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Research Article

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Synthesis of Silver Nanoparticles Using Plant Extracts from Legeneraria Siceraria Fruits and Assessment of their Anti-Bacterial and Anti-Oxidant Activities

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ABSTRACT
Nanotechnology is a key subject of science, engineering, and technology that is experiencing enormous growth in the current century. In this study, fruits of the legeneraria siceraria plant, this is widely used in traditional medicines in Ethiopian rural areas, used as reducing and capping agent for AgNPs synthesis. The legeneraria siceraria fruits -mediated synthesized AgNPs characterized by ultraviolet-visible (UV-VIS.) spectrophotometry, Fourier transform-infrared (FT-IR) spectroscopy, X-ray diffractometer (XRD), scanning electron microscopy (SEM), dynamic light scattering (DLS). The optimal synthesization parameters for AgNPs in this study were temperature 60 °C, stirring time 60 minutes, pH 11, and 2 mM silver nitrate concentration. Characteristic and well-defined SPR bands for AgNPs were confirmed at around λmax = 426 nm. Synthesized AgNPs of calculated mean crystallite size were 10.21 nm. Scanning electron microscopy (SEM) revealed the synthesized AgNPs have a spherical structure. The antibacterial activity of AgNPs synthesized using legeneraria siceraria fruits medicinal plants demonstrated that Gram-positive and Gram-negative bacteria inhibited. Additionally, the AgNPs showed minimal toxicity to human cells. AgNPs were useful antioxidants for health preservation against different oxidative stress associated with degenerative diseases. In this work, it acts at scavenging against DPPH.

Keywords: Legeneraria Siceraria, Leaf Axil Extract, Antibacterial, Nanotechnology, Scavenging, Antioxidant

Introduction

Nanotechnology is a significant field of science, engineering, and technology that is gaining tremendous impetus in this era. It produces nanomaterials with diameters smaller than 100 nm. Nanoparticles are smaller (nm) and have a larger surface area-to-volume ratio, which improves their physical, chemical, and biological properties compared to non-nano materials. The improved properties include catalytic reactivity, thermal conductivity, antibacterial activity, chemical stability; etc [1]. Many kinds of nanoscale metals have wide applications in biology, medicine, and engineering. Ag-NPs inhibit the growth and activities of both gram-positive and gram-negative bacteria [2]. There are several methods for synthesizing nanoparticles, including physical approaches employing mechanical operations, chemical methods using various organic or inorganic chemicals, and biological protocols involving live organisms. Physical and chemical methods were increasingly replaced by green synthesis methods due to difficulties such as low yield, high-energy consumption, the production of toxic and hazardous substances, and the application of sophisticated equipment and synthesis conditions. In this context, green synthesis employs natural and environmentally friendly materials as reducing agents. This method reduces energy consumption; and avoids the use of toxic and harmful reagents. To date, in the green synthesis method, plants, fungi, or bacterial extracts employed as reducing and capping agent for nanoparticle synthesizing. In this case, plant extract mixed with the solution contains metal ions; the bioactive molecules of the extracts reduce the metal ion to elementary metal, and then it precipitated in alcohol [1,3]. Green synthesis for AgNPs involves the mixing of silver nitrate solution with reducing substances extracted from plants [4,5].

Plant parts like leaves, roots, latex, bark, stem, and seeds have been considered the best candidates for the synthesis of AgNPs. This is due to the presence of phytochemicals in its extract, which

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acts like stabilization, and reducing agent [6]. In this study, fruits of the *legeneraria siceraria medicinal* plant, which is widely used in traditional medicines in Ethiopian rural areas, were used as plant extracts for AgNPs formulation. Using medicinal plants is advantageous as their therapeutic properties are added to the nanoparticles during synthesis [7]. AgNPs have proved to be one of the most effective antimicrobial agents among different types of nanomaterials owing to their biocidal properties against bacteria, viruses, and other eukaryotes [8].

Methods and Materials

Chemicals and Instruments

All chemicals and reagents used for this research work were of analytical grade and used without further purification. Silver nitrate ($AgNO_3$, 99%) was purchased from Sigma-Aldrich, India; methanol (CH_3OH, 100%); and glycerin ($C_3H_8O_3$, laboratory-grade) from Dallul Pharmaceutical Plc., Ethiopia. Ethanol (CH_3CH_2OH, 99.8%) from Fine Chemicals General Trading Plc., Ethiopia, is a nutrient broth powder. Mueller Hinton Agar powder was purchased from Merk, Germany. Acetone (C_6H_6O, 99%) from Som Datt Finance Corporation Ltd. (SDFCL), India; sodium hydroxide (NaOH, 97%); sulphuric acid (H_2SO_4, 98%); hydrochloric acid (HCl, 35%); and chloroform ($CHCl_3$, 99.8%) were purchased from Loba Chemie Pvt. Ltd., India. Bromine water (Br_2, concentrated) from Bulux Laboratories, India; ferric chloride ($FeCl_3$, 98%) from Sisco Research Laboratories plc. India; and acetic anhydride ($(CH_3CO)_2O$, 97%) from Carlo Erba reagents SAS, France.

Characterization was performed by, UV-Vis (JASCO V-770, UK), Dynamic Light Scattering (DLS, Malvern, UK), Fourier transform infrared spectrometer (FT-IR, iS50ABX Thermo Scientific, Germany), scanning electron microscope (SEM, JCM-6000 PLUS, and Japan), and X-ray diffraction spectrometer (XRD-700 X-ray, Germany).

Methodology

Plant Sample Collection and Pretreatment

The leaf axils of *legeneraria siceraria* were harvested in the Furi wild garden in Addis Ababa, Ethiopia. Taxonomists at Addis Ababa University's Department of biology certified the validity of plant sample and a voucher specimen was deposited at the herbarium at biology department. After collection, the fruits was continuously cleaned with distilled water to get rid of any contaminants and then allowed to drain. Following that, it was cut using a knife and put away for subsequent use.

Extraction of Reducing Compounds

legeneraria siceraria fuits was chopped into smaller pieces, and 20 g of chopped fuits was weighed and placed into a juice presser. Then, the juice was filtered, and the juice extract was collected and dried using a rotary evaporator at about 40 °C and 87 RPM. The percentage yield of extracts could be calculated using equation 2.1 [9].

$$\text{Percentage yield of extract} = \frac{\text{Amount of extract obtained}}{\text{Amount of intial sample}} \text{X } 100$$

Qualitative Phytochemical Tests for the Extracts

The phytochemical constituents of the extract were analysed for their primary as well as secondary metabolites by protocols described by several groups, as shown in Table 1.

Table 1: Phytochemical test for the extracts

Phytochemical	Procedures	Observation	Reference
Phenols	2ml extract + 0.5 ml FeCl3	Intense colour formation	10
Alkaloids	2ml extract + a few drops of Dragendorff's reagent	Orange precipitate formation	11
Flavonoids	1ml extract + a few drops of FeCl3	Blackish red colour formation	12
Saponins	0.5 DW + 1ml extract shake for 10 minutes	Froth formation	11
Terpenoids	2ml extract + 2ml (CH3CO)2O + a few drops conc. H2SO4	Ring formation	13
Glycosides	1ml distilled water + 1ml extract + aqueous NaOH	Yellow colour formed	14
Tannins	3ml extract +2ml 10% alcoholic FeCl3	Dark blue color formation	15

Optimization of AgNPs Synthesis Parameters

The effect of temperature (40, 60, and 80 °C), stirring time (10, 20, 30, 40, 50, 60, and 70 min), pH (2, 5, 7, 8, 11, 12), concentration of AgNO3 (1, 2, 3, 4, and 5 mM), and concentration on particle size were obtained by varying volume ratios of reactants (extract to metal salt) (V: V; 1:9, 1:4, 3:7, 2:3, 1:1) in a 10 ml total volume of solution with constant stirring at 870 rpm by varying one

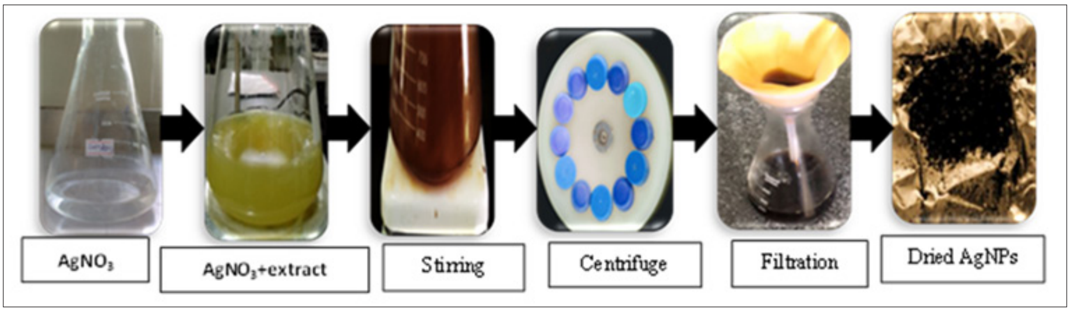

Figure 1: AgNPs synthesis from silver nitrate solution and plant extract

experimental parameter at a time and keeping others constant. The overall synthesis of AgNPs is summarized in Figure 1.

Antibacterial susceptibility testing

To determine the susceptibility of the S. aureus and E. coli isolates to AgNPs and extracts, it was applied based on the methodology mentioned by previous authors [16]. The agar well diffusion standard method was used to collect results in an accessible and easy-to-interpret manner. The antimicrobial activities of synthesized AgNPs and the extract were evaluated by adding a volume of 50 µL/well of 25 mg/ml extract, 25 µg/ml AgNPs, 2mg/L of amoxicillin, and distilled water as a negative control to bacteria containing Mueller Hinton Agar plates. Finally, the plates were incubated for 24 hours at 37 °C, and the inhibition zones were measured for each sample.

Antioxidant Activity of AgNPs

The antioxidant activity of the synthesized nanoparticles was determined by scavenging free radicals from DPPH [7]. The DPPH (0.015 µg/mL) solution was prepared by dissolving in methanol, and then mixed with AgNPs solutions to achieve final concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/mL, which were then mixed and reacted at 30°C for 30 minutes. The absorbance of each test tube was determined using an ultraviolet-visible spectrometer at 517 nm. Ascorbic acid was used as a positive control and the sample containing only DPPH was used as blank. The DPPH radical scavenging activity was calculated using the following equation.

$$\text{DPPH scavenging effect} = \frac{A_0 - A_t}{A_0} \text{ X } 100\ \%$$

where A_0 is the absorbance of the control and At is the absorbance of the sample.

Results and Discussion

Qualitative Phytochemical Tests

To identify the major types of chemicals (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, and terpenoids) present in the extracts, a qualitative phytochemical screening of legeneraria siceraria plant extracts was performed in this work using standard methods. The test assay average result for the extract is shown in Table 2. To keep silver nanoparticles from aggregating and to provide them stability, those phytochemicals are in charge of reducing and capping of the particles.

Table 2: Phytochemical test of plant extract

Plant extract	Alkaloid	Terpenoid	Glycosides	Flavonoid	Saponins	Phenols	Tannins
C. macrostachys	---	+ + +	+ + +	++ + +	+ + +	+ + +	+ + +

a +Presence; −Absence.

Parameters Optimization

Reaction Temperature

The temperature has a specific effect on the synthesizing of NPs. The temperature of the reaction medium determines the nature of the nanoparticles formed. Increased reaction temperature led to a rapid reduction of Ag^+ and subsequent homogeneous nucleation of Ag nuclei. The synthesis of nanoparticles using green technology requires temperatures less than 100°C [10]. In this study, the temperature could be adjusted to 40, 60, and 80 °C. A temperature of 60 °C was optimal for the synthesis of AgNPs based on the UV-Vis spectra shown in Figure 2. However, as the temperature of the reaction mixture increases above the optimum condition, the uniformity of AgNPs decreases. Based on the current experimental results, higher temperatures above 60 °C led to the formation of non-uniformly sized AgNPs. Published works support this result [11].

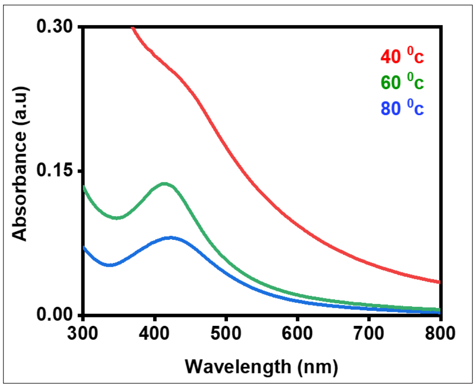

Figure 2: UV-visible absorption spectrum of synthesized AgNPs by legeneraria siceraria fruits extract at different temperatures.

Stirring Time

In this study, the stirring time varied from 10 to 70 minutes. As shown in Figure 3, 60 minutes of stirring time was found to be better for synthesizing AgNPs using *legeneraria siceraria fruits* extract. However, the stirring time of the reaction in this study was varied from 10 to 70 minutes. As shown in Figure 3, 60 minutes of stirring time was found to be better for synthesizing AgNPs using legeneraria siceraria fruit extract. However, as the stirring time of the reaction mixture increases above the optimum condition, the uniformity of NPs decreases. Thus, stirring time affects the interaction of $AgNO_3$ and plant extract [12]. Based on the current experimental results, a longer stirring time above 60 minutes led to the formation of non-uniformly sized NPs based on UV-Vis spectra.

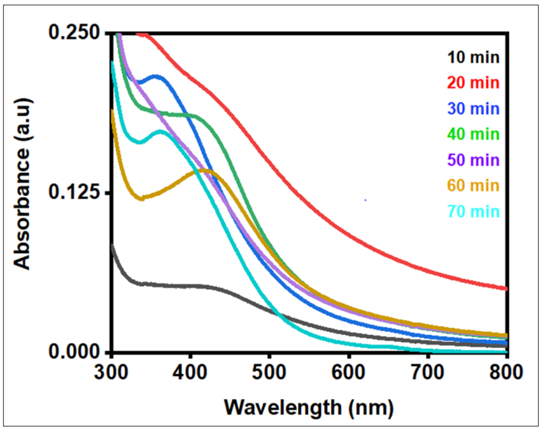

Figure 3: UV-visible absorption spectrum of synthesized AgNPs by legeneraria siceraria fruits extract at different stirring times.

Effect of pH

During optimization in this work, pH could be tested from 2 to 11. At an acidic pH, the UV-Vis absorption peaks are very broad, as shown in Figure 4. As seen from the figure, SPR at pH 2 was poorer than that at pH 11, both of which were observed in UV-visible spectra measurements. In this work pH 11 was used for the synthesis of AgNPs. According to the literature, alkaline pH results in smaller size and uniform AgNPs [13]. In addition to this, if the pH of the reactant is acidic; the transition from light green to dark brown will take a much longer time, indicating that the acidic medium is not appropriate for NPs synthesis.

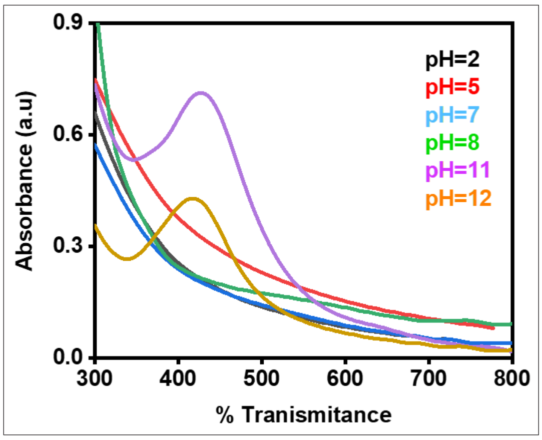

Figure 4: UV-visible absorption spectrum of synthesized AgNPs using legeneraria siceraria fruits extract at different pH conditions.

Silver Nitrate Concentration

The concentration of $AgNO_3$ was measured in the range of 1 mM-5mM in this study. Throughout this work, 2 mM was best studied and suggested for synthesizing AgNPs as shown in Figure 5. Therefore, a very small quantity of the reactant is required for the reaction to occur. If the concentration of the reactant is increased, the reduction of Ag^+ will not be successful, and the accumulation could be noticeable.

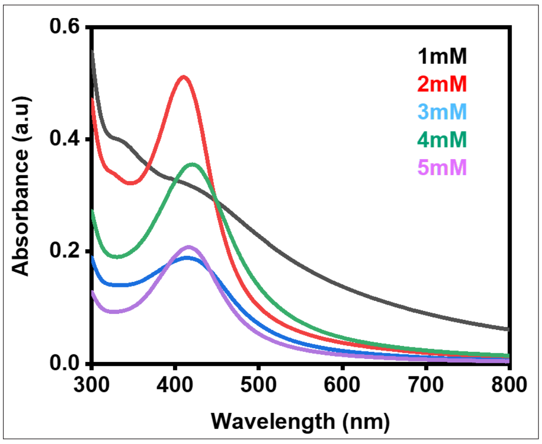

Figure 5: UV-visible absorption spectrum of synthesized AgNPs by legeneraria siceraria fruits extract at different concentrations of $AgNO_3$.

The Concentration of Extracts

The concentration of extract also affects the processing of NPs and affects the time required for silver nanoparticle formation. Because extracts are a major part of the reaction for the reduction of silver ions, their concentration up to a certain quantity is efficient in the formation of AgNPs. In general, the 1, 2, 3, 4, and 5 ml of both extracts in a total of 10 ml solution could be checked for effect in our work shown in Figure 6. Among these, 1ml (V:V, 1:9) of extract was found to be optimum for the synthesis of AgNPs legeneraria siceraria fruits extract.

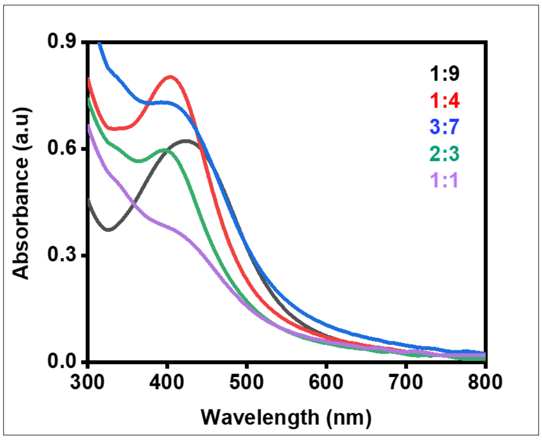

Figure 6: UV-visible absorption spectrum of synthesized AgNPs by legeneraria siceraria fruits extract at different volumes of extracts.

Characterization of AgNPs

UV-Vis Spectra Analysis

Noble metals exhibit distinctive optical features because of the characteristic known as surface plasmon resonance (SPR). The color in the silver nanoparticle was due to the reduction of Ag+ to Ag0 via the active biomolecules present in the extracts, such as alcohol, phenols, polysaccharides, and proteins. Increasing the concentration of extracts of biomolecules used as reducing and capping agents can protect the nanoparticles from aggregation. Between 400 and 450 nm, a distinct absorbance peak is produced by silver nanoparticles. In this work, extracts from the leaf axils of legeneraria siceraria fruits were used to produce AgNPs. The SPR bands that are typical and well-defined for AgNPs produced in this investigation were confirmed at about the maximum wavelength = 426 nm, as shown in Figure 7.

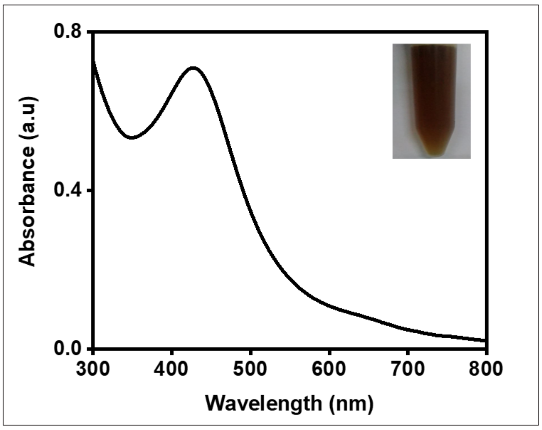

Figure 7: UV-Vis spectra of AgNPs synthesized legeneraria siceraria fruits axil extract.

DLS analysis AgNPs

The diameter of nanoparticles scattered in a liquid is determined using the DLS. Additionally, it determines the distribution and size of particles in physiological fluids. As shown in Figure 8, the DLS supported that 98.7 % by volume of the tested sample has a size (d.nm) of 38.18 nm for AgNPs synthesized using legeneraria siceraria fruits leaf axil extract.

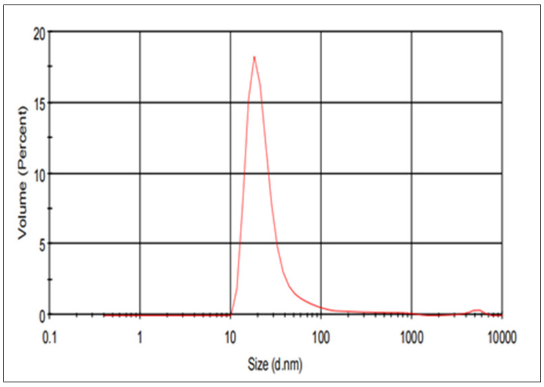

Figure 8: Size distribution of AgNPs synthesized from legeneraria siceraria fruits extract.

Zeta Potential Measurement

The colloidal dispersion's storage stability is predicted by the zeta potential values [14]. The zeta potential values for AgNPs synthesized with legeneraria siceraria fruits extract are -35.4 mV in this instance (Figure 9).

The negative zeta potential indicates the repulsion among the green synthesized AgNPs and increases the stability of the NPs. The NPs are prevented from aggregating together by the electrostatic repulsive force between them, which also reveals an antibacterial effect. These outcomes corresponded with the previous findings [15].

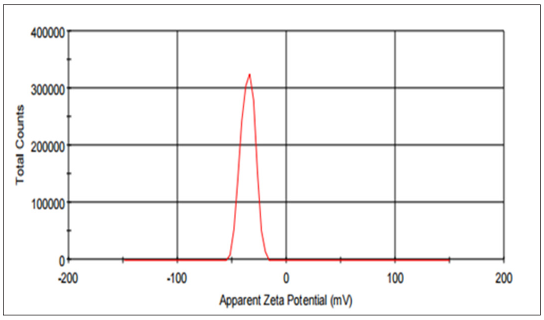

Figure 9: Zeta potential value of AgNPs synthesized from legeneraria siceraria fruits extract.

FTIR Spectra Analysis Plant Extracts, Synthesized AgNPs

The FTIR spectra of legeneraria siceraria fruits extract and synthesized AgNPs are shown in Figures 10(a and b), respectively. The broad spectrum between 3200 and 3300 cm^{-1} was corresponding to the O-H bond stretching in leaf axil extract. The extract's peak at 1630 cm^{-1} was ascribed to a carboxyl group (C=O) stretching vibration. Spectral peaks at 618 cm^{-1} and 532 cm^{-1}, identified the bending area of the aliphatic chain. AgNPs and those chemical functional groups may have interacted to produce the additional peaks in the AgNPs spectral profile (1412 cm^{-1}, 1119 cm^{-1}, 1045 cm^{-1}, 872 cm^{-1}, and 618 cm^{-1}). The findings demonstrate that certain metabolite functional groups, including alcohols, ketones, and aldehydes, were responsible for the creation of AgNPs by plant extracts.

SEM Image Analysis of AgNPs

The AgNPs synthesised using legeneraria siceraria fruits extract were observed to predominately adopt a rod shape with a rough surface topology under the SEM (Figure 11). This is the first study on AgNPs synthesised with fruit extract from legeneraria siceraria fruits. Its rough surface and shape are comparable to those of AgNPs synthesized from other plant extracts, nevertheless.

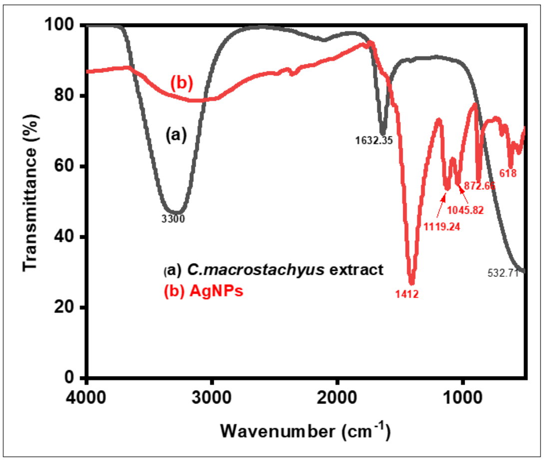

Figure 10: FTIR spectra of legeneraria siceraria fruits extract (a) and capped AgNPs (b).

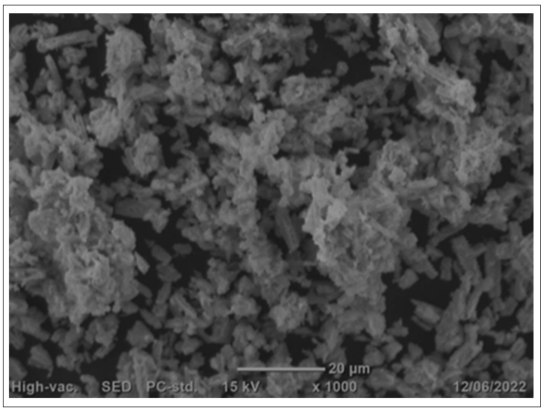

Figure 11: SEM image of AgNPs synthesized using legeneraria siceraria fruits extract.

XRD Studies

XRD patterns of AgNPs synthesized using leaf axil extract of legeneraria siceraria fruits show the face center cubic structure of Ag crystal, having diffraction peaks at 32.25° & 38.38°, 44.33° & 46.18° 64.70° & 67.67°, and 77.27° corresponding to (111), (200), (220) and (311) planes respectively. These results also confirmed the nature of AgNPs as crystalline face-centered cubic [15]. The diffraction peak at 38.38° had a robust diffraction intensity indicating the preferential orientation of Ag crystal along the (111) plane. The unassigned peaks at 2θ = 23.8° 27.97°, 54.89°, and 57.51°, denoted by (*) in Figure 12 are thought to be related to crystalline and amorphous organic substances. Synthesized AgNPs of calculated mean crystallite size was 10.21 nm.

Antimicrobial assay of AgNPs and extracts

The antibacterial action of the AgNPs produced by the legeneraria siceraria fruits extract and the extract alone, in combination with distilled water as a negative control and amoxicillin as a standard, as demonstrated in Figure 13. Gram-positive and gram-negative bacteria had distinct outcomes. There is a comparable inhibition zone with the AgNPs on the well filled with amoxicillin against both bacteria (Table 3). However, the absence of an inhibitory zone surrounding the distilled water-filled well suggests that the biosynthesized AgNPs, which exhibit robust antimicrobial action, are the source of the antimicrobial activity. These medicinal plants were used to synthesize AgNPs, which showed antibacterial action against both Gram-positive and Gram-negative bacteria. The results corresponded with previous comparable research conducted by Momeni et al [17].

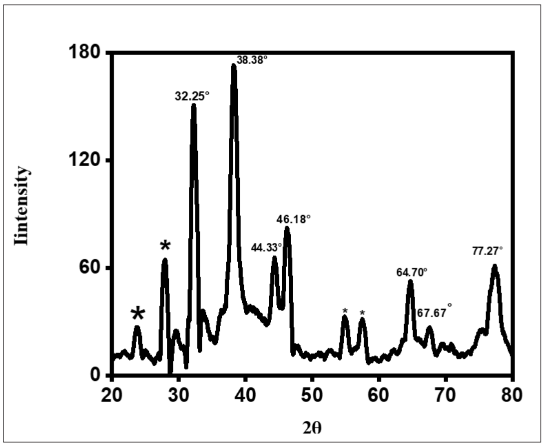

Figure 12: XRD pattern of AgNPs synthesizes using legeneraria siceraria fruits extract.

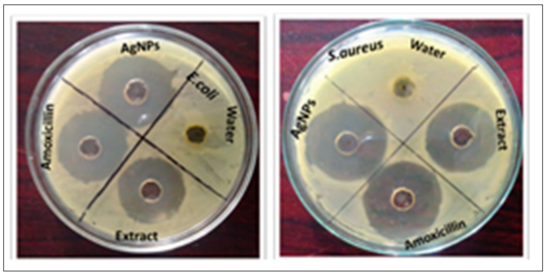

Figure 13: Antibacterial activity of AgNPs and legeneraria siceraria fruits extract against multidrug-resistant (MDR) strains of E. coli and S. aureus using amoxicillin as positive control and distilled water as a negative control.

Table 3: Diameter of zone of inhibition by distilled water amoxicillin, extracts, and biosynthesized AgNPs against multidrug-resistant pathogenic bacteria.

Strain	**ZOI (mm)**			
	legeneraria siceraria fruits	AgNPs synthesized using legeneraria siceraria fruits extract	Amoxicillin	Distilled water
S. aureus	24 mm	29 mm	28 mm	0
E. coli	22 mm	26 mm	26 m	0

Antioxidant Activity AgNPs

Because it accepts fewer electrons from donors, DPPH is a more stable and well known free radical. Figure 14 demonstrates how the DPPH scavenging experiment effectively inhibited the activity of AgNPs in comparison to conventional ascorbic acid. The DPPH activity of the AgNPs was seen to rise in a dose-dependent manner. The utilization of AgNPs as effective natural antioxidants for health preservation against various oxidative stressors linked to degenerative disorders is highly advised by the results.

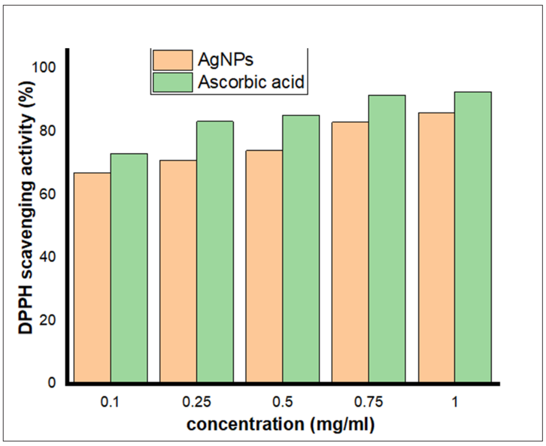

Figure 14: DPPH scavenging activity of AgNPs

Conclusion

This work established an eco-friendly, easy and one-step method for producing AgNPs from the aqueous extracts of the leaf axil of the legeneraria siceraria fruits plant. Characteristic and well-defined SPR bands for AgNPs were confirmed at around λmax = 426 nm. The FTIR spectra's observation of peak shifting suggested that various functional groups of plant secondary metabolites may play a role as stabilizing and capping agents. The XRD and SEM, respectively, determined the particles' crystallinity and spherical shape. Synthesized AgNPs of calculated mean crystallite size was 10.21 nm. It was discovered that the synthesized AgNPs exhibited more antibacterial activity than the aqueous extract. AgNPs has DPPH scavenging activity, which indicates that, its useful natural antioxidants for health preservation against different oxidative stress.

Conflict of Interests

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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