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Syzygium cumini Mediated Green Synthesis of Silver Nanoparticles for Reduction of 4-Nitrophenol and Assessment of Its Antibacterial Activity

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Abstract

The biosynthesis of silver nanoparticles (AgNPs) has become more significant in the recent years owing to its applications in catalysis, imaging, drug delivery, nano-device fabrication and in medicine. We propose the synthesis of silver nanoparticles from the plant extract of *Syzygium cumini* and evaluation of its antibacterial and chemocatalytic potential. Synthesis of AgNPs carried out by using aqueous silver nitrate. The UV-Vis absorption spectrum of the synthesized AgNPs showed a broad absorption peak at 470 nm. TEM analysis shows the morphology of AgNPs as a hexagonal matrix with average particle size is about 50 nm. XRD analysis displays the crystalline structure of AgNPs. The presence of elemental silver was confirmed with EDX analysis. FTIR analysis shows that amide groups present in proteins are dominant reducing agents and play an important role in the bioreduction of Ag^+ ions to Ag^0 . The bioreduced AgNPs demonstrated significant catalytic properties in a reduction reaction of 4-nitrophenol to 4-aminophenol using NaBH_4 in an aqueous condition. The biosynthesized AgNPs have potent antibacterial activity against common clinical pathogens. Considering the remarkable antibacterial activity against common pathogenic microorganisms, AgNPs can be used in the pharmaceutical industries.

Keywords: *Syzygium cumini*, Silver nanoparticles, Antibacterial activity, Catalytic reduction

1. Introduction

Nanobiotechnology is an economic alternative to chemical and physical methods for the synthesis of nanoparticles [1]. The nanoparticles are extensively used in cosmetics, tires, textiles, food industries and medicines [2]. Recently, nanotechnology

has gained significant attention due to its unique and different properties such as catalytic, electrical, optical, magnetic and thermal, which have wide varieties of applications [3].

Numerous approaches are in practice to generate AgNPs such as chemical, electrochemical, photochemical and radiation. The significance of NPs is recognized when researchers found that size can persuade the physico-chemical properties of a substance [4]. In the past few decades, tremendous awareness and extensive research efforts were intended toward the metallic nanoparticles derived from noble metals, such as silver and gold [5]. However, there is still a need to enhance and develop high yield, low cost, non-toxic and environmentally friendly procedures. Therefore, the biological loom for the synthesis of NPs becomes crucial.

Nanotechnology has focused much more attention in recent years in several research fields. Due to their advanced optical properties, metal NPs find applications in the areas of biological, chemical and electronic sciences [5, 6]. The AgNPs have a well-built report on antimicrobial, anti-inflammatory, anti-viral, anti-angiogenesis and anti-platelet activity [7, 8]. Currently, the green synthesis of AgNPs finding medical application in the area of continued significance [9]. The new drug synthesis from AgNPs can fight against cancer and kill pathogens like bacteria, fungi, and viruses [10]. There are diverse natural sources like plants, bacteria, yeast, and fungi used to synthesize Au and AgNPs [11, 12]. The use of leaf extract for nanoparticle synthesis is low-cost and eliminates the need for culture preparations and maintenance of aseptic conditions required for microorganisms [13]. At present, plant-mediated synthesis of nanoparticles is gaining more attention due to its simplicity, rapid rate of synthesis and eco-friendliness [14]. Diverse bio-molecules such as, carbohydrates, proteins and co-enzymes are existing in plant that reduce the metallic salt into nanoparticles [1]. The phytosynthesis of nanoparticles is advantageous over chemical synthesis concerning the adverse effect of harmful chemicals on the environment [15].

Syzygium cumini is a plant recognized for its antifungal, antioxidant, anti-inflammatory, hypolipidaemic, hypoglycaemic and pharmacological properties, due to the presence of bioactive compounds in various parts of the plant [16, 17]. The fruits of *Syzygium cumini* have various medicinal purposes and currently have a huge market for treating chronic diarrhea and other enteric infections [18]. However, the remedial outcome of medicinal plants has been consistently queried due to little bio-availability of the chief constituents subsequent to metabolic conversion in the liver [19]. Therefore, the use of nanoparticles confirmed to be a valuable substitute, as they are biodegradable, biocompatible and can allow the sustained release of specific drug [20].

The antimicrobial prospective of nanoparticles is pertinent in the massive area of biology and medicine to prevent infections in burns and open wounds [21, 22]. Therefore, this manuscript describes the antibacterial activity of nanoparticles against common human pathogenic bacteria like *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Klebsiella pneumonia*. Additionally, we revealed the catalytic potential of biosynthesized AgNPs in the reduction of 4-nitrophenol to 4-aminophenol. The reduction of 4-nitrophenol to 4-aminophenol by NaBH_4 is one of the leading model catalytic reaction since it allows easy and reliable assessment of catalysts using the kinetic parameters [23].

Herein, we report the simple, facile, rapid and efficient process for the synthesis of AgNPs using the aqueous leaf extract of *Syzygium cumini* and their applications in antibacterial activity and catalytic reduction of 4-nitrophenol.

2. Materials and methods

2.1 Preparation of plant extract

Syzygium cumini leaves were thoroughly washed in running tap water for 15 min and then shade dried for two days at room temperature. Dry leaves were ground into fine powder in a mortar and pestle. The extract obtained was filtered through Whatman filter paper No.1. The filtrate was collected and stored at 4°C, which was further used for all experiments [24].

2.2 Synthesis of AgNPs

The well-grinded material was mixed with 100 mL of double distilled water and then transferred in 500 mL Erlenmeyer flask followed by continuous stirring on the magnetic stirrer for 10 min. The content was centrifuged at 10,000 rpm for 10 min for the removal of cell debris. 50 mL of aqueous silver nitrate (1 mM) was added to 10 mL of the leaf extract with continuous stirring. A color change from colorless to yellowish-brown, visually confirms the formation of AgNPs [25].

2.3 Characterization

The resulting solution was then diluted by using double distilled water and characterized using UV-Visible spectroscopy, X-ray diffraction, Energy dispersion spectroscopy, FT-IR and Transmission electron microscopy [26].

2.3.1 UV-visible spectroscopy

Silver nanoparticles were characterized by using Systronics UV-Vis spectrophotometer. The bio-reduction absorption spectra were monitored in 300–700 nm range.

2.3.2 X-ray diffraction spectroscopy

The biosynthesized AgNPs using *Syzygium cumini* leaf extract were lyophilized to a powder. The powdered or dried AgNPs were coated on the XRD grid, and the spectra were recorded using Rich Seifert p 300 instruments.

2.3.3 Transmission electron microscopy and energy dispersive spectroscopy

In order to know the morphology of the biosynthesized AgNPs, transmission electron microscopy (TEM) studies were carried out. The size and shape of the AgNPs were recorded by using the FEI (Netherland) model TECNAI-G2U twin operated at an accelerating voltage of 200 KV. EDS analysis was carried out at the same time by the EDS compatible with TEM.

2.3.4 Fourier transform infrared spectroscopy (FTIR) analysis

After the biosynthesis, AgNPs were centrifuged for 15 min at 10,000 rpm. The obtained pellet was re-dispersed in double distilled water to ensure the removal of any uncoordinated bio-molecules. In order to obtain the better separation of nanoparticles, the process of centrifugation was repeated twice. The purified pellet

was then subjected to FTIR analysis (Shimadzu IR). AgNPs were mixed with KBr and subjected to IR source 500–4000 cm^{-1} .

2.4 Catalytic reduction of 4-nitrophenol

The catalytic reaction was studied as mentioned by Ghosh et al. with slight modification. Briefly in standard quartz cuvette 1 mL of 0.1 mM aqueous NaBH_4 solution mixed with 1.5 mL of 4-nitrophenol aqueous solution (0.25 mM). 100 μL of an aqueous suspension of AgNPs of *Syzygium cumini* (in double-distilled water) was added into the same and time-dependent absorption spectra were recorded every 5 min in the range of 260–520 nm at 28°C [27].

2.5 Antimicrobial activity

The antimicrobial activity of the biosynthesized AgNPs was tested against pathogenic bacteria such as *Serratia marcescens* (NCIM 2078), *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 5029), *Salmonella typhimurium* (NCIM 2501) and *Klebsiella pneumonia* (NCIM 2957), etc. The organisms were collected from National Chemical Laboratory (NCL), Pune. Uniform spreading of bacterial cultures was carried out in the individual plates using a sterile glass spreader. Wells were made on the agar plates using a cork borer to about 10 mm diameter in nutrient agar medium. 100 μg of lyophilized AgNPs were added in 100 μL of distilled water. 50 μL dispersed solution was added to the well. The diameters of the inhibition zone surrounding the wells were measured in millimeters after 24 h. The antimicrobial effect of the biosynthesized AgNPs is directly proportional to the size of the spherical inhibition zone against microbial pathogens [28–30].

3. Result and discussion

3.1 Biosynthesis of AgNPs

The reduction of Ag^+ to Ag^0 NPs was carried out by using aqueous leaf extract of *Syzygium cumini*. The color change of the solution observed from colorless to yellowish-brown indicates that the synthesis of AgNPs shown in **(Figure 1(i))**. The UV–Vis absorption spectrum of the biosynthesized AgNPs demonstrated a characteristic absorption peak at 470 nm, which is a typical band for the silver shown in **Figure 1(ii)**. No other peak was observed in the spectrum, which confirmed that silver only [31]. The formation of AgNPs was further confirmed by using X-ray diffraction (XRD), FT-IR, EDS and transmission electron microscopy (TEM) analysis.

3.2 Characterization

3.2.1 Transmission electron microscopy analysis

The TEM image of the AgNPs is shown in **Figure 2**. TEM has been used to describe the size, shape and morphology of the biosynthesized AgNPs. From the figures, it is observed that the morphology of AgNPs is a hexagonal matrix. **Figure 2** shows the average particle size measured from the TEM image is 50 nm, which are in good agreement with the particle size calculated from XRD analysis.

Figure 1.
(i) Color change observed (a) before and (b) after formation of AgNPs. (ii) UV-visible spectra of the synthesized AgNPs.

Figure 2.
TEM image of AgNPs.

3.2.2 X-ray diffraction analysis

The presence of Ag crystal in the sample was confirmed by using an X-ray diffractometer. In XRD pattern, the Bragg reflections were observed at 2θ value 38.0° , 44.8° , 47.5° , 64.6° and 78.0° confirm the presence of AgNPs (**Figure 3**). A strong diffraction peak located at 38.0° was ascribed to the (111) facets of Ag. The XRD pattern thus clearly indicated that the AgNPs are in crystalline form. No impurities were observed in the XRD pattern.

Figure 3.
XRD pattern of synthesized AgNPs.

3.2.3 Energy dispersive spectroscopy analysis

EDS analysis of synthesized particles showed the presence of elemental silver, which correlates with XRD analysis (**Figure 4**). We identified the signal energy peaks for hexagonal-shaped AgNPs produced by using *Syzygium cumini* leaf extract. The NPs showed the prominent silver energy emission peak in a range of 2–4 keV in the spectrum.

Figure 4.
(a) SAED pattern (b) EDS analysis of AgNPs.

3.2.4 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR absorption spectra of AgNPs are shown in (Figure 5). The different possible functional groups at various positions will be determined by using FTIR analysis. The band at 1559 cm^{-1} indicates the presence of amide group [32] arises due to carbonyl stretch in proteins. It can be stated from FTIR analysis. The band at 1610 cm^{-1} is attributed to the stretching vibration of (NH) C=O group. That amide groups present in carbohydrates, proteins are dominant reducing agents and play an important role in the bio-reduction of Ag^+ ions to Ag^0 leads to nanoparticles synthesis.

3.3 Catalytic reduction of 4-nitrophenol

In order to study the efficiency of bio-synthesized AgNPs, the catalytic reduction of 4-nitrophenol was carried out in an aqueous medium by using NaBH_4 as a reductant at room temperature [33–35]. The 4-nitrophenol (0.1 mM) shows an absorption peak at 400 nm in the visible region with NaBH_4 (Figure 6). In a control experiment, it can be concluded that the reduction does not occur in the absence of AgNPs, even after the addition of excess NaBH_4 . After the addition of AgNPs, the gradual decrease in intensity of the absorption peak at 320 nm was observed due to the formation of 4-aminophenol. The complete reduction of p-nitrophenol was also supported by a change in color from yellow to colorless.

3.4 Antibacterial activity

Antibacterial activity of biosynthesized AgNPs was investigated against human pathogens. The biosynthesized AgNPs showed a high inhibitory effect on bacteria, and it may serve as an option for decreasing bacterial infections [36]. The zone of inhibition was found to be as per Table 1.

Figure 5.
FTIR image of AgNPs.

Figure 6.
The UV-vis spectrum of 4-nitrophenol reduction catalyzed by AgNPs using NaBH₄.

Sr. No	Name of organism	Diameter of zone of inhibition (mm)
1.	<i>Pseudomonas aeruginosa</i> NCIM 5029	14
2.	<i>Serratia marcescens</i> NCIM 2078	18
3.	<i>Staphylococcus aureus</i> NCIM 5021	22
4.	<i>Salmonella typhimurium</i> NCIM 2501	16
5.	<i>Klebsiella pneumonia</i> NCIM 2957	12

Table 1.
Antibacterial activity of *Syzygium cumini* AgNPs measured as the zone of inhibition in (mm).

4. Conclusion

The *Syzygium cumini* leaf extract reduced Ag⁺ metal ions and led to the formation of AgNPs with fairly well-defined dimensions. This green approach for the synthesis of AgNPs has many advantages, such as the simplicity with which the process can be commercialized. The spherical AgNPs with unusual shapes like nano prism, hexagons and trapezoids were synthesized. The synthesized AgNPs showed excellent catalytic properties in a reduction reaction of 4-nitrophenol to 4-aminophenol by NaBH₄ in the aqueous phase. Thus, this rapid, eco-friendly and economical route can be used to synthesize AgNPs with wide biotechnological and chemical applications. The anti-microbial screening demonstrated that the synthesized AgNPs had a high inhibitory effect on bacteria. These observations may serve as a guide for studying the controlled release of AgNPs, in the field of controlling infectious diseases.

Conflict of interest

The authors of this have declared there is no conflict of interest.

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