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Green Synthesis of Silver Nanoparticles Using *Abutilon Grandifolium* Aqueous Leaf Extract, Characterization and Evaluation of their Anti-Bacterial, Anti-Oxidant and Anti-Inflammatory Properties

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ABSTRACT

Developing dependable and environmentally friendly methods for the synthesis of nanoparticles is a critical step in the field of nanotechnology. Silver nanoparticles (AgNPs) are significant due to its superior chemical, physical and biological properties, as well as their numerous applications. The present article reports an environmentally benign method for synthesizing AgNPs using the aqueous leaf extract of the plant *Abutilon grandifolium*, their characterization and evaluation of its anti-bacterial, anti-oxidant and anti-inflammatory activities. The synthesized nanoparticles were characterized by Ultraviolet-Visible (UV-Vis) spectroscopy, which shows an absorption peak at 415 nm due to the Surface Plasmon Resonance (SPR) property. The Energy Dispersive X-Ray (EDAX) spectroscopy shows that the synthesized AgNPs obtained were pure in form. The zeta potential value was found at -36.4 mV. High-Resolution Transmission Electron Microscopy (HR-TEM) showed that the nanoparticles were spherical. The synthesized nanoparticles were checked for the anti-bacterial activity of various gram-positive and gram-negative bacteria. The results obtained show perfect activity against *Bacillus subtilus*. DPPH assay and albumin denaturation assay determined the anti-oxidant and anti-inflammatory activity. The results show that the synthesized AgNPs possess excellent anti-oxidant and anti-inflammatory activities.

Keywords

Abutilon grandifolium; HR-TEM; EDAX; Albumin; AgNPs.

1. Introduction

Nanochemistry is a new subfield of the chemical and materials sciences concerned with developing new methods for fabricating nanoscale materials [1]. A nanoparticle family of materials has a feature that makes them apart from their bulk and molecule counterparts [2]. Noble metal nanoparticles are increasingly in demand for their appealing applications in various industries, including antimicrobials [3], medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion [4]. Silver has been an antimicrobial agent since ancient times and silver-based compounds are much less expensive than gold-based compounds [5]. The characteristics of AgNPs include a large surface area, a tiny size (< 20 nm) and a high dispersion property [6]. Humans and other eukaryotic cells are not poisonous to AgNPs, but prokaryotic cells, such as bacteria, viruses and fungi, are very

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toxic [7]. In several scientific fields, the development in the synthesis of AgNPs has had a significant impact. Physical and chemical procedures are not suited for manufacturing AgNPs due to low yield and the usage of hazardous chemicals, respectively [8]. The green chemistry method shows that using natural organisms has provided a dependable, simple, nontoxic and environmentally friendly solution [9, 10]. As a result, in recent years, researchers have turned to biological systems for nanoparticle synthesis [11]. The use of microorganisms, enzymes or plant extracts in the synthesis of nanoparticles has been proposed as an eco-friendly alternative to chemical and physical methods [12, 13]. The biosynthesis of nanoparticles by plants is more efficient than other methods of producing nanoparticles, reducing the complicated process of maintaining cell culture [14]. *Abutilon grandifolium* belongs to the family *Malvaceae*. For the treatment of measles, decoctions of the leafy or fruiting stems are used as an enema. Internally and externally, the leaves and stems are used to treat insect bites [15].

2. Materials and Methods

Fresh and healthy leaves of *Abutilon grandifolium* were collected from Pechipparai, Kanniyakumari District, Tamilnadu. Silver nitrate ($AgNO_3$) analytical grade reagent from Sigma-Aldrich and deionized water are used to synthesize AgNPs from the aqueous leaf extract of the plant *Abutilon grandifolium*.

2.1. Preparation of aqueous leaf extract of *Abutilon grandifolium*

The leaves of the plant *Abutilon grandifolium* were well cleaned using tap water followed by double distilled water. Around 10 g of the fresh leaves were boiled with 100 mL of deionized water for 30 minutes at 60°C and the extract was filtered using Whatmann No.1 filter paper.

2.2. Synthesis of AgNPs using aqueous leaf extract of *Abutilon grandifolium*

40 mL of the prepared aqueous leaf extract was added to 60 mL of 1 mM $AgNO_3$ solution in a conical flask. Then the mixture was kept on a hot plate with a magnetic stirrer at 60°C. After 15 minutes reduction of silver ions was observed by the change in color of the solution. The mixture is allowed to stir for 15 more minutes and kept aside for 15 hours. The synthesized AgNPs were collected by centrifugation and washed thrice with deionized water.

2.3. Characterization of synthesized AgNPs

The biosynthesized AgNPs were monitored by a JASCO V-650 spectrophotometer within the range 200-800 nm. Jeol/JEM 2100 high-resolution transmission electron microscope (HR-TEM) was used to study the morphology and size of the nanoparticles. Zeta sizer nano series (Malvern) was used to find the stability of the nanoparticles with water as a dispersant.

2.4. Anti-bacterial activity

The sample-loaded disc was placed on the surface of the Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with a transparent ruler in millimetres.

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2.5. Anti-oxidant activity

2.5.1. DPPH radical scavenging assay

For the DPPH assay, ascorbic acid was used as a reference standard. A 60µM solution of DPPH in methanol was freshly prepared and a 200µL of this solution was mixed with 50µL of test sample at various concentrations (6.25, 12.5, 25, 50,100 µg/mL). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula.

Percentage of inhibition = [(Abs of Control at 0 min – Abs of test)/ Abs of Control at 15 min] × 100

2.6. Anti-inflammatory activity

2.6.1. Protein denaturation inhibition assay

The reaction mixture (0.5 mL) consisted of 0.4 mL bovine serum albumin (3% aqueous solution) and varying test sample concentrations. The samples were incubated at 37ºC for 20 minutes and 2.5 mL phosphate buffered saline (pH 6.3) was added to each tube and then heated at 80ºC for 10 minutes. The absorbance was measured using a spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated as follows.

Percentage of inhibition = [(Abs Control - Abs Sample)/Abs Control] ×100

3. Results and Discussion

The current investigation shows the formation of the AgNPs by reducing the silver metal ions during exposure to the aqueous leaf extracts of the plant *Abutilon grandifolium*. The result shows that the formation of AgNPs occurs within 20 min of the reaction time at room temperature. Initially, the reaction mixture turns turbid after adding aqueous leaf extract to $AgNO_3$. With continuous stirring at 60°C after 15 min, the color of the solution turns to mild brown, which turns more brownish with an increase in time. The color is due to the Surface Plasmon Resonance of AgNPs.

3.1. Characterization of AgNPs synthesized using *Abutilon grandifolium* leaf extracts

3.1.1. UV-Visible Spectroscopy

The formation of AgNPs was confirmed by UV-Visible spectroscopy. The respective Surface Plasmon Resonance peaks were recorded between 200 – 800 nm. A distinct absorption peak was obtained at 415 nm (Fig.1).

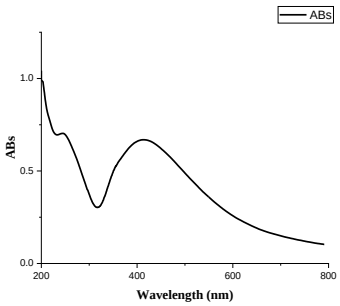

Figure 1: UV-Visible spectrum of AgNPs from *Abutilon grandifolium*

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3.1.2. EDAX Analysis

Energy Dispersive X-ray (EDAX) Spectroscopy shows a strong metal signal for Ag, which confirms the presence of pure Ag metal as the major constituents. In the present investigation, the synthesized AgNPs show strong absorption at around 3 keV (Fig.2) with 88.85% of silver and 11.15% of carbon (Table.1).

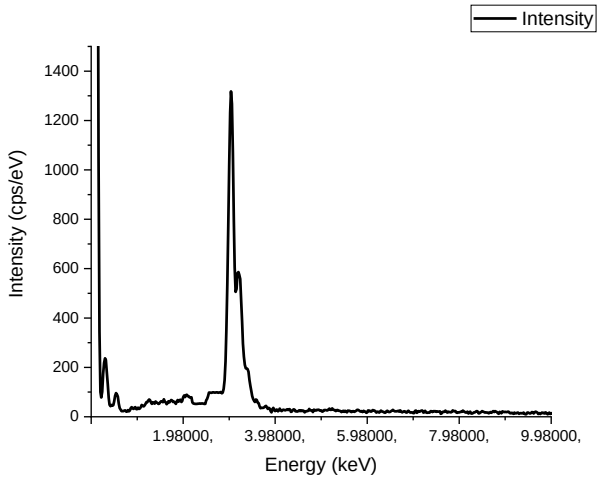

Figure 2: EDAX spectrum of AgNPs from *Abutilon graandifolium*

Table 1: Elemental composition of AgNPs from *Abutilon grandifolium*

Element	Weight %	Atomic %
Ag	88.85	98.17
C	11.15	1.83

3.1.3. High-Resolution Transmission Electron Microscopy (HR-TEM)

High-Resolution Transmission Electron Microscopy (HR-TEM) has been used to identify the nanoparticle's size, shape and morphology. The synthesized AgNPs were spherical in shape and are polydispersed in nature with no aggregation (Fig.3).

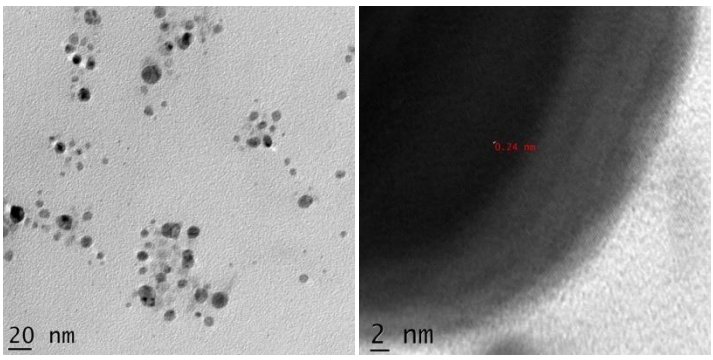

Figure 3: HR-TEM images of AgNPs from *Abutilon grandifolium* with clear lattice fringes

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3.1.4. Zeta Potential

Zeta potential was used to determine the surface potential of synthesized AgNPs solutions and their stability. The zeta value of synthesized AgNPs was found at -36.4 mV (Fig.4). This value shows that the generated AgNPs were highly stable in colloidal solution.

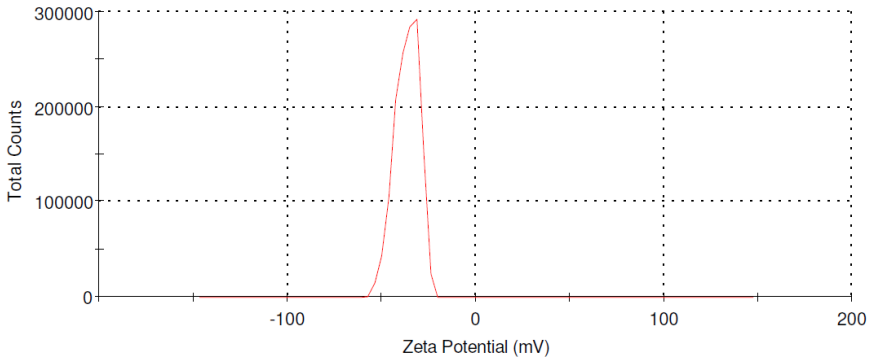

Figure 4: Zeta potential of AgNPs from *Abutilon grandifolium*

3.2. Biological Applications

3.2.1. Anti-bacterial activity of synthesized AgNPs using *Abutilon grandifolium* extract

The anti-bacterial activity of synthesized AgNPs was studied with different gram-positive and gram-negative bacteria's is listed in the below table 2. The results confirmed the existence of anti-bacterial activity. The synthesized AgNPs show a maximum zone of inhibition of 13 mm for the gram-positive bacteria *Bacillus subtilis*.

Table 2: The anti-bacterial activity of AgNPs from *Abutilon grandifolium*

Organism	Standard Value (mm)	AgNPs (mm)
Escherichia coli	20	11
Staphylococcus aureus	25	10
Pseudomonas aeruginosa	25	11
Klebsiella pneumoniae	20	12
Bacillus subtilis	25	13
Enterococcus faecalis	20	-
Lactobacillus salivarius	22	10
Proteus mirabilis	20	11

3.2.2. Anti-oxidant activity:

Oxidative stress is a significant risk factor for cancer. Free radicals are known to cause oxidative damage in biomolecules and to play a significant role in ageing, cardiovascular disease and immune system imbalance [16]. Anti-oxidant activity manifests itself in a wide range of actions, including oxidation enzyme inhibition, transition metal chelation and enzyme detoxification of reactive oxygen species [17]. The DPPH radical scavenging activity of AgNPs synthesized using the aqueous leaf extract of *Abutilon grandifolium* was studied. The percentage of inhibition differs while increasing the

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concentration of AgNPs, as shown in the below table 3. The results showed that the synthesized AgNPs possess good anti-oxidant activity.

Table 3: The radical scavenging activity of AgNPs from *Abutilon grandifolium*

S.No.	Concentration (µg/mL)	Inhibition of DPPH (%)
1.	6.25	3.57
2.	12.5	15.47
3.	25	28.57
4.	50	42.85
5.	100	61.90

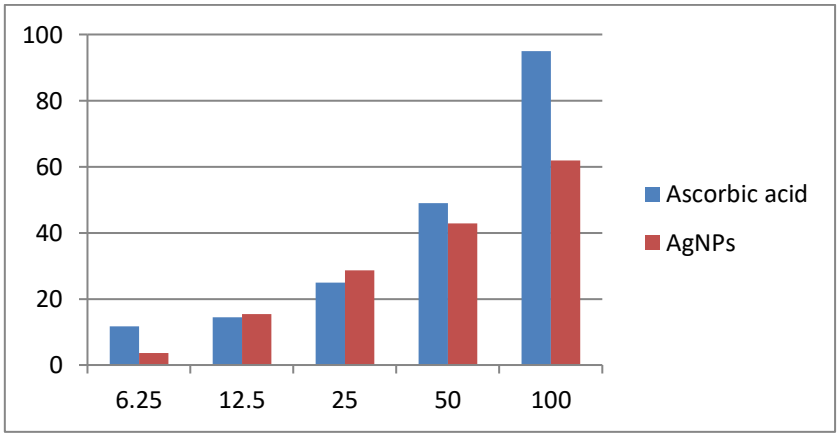

Figure 5: DPPH radical scavenging activity of standard Ascorbic acid and AgNPs from *Abutilon grandifolium*

3.2.3. Anti-inflammatory activity:

Inflammation is also known as the local microcirculation protective mechanism against tissue injury caused by physical trauma, noxious stimuli from chemical agents, heat, antigen-antibody reaction and microbial effects [18]. Any substance that inhibits protein denaturation by more than 20 per cent could be considered a potential anti-inflammatory drug [19]. Protein denaturation is one of the causes of inflammation. Therefore, agents that can prevent protein denaturation can be used to treat inflammatory diseases. Anti-inflammatory activity of synthesized AgNPs was carried out using protein denaturation assay with diclofenac as a standard drug. The maximum denaturation assay was observed as 69.12% at 100µg/mL. The percentages of inhibition observed at different concentrations are listed in the below table 4.

Table 4: The albumin denaturation activity of AgNPs from *Abutilon grandifolium*

S.No.	Concentration (µg/mL)	Inhibition of albumin denaturation(%)
1.	6.25	5.90
2.	12.5	15.16
3.	25	27.11

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4.	50	33.82
5.	100	69.12

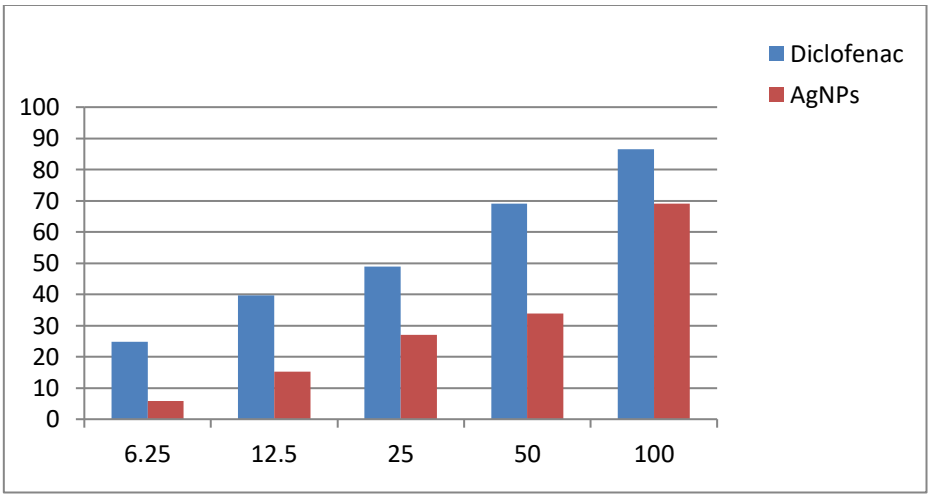

Figure 6: Inhibition of albumin denaturation activity of standard Diclofenac and AgNPs from *Abutilon grandifolium*

4. Conclusion:

A simple method of synthesis of AgNPs using the aqueous leaf extract of the plant *Abutilon grandifolium* was reported in this study. The reaction takes place within 15 minutes of reaction time without the use of any toxic chemicals. The bio-molecules present in the leaf extract itself act as the reducing and capping agents. The zeta potential value obtained at -36.4 mV shows that the synthesized nanoparticles were highly stable. The HR-TEM images confirm that the particles were spherical in shape with no aggregation and are polydispersed in nature. The EDAX result shows that the synthesized AgNPs were pure in form. Among the bacteria studied, AgNPs possess excellent activity against the bacteria Bacillus subtilis. The anti-oxidant and anti-inflammation activity results show that the synthesized AgNPs possess excellent activity, which can be used as a potential drug in future. The AgNPs synthesized in this method are environmentally friendly as it uses no harmful chemicals. Most of the silver in silver nitrate is reduced to silver nanoparticles. Therefore the waste products obtained are less harmful to the environment.

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