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Invitro Antimicrobial Properties of Gold Nanaoparticles Biosynthesized by Medicinal Plant Extracts: *Croton Megalocarpus*

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Abstract: Nanoparticles are small particles with distinctive characteristics of chemical, biological and physical properties as compared to the bulk matter. Great interest to explore the potential and application of this nanoparticles have gain momentum in the field of science. This research in particular was designed to explore alternative way of producing these nanoparticles. Green synthesis used in this research was prefer as the materials are readily available, the method is ecofriendly and potentially efficient. The study utilized *croton megalocarpus* leaves extract as reducing and capping agent of gold solution (1 mM gold (III) chloride trihydrate).Formation of AuNPs was done using Uv vis spectrophotometer while characterization was done using HRTEM,the active biomecules present in the plant extract was screened using FTIR. From the results the stirring time was six minutes and the color of the solution turned from right yellow to dark brown. The uv vis spectrophotometer surface plasmon resonance (SPR) was λ_{max} 545 nm with the intensity of 1.0 after six minutes. The HRTEM results indicated that the resultant nanoparticle were mostly spherical with non-uniform surface with an average diameter of 26.5±1.2nm. The FTIR indicated O-H stretching at 3273.26 cm^{-1+} absorbance peak which corresponds to alcohol and phenol, carbonyl (C=O) groups stretching at an absorbance peak of 1637.59 cm^{-1} corresponding to carbonyl-containing organic species. The nanoparticles produced showed great antimicrobial properties especially on gram negative bacteria, with *E.coli* having highest inhibition of 15 ±0.41nm the least affected was *B.sabtilis* a gram positive bacteria with 8.5 ± 0.73 and *E.feacalis* which was resistant to AuNPs inhibition. Synergistic antimicrobial effect was observed on *E.feacalis* where coating an inhibition of 16.5nm as compared to drug alone which had an inhibition of 14.5nm. These findings suggest that AuNPs synthesized from *Croton megalocarpus* could be valuable in the development of nanotechnology-based antimicrobial drugs.

I. Introduction

Drug resistance is a phenomenon where carcinoma cells or microorganisms such as bacteria, viruses, and parasites such as mosquitoes fails to respond to the drugs that they were previously sensitive (1). Drug resistance has been on the rise due to overuse and misuse of the current antimicrobial. Without the effective antimicrobial practice of modern medicine such as surgery become impossible to perform. There has been steady rise in antimicrobial resistance globally across all microorganisms more especially antibiotics (2,3, 4). This has led to rise in mortality and morbidity from previously treatable infection a good example is Tuberculosis in which some strains have developed total drug resistance. Therefore there is a great need to develop new antimicrobials to assist and reduce the pressure to the current ones (5,6).

Nanoparticles are particles smaller in size with an average size of less than 100nm (7).Metal nanoparticles are invisible to the naked eye but exhibit different physical and chemical properties as compared to the bulk metal. (8,9) The nanoparticles have diverse applications in biology and medicine, recently nanoparticles engineered to attach markers have been deployed to deliver drugs, heat, light, and other substances to the target cells for efficient treatment, for example cancer(10.11). Other application includes in medical machinery such as MRI in the detection of cancer cell, ultrasound .optical imaging among others (12).

Recently nanoparticles have shown promising antimicrobial activities through various research. For instance *Nagalingam M et al.* has shown that, aqueous leaf extract of *A. bettzickiana* had great antimicrobial activities against *B. subtilis* with a zone of inhibition of 10 ± 0.17nm ,*S. aureus* with , 14 ± 0.15nm , *S. typhi*, with 16 ± 0.23nm *P. aeroginosa*, with 14 ± 0.4nm among others.

Although nanoparticles have shown great potential the current standard methods of manufacturing which involve mechanical and chemical methods, have proven to be toxic, expensive and unfriendly to the environment (8,13 ,14). N-n-dimethylformamide and methoxy polyethylene the chemical used in the synthesis have been link to various human diseases, especially nervous system diseases (14). Even though physical methods produces stable nanoparticles, they are expensive and environmentally unfriendly and the nanoparticles size cannot be controlled.

In this study we used *Croton megalocarpus* , a plant known for its medicinal properties, has demonstrated considerable potential for the green synthesis of silver nanoparticles (AgNPs) in previous studies (15,16). The suitability of this plant arises primarily from its rich phytochemical composition, which includes compounds like flavonoids, terpenoids, and saponins, known to facilitate nanoparticle formation and stabilization (15, 16.17). These compounds serve as both reducing and capping agents in the synthesis process, reducing silver ions (Ag^{+}) to elemental silver (Ag^{0}) and helping prevent particle aggregation, resulting in a stable colloidal solution of AgNPs (Sharma et al., 2019; Benakashani et al., 2016).

Additionally, studies have shown that AgNPs synthesized using *C. megalocarpus* extracts exhibit strong antimicrobial properties, which is attributed to the synergy between silver and the plant's bioactive molecules. The eco-friendly synthesis approach not only minimizes the need for harmful chemicals but also enhances the biocompatibility of the nanoparticles, making them suitable for medical applications (Mandal et al., 2020; Otari et al., 2016). This demonstrates *C. megalocarpus's* suitability as a sustainable, cost-effective source for synthesizing Nanopartcles, with promising implications for biomedical and environmental applications.

Studying the synthesis of gold (AuNPs) *Croton megalocarpus* leaves offers a sustainable, green alternative to chemical methods, leveraging the plant's abundant phytochemicals to reduce metal ions without toxic byproducts. *C. megalocarpus*.(Sharma et al., 2019; Mandal et al., 2020). Utilizing this plant can reduce environmental impacts compared to conventional synthesis methods, making it an appealing option for nanoparticle production (Ahmed *et al.*, 2016).

This study is especially justified for its potential applications in treating antibiotic-resistant pathogens, as the synergistic effects standard antimicrobial and AuNPs can reduce the required concentration of conventional antibiotics, minimizing side effects and slowing resistance development

II. Material and Method

Plant extract preparation

Croton megalocarpus plant was collected from Kiambu County where it naturally grow. The leaves were then dried under the shade then ground to a fine powder. The preparation of plant extract solution involved addition of 100ml of distilled water to a ten gram s of extracts powder. After thorough mixing, the mixer was boiled on a water bath at 60ºC for one hour (18, 19). The resulting mixer was filtered and stored at 4ºC for the synthesis of gold nanoparticles.

Synthesis of AuNPs and monitoring UV vis spectrophotometer of AuNPs

Synthesis of gold nanoparticles entailed the addition of 1 mM gold (III) chloride trihydrate to the plant extracts in the ratio of 9:1.the mixture was then incubated until AuNPs were formed, the time taken for the color change denoting the formation of AuNPs was noted. Apart from color change, UV VIS spectrophotometer was used to monitor the formation of AuNPs between ranges of 400 to 800 nm. (19, 20, 21)

High Resolution Transmission Electron Microscope (HRTEM) analysis

The characterization of gold nanoparticles produced was done by use of high resolution transmission electron microscope. HRTEM was used to understand the morphology of the nanoparticles produced. The parameters that were determined includes the size distribution of nanoparticles synthesized, the shape, the surface and their distribution

The screening of bioactive molecules was done by use of Fourier Transform-Infra Red (FT-IR) Spectroscopy. This was done by taking a small volume of the concentrated reaction mixture and measuring it in the transmittance mode at 400 to 4000 cm^{-1}(19, 20)

III. Results and Discussion

Visual Observation

Gold ion is a strong oxidizing agent and take shorter period to be reduced in the presence of strong reducing agent. In this process gold ion solution is reduced from Au3+ to Au metal.

$$Au^{3+} + {}^{-}e \longrightarrow Au$$

When the plant extract was added to a solution of gold ions. It took the reaction took approximately six minutes for complete nucleation. The color of the solution changed from light yellow solution through brown to dark brown after which no other visible color change was observed. Previous studies have attributed the color change to the excitation of surface Plasmon resonance (SPR) as a result of the reduction of gold ions to gold metal nanoparticles by the plant extracts. (21) The short stirring time can be attributed to the high concentration of phytochemicals with strong reducing potential in the plant extracts. (22)

Figure 1.0: Showing the color development during the formation of gold nanoparticles by use of *croton megalocarpus* leaves as the reducing agent.

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UV VIS spectrophotometer of AuNPS synthesized using *c. megalocarpus* leaves extracts.

Uv vis spectrophotometer was used to monitor the formation of AuNPs between the absorbance 400-800 nm. The AuNPs possess a distinct optical feature known as surface plasmon resonance (SPR).This occurs as a result collective oscillation of free electrons in the conductive band of AuNPs. SPR is what can be measured using a spectrophotometer (19). The shape of the curve depends on the size and shape of the nanoparticles the peak increases with the diameter of nanoparticles the peak absorbance density (OD) depends on the concentration of nanoparticles. In this study there was a steady but gradual increase of the aptitude of the curve, after the addition of plant extract up to 6 minutes, where there was no change in the aptitude of the curve. The absorbance peak increased steadily with time due to the increase in the concentration of AuNPs formed as gold (III) chloride trihydrate was reduced to Au^0 by plant extracts. However, after 6 minutes the increase in time did results in the increase of SPR peak. The absorbance peak at 6 minutes was λ_{max} 588 nm with an intensity of 3.1. From the study increase in the intensity of the peak with time signify the gradual formation of nanoparticles from gold ions. The results are consistent with Bappi Paul, B. P. (2015). Based on the previous studies the shape, size, and surface absorbed dictates the shape and the position of the peak (23).The curve shifted more too red indicating presence of mono-dispersed nanoparticle. The red shift also indicate the presence of uneven sized gold nanoparticles as shown in the figure below.

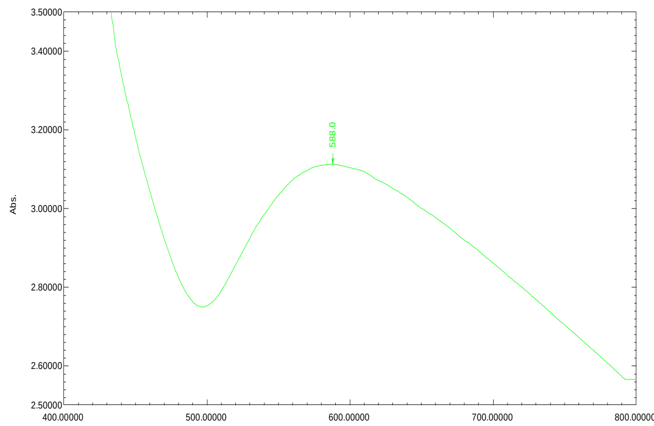
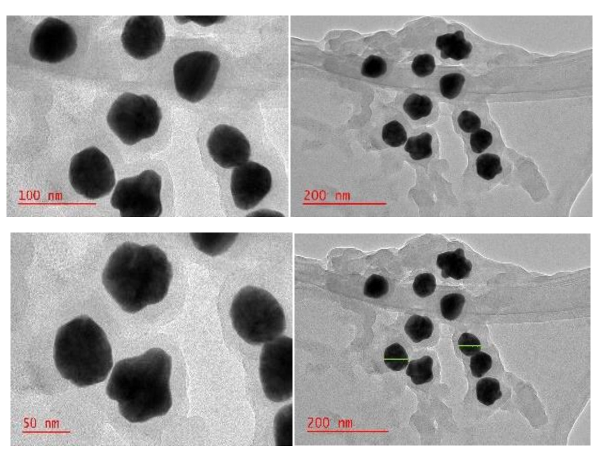


Fig 2.0: showing UV vis spectrophotometer of AuNPs the SPR absorption peaks was λ_{ma}x 540 nm

High Resolution Transmission Electron Microscope (HRTEM) analysis

HRTEM is an important tool for understanding the morphology and the size of the particle, it was also used to determine if the particles were agglomerated. In other studies HRTEM is important for monitoring the cellular uptake of nanoparticles. The size and morphology of the particles are the key determinants of the optical and chemical properties of nanoparticles (24, 25). In this study the nanoparticles synthesized using *C.megalocarpus* leaves were majorly spherical with non-uniform surfaces as shown in figure 3.0. The micrograph in the figure below indicates that; the nanoparticles were well distributed and monodispersed with no agglomeration, these results are similar to the once obtained by Jili *et al*: (26), during the green synthesis of AuNPs using Ginkgo biliba leaves and the once obtained by Eskandari.M *et al;,(*58) using microwave and edible mushroom, where the sizes of nanoparticles ranged between 10nm to 50nM the nanoparticles were however larger compared to the ones obtained from the work of Abimbola.A. *et al* (28). When nanoparticles surfaces are covered by phytochemical which created a cladding layer that prevents the particles from aggregation, (28).The selected area electron of diffraction micrograph showed shinny spots, an indication that the AuNPs synthesized were crystalline. The particle sizes were widely distributed, ranging from 10nm to 50 nm. However, the highest frequency of AuNPs sizes ranged between 20nm to 29nm, this brought the total mean average sizes to 26.5nm±1.2nm. The results concur with the UV-vis spectrophotometer which showed absorbance peaks of λ_{max} 545 nm.



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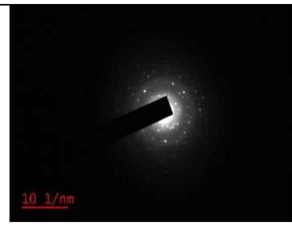

FIG 3.0: Micrograph A, B, C showing HRTEM of AuNPs synthesized through *C.megalocarpus* leaves extract D) SAED micrograph showing the nanoparticles formed were crystalline.

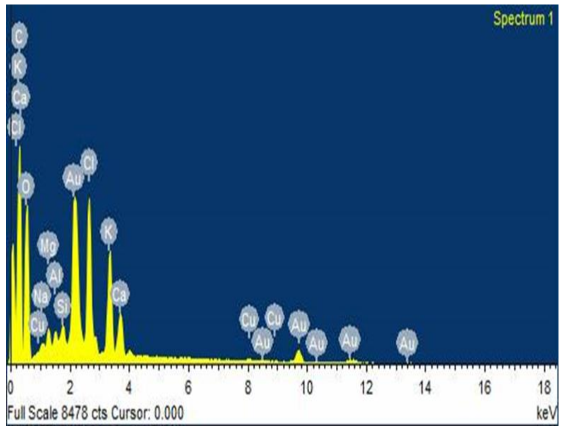

Figure 4: EDX of AuNPs synthesized using *C.megalocarpus* leaves

Energy Dispersive X-Ray (Edx) Analysis for Aunps Synthesized Using *C.Megalocarpus*

EDX was used to analyze chemical characteristics/ element composition in the solution during the synthesis of gold nanoparticles. The analysis of gold nanoparticles synthesized using plant extracts revealed the presence of various minerals, including carbon, potassium, calcium, chlorine, oxygen, copper, magnesium, sodium, aluminum, and silicon. These minerals likely originated from the plant extract, reagents, or environmental contaminants, indicating a complex synthesis process with interactions between plant material, reagents, and environmental factors. Additionally, the detection of carbon and oxygen highlights the involvement of phytochemicals from the plant extract, which act as reducing and stabilizing agents in the nanoparticle synthesis process. Carbon-based compounds serve as reducing agents, facilitating the reduction of gold ions, while oxygen-containing functional groups contribute to nanoparticle stabilization, preventing agglomeration. Understanding the composition and role of these components is crucial for optimizing the synthesis process and tailoring the properties of the nanoparticles for various applications. Further analysis and purification steps may be necessary to isolate silver nanoparticles with desired characteristics.

Based on the analysis it was determine that pure gold nanoparticles were present.as shown in the figure 4.0 where EDX spectrum was observed at 3.0 KeV.

Fourier Transform Infra-Red (Ftir) Analysis

Fourier-transform infrared spectroscopy (FTIR) is a powerful analytical technique used to characterize the chemical composition of synthesized nanoparticles by identifying specific functional groups present in their structure. In this study, Gold nanoparticles using plant extracts and employed FTIR spectroscopy to analyze their chemical composition.

The FTIR spectrum of AuNPs synthesized by *C.megalocarpus* revealed distinctive absorption peaks at 3273.26 cm^-1 and 1637.59 cm^-1, indicating the presence of specific functional groups pivotal to the synthesis mechanism.

The absorption band at 3273.26 cm^-1 is attributed to the stretching vibration of hydroxyl (-OH) groups, indicative of the involvement of hydroxyl-bearing compounds within the plant extract. Plant extracts are rich sources of various phytochemicals such as phenolics, flavonoids, and terpenoids, many of which contain hydroxyl group's .These compounds potentially serve as reducing agents or capping agents, facilitating nanoparticle nucleation and stabilization.

The absorption peak observed at 1637.59 cm^-1 corresponds to the stretching vibration of carbonyl (C=O) groups, implying the presence of carbonyl-containing organic species within the extract. These organic moieties likely play a vital role in mediating the reduction of gold ions and subsequent stabilization of the formed nanoparticles.

Therefore, the discernible FTIR features signify the orchestrated interplay between hydroxyl and carbonyl functionalities in the synthesis process, elucidating the intricate molecular dynamics underlying the formation of gold nanoparticles using plant-mediated routes.Previous studies e.g. That done by Alfred M *et al;.(*29), on the phytochemistry, pharmacology, and medicinal

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potential of *C.megalocarpus* leaves and bark reveals the presence of tannins, alkaloids, flavones, flavonoids, glycosides, reducing sugars among other phytochemicals. These phytochemicals are crucial in synthesis and providing stability to nanoparticles.

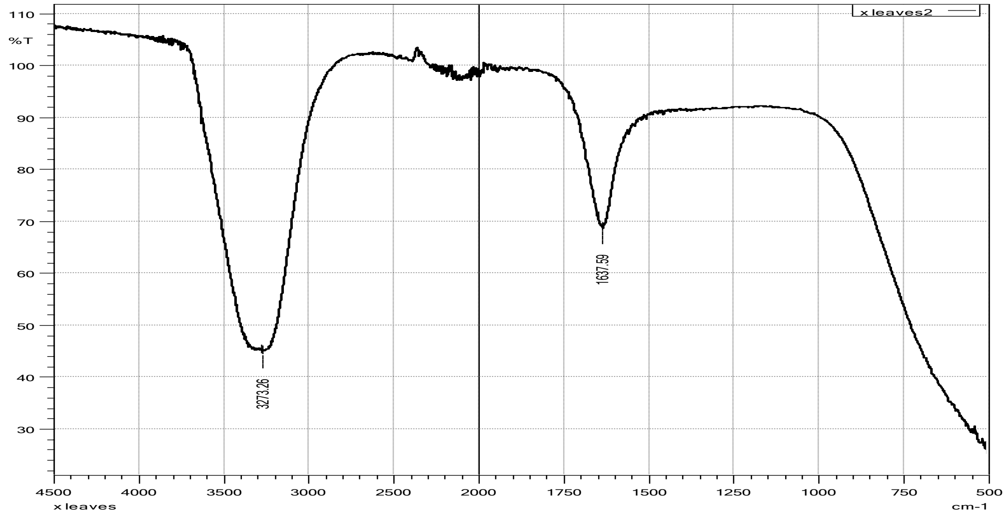

Figure 6.0: FT-IR Spectrum of Aunps Synthesized By *C.Megalocarpus* Leaves.

Antimicrobial Properties of AuNPS

The antimicrobial properties of nanoparticles are determined by the size, surface of nanoparticles and shaped, smaller nanoparticles are known to have higher antimicrobial properties due to their high surface area to volume ratio (30). The nanoparticles interfere with cell physiology by creating oxidative stress in microorganisms leading to cell toxicity and death. To understand the antimicrobial properties of gold nanoparticles synthesized using *croton megalocarpus* leaves extracts, both gram negatives and gram positives bacteria were used.

The gram negative bacteria used included normal gut flora *E.coli* and food poisoning causing bacteria *S.paratyphi*. For the gram positive microorganism, *S.aureus*, *E.feacalis* and *B.sabtilis* gram positive bacteria were used. For gram negative AuNPs showed a significant inhibition, *E.coli* had an inhibition zone of 15 ±0.41nm while *S.paratyphi* was 12.5 ±0.71nm.Similar results was reported by Sadhasivam (*et al.*,2012).

Sample	Method	Zones of inhibition in mm				
		E.coli	*S.paratyphi*	*S.aureus*	*E.feacalis*	B.sabtilis
AuNPs	Water extraction	15±0.41[a]	12.5±0.71[b]	9.5±0.42	0	8.5±0.73
Plant extracts		8.5	9.5	8.6	0	0
Antibiotic coated with AuNPs		18.78±0.14	20±0.41	17.4±0.32	16.54±0.23	18.23±0.14
Ciprofloxacin		20.5	24.25	17.5	14.45	23

Figure 7.0: Showing antimicrobial activity of AuNPs synthesized using *C.megalocarpus* leaves

Where spherical gold nanoparticles of an average size of 25nM had an inhibition of between 14mm to 16mm against gram negative bacteria. In gram positive *S.aureus* had an inhibition zone of 9.5 ± 0.42mm, *B.sabtilis* had 8.5 ± 0.73, and however *E.feacalis* did not show sensitivity to AuNPs. During invitro study by Zawrah MF (25). It was observed that, 25 nm gold nanodiscs inhibit the gram positive to the larger extent than the 30 nm sized gold nanocrystals.

These means; size and shape of nanogold greatly influence their antimicrobial potency. Plant extracts also showed antimicrobial effect although significantly lower compared to AuNPs. For instance *E.coli* (8.5mm), *S.paratyphi* (9.5mm), *S.aureus* (8.6mm) but did not show any antimicrobial effects on *E.feacalis* and *B.sabtilis*. Coating ciprofloxacin with AuNPs in the ratio of 1:1 showed synergistic effects on *E.feacalis* with an inhibition of 16.5nm as compared to drug alone which had an inhibition of 14.5nm.

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Nanoparticles possess unique properties due to their small size, which can influence their interactions with biological systems. AuNPs, with their precisely controlled sizes, exhibit high surface area-to-volume ratios, enhancing their interactions with bacteria (30). Moreover, their antibacterial mechanisms, including membrane disruption and reactive oxygen species (ROS) generation, contribute to their effectiveness against a broad spectrum of bacteria.while gram-negative bacteria typically present greater resistance due to their outer membrane, AuNPs demonstrate efficacy against both gram-negative and gram-positive bacteria. However, there's a noted variation in susceptibility, as evidenced by the smaller inhibition zones observed for gram-positive bacteria such as *S. aureus* and *B. subtilis*.

Surface functionalization of nanoparticles can further enhance their antibacterial activity by modulating their interactions with bacteria. This includes attaching molecules to the nanoparticle surface to improve stability, biocompatibility, and targeting specificity.

Characterization techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS) provide crucial insights into nanoparticle properties, such as size, morphology, and stability. These analyses help understand nanoparticle behavior and optimize their design for antibacterial applications.

While the results show promise, challenges remain in the clinical translation of nanoparticle- based therapies. Issues related to scalability, regulatory approval, and cost-effectiveness need to be addressed for widespread clinical use. Nevertheless, ongoing research efforts aim to overcome these hurdles and advance nanoparticle-based antibacterial therapies toward clinical applications.

IV. Conclusion

Visual inspection and characterization indicate successful synthesis of gold nanoparticle from Croton megalocarpus leaves extracts. The stirring time is short and convenient for fast mass production. The produced nanoparticles had longer shelf life without requirement of special storage conditions. The nanoparticle produced did not show any agglomeration and the size of 26nm and the morphology provide a wide range biological applications. The Nanoparticles characteristically had good antimicrobial properties and combining nanoparticle with drug resulted into synergistic effects. Therefore it can be concluded that *croton megalocarpus* leaves extract is suitable for the production of gold nanoparticles. It provide, efficient, easy, less costly and environmental friendly way of synthesis. This research therefore achieved its objectives and the the knowledge will contribute to the body of science with the hope that the nanoparticles produced will have great impact in solving antimicrobial resistance.

Conflicts of Interest

There is no conflict of interest by any party involved in this study. This journal is an original work that has not been published or in process of being published in any by any other publisher. On behalf of all Co-Authors, the Author shall bear full responsibility for the submission.

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