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Research Article

Green synthesis of silver nanoparticles using leaf extracts of *Withania coagulans*

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Abstract

The "Green Synthesis" method is the most cost-effective and sustainable approach to synthesizing nanoparticles using naturally occurring microbes and plants. The advantages of green synthesis include the ability to produce nanoparticles with consistent shapes and sizes, eliminating harmful chemicals, reducing waste production, and ease of application. *Withania coagulans*, commonly known as Indian rennet, is a remarkable plant used in both Unani and Ayurvedic medicine to treat various diseases. It is a rich source of bioactive compounds called withanolides. *W. coagulans* offers several advantages for the green synthesis of nanoparticles, including economic feasibility, prevention of microbial contamination, effective capping and reduction properties, and the absence of harmful chemicals. This research focused on synthesizing AgNPs using both methanolic and aqueous extracts of *W. coagulans*. AgNPs were successfully synthesized from the leaf extracts of *W. coagulans*, with a qualitative analysis of phenolic compounds conducted using the $FeCl_3$ test. The characterization of the AgNPs involved UV-VIS spectroscopy and SEM/EDS. The methanolic extract of *W. coagulans* showed positive results, indicating the presence of phenolic compounds. In contrast, the aqueous extract appeared to contain only a negligible amount of phenolics. The nanoparticles were predominantly spherical and exhibited a polydisperse size distribution, ranging from 50 to 80 nanometers. EDS analysis confirmed the presence of elemental silver. These findings underscore the potential of *W. coagulans*, particularly its methanolic extract, as an effective green synthesizing agent for AgNPs.

Keywords: Nanoparticle Synthesis, *Withania Coagulans*, AgNPs, Phenolics, SEM/EDS.

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Introduction

The emergent technology primarily concerned with the nanomaterials to overcome the limitations of size and transform the global perception of science is termed "Nanotechnology" [1]. This novel technology develops the substances, frameworks, and instruments on the nanoscale and can be applied industrially in agriculture, pharmaceutical, and food sectors [2]. Silver nanoparticles (AgNPs) are the most prevalent component discovered through nanotechnology. These nanoparticles consist of pure silver with diameters ranging from 1 to 100 nm, exhibiting exceptionally high energy, catalytic activity, and surface area [3]. AgNPs have an extensive range of applications, especially in Agri-biotechnology, to improve the germination of seeds, the growth of plants, the efficiency of photosynthesis, and their use as competent fertilizers and nano-pesticides. Owing to their antimicrobial properties, AgNPs can be commonly used in numerous industries, including fabric, cosmetics, healthcare, etc [1]. Many approaches can be used to synthesize AgNPs, such as laser ablation, microwave processing, chemical reduction, electron irradiation, green synthesis, biological synthetic procedures, chemical and photochemical methods, and γ irradiation [4].

The "Green Synthesis" method is the most economical and sustainable way to synthesize nanoparticles using naturally occurring microbes and plants. Additionally, it assists in producing nanoparticles through various chemical, physical, biological, and hybrid methods [4]. The synthesis of AgNPs using green technology primarily involves plants, bacteria, algae, and fungi to reduce Ag^+ to Ag^0, either through extracellular or intracellular sources [5]. The synthesis of plant-derived nanoparticles has gained significant interest due to the variety and abundance of plant species, which offer a rich source of bioactive composites for synthesizing nanoparticles. Seeds, roots, and leaves were the parts of plants that have been deliberated for the development of nanoparticles by reducing the metal ions [6]. The benefits of green synthesis include accurate and constant shapes and sizes of nanoparticles, preventing the use of destructive chemicals, minimizing the production of waste, and effortless application [6]. *Withania coagulans* (Indian rennet) is a remarkable plant used in both Unani and Ayurvedic medication, comprising a rich source of bioactive compounds, known as withanolides [7]. The entire plant and its parts are used for the treatment of several diseases. The *W. coagulans* seeds are used to reduce the inflammation, the flower buds act as an anthelmintic agent, and the ripe fruit is used to treat dyspepsia, wounds, asthma, and to induce sedation. The phytochemical research on *W. coagulans* has discovered an extensive range of secondary metabolites such as saponins, alkaloids, amino acids, steroids, organic acids, tannins, volatile oils, and fatty acids. Equally, the aqueous and alcoholic extracts of every part of this plant, mainly fruits, are widely reported for their antimicrobial, neuroprotective, antioxidant, antidiabetic, anti-inflammatory, anticonvulsant, antitumor, and antihyperlipidemic activities [8].

There are numerous advantages of *W. coagulans* for the green synthesis of nanoparticles, which include being economically feasible, preventing microbial contamination, efficient capping and reducing agent, and a lack of harmful chemicals [7]. In the last few years, scientists have synthesized AgNPs from the leaf extracts of *Vernonia amygdalina* [9], *Acer oblongifolium* [10], *Conocarpus lancifolius* [11], *Ammi visnaga* [12], *Ruta, Eucalyptus, Basil, Melia, Geranium* [13], *Terminalia arjuna*, and *Euclayptus cameldulensis* [14], and investigated their potential for application as an antimicrobial [9 - 12], antiproliferative [10], antic-ancerous agents [11 - 12], and cytotoxic agents [14], as well as in wastewater treatment [12], and the development of phenol nanosensor [13]. This research aimed to synthesize AgNPs using methanolic and aqueous leaf extracts of *Withania coagulans*, then characterized through UV-spectrometry and SEM-EDS.

Experimental

Materials

W. coagulans leaves were purchased from a local supplier located at the Water Pump in Karachi, Pakistan. Chemical of silver nitrate was purchased from Merck Chemicals. All reagents used in the study were of analytical grade.

Preparation of *Withania coagulans* extract

Leaf extracts of *W. coagulans* were prepared in methanol and distilled water individually. Powdered *W. coagulans* (4.17 g) was added to each flask containing 25 ml distilled water and 25 ml methanol respectively. Flasks were kept in shaking water bath at 50˚C and 120 rpm for 30 minutes. Both the extracts were filtered using Wattman filter paper no. 1, and centrifuged at 7,000 rpm. Supernatants were stored at 4˚C until further use [25].

Silver nanoparticles (AgNPs) synthesis

$AgNO_3$ (0.1M) solution was added to each of the dark bottles containing 2 ml of each plant's extract in 2:1 ratio. While distilled water was added in bottles containing each of extracts instead of $AgNO_3$ solutions, as control. All the bottles were kept at room temperature for 24 hours in dark. Then, solutions were centrifuged twice at 12,000 rpm for 10 minutes, and supernatants were collected for further characterization.

Qualitative analysis of phenolics in plant extracts

Phenolic compounds are found to make up most of the reducing strength in plant extracts. They play an important role in metal ion reduction. Thus, ability of different plant extracts in fabricating nanoparticle can be correlated to their total phenolics content. For that, $FeCl_3$ test was performed. Each of the plant extracts were added in individual labeled test tubes and a 2% $FeCl_3$ solution was added drop wise. Bluish green or black coloration indicated presence of phenols.

Characterization of AgNPs

UV-VIS spectroscopy

For preliminary determination of AgNPs, change in color of solutions was observed and UV-Vis Absorption spectra were obtained in the range of 200-800nm by a UV-Vis spectrophotometer DU-7.

Scanning electron microscopy (SEM)

SEM analysis was performed to determine the size and morphology of silver nanoparticles formed in each of the test solutions. Energy dispersive spectroscopy (EDS) spectrums were taken to confirm the existence of Ag element on the prepared slides. Smears of all the solutions were prepared on clean dried slides and air dried. These slides were coated with gold up to a thickness of 300˚A. The slides were then observed in SEM/ EDS.

Results and Discussion

Plant resources are abundant and easy to handle as compared to other biological resources, rendering them a better system to work with [15]. Plant extracts contain biologically active chemical constituents like phenolics, flavonoids, alkaloids, saponins, triterpenoids, tannins, and steroids [16]. Nanoparticle synthesis is affected by the plant source, its organic compounds, temperature, salt concentration, as well as the pigments in the leaf extract [17]. Phenolic components undergo redox reactions, behaving as metal chelating agents, reducing agents, hydrogen donors, and oxygen quenchers. Thus, their presence in plant extracts could be liable for metal ion reduction and nanoparticle formation [18].

We have exploited the benefits of its phytochemicals and tried to reduce Ag+ ions into Ag nanoparticles in this study. Methanolic and distilled water extracts were prepared, and silver nanoparticles were synthesized thereafter. Many researchers have associated phenolics in plant extracts with silver-reducing ability. Therefore, total phenolics were tested using the $FeCl_3$ method [19]. Methanolic extract of *W. coagulans* gave positive results, indicating the presence of phenolic compounds in the extracts. While D/W extract of *W. coagulans* might not have a significant amount of phenolics, and hence, it did not show black coloration on the addition of $FeCl_3$ solution.

Each extract was prepared as described earlier, and the extracts were then incubated with 0.1M $AgNO_3$ for 24 hours and centrifuged. The colour of the solutions turned darker, indicating that a reduction reaction of Ag^+ into AgNPs has occurred. For confirmation, the UV-Vis spectrum of aliquots was recorded in a range of 300-600nm. Due to their Surface Plasmon Resonance, AgNPs show a peak between 350-350nm. UV- Vis spectra of samples are displayed in Figure 1 and Figure 2.

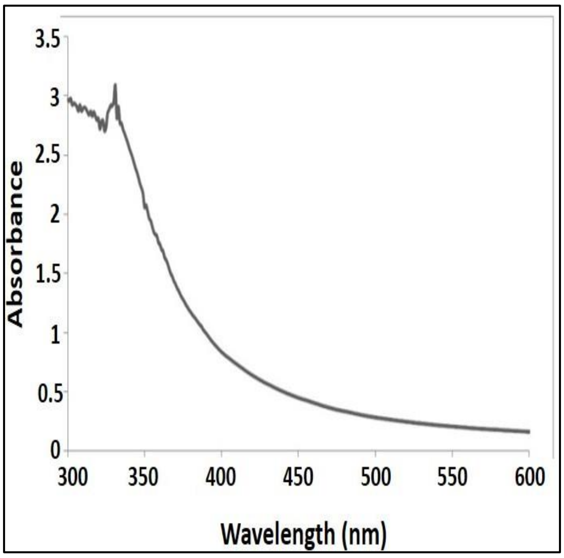

Figure 1: UV-Vis spectrum of AgNPs synthesis in methanolic extract of *W. coagulans.*

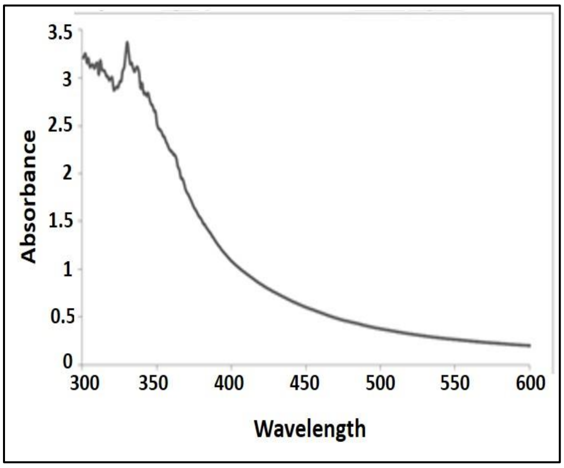

Figure 2: UV-Vis spectrum of AgNPs synthesis in D/W extract of *W. coagulans.*

Both extracts displayed maximum absorbance in the range of 300-350 nm. The study reported that the AgNPs prepared from the *Chromolaena odorata* leaf extract at room temperature showed the highest peak at 250-300 nm in a UV-Vis spectroscopy from 60-105 minutes [20]. Another research study tested the AgNPs synthesized by the aqueous leaf extract of *Calotropis procera* from a 1–5-hour time interval and showed that all the peaks were observed at 400 nm wavelength, but the highest peak was observed in 4 hours [21]. Additionally, one more research study conveyed that the AgNPs produced by the *Ajuga iva* leaf extract showed a significant peak around 400 nm in a UV-Vis spectroscopy [22]. Comparing all these studies revealed slight differences in their peaks, which are attributed to the types of plants and the solutions used for the synthesis of AgNPs. Smears were prepared from the extracts on glass slides. These smears were air dried and coated with a uniform layer of Gold up to 300˚A in an auto coater, and each slide was individually scanned at different magnifications in a Scanning Electron Microscope (SEM) to observe the presence of metallic nanoparticles. For further confirmation that the nanosized particles were of silver, the same slides were observed in Energy Dispersive Spectroscopy (EDS), and spectra were generated. The spectral graph showed X-ray energies (usually in channels 10 or 20 eV wide) on the x-axis, while the y-axis represents the number of counts per channel. The higher the number of counts, the higher the amount of AgNPs in the region under observation. SEM images and EDS scans for each of the plant extract and silver nanoparticle suspension are displayed in Figure 3-6.

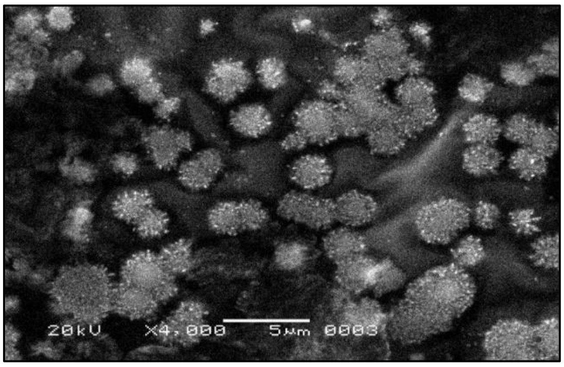

Figure 3: SEM image of AgNPs synthesis using methanolic extract of *W. coagulans*.

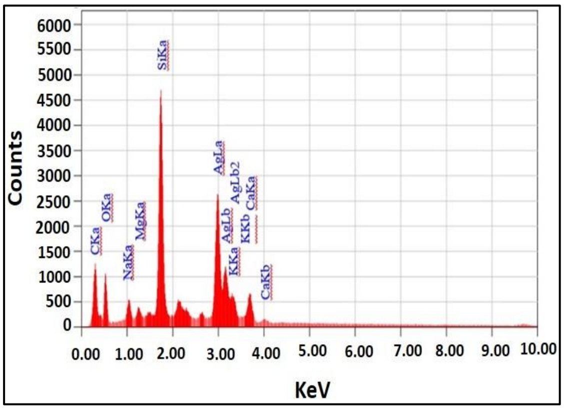

Figure 4: EDS scan of AgNPs synthesis using methanolic extract of *W. coagulans.*

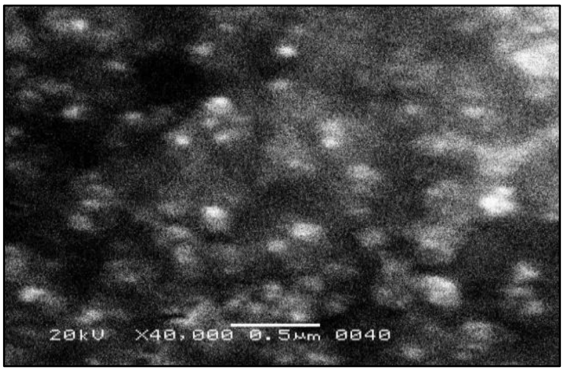

Figure 5: SEM image of AgNPs synthesized using D/W extract of *W. coagulans.*

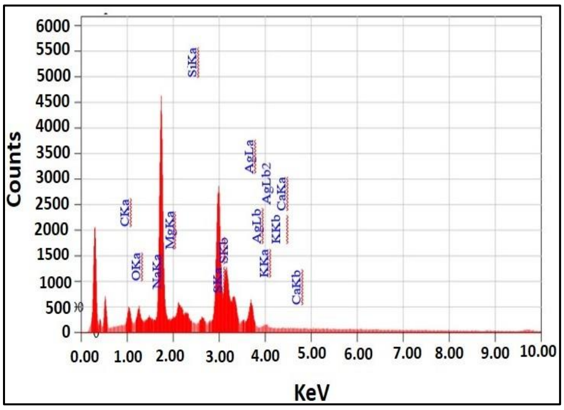

Figure 6: EDS scan of AgNPs synthesized using D/W extract of *W. coagulans.*

SEM micrographs of the samples proved that the morphology of AgNPs synthesized by using plant extracts was approximately spherical. Particles in both samples were found to be polydispersed, having sizes ranging between 50-80nm and a broad distribution. In the analysis by EDS of the AgNPs synthesized by plant extracts, the presence of an elemental silver peak at ~3 keV provides supportive evidence for the existence of silver nanoparticles in each of the suspensions in sufficient quantity. The optical absorption peak at ~3 keV is specific for metallic silver nanocrystals in EDS spectra due to their Surface Plasmon Resonance. A research group from Lithuania synthesized the AgNPs from the aqueous plant extracts of *Thymus vulgaris*, *Artemisia absinthium*, and *Humulus lupulus*. The SEM results reported that the size of nanoparticles prepared from *Thymus vulgaris* is ~45 nm with a spherical shape, *Artemisia absinthium* is ~50 nm with a spherical shape, and *Humulus lupulus* is ~40 nm with a spherical shape. The presence of strong signals of AgNPs was observed at ~3 keV in the EDS investigation [23]. Similarly, another study described the synthesis of AgNPs from the seed extract of *Ocimum basilicum* and reported that the morphology and size of the synthesized NPs were spherical, with different sizes of 10-80 nm at 2000–3000X magnification. The elemental configuration of the AgNPs was observed by EDX analysis with the dominant peak at ~3.2

keV [24]. A comparison of all these studies revealed that the shape of AgNPs observed in SEM and the signals detected in EDX are mostly similar. However, there are differences in the size of the nanoparticles due to the varying types of plant extracts utilized in the experiments.

Conclusion

The silver nanoparticles (AgNPs) synthesized in this research using methanolic and aqueous leaf extracts of *Withania coagulans* demonstrated promising results in their production time compared to other bacterial and fungal sources. The methanolic extract was found to contain phenolic compounds, which effectively reduced Ag^{+} ions, as confirmed by changes in color, UV-Vis spectrometry, SEM, and EDS analysis. The nanoparticles were primarily spherical, polydisperse, and ranged in size from 50 to 80 nm. EDS analysis confirmed the presence of elemental silver. These findings highlight the potential of *W. coagulans*, particularly its methanolic extract, as an efficient green agent for synthesizing AgNPs. However, this study has limitations, including a focus solely on nanoparticle synthesis and characterization without assessing their stability, biological activities, or potential toxicity. Additionally, the comparative efficacy of the methanolic and aqueous extracts was not quantified beyond the phenolic content and visual observations. Future research should explore the antimicrobial, antioxidant, and cytotoxic properties of the synthesized AgNPs for practical applications. Furthermore, optimizing extraction conditions, reaction parameters, and scaling up the synthesis process will be crucial for industrial and biomedical uses. Investigating other solvent systems and advanced characterization techniques will also be important for gaining deeper insights into the behavior and functionality of the nanoparticles.

Author’s Contribution

Shafaq Aiyaz Hassan: Project designing and contributing to the execution of experimental work.

Erum Hanif: Contribute to experimental work.

Syeda Sadaf Wajahat: Manuscript preparation, editing, and formatting.

Ehtisham Asif: Manuscript preparation.

Shahneela Siraj: Manuscript preparation.

Saifullah Sahir: Data analysis and critical review of the final manuscript.

Data availability statement

The data that supports the findings of this study is available from the corresponding author upon reasonable request.

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