

Synthesis, Characterization, and Antibacterial Activity of Biosynthesized Gold Nanoparticles

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Abstract: In this study, *Acalypha indica* was utilized to green synthesize gold nanoparticles. The characteristics of the synthesized nanoparticles were observed through UV-Vis, FTIR, TEM, particle size analyzer, and XRD. Furthermore, the nanoparticles were investigated for antibacterial properties. The particle size of gold nanoparticle was around 50 – 100 nm, and the antibacterial property of the nanoparticle was assessed using agar well diffusion, swarming motility, MIC, and protein leakage assay. The gold nanoparticles were observed to be active against *E. coli* alone with MIC at 160 µg, and it was observed to inhibit its swarming motility and to make the cell leak out proteins.

Keywords: *Acalypha indica*; gold nanoparticles; antibacterial activity; protein leakage assay.

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1. Introduction

Nanobiotechnology can be defined as the manipulation of biological living systems at the molecular size between 1 and 100 nm. These nanoparticles have been well exploited in the field of biomedical engineering, biotechnology, pharmaceutical industries, environment, etc. [1-3]. Metal-based nanoparticles are used more these days as they possess various bioactivities [4, 5]. Nanomaterials possess unique properties that have drawn major attention in terms of pharmaceutical applications [6]. However, there are certain disadvantages involved in physiochemical methods that include the usage of noxious chemicals, time consumption, stability, production of hazardous products, and large-sized and aggregated products [7]. Hence, these impediments have called for the advancement of an environmental-friendly, less consumption of energy and green production approach using biological systems (microbes, algae, plants, and plant-derived products) [8, 9]. This environmental and green synthesis approach does not involve the usage of hazardous chemicals as opposed to chemical procedures that make use of hazardous reagents in its practice [10].

Amongst all, the stability of gold nanoparticles is high in comparison with the other metal nanoparticles, which increased their application in photo thermolysis, drug delivery, cancer diagnosis, and treatment [11, 12]. Moreover, their biocompatibility and a strong interface with soft bases such as thiols have increased its use in various bioactivity studies [13]. On most occasions, these gold nanoparticles are produced by the chemical method that makes use of aggressive chemicals like sodium borohydride, hydrazinium hydroxide, cetyltrimethylammonium bromide, but despite their advantage, they possess toxicity [14]. A remarkable emanating recent approach of green synthesis of biogenic nanoparticles from biological sources played a role in cancer due to their attributes dealt with cost efficiency and less toxic in nature [15]. Barabadi *et al.* [16] stated that the biometal nanoparticles synthesized by *Penicillium* sp had a pharmaceutical potential and were investigated for antimicrobial efficacy, which explored the applications of NPs.

The gold nanoparticles produced utilizing various bacteria, fungi [17], and plant extracts that make them useful in most biomedical fields [18]. Khatua *et al.* [19] stated that *Pongamia pinnata* leaf extract was a potential reducing and stabilizing agent in the formation of colloidal AuNPs which fight against fungal species. There was a higher scolicidal activity observed in nanoparticles that were obtained from the solvent aerial extract of *Penicillium aculeatum* that was evaluated against the protoscolices of CHD *in-vitro* [20]. Bio-production of gold nanoparticles from *Terminalia catappa* [21], tea [22] lemongrass [23] has been investigated as well. Lee *et al.* [24] described that numerous surface functionalities of AuNPs permit them to be more vigorous when joined with various biological aggregations or mitigation for improved applications. Keijok *et al.* [25] demonstrated that the *Coffea arabica* plant extract proved as a reducing agent, stabilizer, and functionalizer for the synthesized gold nanoparticles. A research study on *Salix alba* leaf extract was a good bio-reductant for gold nanoparticles synthesis and had prospective for various pharmaceutical applications[26]. Besides, another research revealed that the gold nanoparticles (AuNPs) produced from *Scutellaria barbata* have promising anticancer activity against pancreatic cancer cell lines (PANC-1)[27]. Wongyai *et al.* [28] observed that the gold nanoparticles synthesized from *Cryptolepis buchanani* was profoundly crystalline and monodispersed spherical AuNPs.

Furthermore, green nanoparticles may serve as a novel anticancer agent that could target cancerous cells [29]. According to Saravanan *et al.* [30], nanomedicine developed could be used for HIV treatment by interfering with HIV life cycle. Recently, BMNPs have been appraised as a possible approach to provide resistance against vectors of malaria that could lead to treating malaria disease [31]. Even though metal nanoparticles provide more promising applications, several causes for concern have been appraised in regards to the safety and impact of the nanomaterials for human use. This review had also mentioned the risk of carcinogenesis that may occur as a result of exposure to nanomaterials [32]. Honary *et al.* [33] recommended that silver nanoparticles synthesized by *P. citrinum* could be suitable in emerging a biological process. Further research carried out by Barabadi *et al.* [34] showed the development of a mycosynthesized silver nanoparticle using *P.citrinum* by response surface methodology. Another research study reported by a team of researchers involved the biological synthesis of extracellular iron oxide nanoparticles using *P.waksmanni* isolated from soil [35].

Acalypha indica, which belongs to the family of *Euphorbiaceae*, is commonly seen in tropical Africa, India, Sri Lanka, etc. This plant has been said to exhibit antimicrobial action against bacteria [36]. Very few studies have been reported in utilizing *Acalypha indica* for gold synthesis, and therefore, using this plant might give versatile gold nanoparticles. Hence, in this

current study, leaves of *Acalypha indica* was utilized to produce gold nanoparticles, followed by characterization using UV-Vis spectroscopy, FTIR, XRD, Particle size analyzer, TEM, and EDX. Subsequently, synthesized gold nanoparticles were studied for its antimicrobial property against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus*.

2. Materials and Methods

2.1. Synthesis of gold nanoparticles.

2.1.1. Collection and identification of plant material.

The plant material was obtained from Karunyanagar, Coimbatore, Tamil Nadu, India. The plant material was recognized as *Acalypha indica* by Dr. A. Annamalai, Associate Professor, Department of Biotechnology, Karunya Institute of Technology and Sciences.

2.1.2. Preparation of plant extract.

Plant leaves were collected, cleaned first in tap water, and then later by distilled water. Leaves were finely chopped and weighed 10 g of leaves was heated with 100 mL MilliQ water at 55 °C for 1 h. The obtained mixture was then filtered using Whatman filter.

2.1.3. Synthesis of gold nanoparticles.

75 mL (15 %) of plant extract was made into 500 mL by adding MilliQ water and added with 0.19 g chloroauric acid. The solution was kept undisturbed at room temperature in dark conditions. It was noted for change of color after 20h. The formation of gold nanoparticles was observed by subjecting the solution to UV-vis spectra at 20th h and 40th h.

2.2. Characterization of gold nanoparticles.

FTIR analysis was carried out for the particle as well as the extract to find the role of phytoconstituents in gold nanoparticle formation. UV-Vis and Particle size analyses were done using UV-2910 Hitachi spectrophotometer and particle size analyzer (Malvern Zetasizer Nanosizer), respectively. The particles were also subjected to TEM (Transmission electron microscopy) and XRD (X-ray diffraction) studies.

2.3. Evaluation of antimicrobial activity.

The obtained gold nanoparticles were evaluated for antibacterial property against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* by agar well diffusion method [37]. MIC and Swarming motility against the sensitive organisms was done using the method of Pal *et al.* [38] and Samrot *et al.* [4], respectively. Protein Leakage [4, 39, 40] was also performed for the gold nanoparticles against the bacteria, which showed sensitivity to the particle.

3. Results and Discussion

Plant extracts possess a high amount of carbohydrates and protein biomolecules that exhibit reducing agent properties, which prompt the development of metal nanoparticles [41].

Furthermore, the proteins associated with a functional cluster of amino groups ($-\text{NH}_2$) are present in plant extracts and can take part as a vital role in the reduction of metal ions [42]. The presence of active groups (such as $-\text{C}-\text{O}-\text{C}-$, $-\text{C}-\text{O}-$, $-\text{C}=\text{C}-$, and $-\text{C}=\text{O}-$) in phytochemical compounds such as flavones, alkaloids, phenols, and anthracenes can assist in the promotion of metal nanoparticles. This method is considered as one of the simplest approaches as they do not call for an aseptic environment. The reaction (economic) can be easily initiated through interaction with the starting material that does not require high temperature or pressure—moreover, *Acalypha indica* act as a novel source of bio-reductants. The formation of gold nanoparticles produced using *Acalypha indica* was evidenced by the color change to purple-red. Equivalent results were observed by Krishnaraj *et al.* [43] where he used *Acalypha indica* to produce gold nanoparticles that were characterized by various methods, and their results are as follows.

3.1. FT-IR analysis.

FT-IR results of the leaf extract revealed prominent absorption bands at 3726.47, 3522.02, 2927.94, 2357.01, 2312.65, 1681.93, 1519.91 cm^{-1} , and various cluster peaks ranging between 1500 and 1000 cm^{-1} were also observed. The peaks at 2927.94, 2357.01, 2312.65 cm^{-1} corresponded to the phosphine functional group (P-H stretch) and alkanes functional group (C-H stretch). The cluster of peaks from 1458.18 to 1033.85 corresponds to the presence of various alkenes (C-H plane bend) (Figure 1a). Gold nanoparticle showed prominent absorption bands at 3359.56, 3730.33, 2353.16, 2312.65, 1788.01, 1739.79, 1516.05 cm^{-1} (Figure 1b). There were shifts in wavenumbers from the extract to gold nanoparticles, which confirmed the role of extract in the formation of gold nanoparticles [44]. This study is in accordance with Barbadi *et al.* [45], who reported that the Fourier transform infrared (FT-IR) study of AuNPs synthesized using *Penicillium aculeatum* revealed the existence of possible functional groups that accounts for bioreduction and capping.

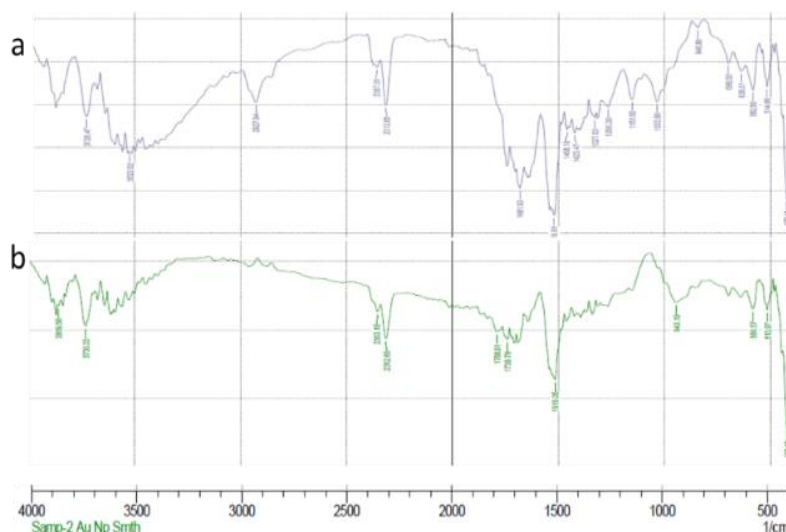


Figure 1. FTIR spectra of (a) *Acalypha indica* leaf crude extract; (b) gold nanoparticles synthesized using *Acalypha indica* extract.

3.2. UV-Vis analysis of gold nanoparticles.

Figure 4 displays the UV-visible absorption spectra recorded. A strong peak was observed at 542.5 nm in particles subjected for reduction for 40 h. Ruby red-colored gold

nanoparticles were reported to give absorbance maximum between 530 and 550 nm [46, 47]. There was a presence of broadness of peak (Figure 2), and normally a narrow peak with a decreased bandwidth stands for bigger sized particles [48].

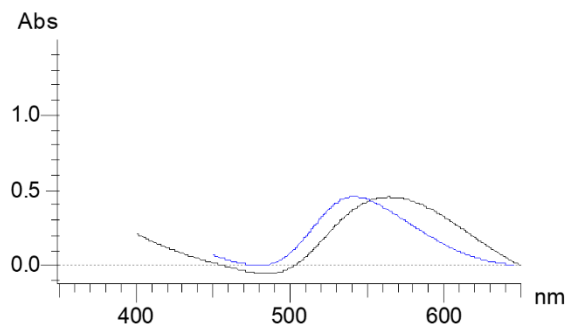


Figure 2. UV-Vis spectrum of gold nanoparticles synthesized using *Acalypha indica*; Black line – absorbance was taken at 20th h, blue line absorbance taken at 40th hour.

3.3. Particle size analysis.

Particle size analysis revealed the size of the particle distribution to be around 100 nm (Figure 3). This might be due to the accumulation of gold nanoparticles for a prolonged period of time. The particle size analysis of GNPs synthesized by *Penicillium crustosum* revealed the presence of spherical shaped particles with a mean average size under 100 nm that correlates with our results [49].

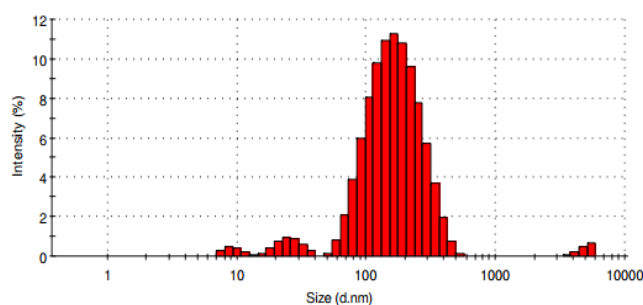


Figure 3. particle size analysis of gold nanoparticles.

3.4. TEM analysis.

Au NP cellular distribution and impacts were revealed using transmission microscopy, where the size was observed to be around 50 nm (Figure 4). Krishnaraj *et al.* [43] also reported the size of the gold nanoparticle to be around 30 nm that was obtained using the extract of *Acalypha indica*.

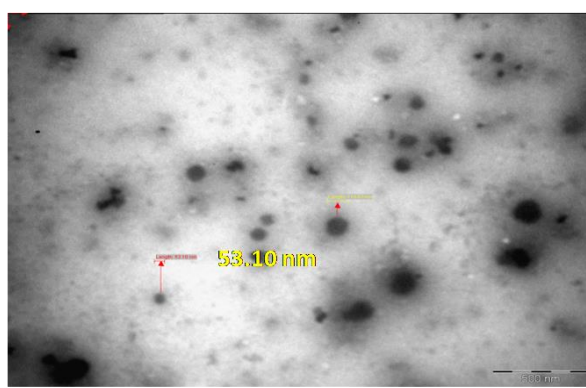


Figure 4. TEM images of gold nanoparticles synthesized using *Acalypha indica* leaf extract.

3.5. EDX analysis.

EDX analysis showed predominant peaks for gold (Figure 5), and the presence of other elements might be due to the addition of chemical/salt present in water. This is in accordance with Khatua *et al.* [50], who reported similar results that displayed strong signals in favor of Au, confirming the formation of AuNPs using *P. pinnata* leaves.

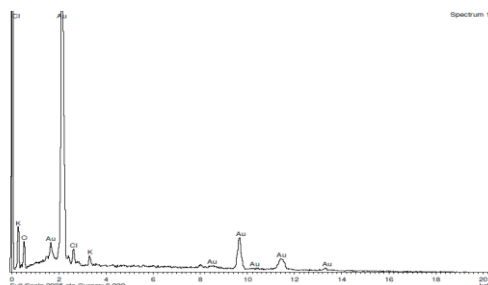


Figure 5. EDX pattern of Au NPs synthesized by *Acalypha indica* leaf extract.

3.6. X-Ray diffraction (XRD) analysis.

XRD analysis was found with four sharp peaks (Figure 6) in the XRD pattern at 2 theta values 38.4788 (111), 65.0189 (200), 44.6277 (220), and 77.8432 (311), which is on par with JCPDS card no 65- 2870 and belong to be cubic face-centered structure [5, 50]. Krishnaraj *et al.* [43] could also produce face-centered structured gold nanoparticle using the extract of *Acalypha indica*.

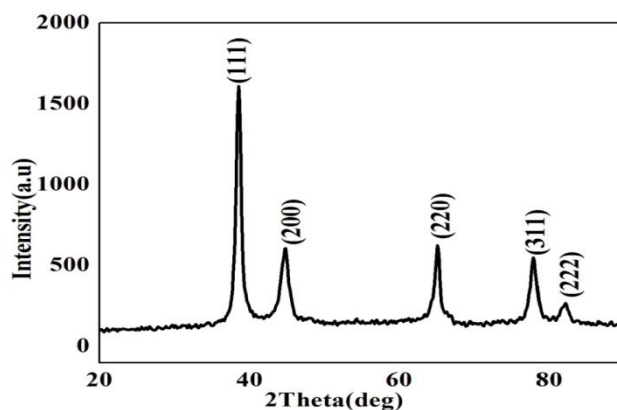


Figure 6. XRD pattern of gold nanoparticles synthesized using *Acalypha indica* leaf extract.

3.7. Antimicrobial activity.

The cell walls of both Gram-positive and Gram-negative bacteria consist of an anionic cell surface [51]. Due to the electrostatic interaction, the cationic NPs are drawn towards the surface of the opposite charged bacterial cell walls, and hence, the cationic metal nanoparticles institute a solid bond with the membranes that result in the disturbance of cell wall and increases permeability. Subsequently, the nanoparticles are capable of releasing metal ions from the extracellular matrix and thus, penetrating the cell and causing interference to the biological processes [52].

In the current study, the zone of inhibition was observed against *E.coli* alone (Table 1) at 160µg concentration, and minimal inhibitory concentration (MIC) was at 160 µg (Table 2).

It was even inhibiting the *E.coli* movement, which was evidenced by swarming motility (result not shown here).

Table 1. Antibacterial activity test against various microorganisms.

Organism	Zone of inhibition (mm)			
	40 µg	80 µg	120 µg	160 µg
<i>Escherichia coli</i>	-	-	-	2 mm
<i>Salmonella typhi</i>	-	-	-	-
<i>Shigella dysenteriae</i>	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-

Table 2. Determination of MIC.

Organism	40 µg	80 µg	120 µg	160 µg	200 µg
<i>Escherichia coli</i>	+	+	+	-	-

+ - presence of growth, - - no growth

Paul *et al.* [53] reported *Parkiarox burghii* mediated synthesized gold nanoparticles to have high antibacterial property against gram-positive bacteria compared to gram-negative bacteria. The organism was leaking out more protein when treated with gold nanoparticles (Figure 7). Metal nanoparticles have the ability to break the outer cell wall and leak out the cellular materials [54, 55]. Thus there was an increase in the absorbance. The understanding of the biomedical advantage of biogenic metal nanoparticles (MNPs) is significant in order to be familiar with the hazards associated with the use of biogenic MNPs [56].

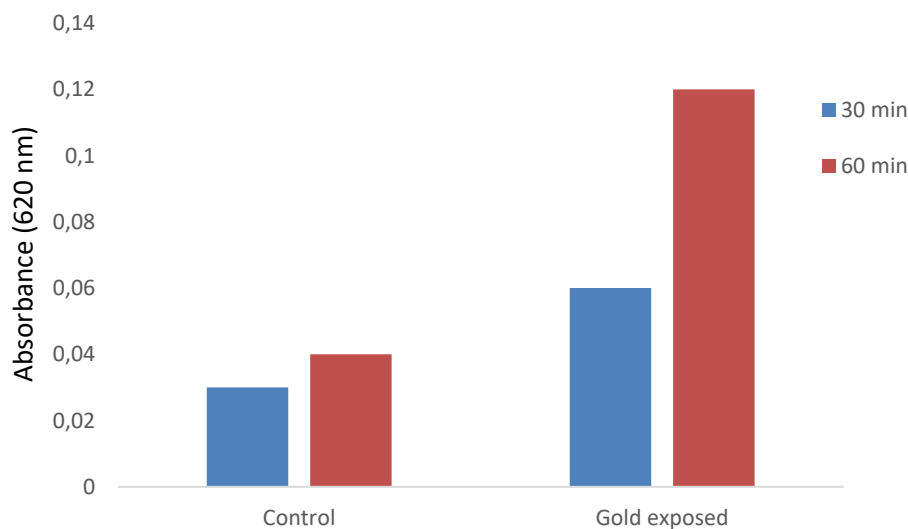


Figure 7. Protein leakage assay of gold nanoparticles.

4. Conclusions

The current study revealed that the gold nanoparticles synthesized from *Acalypha indica* had shown inhibitory against *Escherichia coli*. Therefore, gold nanoparticles were subjected to MIC and protein leakage assay to determine the antibacterial activity. The gold nanoparticles had MIC at 160 µg, and the particles were making the cells to leak out protein at 160 µg. Moreover, the characteristics of green synthesized gold nanoparticles were studied by SEM, XRD, FTIR. The observed size of the particle was around 50 – 100 nm. The FTIR results exhibited the functional groups found in AuNPs. Hence the current study indicated that the gold nanoparticles synthesized could be considered as a potential bactericidal agent to treat

pathogens. Nevertheless, supplement studies are statutory to understand the effectiveness of AuNPs in the animal model.

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Conflicts of Interest

None of the authors of this paper have any conflict of interest.

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