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Green Synthesis of Silver Nanoparticles from *Glycyrrhiza glabra* Root Extract for the Treatment of Gastric Ulcer

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Abstract

Glycyrrhiza glabra is a traditional herb which grows in various parts of the world, which have been used for the treatment of various diseases like gastric ulcer. Many reports have been published about the biogenesis of silver nanoparticles using *Glycyrrhiza glabra*, but green synthesized silver nanoparticles from *Glycyrrhiza glabra* has not yet been investigate the *in-vitro* anti-ulcer activity against *H. pylori*. In the present study was aimed to investigate the *in-vitro* anti-ulcer activity of green-synthesised silver nanoparticles (AgNPs) from *Glycyrrhiza glabra* root extract. The green synthesized of silver nanoparticles were characterized by UV-Visible Spectroscopy, X-ray diffraction (XRD), TEM, and FT-IR Analysis. UV-VIS Spectral analysis of the green synthesised nanoparticles was observed a sharp peak at 404 nm indicates the formation of silver nanoparticles. We successfully synthesized uniformly dispersed silver nanoparticles with a uniform size and shape in the range of 7 nm to 45 nm with an average size of 19 nm. The crystalline natures of Ag nanoparticles were confirmed from the XRD analysis. FTIR analysis was carried out to identify the possible biomolecules in *Glycyrrhiza glabra* root responsible for capping leading to efficient stabilization of the silver nanoparticles. The *in-vitro* antiulcer activities of synthesized silver nanoparticles were studied by Agar disc diffusion method and Micro broth dilution method. In Agar disc diffusion method showed the activity against *H. pylori* at the concentration of 500 µg/ml, which exhibit the most potent concentration of silver nanoparticles of gastric Cytoprotective anti-ulcer. In micro broth dilution method, The Minimum Inhibitory Concentration (MIC) of silver nanoparticles by visual examination was found to be 250 µg/ml.

Keywords: Green-synthesis; *Glycyrrhiza glabra*; Silver nanoparticles; TEM; XRD; FTIR; *H. pylori*

Abbreviations: AgNPs: Silver Nanoparticles; XRD: X-ray Diffraction; TEM: Transmission Electron Microscopy; FT-IR: Fourier Transform Infrared Spectroscopy.

Background

Green synthesis of silver nanoparticles is evolving into an important branch of nanotechnology. Green chemistry to improve and/or protect our global environment is focal issues in many fields of research [1]. The development of cost efficient and ecologically benign methods of synthesis of nanomaterial's still remains a scientific challenge as metal nanoparticles are of use in various catalytic applications, via electronics, biology and biomedical applications, material science, physics, environmental remediation fields [2,3]. Nanoparticles were sub-nanosised structure composed of synthetic and semi-synthetic polymers. Nanoparticles may define as particulate dispersion or solid particles within the size range of 10-1000 nm [3,4]. The environmental friendly synthesis of nanoparticles process is a revolutionary step in the field of nanotechnology. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms. A variety of techniques including physical and chemical methods have been developed to synthesise silver nanoparticles [5-7]. Biological methods of greener synthesis of nanoparticles and these have proven to be better methods due to slower kinetics; they offer better manipulation and control over crystal growth and their stabilization. Plant extracts as reducing and capping agents, has received special attention among others, due to maintaining an aseptic environment during the process [8,9]. Many reports have been published about the biogenesis of silver nanoparticles using various plants such as *Rumex hymenosepalus, Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* etc. [10-14]. Medicinal plants having well established therapeutic importance are being widely used for the size and shape controlled synthesis of silver nanoparticles. Extract from plant may act both as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes or proteins, polysaccharides, and vitamins is environmentally benign, yet chemically complex [15,16].

Glycyrrhiza glabra (Linn.) (Figure 1) is one of the anti-ulcer plants belonging to the family Fabaceae. The plant possess certain chemical constituents such as antioxidant, expectorant, antitussive, demulcent, spasmolytic, anti-inflammatory, anti-allergic, memory stimulant, antimicrobial, antiviral, demulcent, antibacterial, spasmolytic, tonic, diuretic and antistress, antiulcer, liver protective, estrogenic, antidiabetic and anti-depressive actions [17-20].

Methods

Materials

The plant *Glycyrrhiza glabra* was collected from local place of Nemmara, Palakkad. The specimen was authenticated by Dr. P Sujanapal, Scientist, Kerala Forest Research Institute, Peechi and Thrissur. Silver nitrate solution was obtained from Nice chemicals Pvt. Ltd, Kochi.

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Figure 1: *Glycyrrhiza glabra* root.

Study Centre

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Synthesis of silver nanoparticles

Glycyrrhiza glabra root was washed several times with de-ionized water. 50 g of the root was finely cut and boiled with 150 ml de-ionized water at 100°C for 5 minute. After that the aqueous leaf extract was filtered through the Whatmann. No.1 filter paper, and the filtrate was stored. 2 ml of aqueous leaf extract was added to the Erlenmeyer flask containing 98 ml of $AgNO_3$ (10^{-3} M) and the mixture was incubated at room temperature.

Characterization of Silver Nanoparticles

UV-Vis spectral analysis

The reduction in Ag^+ ions was monitored by UV-VIS spectra of the silver nanoparticles solution after diluting a small aliquot of the sample into distilled water and the UV-VIS spectra were recorded by Shimadzu UV-1800 Spectrophotometer from 200-800 nm.

TEM analysis

TEM technique was employed to visualize the size and shape of Ag nanoparticles. HR-TEM measurements were made using a 200 kV Ultra High Resolution Transmission Electron Microscope (Joel/JEM 2100). TEM grids were prepared by placing a drop of particle solution on a carbon-coated copper grid and drying under lamp.

XRD analysis

XRD analysis of the prepared sample of Ag nanoparticles was done using a Bruker AXS D8 Advance diffractometer, using Cu-Kα X-rays of wavelength (λ)=1.54056 Å as source and operated at a voltage of 40 kV and a current of 35 mA. The sample was scanned in the 2θ ranging from 10° to 80° with a step size 0.02° and step time 32.8 s. XRD patterns were analysed to determine peak intensity, position and width. Full width at half-maximum (FWHM) data was used with the Scherer's formula to determine mean particle size. Scherer's equation is given by

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

Where d is the mean diameter of the nanoparticles

λ is wavelength of X-ray radiation source

β is the angular FWHM of the XRD peak at the diffraction angle θ[45].

FT-IR analysis

The formed silver nanoparticles from the flower extract were centrifuged at 10,000 rpm for 20 minutes and then the pellet obtained is washed thrice with distilled water. Then it is dried in an oven at 60°C in an oven for an entire day. This powdered sample of the formed silver nanoparticles from the root extract was subjected to FT-IR analysis using Bruker Alpha FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany).

In vitro Antiulcer Studies of Synthesized AgNPs

In-vitro antiulcer activity against *H. pylori*

Agar disk diffusion assay: For the disk diffusion assay, serial dilutions of the green synthesized silver nanoparticles, was prepared, in order to obtain the following doses: 62.5; 125; 250; 500 and 1000 µg/disk. The sterile disks utilized (6 mm - Tarson®) should be imbibed in 25 µL of each dose of green synthesised silver nano particle. The silver nanoparticles imbibed disks was deposited on the surface of the plate inoculated with *H. pylori*, in a suspension of 5×10^8 CFU/mL (McFarland turbidity standard), using clarithromycin (15 µg) as the standard drug, incubated at 37°C under microaerophilic conditions in an atmosphere of 5 to 10% CO_2 for 3-5 days. After this period, the diameter of inhibitory zone was measured in duplicate and mean values ≥ 10 mm are considered active [21,22].

Broth micro dilution: The broth micro dilution assay allows the determination of the Minimum Inhibitory Concentration (MIC). To each well in the microplate should be added 100 µL of Mueller-Hinton broth, supplemented with 10% foetal calf serum inoculated with 5×10^8 *H. pylori* (McFarland turbidity standard), 100 µL of green synthesized silver nanoparticles, should be also added to reach the final concentrations of 62.5; 125; 250; 500 and 1000 µg/ml. Clarithromycin (5 mg/mL) is used as the standard drug for growth inhibition. Next, the microplate should be incubated at 37°C under microaerophilic atmosphere of 5 to 10% CO_2, for 3-5 days. After incubation, the plates should be visually examined and each well should be replicated in blood agar (Mueller-Hinton agar with 5% sheep blood), to determine whether growth had occurred, with the MIC defined as the lowest concentration to cause complete bacterial growth inhibition (bactericidal activity).

Results and Discussion

Silver nanoparticles were synthesised by root extract of *Glycyrrhiza glabra*, it was observed there was a colour change from pale yellow to brownish red which indicates that the reduction of silver particle in presence of plant extract and the stable product was obtained in dark brown colour within 7 hours as shown in Figure 2.

The UV-VIS Spectral analysis of the green synthesised nanoparticles was observed a sharp peak at 404 nm indicates the formation of silver nanoparticles was shown in Figure 3. The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, composition and aggregation of sample. Scattering from a sample was typically very sensitive to the aggregation state of the sample, with the scattering contribution increasing as the particles aggregate to a greater extent. The optical properties of silver nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared

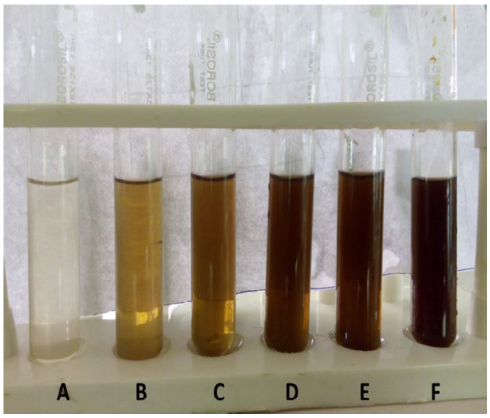

Figure 2: Visual observation of silver nanoparticles (a) Pure 1 mM $AgNO_3$ solution (b) Aqueous extract (c) 1mM $AgNO_3$ and root extract solution after 2 hrs (d) after 3 hrs (e) after 4 hrs (f) after 24 hrs.

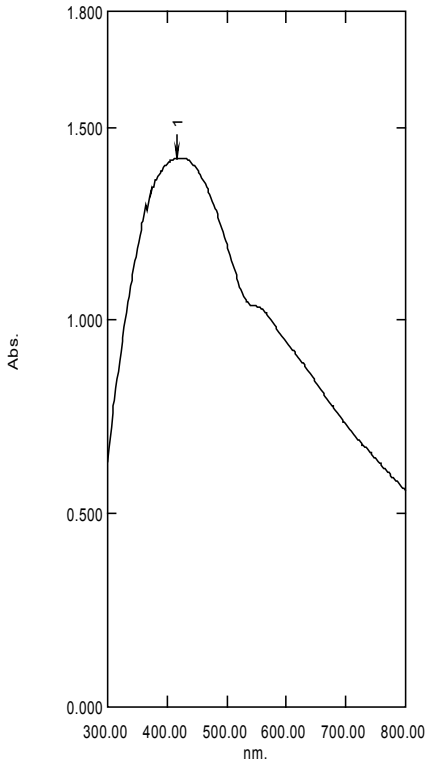

Figure 3: UV-VIS Spectral analysis of the green synthesized nanoparticles.

amongst neighbouring particles. The surface Plasmon resonance shifts to low energies, causing the absorption and scattering peaks to red-shift to longer wavelengths.

Transmission electron microscopic studies revealed that the synthesized silver nanoparticles were spherical in shape. The particle size ranges from 7 nm to 45 nm and the average particle was found to be 19 nm as shown Figure 4. Transmission electron microscopy was a vital characterization tool for directly imaging nano materials to obtain quantitative measures of particle size, size distribution and morphology.

The crystalline nature of Ag nanoparticles was confirmed from the X-ray diffraction (XRD) analysis. Two diffraction peaks observed at 24.281 and 37.977 in the 2θ range 20-70° can be described to the (110) and (111) reflection planes of face- centered cubic (fcc) structure of Ag phases was shown in the Figure 5.

The FT-IR spectrum of silver nanoparticles showed principal peaks at 3242 cm^{-1} for OH stretching, 3050 cm^{-1} for C-H stretching of aromatic, 2860 cm^{-1} for C-H stretching of CH_2, 1670 cm^{-1} for C=0 stretching and 1392 cm^{-1} for C-O-C stretching of ether linkage. The above evidence has proposed that silver nanoparticles may be coated with Glycyrrhizin. The FT-IR spectra of mixture of drug and polymers (carbopol-934 and sodium alginate) shows the peaks at 3234 cm^{-1} for OH stretching, 2900 cm^{-1} for C-H stretching of aromatic, 2840 cm^{-1} for C-H stretching of CH_2, 1696 cm^{-1} for C=O stretching and 1026 cm^{-1} for C-O-C stretching of ether linkage. The FT-IR study revealed no physical or chemical interactions are shown in Figure 6. FTIR analysis corresponding to vibrational bands such as –C=O,-C-O,-C-O-C are derived from compounds such as phenols, flavonoids and terpenoids present in *Glycyrrhiza glabra* root. Hence it may be assumed that these biomolecules are responsible for capping and efficient stabilization. The presence of reducing sugars in the extract could be responsible for the reduction of silver ions and formation of the nanoparticles. This rapid and environmentally benign method is a faster synthesis comparable to chemical reduction methods [7].

The *in-vitro* antiulcer activities of synthesized silver nanoparticles were studied by Agar disc diffusion method and Micro broth dilution method. In Agar disc diffusion method showed the activity against *H. pylori* at the concentration of 500 µg/ml was shown in Figure 7, making it was the most potent concentration of silver nanoparticles of gastric Cytoprotective antiulcer. In micro broth dilution method, The Minimum Inhibitory Concentration (MIC) of silver nanoparticles by visual examination was found to be 250 µg/ml was shown in Figure 8.

Conclusion

Biosynthetic methods such as bacteria and fungus or plant extract have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nanomaterials. Extract from plant may act both as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes or proteins, amino acid, polysaccharides, and vitamins is environmentally benign, yet chemically complex.

In the present work, Silver nanoparticles were successfully formulated from bioreduction of silver nitrate solutions using

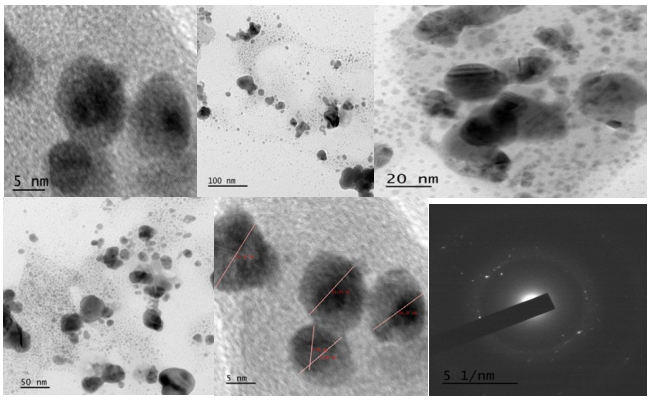

Figure 4: HRTEM micrograph of silver nanoparticles synthesized from 1 × 10^{-3} M $AgNO_3$ solution and Glycyrrhiza glabra root extract.

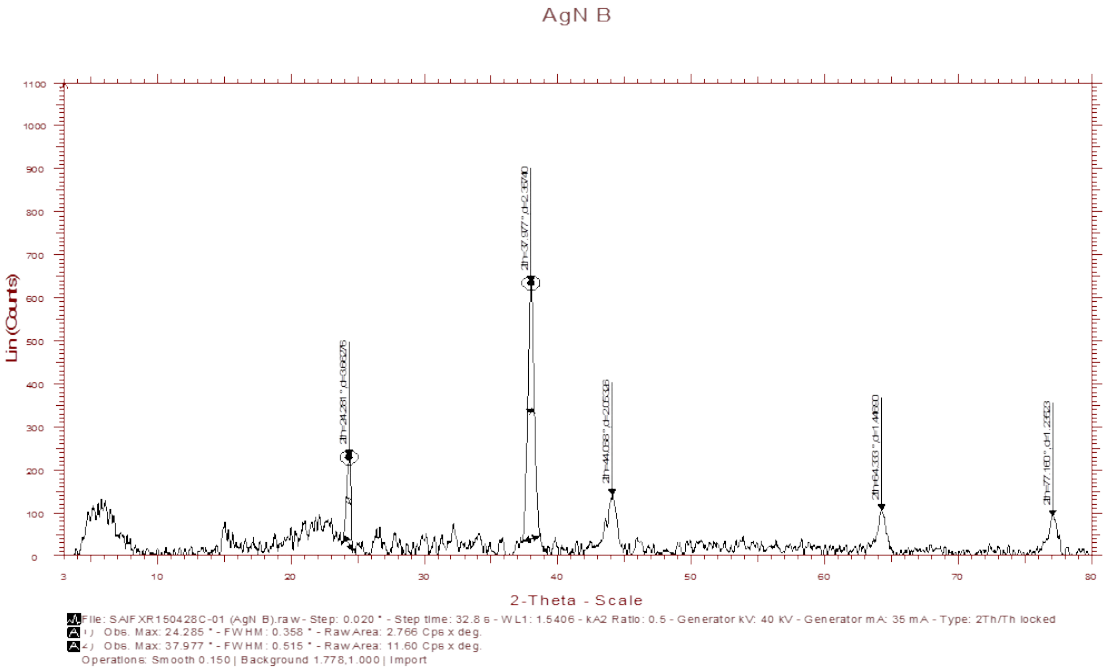

Figure 5: XRD pattern of synthesized silver nanoparticles.

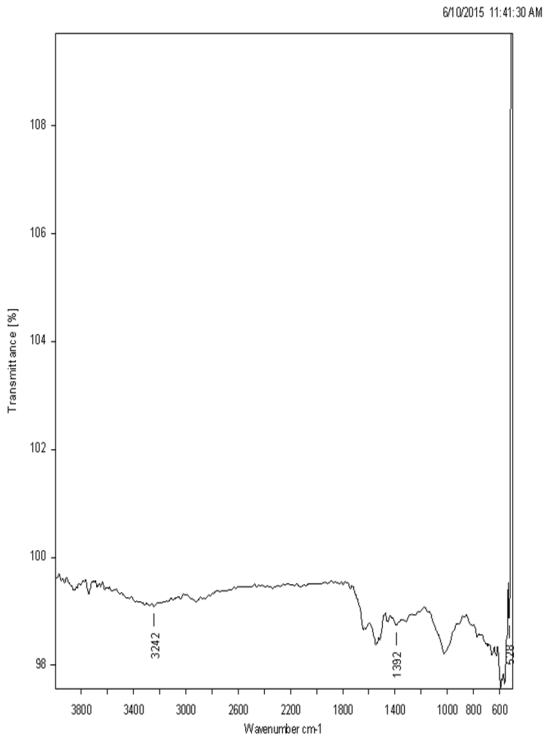

Figure 6: FT-IR spectrum of silver nanoparticles.

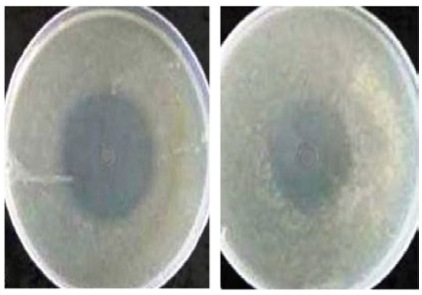

Figure 7: Agar disc diffusion plates.

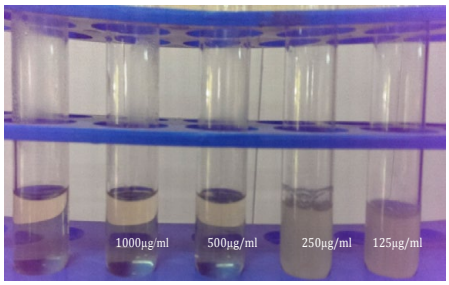

Figure 8: The Minimum Inhibitory concentration (MIC) of the green synthesized nanoparticles by visual examinations.

Glycyrrhiza glabra root extract. Silver nanoparticles have been appropriately characterized using UV-Visible spectroscopy, TEM, XRD and FTIR analysis. The Anti-ulcer activity was done by Agar disc diffusion assay and Broth micro dilution. We have developed a biosynthetic method to develop AgNPs using aqueous root extract of *Glycyrrhiza glabra*, which acts as a reducing as well as stabilizing agent. Particles formed are mostly poly- dispersed in shape. The green synthetic method is a fast, low cost and eco-friendly process in the field of nanotechnology. The study revealed that the green-synthesized silver nanoparticle provides a promising approach for gastric ulcer therapy. So we conclude that the novel formulation may expect to be having effect on *H. pylori* eradication for the treatment of gastric ulcer.

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