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Green Synthesis and Characterization of Gold Nanoparticles for Antibacterial and Antifungal Activities Using Leaf Extracts of Annona Muricata

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Abstract

The necessity of disclosing the expeditious synthesis, non-hazardous nature, cost-effectiveness, and environmentally friendly approaches for synthesizing nanoparticles using plants is highly significant. The observed phenomenon can be attributed to the elevated toxicity levels inherent in the chemical approach employed for nanoparticle synthesis. The use of plants for synthesizing metal nanoparticles has garnered significant interest among researchers, owing to the distinctive characteristics of these nanoparticles and their wide-ranging applications across several sectors. This paper aimed to assess the efficacy of the synthesized Gold Nanoparticle (GNP) against certain human diseases. The synthesis of GNP has been accomplished by the reaction between a gold chloride solution and a leaf extract derived from Annona muricata. The GNP was characterized using a Transmission Electron Microscope (TEM) and Fourier Transformed Infrared spectroscopy (FTIR). The morphology, size, and structural characteristics of the GNP that have been synthesized are assessed using TEM analysis. The research revealed that the nanoparticles exhibited a spherical shape and were uniformly distributed, with a mean particle dimension of 26.5 nm. The FTIR analysis demonstrates that peaks within the mid-range wavenumbers, namely in the region of 1700-1300 1/cm, indicate the potential existence of organic molecules such as phenols, aliphatic amines, and carboxylic acids. These functional groups play a crucial role in the capping and stabilizing processes of the GNP that were produced. The synthesized GNP with sustainability were assessed for their antibacterial and antifungal properties. The results demonstrated a modest level of antibacterial activity and antifungal activity. The leaf extract of Annona muricata, rather than being discarded, may be effectively utilized to produce GNPs. These nanoparticles can serve as a natural source of antibacterial and antifungal agents.

Keywords: *Green synthesis, Gold nanoparticles, antibacterial, antifungal, Annona muricata.*

Introduction

Nanotechnology is a scientific discipline that has garnered significant attention for its rapid growth and diverse applications in several fields, including pharmaceuticals, manufacturing, environmental science, electronics, energy, and mechanical engineering. The significance of nanoparticles stems from their size, typically within the 1 to 100 nm range. This characteristic has garnered significant interest in utilizing nanoparticles across numerous disciplines. At this scale, materials display exceptional qualities that surpass those of other substances due to their size and shape-dependent characteristics.

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Nanoparticles are widely recognized for having a high surface area to volume ratio, resulting in increased surface energy [1]. The current focus of research in nanotechnology revolves around the pursuit of more environmentally friendly methods for producing nanoparticles. The utilization of this technology has several advantages in terms of safety, speed, ease of use, cost-effectiveness, non-toxicity, and environmental friendliness, in contrast to conventional approaches that rely on the use of a greater quantity of very toxic chemicals, which pose risks to human health [2].

The green chemistry strategy often uses natural sources, such as plants, fruit wastes, and microbial organisms [3]. Plant extracts are commonly employed in manufacturing Metal Nanoparticles (MNPs) due to their significant utility. The reducing properties of this substance can be attributed to its bioactive constituents, including alkaloids, phenols, flavonoids, proteins, and vitamins. These materials possess antioxidants that possess reducing characteristics, primarily responsible for reducing metals into their corresponding nanoparticles. Additionally, they serve as capping agents to ensure the stability of these MNPs.

Furthermore, it has been suggested that utilizing these materials might facilitate the integration of MNPs into the human body, minimizing the risk of immune system recognition and subsequent assault [4]. Numerous scholars have conducted investigations on the production of diverse noble MNPs, including gold (Au), silver (Ag), platinum (Pt), and palladium (Pd), utilizing natural sources derived from plant seeds, peels, leaves, and root extracts. These studies have provided evidence to support the notion that noble MNPs play a substantial role in numerous domains, including medicine, industry, and the environment.

Due to the emergence of MNPs, there has been a significant focus on the potential of Silver Nanoparticles (AgNPs) due to their antimicrobial and anti-inflammatory capabilities. The optical characteristics of AgNPs have rendered them valuable for investigating many applications in medicines, cosmetics, medical devices, and textile industries. In recent studies, researchers have investigated the potential of AgNPs to selectively target cancer cells while minimizing harm to healthy cells [5]. In addition to platinum (Pt), palladium (Pd), and other metal nanoparticles, GNPs (AuNPs) have made significant contributions to the field of nanotechnology. Homogeneous or heterogeneous catalysts are commonly employed in catalytic applications. Furthermore, several applications have been conducted on these nanoparticles in medicine and industry, as well as in developing hydrogen sensors and storage systems. The user has provided a numerical reference, indicating they may refer to a specific source.

GNPs (AuNPs) have garnered significant interest among the scientific community due to their exceptional physicochemical and optical characteristics. These attributes have found widespread applications across several domains of human endeavor. AuNPs have a high sensitivity, stability, reliability, and comparatively lower toxicity compared to other types of MNPs. One crucial characteristic of AuNPs is their ability to interact harmoniously with living beings, rendering them highly valuable in several biological domains such as bio-imaging, medication transport, diagnostics, and other related applications. GNPs have demonstrated the ability to exhibit antibacterial, antioxidant, and catalytic properties. They are derived from natural sources and have effectively treated several cancer cell lines [7].

The utilization of Annona muricata leaf extracts in the green synthesis of GNP presents a viable and ecologically conscious method for the production of nanoparticles. This technique has promise for many applications in antibacterial and antifungal therapy. The present study emphasizes the need to employ green synthesis techniques and stresses the significance of investigating natural resources as potential sources for innovative nanomaterials with favorable biological characteristics.

Literature Survey

Due to its low ecological impact and potential use in many fields, "green synthesis," producing nanoparticles using environmentally friendly methods, has garnered attention. This study examines the green synthesis of gold nanoparticles (AuNPs) using leaf extracts from Annona muricata, also known as soursop or Graviola. This study also investigates nanoparticles' antibacterial and antifungal properties.

Khan et al. (2019) summarize and analyze a decade of plant-based gold nanoparticle research [8]. This process involves reviewing the literature, collecting data, and integrating key findings. The output values explain plant-derived gold nanoparticles' many methods, processes, and applications. The advantage of this approach is its comprehensive view of this growing field, which helps identify patterns and knowledge gaps. However, relying on previously published research, which may vary in quality and consistency, is a significant drawback.

Sunderam et al. (2019) combined Anacardium occidentale leaf extract with gold nanoparticle production to create an environmentally friendly method. The experiment involved extracting bioactive chemicals from leaves and reducing gold ions to make nanoparticles [9]. After production, the nanoparticles were characterized using various methods. The antimicrobial and anticancer properties were tested in vitro. The results included nanoparticle size, shape, stability, and antibacterial and anticancer properties. The use of natural botanical extracts in nanoparticle production may reduce environmental impact. However, plant extract heterogeneity may be a drawback.

Khandanlou et al. (2018) synthesized and characterized gold-conjugated Backhousia citriodora nanoparticles [10]. The study synthesized and characterized nanoparticles using TEM and XRD. The anticancer effects were tested on MCF-7 breast and HepG2 liver cancer cell lines. The results provided detailed information on the nanoparticles' physicochemical properties and potential use as cancer treatments. The synthesis of nanoparticles using a botanical source has many advantages. However, process scalability and toxicity issues may be issues.

Al-Radadi (2021) developed a green biosynthesis method for flaxseed-based gold nanoparticles (Au-NPs). The process involved extracting beneficial chemicals from flaxseed and reducing gold ions to form nanoparticles. Anticancer efficacy against breast cancer cells was assessed [11]. The output values included nanoparticle characteristics and cancer-fighting potential. The use of plant-derived sources may have health benefits. It is important to note that this approach may have drawbacks, such as extract composition changes.

Donga et al. (2020) synthesize gold nanoparticles from Mangifera indica seed aqueous extract [12]. The study characterized nanoparticles and assessed their antibacterial, antioxidant, and anticancer properties. The output values included nanoparticle characteristics and potential multifunctional uses. Fruit seed extracts have many benefits, but their inherent variability may limit their use.

Folorunso et al. (2019) used Annona muricata leaf extracts to make gold nanoparticles [13]. The implementation process included nanoparticle characterization and antibacterial efficacy testing. The output values contained nanoparticle characteristics and antimicrobial activity. The use of a therapeutic botanical source has many benefits. However, some constraints must be considered, such as the variability in extracted substance composition and the challenges of scaling up production.

The studies in this review emphasize the need for eco-friendly nanoparticle synthesis. These methods use bioactive, chemical-rich natural sources and reduce environmental impact. The methods under consideration involve extracting phytochemicals from Annona muricata leaves to reduce and stabilize gold nanoparticles. GNPs are characterized using various analytical methods to determine their dimensions, morphology, and robustness. The study explores the antibacterial and antifungal properties of GNPs.

Green Synthesis and Characterization of Gold Nanoparticles Using Leaf Extracts of Annona Muricata

The production of nanoparticles using environmentally friendly and sustainable techniques, commonly known as "green synthesis," has garnered considerable interest owing to its diminished ecological footprint and potential utility across diverse disciplines. The primary objective of this investigation is to examine the green fabrication process of GNP through the utilization of leaf extracts derived from Annona muricata, a plant commonly referred to as soursop or Graviola. Additionally, this study aims to investigate the potential antimicrobial attributes associated with the aforementioned synthesized nanoparticles.

Materials and Method

The leaves of the Annona muricata species were gathered from the grounds of the botanical department at Obafemi Awolowo University, Ile-Ife, Nigeria, and afterward underwent authentication. The leaves were thoroughly washed, followed by air drying, and were crushed using a blender grinder. Fig. 1 displays the dried and powdered leaves of Annona muricata. A total of 15 grams of crushed leaves from the Annona muricata plant were carefully placed into a 150-millilitre Erlenmeyer flask. Subsequently, 100 milliliters of de-ionized water were added to the flask, and the mixture was subjected to boiling for 15 minutes. The plant extract subjected to boiling was subsequently cooled to ambient temperature and underwent filtration. As mentioned above, the extract was subsequently employed in the production of GNP.

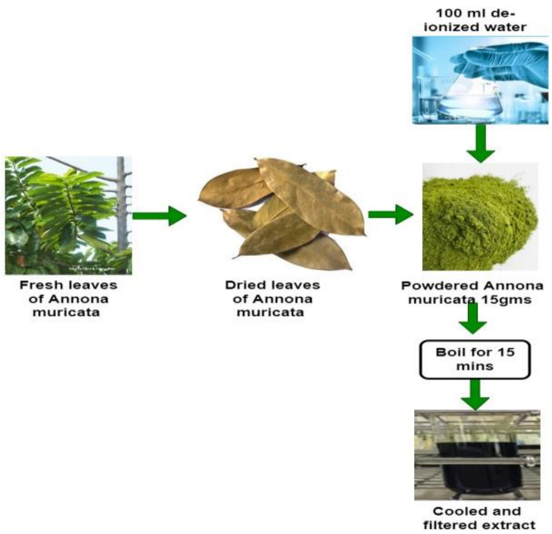


Figure 1: Process of obtaining Annona muricata extract for green synthesis of GNP.

Green Synthesis of GNP

The GNP was synthesized using the method described in reference [14]. A volume of 1.5 milliliters of the leaf extract was introduced into a solution containing gold chloride with a concentration of 1.5 millimolars. The resulting mixture was thoroughly agitated at ambient temperature until the characteristic yellow hue of the extract transitioned to a purple tint, validating the successful synthesis of GNP. The solution was subjected to an incubation period of 21 hours to achieve complete bio-reduction of gold chloride into GNP using a leaf extract. Converting gold chloride into GNP was seen using a UV–vis spectrometer. The absorption spectra in the UV–Vis range were measured at different time intervals to track the progress of the reduction reaction. UV-visible spectroscopy is a widely used analytical technique that involves the measurement of the absorption of UV and visible light.

UV–visible Spectroscopy

The UV-Vis spectrometer (UV-245 Shimadzu) was employed to determine the optimal concentration of plant extracts by measuring at different time intervals. The presence of the purple color indicated the successful production of the GNP. The confirmation of the production of the GNP was achieved by scanning the absorption maximum of a recently created sample of GNP. This scanning process was conducted using quartz cuvettes with a path length of 1 cm, within a temperature range of 25-29 °C, and throughout a wavelength range of 445-845 nm.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR study used the Nicolet iS50 FTIR spectrometer manufactured by Thermo Fisher Scientific in Waltham, MA, USA. The freeze-dried extract of gold nanoparticles was combined with KBR (FTIR grade) and subjected to scanning using FTIR (Fourier Transform Infrared) in the spectral range of 4100–400 cm^{-1} with a resolution of 10 cm^{-1}. FTIR analysis was employed to identify the functional groups present and investigate the interactions between these functional groups as a potential source of reducing agents on the surface of the produced nanoparticles.

Transmission Electron Microscopy (TEM)

TEM is a powerful imaging technique that utilizes a beam of electrons to visualize a specimen's internal structure and morphology at high resolution. The gold nanoparticles' size and surface morphology were assessed using a JEOL model 1200EX TEM operating at an accelerating voltage of 80 kilovolts. The GNP samples were generated by depositing a small volume of the nanoparticle solution onto Lacey carbon grids with a mesh size of 350. The grids were then let to dry before measurement.

Antibacterial Properties of GNP Produced Using Green Synthesis

This study aims to investigate the antibacterial properties of GNP. The study involved examining the antimicrobial efficacy of the manufactured gold nanoparticles, specifically against certain bacteria strains isolated from clinical samples. This experiment followed the established well diffusion protocols outlined in reference [16]. The recently generated cultures, incubated overnight, were evenly distributed onto sterilized plates containing nutrient agar. The agar plates were perforated using a sterile cork borer to create wells with a diameter of 9 mm. The wells were filled with 45 μl of a gold nanoparticle solution at 2.5 mg/l and 3.5 mg/l concentrations. A positive control consisting of an antibiotic (Streptomycin) at a dose of 2.5 mg/l was included. The plates were incubated at a temperature of 38 °C for 27 hours. The measurement of the inhibition zones surrounding the well-containing gold nanoparticles and the calculation of the percentage zones of inhibition were conducted to assess the antibacterial efficacy of the manufactured gold nanoparticles. The antimicrobial study used triplicate analyses. The formula mentioned below was employed to compute the percentage growth inhibition.

The formula for calculating the percent growth inhibition (PGI) is as follows:

$$PGI = \frac{BDC - BDT}{BDC} * 100 \qquad (1)$$

In this formula, PGI represents the percentage of growth inhibition, BDC represents the diameter of bacteria colonies in the control group, and BDT represents the diameter of bacteria colonies in the treatment group.

Antifungal Properties of GNP Produced Using Green Synthesis

The antifungal efficacy of the gold nanoparticle generated from Annona muricata was investigated using the methodologies outlined in the study by [17]. Sterile Sabouraud Dextrose Agar (SDA) was aseptically put onto a sterile Petri plate. Following the preparation of the medium, a sterile gel puncture technique was employed to create wells with a diameter of 8 mm in the agar plates. A total volume of 45 microliters containing gold nanoparticles at concentrations of 2.5 mg/l and 3.5 mg/l was added to the wells. The fungal discs in each well were injected in an inverted posture. Subsequently, the plates were incubated at a temperature of 27 °C for a duration ranging from 75 to 95 hours. Amphotericin B was employed as the experimental control in the study. The fungal colony's diameter was measured subsequent to its incubation at a temperature of 27 °C. The percentage growth inhibition was determined by comparing the fungal diameter in the experimental condition to that of the control. The antifungal investigation was conducted using triplicate analyses. The following formula was employed to compute the PGI:

$$PGI = \frac{FDC-FDT}{FDC} * 100 \quad (2)$$

FDC denotes the fungal colony diameter in the control group, and FDT represents the fungal colony diameter in the treatment group.

Results and Discussion

The dimension, structure, and dispersion of produced GNP were determined using a TEM. The TEM image of the GNP obtained from Annona muricata leaves reveals the existence of uniformly shaped nanoparticles with a spherical morphology. The particle size distribution ranges from 28 to 33 nm, with a mean nanoparticle size of 26.5 nm, as seen in Fig. 2.

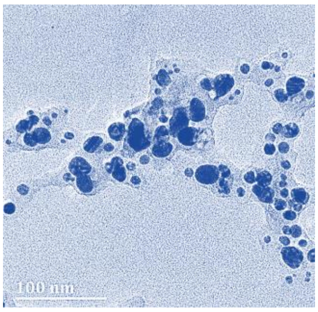


Figure 2: TEM micrograph of GNP synthesized using Annona muricata

The FTIR spectrum (shown in Fig. 3) obtained from the green production and characterization of AuNPs utilizing leaf extracts derived from Annona muricata offers significant insights into the chemical composition and functional groups implicated in the synthesis procedure. The spectrum exhibits discrete peaks at different wavenumbers, with each peak corresponding to individual chemical vibrations. At the outset, within the range of higher wavenumbers (e.g., 4000-3800 1/cm), there is a notable prevalence of transmittance, signifying a limited degree of absorption of infrared light. As the wavenumbers decline, there is a noticeable decrease in transmittance, suggesting an enhanced absorption by the functional groups found in the leaf extract.

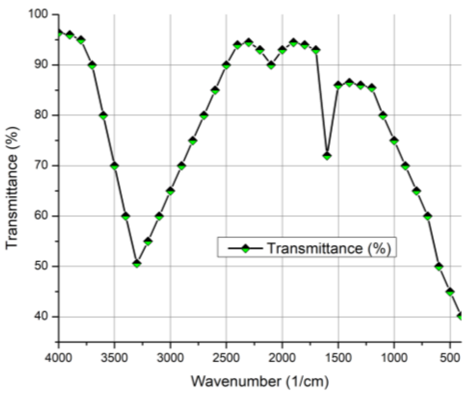
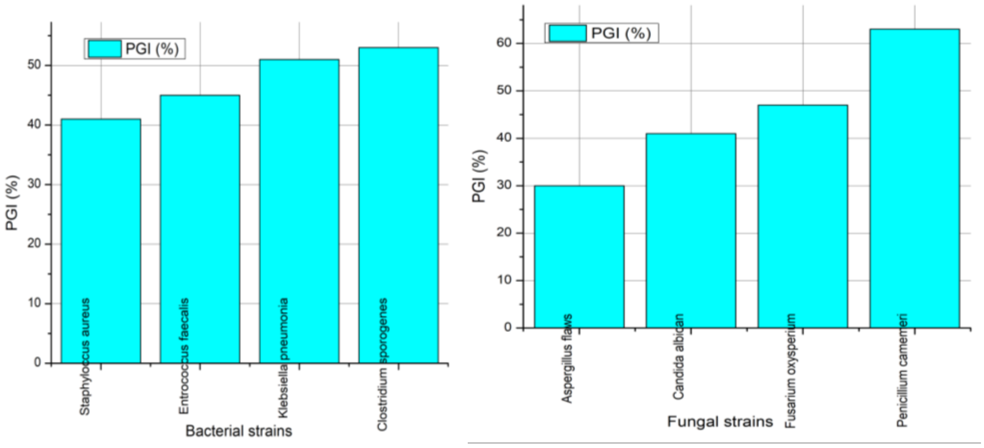


Figure 3: FTIR spectrum for green synthesis and characterization of gold nanoparticles using leaf extracts of Annona muricata

It is worth mentioning that the occurrence of peaks within the mid-range wavenumbers, namely in the region of 1700-1300 1/cm, indicates the potential existence of organic molecules such as phenols, aliphatic amines, and carboxylic acids. The aforementioned functional groups are thought to have a significant impact on the reduction and stability of gold ions in the process of synthesizing nanoparticles. The FTIR spectrum offers significant information about the participation of distinct biomolecules in the green synthesis process. This data contributes to an enhanced comprehension of the underlying processes in creating AuNPs mediated by Annona muricata.



(a) (b)

Figure 4: Antibacterial and antifungal activities of GNP produced using green synthesis

Fig. 4 shows gold nanoparticles' antibacterial and antifungal activities produced using green synthesis. It displays the PGI values of GNP that were synthesized by a green synthesis method using leaf extract of Annona muricata. These values represent the effectiveness of the GNP against different strains of bacteria and fungi. The PGI values measure the inhibitory effect of the generated GNPs on the development of the microorganisms. It is worth mentioning that the GNPs demonstrate noteworthy antibacterial properties, as indicated by the PGI values that range from 41% to 53% against various bacterial strains such as Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, and Clostridium sporogenes (Fig. 4a). Furthermore, these substances also exhibit notable antifungal effectiveness, as seen by PGI values that span from 30% to 63% when tested against several fungal species including Aspergillus flavus, Candida albicans, Fusarium oxysporum, and Penicillium camemberti (Fig. 4b). The results of this study emphasize the potential of green-synthesized GNPs as very efficient antibacterial and antifungal agents. These findings demonstrate the adaptability of GNPs in effectively combating a wide range of bacterial and fungal diseases.

Conclusion

The primary aim of this study was to evaluate the effectiveness of the produced GNP in combating specific human disorders. The production of GNP has been successfully achieved by the chemical interaction between a solution of gold chloride and a leaf extract obtained from Annona muricata. The GNP was subjected to characterization utilizing a TEM and FTIR. The assessment of the shape, size, and structural features of the produced GNP is conducted by the utilization of TEM analysis. The study's findings indicate that the nanoparticles displayed a spherical morphology and were evenly dispersed, with an average particle size of 26.5 nm. The FTIR study reveals that peaks in the intermediate wavenumber range, namely between 1700-1300 1/cm, suggest the possible presence of organic compounds such as phenols, aliphatic amines, and carboxylic acids. The aforementioned functional groups are of utmost importance in the capping and stabilizing mechanisms employed during the production of GNP. The antibacterial and antifungal activities of the synthesized GNP with sustainability were evaluated. The findings exhibited a moderate degree of antibacterial and antifungal efficacy.

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