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AZEEZ ADEGOKE

chemazeez@gmail.com

Afe Babalola University, Nigeria

Abiodun Ojo

Afe Babalola University, Nigeria

Basheer Balogun

King Abdulaziz University

Oluwabukola Kudirat Jimoh-Hamza

Al-Hikmah University

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Green Synthesis and Therapeutic Evaluation of Polyphenolic Silver Nanoparticles Derived from *Cymbopogon citratus*

Authors: Azeez Adegoke[a,e], Abiodun Ojo[a], Basheer Balogun[b,d], and Oluwabukola Kudirat Jimoh-Hamza,[c].

a: Afe Babalola University, Nigeria, Ado-Ekiti, Nigeria, Department of Chemical Sciences.

b: Fulcrum Innovation Laboratory, Ilorin, Nigeria, Biomolecular Section.

c: Al-Hikmah University, Adewole Estate, Ilorin, Nigeria, Department of Biological Sciences.

d: Department of Biochemistry, King Abdulaziz University, Jeddah, Saudi Arabia

e: Corresponding author
Corresponding Author Email: chemazeez@gmail.com.

Abstract

Green nanoparticle synthesis has emerged as a sustainable approach in biomedical research, utilizing biological systems such as plants to overcome the limitations of conventional physical and chemical methods. In this study, we biosynthesized silver nanoparticles using plant extract as a capping agent and silver nitrate as a precursor under sunlight. A distinct color change confirmed nanoparticle formation, and UV–visible spectroscopy revealed an absorption peak at 412 nm, while SEM and TEM analyses indicated particle sizes of 1–7 nm. Concurrently, a strain of *Salmonella Typhi* was obtained from the Central Research and Diagnostic Laboratory, Ilorin, Nigeria, and subcultured on selective and differential media. Identification was confirmed by colony morphology, hydrogen sulfide production, Widal test, and Gram staining. Antimicrobial activity was evaluated using the agar well diffusion method, where standardized inocula were exposed to polyphenolic-rich plant extracts, biosynthesized nanoparticles, and standard antibiotics. Plates were incubated at 37°C for 18–24 hours, and inhibition zones were observed.

The results demonstrated enhanced antimicrobial efficacy of the biosynthesized nanoparticles, validating their eco-friendly synthesis and potential biomedical application against *Salmonella Typhi*.

Keywords: Nanoparticles, Precursor, Capping agent, SEM, TEM, Antimicrobial activities.

Introduction

Green synthesis of nanoparticles has emerged as a sustainable frontier in biomedical innovation, offering an eco-friendly alternative to conventional physical and chemical methods that often depend on hazardous reagents and high energy consumption. Biological systems, including microorganisms, fungi, and plants, serve as both reducing and stabilizing agents, enabling the production of nanoparticles with enhanced biocompatibility and therapeutic potential (Huo *et al.,* 2018). Among these, silver nanoparticles (AgNPs) synthesized via green routes have demonstrated superior safety profiles and efficiency compared to their chemically derived counterparts, underscoring their relevance in biomedical applications.

Nanotechnology further addresses critical pharmacological challenges, particularly those related to drug absorption and bioavailability. According to Lipinski's principle, hydrophilic compounds face significant limitations in penetrating lipophilic cell membranes, thereby reducing therapeutic efficacy. Nanoparticles, through surface modification and nanoscale engineering, provide a strategic solution to these barriers. They function as versatile carriers that improve drug delivery, optimize pharmacokinetics, and broaden the scope of biomedical applications. This dual advantage of environmental sustainability and clinical efficacy positions green-synthesized nanoparticles as a transformative tool in modern nanomedicine.

MATERIALS AND METHODS

Materials: Silver nitrate salt, dried lemongrass sample, ethanol, distilled water, centrifuge, conical flasks, and beakers.

Plant Material and Extraction Procedure

1. Plant samples were collected from Equity Estate, Adewole, Ilorin, Kwara State, Nigeria, and subsequently submitted to the Department of Biological Sciences, University of Ilorin, for taxonomic identification. The samples were thoroughly rinsed with distilled water to eliminate surface contaminants, air-dried at room temperature, and ground into fine powder. A total of 1000 g of powdered plant material was macerated in 1 L of 70% ethanol for 48 hours. The mixture was sieved to remove coarse residues, and the filtrate was concentrated using a water bath. The resulting extract was subjected to total polyphenolic screening.

Reducing/Capping Agent Preparation

2. 10 g of the extract was dissolved in 100 mL of distilled water; it was mixed using a magnetic stirrer and filtered using a muslin cloth. The filtrate was centrifuged at 4000 rpm for 15 min. The supernatant was collected for further use (Oladipo *et. al*., 2025).

Precursor Preparation

3. A 1 mM silver nitrate solution was prepared by dissolving 0.17 g of silver nitrate salt in 1000 mL of distilled water. The preparation was conducted in an amber bottle wrapped with foil to prevent photo-reduction of silver nitrate; the solution was kept in the bottle for further use.

Green method biosynthesis of the nanoparticles

4. The biosynthesis of the AgNPs was conducted according to the modified method by Oladipo *et. al*. (2025). In the method, 2 mL of the capping agent was added to 40 mL of the precursor; the reaction was carried out in the presence of light, and the color change was observed at 15 min, 25 min, and 1 hour. The color change to dark brown confirms the synthesis of nanoparticles, which will be confirmed by further analysis.

Characterization of synthesized AgNPs

The synthesized silver nanoparticles (AgNPs) were subjected to comprehensive physicochemical characterization using standard analytical techniques. Ultraviolet–visible (UV–Vis) spectroscopy was performed with a UV–Vis spectrophotometer to monitor the surface plasmon resonance (SPR) band, thereby confirming nanoparticle formation and evaluating their optical stability. The particle size distribution, morphology, and structural features were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), providing high-resolution images of the nanoparticles. Fourier-transform infrared (FTIR) spectroscopy was employed to identify functional groups associated with biomolecules from the plant extract, elucidating their role in the reduction of silver ions and stabilization of the nanoparticles.

Antimicrobial Activity

5. The biosynthesized nanoparticles were tested for their antimicrobial activity against the Salmonella typhi bacterium using the Agar well diffusion method. In the assay, a suspension of the organism to be tested was prepared in a saline solution and adjusted to a 0.5 McFarland standard (~1 × 10^8 colony-forming units (CFU)/mL). The culture was swabbed onto standard Mueller-Hinton Agar (MHA) medium with a sterile cotton swab. A well approximately 7 mm in diameter was made in an MHA plate using a gel puncture. A volume of 60 uL of synthesized particles was inoculated into the well. Then the plates were incubated in an incubator at 37 ℃ for 24 hours. The zone of inhibition was observed.

6. **Antioxidant Screening assay**

The antioxidant potential of the synthesized silver nanoparticles (AgNPs) was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, following the modified protocol of Shirwaikar *et al.* (2006). Briefly, 2 mL of 0.1 mM DPPH solution prepared in methanol was mixed with 2 mL of varying concentrations of AgNPs, plant extract, or ascorbic acid (used as the standard reference antioxidant). The reaction mixtures were incubated in the dark at 30 °C for 20 minutes to prevent photodegradation of the radical. After incubation, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. All experiments were performed in duplicate to ensure reproducibility.

The percentage radical scavenging activity was calculated using the following equation:

% DPPH radical scavenging activity= [(Abs Control−Abs sample) / (Abs control) ×100]

Where:

7. Abs control = absorbance of DPPH solution with methanol (blank)

8. Abs sample = absorbance of DPPH solution with test sample or standard

RESULTS AND DISCUSSION

Nanoparticles Synthesis

Upon exposure to sunlight, the reaction mixture containing silver nitrate and the plant extract exhibited a distinct color progression indicative of nanoparticle formation. Initially, the solution appeared pale yellow. Within the first 10 minutes of sunlight irradiation, the mixture gradually darkened, suggesting the onset of silver nanoparticle nucleation. After 20 minutes, the reaction stabilized, yielding a characteristic dark brown coloration. This final color change confirmed the successful reduction of silver ions and the formation of stable silver nanoparticles, as illustrated in Figure 1.0.

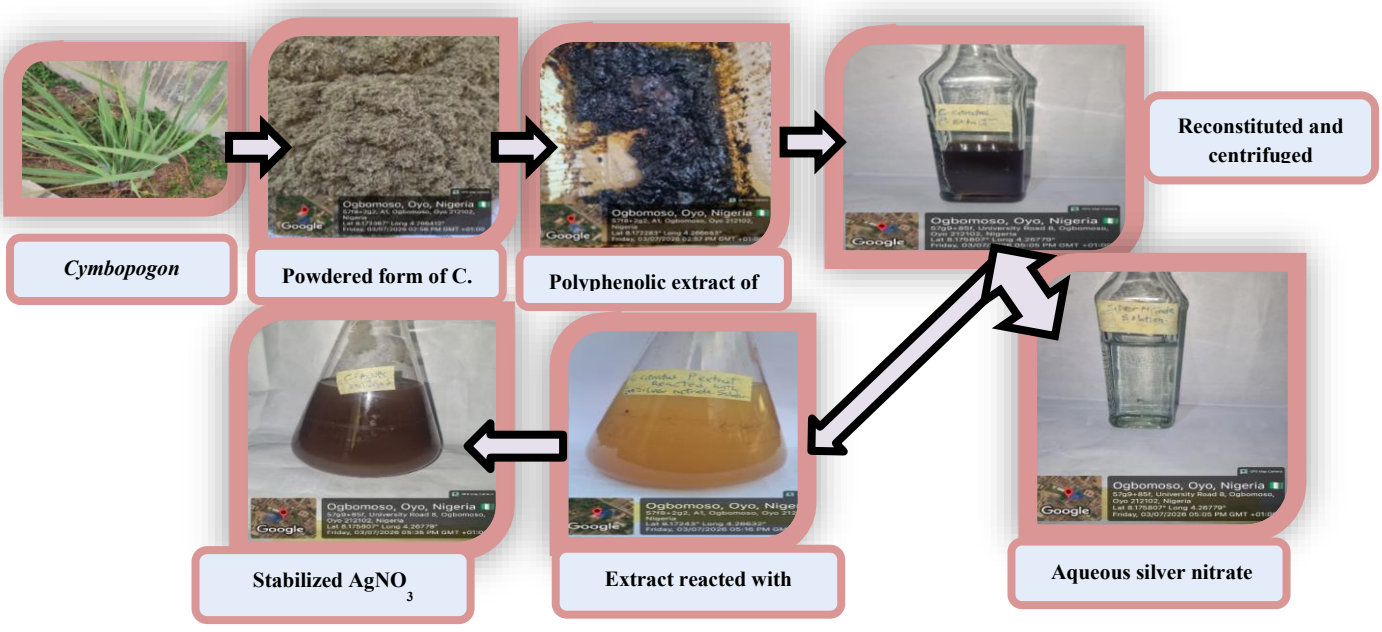


Fig. 1.0 The Pipeline shows the green method for biosynthesizing AgNPs from the polyphenolic extract of *C. citratus*.

Characterization of Nano-silver Samples

UV-VIS analysis

According to Mie scattering theory, the absorption spectrum of the synthesized spherical nano-silver particles exhibited a distinct peak ranging from 400-450 nm, which corresponds to the characteristic ultraviolet spectroscopic signature of silver nanoparticles (Ahmad *et al*., 20003). The symmetrical nature of the curve observed in Fig. 2.0 indicates that the nanoparticles possessed excellent dispersibility, confirming the successful synthesis of well-dispersed spherical nano-silver particles.

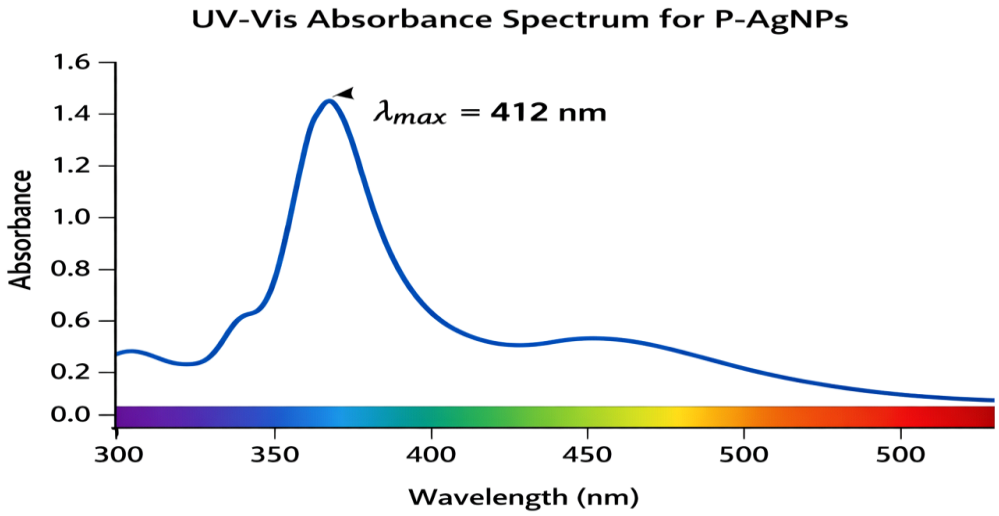


Fig. 2.0 The UV-Vis absorbance spectrum for Synthesized Polyphenolic Silver nanoparticles

FTIR analysis

The Fourier Transform Infrared (FTIR) spectrum of the synthesized silver nanoparticles, shown in Fig. 3.0, was recorded in the frequency range of 3398–605 cm^{-1} (% transmittance). A slight shift was observed in the characteristic peaks at 3398, 1704, 1216, and 605 cm^{-1}. The band at 3398 cm^{-1} corresponds to the O–H stretching vibration, indicating the presence of alcohol and phenolic groups. Hydroxyl groups (O–H) are known to exhibit strong binding affinity with silver ions, thereby facilitating the reduction process. The observed peak shifts suggest the involvement of various functional groups in the bio-reduction of silver ions to silver nanoparticles, confirming the role of biomolecules in nanoparticle stabilization (Iravani, 2011; Zhang et al., 2016).

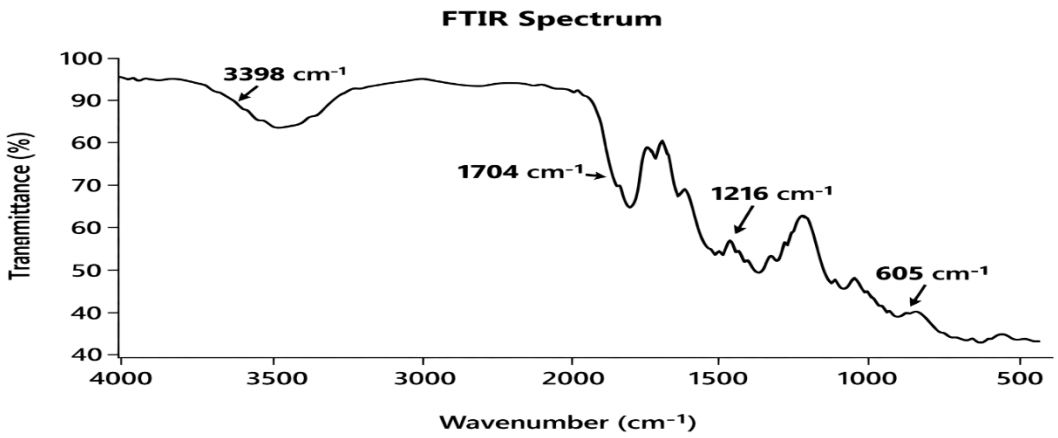
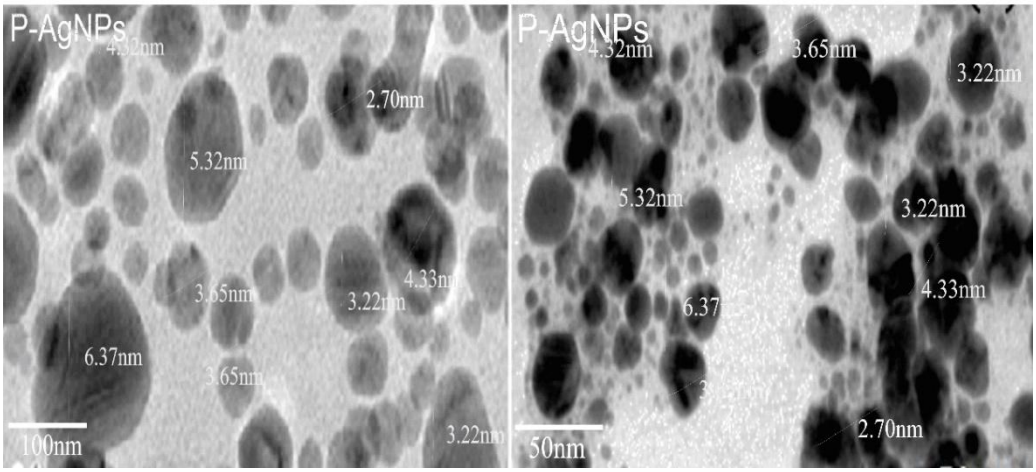


Fig. 3.0 The FTIR spectrum of the synthesized Polyphenolic Silver nanoparticles

TEM analysis

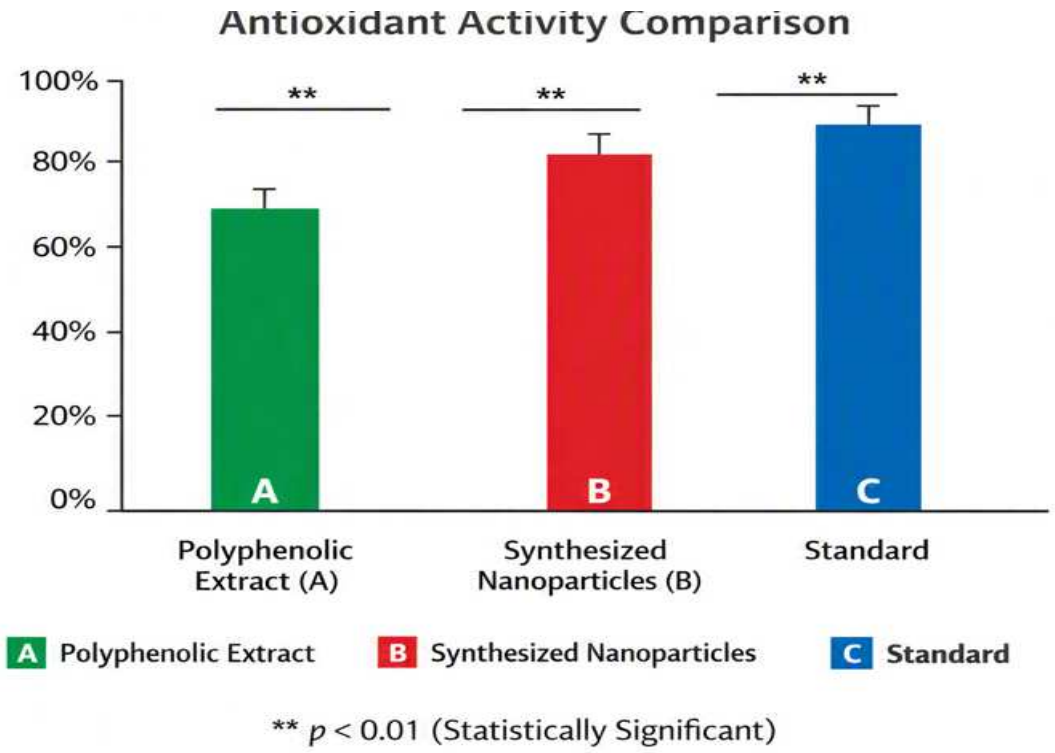
Figure 4 shows the result of field emission transmission electron microscopy of the synthesized silver nanoparticles. Fig. 4 (a) and (b) show the morphologies of the spherical nano-silver particles at 100 nm and 50 nm scale, respectively.



9. 100nm (b) 50nm

Fig. 4. Field emission transmission electron microscopy.

In Vitro Antioxidant Activities of Biosynthesized Silver Nanoparticles



Antimicrobial Activity of AgNPs and Polyphenolic Extracts of Cymbopogon citratus

The findings revealed that both the polyphenolic extract and the green-synthesized silver nanoparticles (AgNPs) of *Cymbopogon citratus* exhibited antibacterial activity against the test

organism, *Salmonella Typhi*. The presence of inhibition zones around the discs confirmed bacterial sensitivity. Ciprofloxacin solution was used as a positive control to benchmark the antibacterial efficacy of the AgNPs. The results demonstrated an enhancement in the diameter of the zone of inhibition (ZOI) against *S. Typhi*, with measurements on Mueller–Hinton agar (MHA) showing 10 mm for the polyphenolic extract and 12 mm for the AgNPs. In comparison, the antibiotic strip colistin produced a ZOI of 15 mm, serving as a reference standard.

The observed increase in ZOI diameter for AgNPs indicates superior antibacterial activity compared to the crude extract. This enhanced efficacy can be attributed to the strong affinity of Ag^{+} ions released from the nanoparticles, which readily bind to phosphorus, proteins, and sulfur-containing biomolecules such as RNA and DNA. Their ability to penetrate bacterial cell walls disrupts vital cellular processes, ultimately leading to bacterial death. These results support the hypothesis that green-synthesized AgNPs possess improved antimicrobial potential over plant extracts alone, highlighting their promise as eco-friendly alternatives in combating pathogenic bacteria.



Fig. 5. Agar well diffusion method for the bacterial susceptibility test.

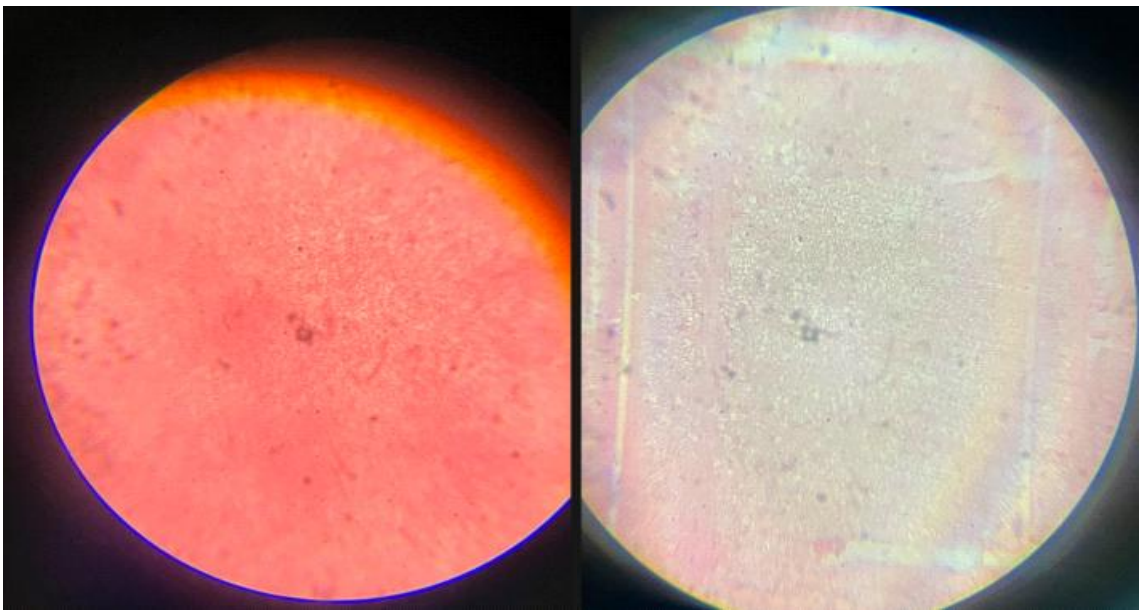


Fig. 6. Microscopy Morphology of *Salmonella typhi* bacteria

CONCLUSION

In conclusion, the absorption at 412 nm confirmed the successful synthesis of the nanoparticles, as did the SEM and TEM results. The diffusion and formation of the inhibition zone on the MHA show that both extracts are susceptible to antimicrobial activity.

Declarations

Ethics Approval: Not applicable. This study did not involve human participants, animal subjects, or human tissue samples requiring institutional ethical board approval.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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Competing Interests: The authors declare that they have no competing financial or non-financial interests.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contributions: All authors contributed to the conceptualization, experimental procedures, data analysis, and drafting of the manuscript. All authors read and approved the final manuscript.

Plant Collection Guidelines: The collection of *Cymbopogon citratus* plant material complied with local and national guidelines. The plant samples were collected from Equity Estate, Adewole, Ilorin, Kwara State, Nigeria. No protected or endangered species were involved, and no special permits or licenses were required.

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