiherbal ayurvedic formulations containing extracts of *G. sylvestre*, such as “Hyponidd” and “Dihar”. They also demonstrated the antioxidant activity by increasing the levels of superoxide dismutase, glutathione, and catalase in rats through in vivo studies.¹⁹

Kang et al²⁰ proved the role of antioxidants from *G. sylvestre* in diabetic rats using ethanolic extracts. Using several antioxidant assays, eg, thiobarbituric acid assay with slight modifications, egg yolk lecithin or 2-deoxyribose (associated with lipid peroxidation) assay, superoxide dismutase-like activity assay, and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay.

The potent anticancer activity of *G. sylvestre* against the human lung adenocarcinoma cell lines (A549) and human breast carcinoma cell lines (MCF7) using alcoholic extracts of the herb has been reported by Srikant et al.²¹ Also, Amaki et al²² reported the inhibition of the breast cancer resistance protein using the alcoholic extract of *G. sylvestre*.

Many plant-derived saponins, eg, ginsenosides, soyasaponins, and saikosaponins, have been found to exhibit significant anticancer activity. The anticancer activity of gymnemagenol on HeLa cancer cell lines in in vitro conditions was determined by the MTT cell proliferation assay for cytotoxic activity of saponins. Using 5 μ g/mL, 15 μ g/mL, 25 μ g/mL, and 50 μ g/mL concentrations of gymnemagenol, the IC₅₀ value was found to be 37 μ g/mL after 96 hours. The isolated bioactive constituent, gymnemagenol, showed a high degree of inhibition to HeLa cancer cell line proliferation, and saponins were not found to be toxic to the growth of normal cells under in vitro conditions.²³

Already many researchers have reported that the leaves of *G. sylvestre* lower blood sugar, stimulate the heart, uterus, and circulatory systems, and exhibit anti-sweet and hepatoprotective activities.^{20,24–31} Administration of *G. sylvestre* extract to diabetic rats increased superoxide dismutase activity and decreased lipid peroxide by either directly scavenging the reactive oxygen species, due to the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules (albumin and uric acid).^{24,30,32}

Therefore, in this study, an attempt was made to synthesize the silver nanoparticles (SNPs) from aqueous extracts of the *G. sylvestre* leaves. These green-synthesized SNPs (GSNPs) of *G. sylvestre* were examined by ultraviolet–visible (UV–vis) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analysis for studying their size and shape. The synthesized and well-characterized nanoparticles (NPs) were tested for their cytotoxicity effect. Our findings clearly demonstrate that it is indeed possible to have a much greener way to synthesize SNPs without compromising their antibacterial properties and thus plant extracts may prove to be a good alternative to obtain such NPs with improved antibacterial and antiviral properties for diabetic wound healing applications. Goix et al³³ and Boholm and Arvidsson³⁴ have pointed out that silver is either beneficial or harmful in relation to four main values: the environment, health, sewage treatment, and product effectiveness. As reported by Barua et al³⁵ poly(ethylene glycol)-stabilized colloidal SNPs showed the nonhazardous anticancer and antibacterial properties. Jin et al³⁶ have reported the therapeutic applications of plant-extract-based scaffolds for wound healing and skin reconstitution studies.

Materials and methods

Collection of plants

Fresh leaves of *G. sylvestre* from plants of same age group of a single population were collected from the experimental Herbal Garden, Tamil University, Thanjavur, Tamil Nadu, India, in July, 2010. The herbarium was prepared for authentication (RefNo: SRM\CENR\PTC\2010\03), and taxonomic identification was done by Dr Jayaraman, Professor, Department of Botany, Madras Christian College, Tambaram, Chennai, Tamil Nadu. The herb sample is maintained in the research laboratory for further reference.

Preparation of aqueous extract

The leaves of *G. sylvestre* were washed with distilled water to remove the dirt and further washed with mild soap solution and rinsed thrice with distilled water. The leaves were blotted with tissue paper and shade dried at room temperature for 2 weeks. After complete drying, the leaves were cut into small pieces and powdered in a mixer and sieved using a 20- μ m mesh sieve to get a uniform size range for further studies. Twenty grams of the sieved leaf powder was added to 100 mL of sterile distilled water in a 500-mL Erlenmeyer flask and boiled for 5 minutes. The flask was kept under

continuous dark conditions at 30°C in a shaker. The extract was filtered and stored in an airtight container and protected from sunlight for further use.³⁷

Qualitative and quantitative phytochemical analysis

The qualitative phytochemical analysis of *G. sylvestre* extracts were performed following the methods of Parekh and Chanda³⁸ to determine the presence of alkaloids (Mayer, Wagner, Dragendorff), flavonoids (alkaline reagent, Shinoda), phenolics (lead acetate, alkaline reagent test), triterpenes (Lieberman-Burchard test), saponins (foam test), and tannins (gelatin).³⁹ The results were qualitatively expressed as positive (+) or negative (–).⁴⁰ The chemicals used for the study were purchased from Sigma-Aldrich (Chennai, India).

Phytochemical quantitative analyses are described briefly in our previous paper.¹³ The total phenolic content was measured using the Folin–Ciocalteu colorimetric method. The flavonoids were estimated using aluminum chloride colorimetric method. Gallic acid was used as standard for the analysis of total antioxidant capacity, and the DPPH radical scavenging activity was done following the methods described by Blis.⁴¹

Synthesis of SNPs

Silver nitrate (AgNO_3) was purchased from Sigma-Aldrich (St Louis, MO, USA), and all solutions were freshly made for the synthesis of SNPs. The aqueous leaf extract of *G. sylvestre* was used for the bioreduction synthesis of the NPs. The SNPs were synthesized by adding 5 mL of plant extract to 15 mL of 1 mM aqueous AgNO_3 solution in a 250-mL Erlenmeyer flask and incubated in a rotary shaker at 150 rpm in dark. The synthesis of NPs was confirmed spectrophotometrically at every 30-minute interval till no reduction was observed. The reduction was observed by the color change in the colloidal solution, which confirmed the formation of SNPs.^{42,43}

Characterization of SNPs

The GSNPs were characterized periodically by measuring the bioreduction of AgNO_3 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 surface morphology of the biofunctionalized SNPs was characterized by high-resolution SEM analysis (JSM-5600LV; JEOL, Tokyo, Japan) and the elemental

compositions were determined by EDAX analysis (S-3400N; Hitachi, Tokyo, Japan). The functional characterization of biomolecules present in the GSNPs from the leaf extract of *G. sylvestre* was done by FTIR spectrometry (RX1; Perkin-Elmer, Waltham, MA, USA).⁴⁴

The crystal lattice and the size of the synthesized NPs were 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, as described in our previous papers.^{17,45–47}

In vitro anticancer activity

Cell line and culture medium

Vero cell line (derived from the normal kidney of adult monkeys) and human adenocarcinoma colon HT29 cells were purchased from the National Center for Cell Sciences, Pune, India. The cells were cultured under standard conditions in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 U/mL of streptomycin in a humidified incubator set at 37°C with 5% CO₂.¹⁷

Cell viability by MTT assay

MTT assay was performed to determine the cytotoxic properties of biofunctionalized SNPs against HT29 cell lines by adding 1×10⁵ cells/well in 12-well plates and incubated with various concentrations of biofunctionalized particles (83 µg/mL, 84 µg/mL, and 85 µg/mL). Vero cells were used as a monolayer for culturing the HT29 cells. The cell lines were seeded in 96-well tissue culture plates and the appropriate concentrations of GSNP stock solutions were added to the cultures to obtain the respective concentration of the NPs and incubated for 48 hours at 37°C. The untreated cells were used as control. The incubated cultured cells were subjected to MTT colorimetric assay. All assays were performed in triplicate, and the aqueous leaf extract of the *G. sylvestre* were also similarly assayed for the anticancer activity for comparison.^{17,48}

Morphological changes

The cytotoxicity effects were observed using an inverted microscope, and the morphological changes were photographed.⁴⁹

Results

Phytochemical screening of *G. sylvestre* leaf extract

The preliminary phytochemical screening of aqueous extracts of *G. sylvestre* revealed the presence of alkaloids, phenols,

Table 1 Phytochemical screening of leaf extract of *Gymnema sylvestre*

Compound	Aqueous extract
Alkaloids	+
Triterpenoids	+
Glycosides	–
Saponins	+
Tannins phenols	+
Flavonoids	+
Steroids	+

Notes: +, present; –, absent.

flavonoids, sterols, tannins, and triterpenes (Table 1). As shown in Table 2, 125.62±26.84 µg/g of total flavonoids, 285.23±1.11 µg/g of total phenols, and 111.53±15.13 µg/g of tannin were present in the aqueous extract of *G. sylvestre*. The flavonoids and phenolic compounds exhibited a wide range of biological activities, such as antioxidant and lipid peroxidation inhibition.¹³

The estimated total antioxidant activity was 9.13±0.04 µg/g and the DPPH radical scavenging activity was 52.14%±0.32% (Table 2).

Characterization of biofunctionalized SNPs

The color change observed in the aqueous silver nitrate solution showed that the SNPs were formed rapidly within 30 minutes of incubation of the plant extract with aqueous AgNO₃ solution. The colorless solution changed to ruby red, confirming the formation of SNPs (Figure 1). The intensity of the red color increased with time because of the excited surface plasmon resonance effect and reduced AgNO₃. The control aqueous AgNO₃ solution (without leaf extract) showed no change of color with time and was taken as the blank reference.

UV–vis spectrometry is a reliable and reproducible technique that can be used to accurately characterize the metal NPs though it does not provide direct information regarding the particle sizes. The surface plasmon bands

Table 2 Estimation of phytochemical compounds of leaf extract of *Gymnema sylvestre*

Bioactive compounds	µg/g
Total antioxidant ^a	9.13±0.04
Flavonoids ^b	125.62±26.84
Tannin ^c	111.53±15.13
Total phenol content ^d	285.23±1.11
Free radical scavenging ^d	52.14±0.32

Notes: All the values given are means of triplicates. Data presented as the mean ± standard deviation. ^aGallic acid equivalent. ^bTannic acid equivalent. ^cQuercetin equivalent. ^dCatechin equivalent.

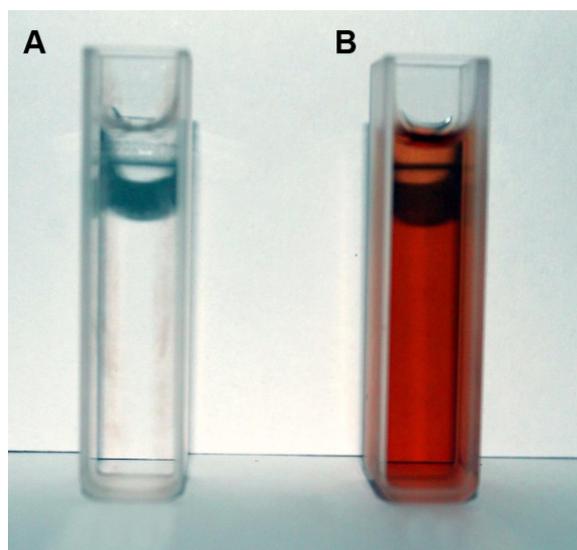


Figure 1 Surface plasmon resonance of silver nanoparticles.
Notes: (A) silver nitrate solution; (B) green-synthesized silver nanoparticles in ruby red color after 30 minutes.

(absorbance spectra) are influenced by the size and shape of the NPs produced, along with the dielectric constant of the surrounding media.

Figure 2 shows the time-dependent intensity of the absorption band, which reached its maximum peak at 12 hours, after which no further change in the spectrum was observed indicating that the precursors had been consumed. Initially, the UV–vis spectrum did not show evidence of any absorption in the region 350–600 nm, but after the addition of extract a distinct band was observed at 432 nm.

When silver nitrate was added to the aqueous plant extract of *G. sylvestre*, it was reduced to SNPs by the aldehyde group present in the flavonoids (125.6 $\mu\text{g/g}$), which was further oxidized to the carboxyl group. Also, the carboxyl groups of

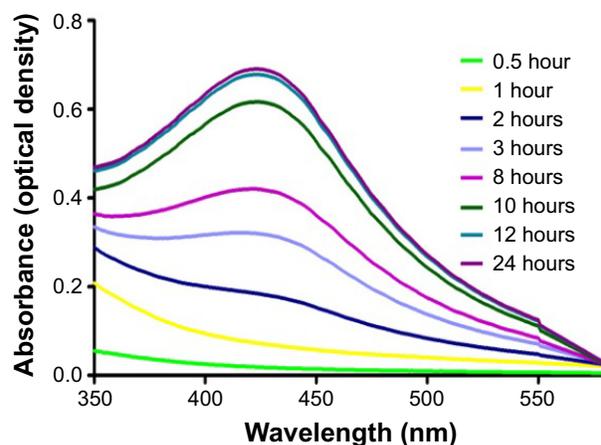


Figure 2 Time-dependent absorption spectra of silver nanoparticles after the bioreduction of silver in the aqueous extract of *Gymnema sylvestre*.

the phenols from the *G. sylvestre* extract (285.23 $\mu\text{g/g}$) acted as a surfactant to attach the major phytochemicals from the plant extract to the surface of the SNPs. Our previous study on the synthesis of SNPs using aqueous extracts of *Memecylon edule*,³⁷ *Memecylon umbellatum*,⁴⁷ *Chrysopogon zizanioides*⁴⁶ and *Indigofera aspalathoides*⁵⁰ showed that the color of the reaction mixture during the formation of GSNPs changed to ruby red color from colorless/straw color. Our results are also comparable with the other available reports for plant-extract-mediated synthesis of SNPs.

From Figure 3, it is clear that the synthesized SNPs were approximately spherical and of different sizes. The SEM images in the figure clearly indicated a thin layer of phytochemicals from the plant extract covering the synthesized SNPs. Mostly the total phenolic content, flavonoids, and tannins were responsible for the bioreduction of the SNPs. In this green synthesis, the phytochemicals from the plant extract acted as a surfactant to prevent the aggregation of the synthesized SNPs. The SEM images of our earlier research had revealed that this biologically eco-friendly synthesis of NPs utilizing the leaf extracts of *M. edule*,³⁷ *C. zizanioides*,⁴⁶ and *M. umbellatum*⁴⁷ showed no aggregation due to the biomolecules from the plant extract. And the mechanism behind this particle formation with no aggregation may be the spontaneous nucleation and isotropic growth of NPs along with the plant extract. As these chains grow in diameter with increasing silver deposition, spherical particles break off from these structures forming nanospherical particles which can be typically observed from this synthesis.⁵¹

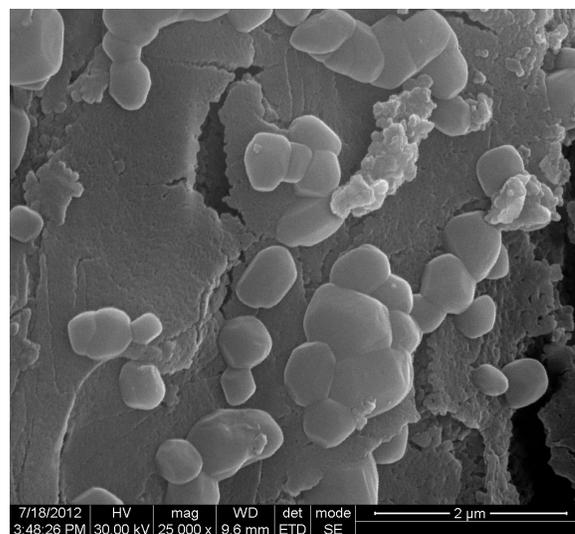


Figure 3 Scanning electron microscopic image of green silver nanoparticles synthesized by reduction of aqueous AgNO_3 ions using aqueous extract of *Gymnema sylvestre*.
Abbreviations: HV, high voltage; WD, working distance; mag, magnification; ETD, Everhart–Thornley detector.

The elemental composition of green-synthesized AgNPs was analyzed through EDAX. These measurements confirmed the presence of the elementary silver signal of the SNPs. The vertical axis displays the number of X-ray counts and the horizontal axis displays the energy in keV.

The EDAX spectrum of the biofunctionalized SNPs in Figure 4 clearly shows the strong signals from silver atoms along with the weaker signals from carbon and oxygen present from biomolecules of the plant extract. The elemental silver peak at 2–4 keV, which is the major emission peak specified for metallic silver, with minor peaks of C and O were also seen due to the capping of Ag NPs by the biomolecules of *G. sylvestre* leaf extract, and the absence of other peaks evidenced the purity of the Ag NPs.

FTIR analysis in Figure 5 shows that the SNPs produced by *G. sylvestre* extract were coated by phytochemicals and secondary metabolites such as saponins, terpenoids, and gymnemagenin derivative of gymnemic acid containing the functional groups of amines, aldehydes, carboxylic acids, and alcohols.

The presence of the amide linkages seen in Figure 5 suggests that the different functional groups of the proteins present in the plant extracts might be capping the NPs and playing an important role in the stabilization of the green NPs formed. The band at $1,443\text{ cm}^{-1}$ was assigned to the methylene scissoring vibrations of proteins, and the bands located at $1,318\text{ cm}^{-1}$ and $1,089\text{ cm}^{-1}$ are due to the C–N stretching vibration of aromatic and aliphatic amines, respectively, which agrees with earlier reports of Suman et al.⁵²

The positions of these bands were comparable to those reported for phytochemicals reported in the *G. sylvestre* extract as total phenols, flavonoids, and tannins (Table 2). Thus, we can confirm that the nanocapping of the phytochemicals from the *G. sylvestre* extract is responsible for the reduction and subsequent stabilization of the SNPs.

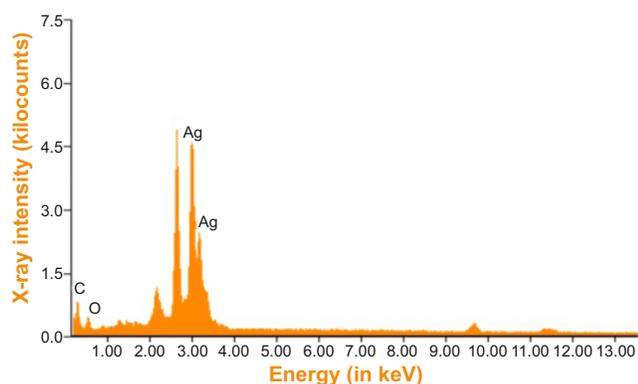


Figure 4 Energy-dispersive X-ray spectrum of green silver nanoparticles.

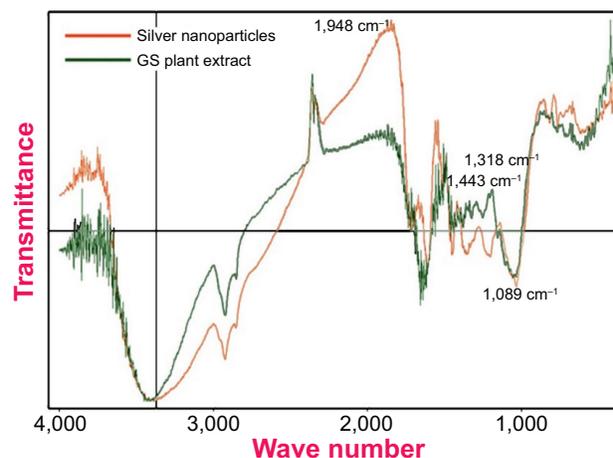


Figure 5 Fourier transform infrared spectroscopy spectrum of green-synthesized silver nanoparticles along with the plant extract of *Gymnema sylvestre* (GS).

The absorption bands that appear in the IR spectrum of the aqueous extract could also be seen in the IR spectra of phyto-capped Ag NPs, confirming the role of the phyto-constituents (mostly gymnemic acid) in protecting the Ag NPs from aggregation.

Also, during our repeated experiments there were no batch-to-batch variations in size, regardless of the isotopic composition, and the particles diameters of the SNPs formed were known to a high degree of accuracy. A detailed study on the large-scale synthesis and elemental composition on the synthesized NPs can be carried out using inductively coupled plasma mass spectrometry to obtain reproducible compositions in every batch. In future, the elemental analysis can be carried out as described earlier by other researchers.^{53,54}

XRD analysis of NPs represented in Figure 6 shows several size-dependent features leading to irregular peak position, height, and width. XRD was mainly carried out to study the crystalline nature of the green-synthesized *G. sylvestre* SNPs. From the figure, the GSNPs are seen to exhibit monocrystallinity. The XRD peaks at 38.2° , 44.5° , 64.7° , and 77.7° can be indexed to the [111], [200], [220], and [311] planes, indicating that the SNPs are highly crystalline. Similar results were reported for *Abelmoschus esculentus*, *Citrus limon*, *Citrus reticulata*, and *Citrus sinensis*^{55,56} and in our previous studies using *C. zizanioides*.⁴⁶

Table 3 shows the characteristic features of the GSNPs using various plant parts of different plant species reported by various researchers along with our previous reports.

In vitro anticancer activity

From Figure 7, it can be observed that, as the concentration of the GSNPs increased, the percentage of viable cells decreased

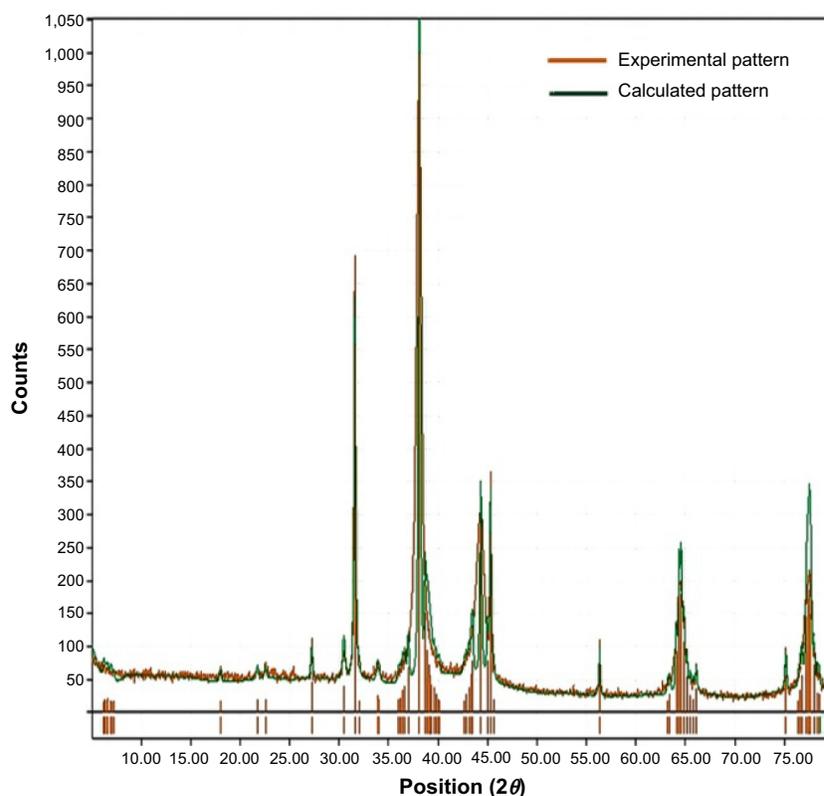


Figure 6 X-ray diffraction spectrum of green-synthesized silver nanoparticles.

Table 3 Comparative chart of the characterization of green-synthesized silver nanoparticles using various plant extracts

Plant name	UV-vis peak (nm)	Color change	Shape	Size (nm)	Reaction time	Reference
<i>Camellia sinensis</i>	436	Colorless to light to dark brown	Spherical	4.06	–	Loo et al ⁵⁷
<i>Dioscorea bulbifera</i>	450	Intense brown coloration	Nano-triangle	8–20	5 h	Ghosh et al ⁵⁸
<i>Pulicaria glutinosa</i>	427	Light yellow to dark brown	Spherical	40–60	20 h	Khan et al ⁵⁹
<i>Curcuma longa</i>	415	Colorless to golden yellow	Spherical	3.66–8.94	24 h	Shameli et al ⁶⁰
<i>Chrysanthemum morifolium</i>	430	Colorless to yellow brown	Spherical	20–50	–	He et al ⁶¹
<i>Rhinacanthus nasutus</i>	437	Colorless to yellowish brown	Irregular	22	24 h	Pasupuleti et al ⁶²
<i>Chrysanthemum indicum</i>	435	Colorless to reddish brown	Spherical	37.71–71.99	–	Arokiyaraj et al ⁶³
<i>Chrysopogon zizanioides</i>	420	Colorless to brown color	Cubic	85–110	24 h	Arunachalam and Annamalai ⁴⁶
<i>Memecylon umbellatum</i>	440	Colorless to brown color	Spherical	15–20	24 h	Arunachalam et al ⁴⁷
<i>Solanum torvum</i>	430	Yellow to brown color	Spherical	5–50	24 h	Ramamurthy et al ⁶⁴
<i>Vitex negundo L.</i>	430	Yellowish brown	Spherical	5–47	4 h	Prabhu et al ⁶⁵
<i>Aloe vera</i>	430	Yellow, golden brown, and brown	Spherical	20	20 min	Zhang et al ⁶⁶
<i>Ixora coccinea</i>	430	Colorless to brown	Spherical	13–57	12 h	Karuppiah and Rajmohan ⁶⁷
<i>Adhatoda vasica</i>	421	Blackish brown colored	Spherical	113–633	–	Nazeruddin et al ⁶⁸
<i>Boerhaavia diffusa</i>	410	Colorless to brown	Spherical	25	–	Vijay Kumar et al ⁶⁹
<i>Prosopis juliflora</i>	420	Colorless to brown	Cubic	11–19	5 min	Raja et al ⁷⁰
<i>Catharanthus roseus</i>	410	Colorless to brown yellow	Spherical	35–55	60 min	Ponarulselvam et al ⁷¹
<i>Moringa oleifera</i>	420	Yellow to reddish brown	Spherical and pentagonal	40	16 h	Vasanth et al ⁴⁸
<i>Morinda citrifolia</i>	413	Light yellow to brown	Oval	32–55	12 h	Suman et al ⁷²
<i>Artemisia nilagirica</i>	–	Clear to yellowish brown	Square	70–90	–	Vijay Kumar et al ⁷³
<i>Indigofera aspalathoides</i>	420	Colorless to yellow	Square	45–69	8 h	Arunachalam et al ⁵⁰
<i>Saraca asoca</i>	424	Colorless to brownish gray	Irregular	87–102	24 h	Meenakumari et al ⁷⁴

Abbreviations: h, hours; min, minutes; UV, ultraviolet; vis, visible spectrum.

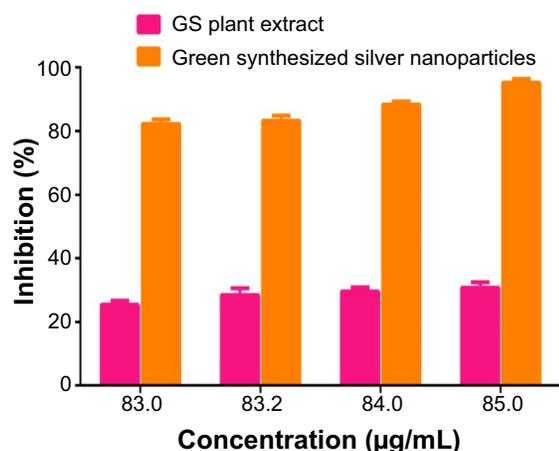


Figure 7 Anticancer activity of the green-synthesized silver nanoparticles. **Abbreviation:** GS, *Gymnema sylvestre*.

in the cytotoxicity studies by MTT assay. The GSNPs were taken up by mammalian cells through different mechanisms such as pinocytosis, endocytosis, and phagocytosis.⁷⁵ Once the NPs enter the cells, they interact with the cellular materials and cause DNA damage and cell death.

The GSNPs at 85 µg/mL concentration showed 95.23% inhibition of HT29 cell growth. The concentration of the NPs was chosen based on the TC ID₅₀ value (results not shown). Another promising result was that *G. sylvestre* plant extracts alone at 85 µg/mL concentration showed 30.77% inhibition of HT29 cell lines growth. From our results, it can be concluded that the GSNPs could have induced intracellular reactive oxygen species generation, which can be evaluated using intracellular peroxide-dependent oxidation, and caused cell death. The control cells were clustered, healthy, and viable cells (Figure 8A), whereas the HT29 cells' proliferation was significantly inhibited by GS (Figure 8B). The SNP-treated cells showed increased apoptotic morphological changes (Figure 8C), also the clearly visible cell debris in Figure 8D is due to cell death by 85 µg/mL SNP treatment.

These results indicate that the sensitivity of HT29 human colon cancer cell line for cytotoxic drugs is higher than that of the Vero cell line for the same cytotoxic agents. Sahu et al⁷⁶ have reported the presence of four new triterpenoid saponins, namely gymnemasins A, B, C, and D, from the leaves of *G.*

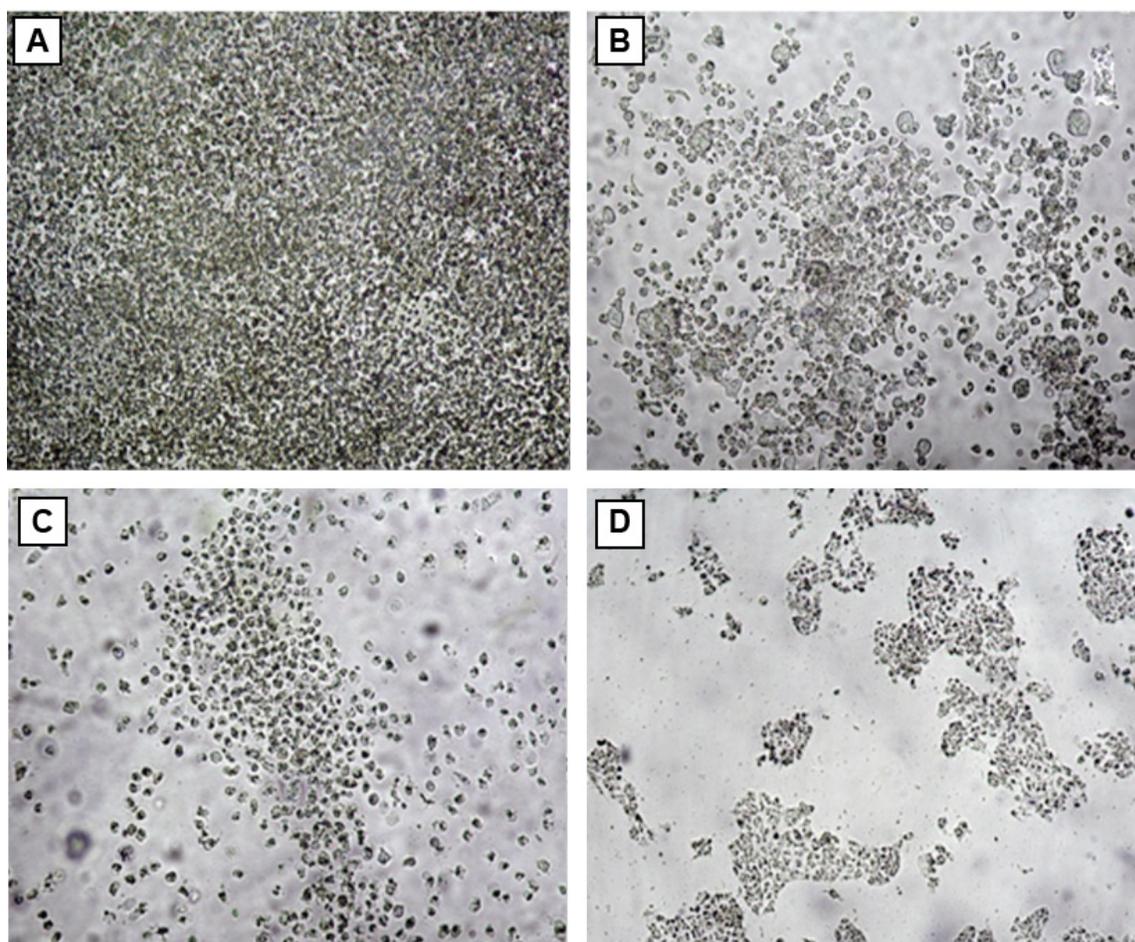


Figure 8 Inverted microscopic image of HT29 (A) control cells, (B) *Gymnema sylvestre*-treated (85 µg/mL), (C) silver nanoparticle-treated (83 µg/mL), and (D) silver nanoparticle-treated (85 µg/mL) cells.

sylvestre, while Chan has identified the presence of acylation with diangeloyl groups at the C21–22 positions in triterpenoid saponins, which is essential for cytotoxicity toward tumor cells.⁷⁷ Tang et al⁷⁸ have reported that saponin could induce apoptosis of U251 cells, and both BAD-mediated intrinsic apoptotic signaling pathway and caspase-8-mediated extrinsic apoptotic signaling pathway were involved in the apoptosis.

The promising saponins were further studied as potential anticancer agents by many researchers. Ai et al⁷⁹ proposed a qualitative method that can be used to recognize the presence or absence of cancer cells with gold NPs for targeted cancer cell imaging and efficient photodynamic therapy. As reported by Raghavendra et al⁸⁰ the size effects and multifunctionality are the main characteristics of NPs, so our method of one-step synthesis of SNPs using the aqueous extract of *G. sylvestre* may serve as a potential anticancer drug for cancer therapy. Further studies have to be carried out to understand the nature of cytotoxicity and the death or proliferation of cells caused by GSNPs from *G. sylvestre* leaf extract.

Conclusion

The green synthesis of biofunctionalized SNPs from the leaves of *G. sylvestre* was economical, nontoxic, and environmentally benign. Due to the reducing and capping nature of the bioactive phytocompounds present in the aqueous extract of *G. sylvestre*, a cap was formed around the silver ions of the biofunctionalized SNPs which were stable. The presence of the functional group of the bioactive compounds was confirmed by FTIR spectra. The particle size and the spherical shape of the SNPs were determined by XRD and SEM analyses. Since the plant extract and the biofunctionalized SNPs showed anticancer activity against cancer cells, *G. sylvestre* may serve as a source for potential anticancer drugs. The present study showed the anticancer activities of both the bioactive compounds of the leaf extract and the biofunctionalized SNPs synthesized against HT29 human adenocarcinoma cells in vitro. Our studies provide an important basis for the application of NPs for in vitro anticancer activity against human colon adenocarcinoma cells. Our earlier reports have also shown the potential antiulcer properties of *G. sylvestre* in mice.¹³ So GSS is a good plant candidate for further studies in alternative medicine due its multifunctional medical properties

Acknowledgment

The authors gratefully acknowledge the management, SRM University, for providing the facilities and supporting the work.

Disclosure

The authors declare no conflicts of interest in this work.

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