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Application of *Acacia modesta* and *Dalbergia sissoo* gums as green matrix for silver nanoparticle binding

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Abstract: A low-cost, efficient, and ecofriendly method for the synthesis of silver nanoparticles (AgNPs) using gums as reducing agent was performed. The obtained nanoparticles were characterized by UV-visible spectroscopy. The antibacterial activity of the prepared nanoparticles was tested against Gram-negative and Gram-positive bacteria. The *in vitro* toxicity was evaluated by performing hemolytic analysis. The mutagenic activity was evaluated using Ames test. The prepared nanoparticles possessed high antibacterial potential and also inhibited the biofilm formation. Under the conditions of this study, it is concluded that the prepared AgNPs were nontoxic and nonmutagenic and possessed pharmaceutical applications.

Keywords: *Acacia modesta*; *Dalbergia sissoo*; green matrix; gums; silver nanoparticles.

1 Introduction

Nanosilver is highly commercialized nanomaterial being produced approximately 320 tons a year [1]. Because of its strong antimicrobial activity, silver nanoparticles (AgNPs) are used in household water filters, cosmetics, clothing, detergents, shoes, laptop keyboards, cutting boards, antibacterial sprays, and respirators [2].

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Many techniques in synthesizing AgNPs, such as the chemical reduction of silver ions in aqueous solutions, have been reported in the literature. Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks [3, 4]. There is a growing need to develop environment-friendly processes for nanoparticle synthesis, which do not use toxic chemicals [4].

The gum of *Acacia modesta* is present in the form of angular fragments or small tears and vermiform. It is a translucent and yellow color gum [5]. *A. modesta* is used as miswak (chewing sticks) in many parts of Pakistan. The fruits, leaves, wood, and bark are mostly used for medicinal, fuel, and timber purposes. It is effective against chronic stomach disorders, gastric troubles, and dental diseases [6]. The gum is also used as a tonic and to cure dysentery [7].

Dalbergia sissoo has many medicinal properties and is used as an abortifacient, aphrodisiac, anthelmintic, antipyretic, and expectorant [8]. The extracts from different parts were reported as anti-inflammatory [9], analgesics [10], and antidysentric [11]. It is used in conditions such as ulcers, emesis, dysentery, leucoderma, skin diseases, and stomach troubles. *D. sissoo* is antidiarrheal, as it affects bacterial virulence.

The aim of the present study was the synthesis of AgNPs using gums as green matrix followed by their characterization, antibacterial, hemolytic, and antimutagenic activities.

2 Materials and methods

2.1 Materials

The *A. modesta* and *D. sissoo* gum was purchased from a local market in Faisalabad, Pakistan, and was identified at the Department of Botany (Dr. Mansoor Hameed, Associate Professor/Taxon), University of Agriculture, Faisalabad, Pakistan. Davis-Mingioli salt (5.5 times concentrated), D-glucose (40%, w/v), bromocresol purple (2 mg/ml), D-biotin (0.1 mg/ml), and L-histidine (0.1 mg/ml). The two

sterile standard mutagens were sodium azide (NaN_3 0.5 µg/100 µl) for *Salmonella typhimurium* TA100 and potassium dichromate ($K_2Cr_2O_7$ 30 µg/100 µl) for *S. typhimurium* TA98.

2.2 Preparation of the reagent mixture

The reagent mixture comprised Davis-Mingioli salt (21.62 ml), D-glucose (4.75 ml), bromocresol purple (2.38 ml), b-Biotin (1.19 ml), and L-histidine (0.06 ml). The reagents were mixed aseptically in a sterile bottle. The reagent mixture, extract, sterile deionized water, strains, and standard mutagens were mixed in several bottles at the amount indicated in Table 1.

Then, 200 µl of the prepared contents were dispensed into each well of a 96-well microtitration plate. The plate was placed in an airtight plastic bag to prevent evaporation and incubated at 37°C for 4 days.

2.3 Purification and extraction of gums

The dried gum samples were powdered to obtain a fine and uniform sample. This was the crude gum. For the purification of gums, the method of Shahid et al. [12] was used. The gums were set aside overnight in water and allowed to swell, and a viscous solution was obtained. The viscous gum solution was stirred on a mechanical stirrer for 6 h at room temperature. The solution was filtered. This solution was slowly added to ethanol. White amorphous precipitates were obtained in the gum solution, which were filtered and purified further by treatment with absolute ethanol. The white precipitates were dried in oven at 40°C and stored for further use as the purified gum.

2.4 Synthesis of gum-based AgNPs

Silver nitrate and the purified gums of *A. modesta* and *D. sissoo* were used for the preparation of gum-based AgNPs. All the solutions were prepared in distilled water. Then, 0.5% (w/v) of homogenous gum solution was prepared and the concentration of silver nitrate was 1 mM. The gum-based AgNPs were synthesized by autoclaving the solution at 121°C for 15 min [13].

2.5 Characterization of AgNPs

The prepared AgNPs were characterized by spectral analysis in the UV-visible region using a spectrophotometer [13, 14].

2.6 Antibacterial activity

The antibacterial activity of gum-based AgNPs was studied against microbes using the disc diffusion method [15, 16].

2.7 *In vitro* toxicity by hemolytic activity

The crude gum solution and prepared AgNPs were used for the hemolytic activity [12]. The samples were prepared in distilled water with the following different concentrations: 2%, 4%, 6%, 8%, and 10%. Human blood (3 ml) was added in heparinized tubes to avoid coagulation and gently mixed, poured into a sterile Falcon tubes, and centrifuged at 850×*g* for 5 min. The supernatant was poured off and red blood cells (RBCs) were washed three times with 5 ml chilled (4°C) sterile isotonic phosphate-buffered saline (PBS; pH 7.4). In 20 ml PBS, washed RBCs were suspended. Erythrocytes were counted on a hemacytometer. Then, 20 µl of the sample were transferred into Eppendorf tubes and 180 µl of the diluted blood cell suspension were added to it. For 30 min at 37°C with agitation, the samples were incubated. After 30 min, the sample mixture was placed on ice for 5 min after centrifugation at 1500×*g*. Then, 100 µl of the supernatant were taken and diluted with 900 µl chilled PBS. In 96-well plates, 200 µl of this mixture were added. Triton X-100 (0.1%) was taken as the positive control and PBS as the negative control. At 576 nm, the absorbance was noted and data are presented as percent lysis of RBCs.

2.8 Mutagenicity test

The Ames test entirely in liquid culture was performed in liquid culture (fluctuation test) [17]. Two mutant strains, *S. typhimurium* TA98 and TA100, were used. These bacteria were maintained on nutrient agar at 4±1°C.

2.9 Interpretation of results and statistical analysis

The blank plate was observed first and the rest of the plates were read only when all wells in the blank plate were colored purple, indicating that the assay was not contaminated. The background, standard, and test plates were scored visually. All yellow, partial yellow, or turbid wells were scored as positive wells, whereas purple wells were scored as negative wells. The extract was considered toxic to the test strain if all wells in the test plate showed purple coloration. For an extract to be mutagenic, the number of positive wells had to be more than twice the number of positive well in the background plate. For statistical analysis, probability was calculated [18].

Table 1: Set-up of the fluctuation assay.

Treatment	Volume added (ml)				
	Mutagen standard	Extract	Reagent mixture	Deionized water	*Salmonella* test strain
Blank	–	–	2.5	17.5	–
Background	–	–	2.5	17.5	0.005
Standard mutagen	0.1	–	2.5	17.4	0.005
Test sample	–	0.005	2.5	17.5	0.005

3 Results and discussion

3.1 Gum-based AgNPs

Silver nitrate and the purified gums of *D. sissoo* and *A. modesta* were used for the preparation of AgNPs. Using *D. sissoo* and *A. modesta* gums, this process results in the green synthesis of AgNPs by the help of autoclaving. This method uses nontoxic, nonhemolytic, natural exudates and renewable gums. These gums act as stabilizing and reducing agents during the AgNP synthesis. The AgNPs became sterile and safe after autoclaving them. The production of gum-based AgNPs is an important requirement for microbial applications. This method of production is according to the requisite of the green chemistry principles [13, 14]. The gums expanded and get swelled to become more available for silver ions to react with the functional groups present on gums during the process of autoclaving. The hydroxyl groups oxidize to carbonyl groups, which are caused by the present silver ions. These silver ions get reduced to elemental silver [13, 19].

3.2 Production of AgNPs

The production was confirmed by the appearance of dark yellow color in the mixture containing gum solution and silver nitrate. This was the clear sign of the formation of gum-based AgNPs. The production of AgNPs using both gums is given in Figure 1.

3.3 Characterization of AgNPs

The prepared AgNPs were characterized by spectral analysis in the UV-visible region using spectrophotometry in the wavelength range of 200 to 800 nm. This is the most extensively used, sensitive, and simple technique for the confirmation of AgNPs. The absorption spectra of gum-based AgNPs and gum solution were recorded to observe the production of AgNPs by UV-visible spectroscopy. The spectrum obtained from both gums is given in Figure 2. The graphs indicate the formation of AgNPs. AgNPs formed from both gums exhibited strong peaks at approximately 410 to 430 nm. It is responsible for conducting electrons present at the surface of AgNPs. The dark yellow/yellowish brown color of AgNPs is due to the surface plasmon resonance transition [20]. There were no peaks at the region of 335 and 560 nm and a confirmation of no formation of nanocluster or aggregation of nanoparticles [13].

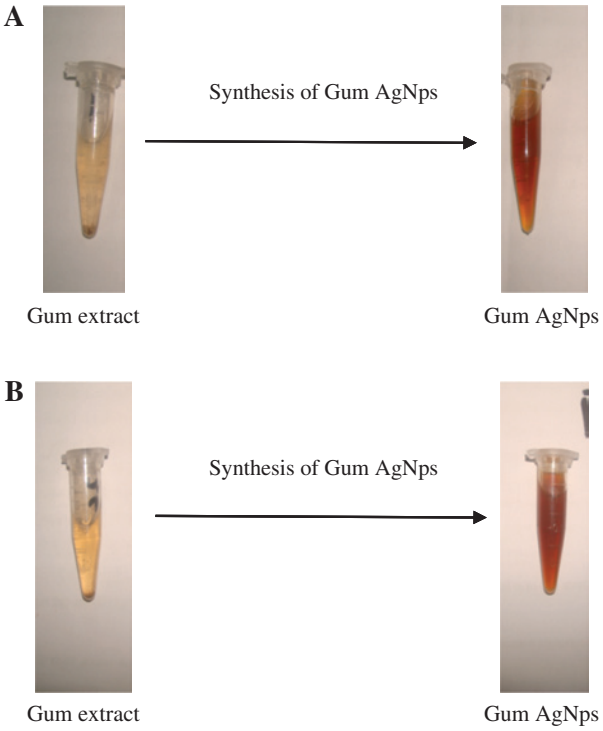

Figure 1: Gum-based AgNPs using *A. modesta* and *D. sissoo* gums as binding matrix: (A) *A. modesta* and (B) *D. sissoo*.

3.4 Antibacterial activity

To study the antibacterial effect of gum-based AgNPs, the disc diffusion method was performed against the bacterial species *Escherichia coli* and *Staphylococcus aureus*. Rifampicin was used as the positive control. The synthesized AgNPs exhibited a significant high antibacterial activity against both species. The diameter of the clear zone formed by the sample was measured. The gum-based AgNPs released compounds that are diffusible and caused the formation of clear zones around the discs in Petri plates. The antibacterial activity by *A. modesta* and *D. sissoo* based nanoparticles is given in Table 2. The results are the mean of two separate experiments, and the mean±SD was calculated.

The antibacterial activity was increased due to the AgNPs. According to the literature survey, no data were reported using these gums. However, there is a report of antibacterial activity of other gums used to form AgNPs by this method [13, 14, 20].

3.5 *In vitro* toxicity by hemolytic activity

According to our literature survey and the best of our knowledge, no information was available about the

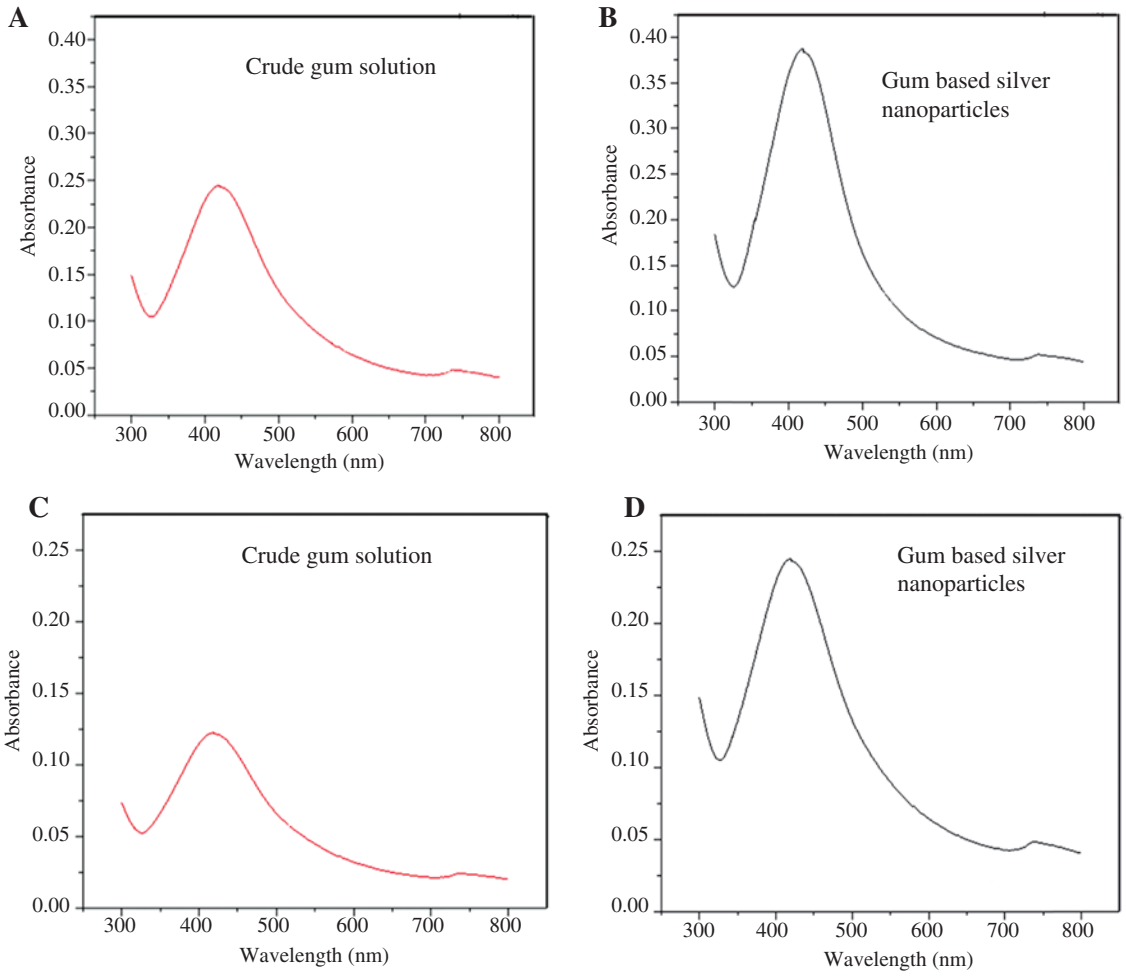

Figure 2: UV-visible spectra of crude and AgNPs using *A. modesta* (A and B) and *D. sissoo* (C and D) gums as green matrix.

Table 2: Antibacterial activity of gum-based AgNPs.

Sample	*E. coli*	*S. aureus*
Crude *A. modesta* (1%)	14±1.4	14±1.2
AgNPs using *A. modesta*	24±0.75	20±1.1
Crude *D. sissoo* (1%)	14±1.2	12±0.9
AgNPs using *D. sissoo*	24±0.89	24±1
Rifampicin (positive control)	25±1.3	25±0.9

toxicity of the crude gums and prepared AgNPs. The hemolytic activity was tested with the help of a rapid assay against human erythrocytes. The negative control (PBS) shows no hemolytic activity. Triton X-100 gives 99.9% lysis of human erythrocytes. The results of hemolytic activity are presented in Table 3.

All used samples were nontoxic, as very low hemolytic activity was observed; therefore, these gums and the

Table 3: Hemolytic activity of gum-based AgNPs.

Samples	Concentration of samples				
	2%	4%	6%	8%	10%
A. modesta					
Crude gum	3.35±0.02	3.37±0.02	3.85±0.06	3.91±0.03	5.26±0.04
AgNPs	3.22±0.02	4.21±0.09	4.41±0.04	4.79±0.02	4.82±0.02
D. sissoo					
Crude gum	3.20±0.04	4.85±0.05	4.97±0.07	5.27±0.06	6.5±0.03
AgNPs	2.33±0.01	3.03±0.02	3.08±0.02	3.15±0.01	3.69±0.01

Table 4: Mutagenic activity of *A. modesta* and *D. sissoo* against TA98 and TA100.

Herbal extracts	Number of positive wells/total number of wells	Results
(a) Mutagenic activity of *A. modesta* and *D. sissoo* against TA98		
Background	20/96	
Standard ($K_2Cr_2O_7$)	92/96	+
A. modesta		
Crude	1/96	-
AgNPs	9/96	-
D. sissoo		
Crude	4/96	-
AgNPs	1/96	-
(b) Mutagenic activity of *A. modesta* and *D. sissoo* against TA100		
Background	25/96	+
Standard (NaN_3)	90/96	-
A. modesta		
Crude	1/96	-
AgNPs	2/96	-
D. sissoo		
Crude	1/96	-
AgNPs	1/96	-

+, Significant increase in the number of positive wells compared to the related control ($p<0.05$). -, No significant effect observed.

prepared AgNPs can be considered for medication use in the treatment of various diseases.

3.6 Mutagenicity test

For the mutagenic activity, the Ames bacterial reverse-mutation test was performed entirely in liquid culture (fluctuation test) [17]. The mutagenicity was tested against two bacterial strains: *S. typhimurium* TA98 and TA100. The blank plate was observed first and the rest of the plates was read only when all wells in the blank plate were colored purple, indicating that the assay was not contaminated. The blank plate was purple in color with no change in color, indicating no contamination. The mutagenicity analysis is given in Table 4 against TA98 and TA100. No samples possess mutagenic potential and were nonmutagenic.

4 Conclusion

The present research reports the green synthesis of gum-based AgNPs. The AgNPs prepared in this research were carried out by a low-cost and ecofriendly method. The used gums were natural plant extracts. The gums acts as reducing agents while reacting with silver nitrate. Based on these findings, it is concluded that gum-based AgNPs have considerable and significant antibacterial activity and were nonhemolytic and nonmutagenic.

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Bionotes

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