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Original Research Paper

Synthesis of silver nanoparticles in an eco-friendly way using *Phyllanthus amarus* leaf extract: Antimicrobial and catalytic activity

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ABSTRACT

In the present study, silver nanoparticles (AgNPs) with a flower-like structure were synthesized through an easy, rapid and eco-friendly pathway using *Phyllanthus amarus* leaf extract. The obtained AgNPs were characterized using ultraviolet–visible (UV–Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). In addition, the antimicrobial and catalytic activities of the bio-synthesized AgNPs were carried out. Our results indicated that the concentration of the Ag precursor and the volume of the leaf extract played key roles in the formation of the flower-shaped AgNPs. Morphology study confirms the shape of the obtained bio-AgNPs as flower like structure. This study also showed the presence of clear capping layers surrounding and apparently interacting with the nanoparticles. Moreover, our studies indicated this interaction to involve bio-organic capping agents in the leaf extract. UV–Vis absorption spectra confirmed the formation of AgNPs with an optimized size. The zeta (ζ) potential of the AgNPs attests the stability of the nanoparticles. FTIR spectra provided evidence for the presence of biomolecules responsible for the reduction as well as capping of the AgNPs. Finally, the bio-synthesized AgNPs were shown to be an excellent microbial activity against the selected pathogens and enhanced catalyst of the reduction of rhodamine B.
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1. Introduction

Among nanomaterials, metal nanoparticles are playing a prominent role in biological and medical fields [1,2]. In particular, advances in the synthesis of silver nanoparticles (AgNPs) have seen the development of NPs with high catalytic activities [3,4]. Consequently, the emergence of well-defined AgNPs with particular shapes and sizes directed towards the significant applications in various fields [3,4]. However, conventional physical and chemical methods are not only costly and require stringent conditions, but are also environmentally unfriendly due to the toxic chemicals that are used [5,6]. Living plants and plant biomass have been recently attracting a great deal of attention for the development of alternative bio-metal nanoparticles. Plant products can be used for the synthesis of metal nanoparticles, as functional groups of plant products reduce metal ions, and hence offer a very simple and eco-friendly route to the production of the metal nanoparticles [7–9]. AgNPs had an immense influence in the medical field: they are used as surgical instruments and are used to coat contraceptive devices to avoid the infection [10]. Moreover, AgNPs are also used as optical receptors, polarizing filters, and bio-labeling [11].

Most of the researchers focused on several plants and plant parts for the synthesis of nanoparticles and used for several applications [12–15]. Among all, *Phyllanthus amarus* is occurs naturally in tropical and subtropical countries [16]. It has been used in the treatment of kidney problems, gonorrhea, diabetes, jaundice and gastro-intestinal disorders [17]. The therapeutic properties of this herb are attributed to the presence of numerous phyto-constituents such as alkaloids, flavonoids, tannins, lignins, tetracyclic triterpenoids and polyphenolic compounds [17]. The use of environmentally benign materials for the synthesis of AgNPs has led to an upsurge in research for better controlling the shapes and sizes of these nanoparticles, which is important for various biomedical applications [18].

The antimicrobial study of AgNPs was performed to enhance the research on well-known activity due to the increase in new

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strains of resistant bacteria and fungi to most potent antibiotics [5,10,15]. The mechanism of the antimicrobial effect of AgNPs as proposed is due to the attachment of AgNPs on the surface of the cell membrane, thus disrupting permeability and respiration functions of the cell [19]. The superior antibacterial and antifungal properties of AgNPs mean that they are frequently present in coatings for surgical devices, dental composites and bone prostheses [20–22], and also in food containers, air/water filters and many other consumer products [23–25]. In addition, AgNPs are well known as redox catalysts, and promote electron transfer through a separate pathway with relatively low activation energy [26,27]. The production of silver nanoparticles is especially important for their many industrial catalytic applications. Here, we report the green synthesis of AgNPs using *P. amarus* leaf extract and their applications in antimicrobial activity against selected pathogens and as a catalyst of the degradation of the rhodamine B (Rh B) dye.

2. Experimental

2.1. Synthesis of bio-silver nanoparticles

First, *P. amarus* leaves were collected and cleaned with Milli-Q water and then dried in the dark, followed by being ground in a mixer. A mass of 2 g of this leaf powder was mixed with 100 ml of Milli-Q water and boiled for 10 min. The resulting solution was filtered through Whatman No. 1 filter paper and the acquired leaf extract was preserved at 4 °C. An AgNP colloid was prepared by adding 0.4 ml of leaf extract to 5 ml (0.001 M) of $AgNO_3$ (Sigma Aldrich, 99.8%), followed by stirring this mixture well at room temperature for 10–15 min. The colloids were also collected by varying the leaf extract volume as 0.8 ml, 1.2 ml, and 1.6 ml, and $AgNO_3$ concentration as 0.004 M, 0.007 M, and 0.01 M, respectively. The schematic synthesis procedure of bio-silver nanoparticles and their final products are depicted in Fig. 1. The obtained nanoparticles were purified by centrifuging the reaction mixtures at 10,000 rpm for 10 min, and the resulting pellets were re-dispersed in deionised water twice and subjected to further studies.

2.2. Antimicrobial assay

The bio-synthesized AgNPs (optimized at 0.007 M of $AgNO_3$ and 1.2 ml of leaf extract volume conditions) were made to adsorb on the Whatman No. 1 paper discs added at different AgNP volumes of 2, 4, 6, 8 and 10 µl and subjected to antimicrobial activity through Kirby-Bauer disc diffusion method against selected pathogenic organisms [28]. The antibacterial assessment of AgNPs was carried out by using *Escherichia coli*, *Pseudomonas* spp. (gram-negative), and *Bacillus* spp., *Staphylococcus* spp. (gram-positive) test pathogens. Similarly, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. were selected as a fungal test organisms for anti-fungal activity. The detail procedure for antimicrobial activity measurements were reported in our previous papers [26]. The selected test-pathogens were collected from the department of microbiology, S.V. University, India. The diameter of the zone of inhibition was measured with the meter ruler around each disk and the mean value of inhibition was calculated and expressed in millimeter.

2.3. Characterizations of the bio-AgNPs

The optical properties and catalytic activity of the bio-synthesized AgNPs were analyzed by using a UV–visible spectrophotometer (Mecasys Optizen POP). The morphology of the AgNPs was characterized by performing transmission electron microscopy (TEM, JEOL, JEM-2100F HR) and the purity of the sample was confirmed using energy dispersive X-ray spectroscopy (EDS) coupled with TEM. The crystalline nature of the nanoparticles was determined by using an X-ray diffractometer (XRD, Rigaku D/Max-2500 model).The stability of the nanoparticles was studied by using a zeta potential instrument (Zetasizer Nano series: ZEN3600). A typical Fourier transform infrared spectroscopy (FTIR) spectrum of bio-AgNPs was recorded with an ATR-FTIR Bruker Vertex-80 spectrometer. To demonstrate the catalytic activity of synthesized Ag colloid, the reduction of Rhodamine B by $NaBH_4$ is selected as a probe reaction. After the addition of Ag colloid the reduction is ascertained by monitoring the UV–Vis spectra.

3. Results and discussion

3.1. UV–Visible analysis

In general, AgNPs exhibit surface plasmon resonance at a wavelength of 421 nm [29]. The synthesis of AgNPs using *P. amarus* leaf extract was thus monitored by using UV–Vis spectroscopy; this technique was specifically used to study the effect of the $AgNO_3$ concentration and leaf extract volume on the formation of the AgNPs. As the concentration of $AgNO_3$ was increased from 0.001 M to 0.01 M, the absorption peak became red-shifted prior to increase of particle size along with increase of intensity (Fig. 2a). This intensity increase was due to more silver ions being reduced to silver nanoparticles. However, the SPR absorbance intensity was nearly independent for 0.01 M $AgNO_3$, indicating that the reaction was close to equilibrium. As the volume of the leaf extract was increased from 0.4 ml to 1.6 ml, the absorbance peak was observed to increase in intensity and to slightly blue-shift (Fig. 2b), due to the decrease of particle size [30]. The UV–Vis analysis indicated

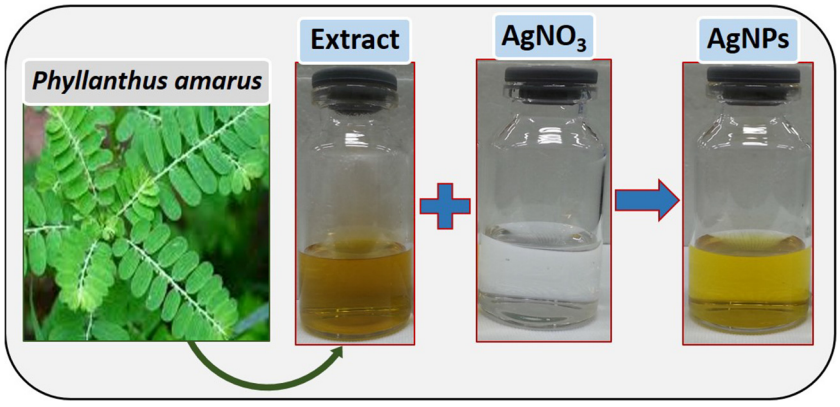

Fig. 1. Schematic synthesis procedure of bio-silver nanoparticles and their final products.

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