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Biogenic Synthesis, Spectroscopic Characterization and Bioactivity of *Cymbopogon citratus* Derived Silver Nanoparticles

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Abstract

The use of plant extracts as a bio-reducer for the synthesis of silver nanoparticles has attracted attention due to its rapid, ecological, non-toxic and economical protocol. The aim of this study was to investigate the possibility of synthesizing silver nanoparticles using *Cymbopogon citratus* leaf extract, and characterizing them by Ultraviolet-visible spectroscopy, X-ray Diffraction and X-ray Fluorescence in order to determine their size and composition, as well as to evaluate them in-vitro bioactivity on selected models. The results of this study show that silver nanoparticles were successfully synthesized, with a size of 29.49 nm. The silver nanoparticles showed bactericidal activity against all three types of bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aerugunosa* ATCC 27853) at the minimum inhibitory concentration (MIC=31.25 µg/mL), larvicidal activity against Anopheles larvae and reasonable antioxidant properties. All these results demonstrate the biopharmaceutical potential of these new products. These nanoparticles, synthesized from plant extracts, could be a promising solution for the treatment of a number of diseases, including malaria, bacterial infections and diseases caused by oxidative stress.

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1. Introduction

Nanotechnology is an evolving field dedicated to the production and valorization of nanoscale resources for a wide range of applications [1]. In recent decades, nanoparticles have grown exponentially due to their numerous applications in fields such as medicine, electronics, industry, the environment, and energy [2-5]. Nanoparticles offer increased effectiveness due to their small size, which allows them to easily penetrate the biological systems of targeted organism. The benefits include a controlled release of active substances, a reduction in the quantity of products required and a reduction in harmful effects on the environment. Thanks to nanotechnology processes, various bioactive compounds are produced to combat several pathogens [6]. There are several physical and chemical techniques that can be used for the synthesis of nanoparticles. However, traditional nanoparticle synthesis involves the use of toxic chemicals that can have adverse effects on the environment and human health. This is why, in recent years, green nanoparticle synthesis has become a promising and environmentally friendly alternative [7, 8]. The use of silver nanoparticles (AgNPs) for their antibacterial and larvicidal properties is increasingly being studied in various fields, such as medicine and vector control [9, 10]. With this in mind, the synthesis of AgNPs from plant extracts is a promising approach as it is economical, environmentally friendly, and produces more stable and biocompatible nanoparticles [4]. Among the plants with significant pharmacological potential, we can cite *Cymbopogon citratus*, which is widely used in Congolese pharmacopeia. It has been reported that the biological properties of this plant are linked to its wealth of active substances, which it contains such as phenolic compounds [11]. In this study, we evaluated the effect of silver nanoparticles synthesized from *C. citratus* extracts on the three bacterial strains and mosquito larvae.

2. Material and Methods

2.1. Biological Materials

2.1.1. Plant

In this study, the plant material used consisted of *C. citratus* leaves. The leaves were collected from Mont Ngafula (Democratic Republic of the Congo). The sample was identified by botanical technician NLANDU from the INERA Herbarium housed at the Faculty of Science, University of Kinshasa. The sample was air-dried at room temperature (±27°C) for a period of two weeks and then ground using an electric grinder (CYCLOTEC 1093 Sample mill FOSS TECATOR) to obtain a fine powder for chemical and biological analysis.

2.1.2. Bacterial Strains

Three bacterial strains were used in this study, namely *P. aeroginosa* ATCC 27853, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922. These bacterial strains were supplied by the microbiology Laboratory, Faculty of Pharmaceutical Sciences, University of Kinshasa.

2.1.3. Anopheles Larvae

The Anopheles larvae used in this study to assess the larvicidal activity of nanoparticles as shown in Figure 1were collected in the stagnant waters, Kinshasa.

Figure 1: Collection of larvae using the dipping technique.

2.2. Methods

2.2.1. Thin Layer Chromatography

The different groups of secondary metabolites were identified by thin-layer chromatographic screening as

previously described by [12, 13].

2.2.2. Secondary Metabolite Quantification

The total polyphenol content of the extracts was determined using the Folin-Ciocalteu method [14]. Total flavonoid content of extracts was estimated by following a spectroscopic method based on the complexation of aluminium trichloride with flavonoids as previously used by [15].

2.2.3. Synthesis of NPAgs

In this study, the method described by [16], was used for biogenic synthesis with some modifications. Experimentally, maceration of 10g of *C. citratus* powder in 100mL of distilled water for 24h, followed by filtration yielded the extract used for particle precipitation and stabilization. Briefly, 5 mL of the *C. citratus* leaf extract was added to 95 mL of 1 mM silver nitrate ($AgNO_3$). The resulting reaction mixture was heated at 60°C for 15 minutes with constant mechanical stirring. After 30 minutes, the change in colour of the solution from yellow to brown denoted the start of metal ion precipitation in nanoparticle form, and nanoparticle formation was monitored using a UV-Vis spectrophotometer. The synthesized silver nanoparticles were centrifuged at 15,000 rpm for 20 minutes, the pellet was washed with distilled water several times to remove impurities and placed in an oven for drying, followed by scraping to obtain the powder used for further characterization and evaluation of biological activities.

2.2.4. Characterization of NPAgs

In this study, we determined the Surface Plasmon Resonance of nanoparticles by scanning wavelengths from 200 to 700 nm using a UV-visible spectrophotometer (Brand: HITACHI U-3900H). The crystal structure and chemical composition of the synthesized nanoparticles were determined by X-ray diffractometer and X-ray fluorescence spectrophotometer.

2.2.5. Evaluation of Antibacterial Activity

The antibacterial activity of the synthesized NPAgs against the three bacterial strains used was evaluated by the Muller-Hinton liquid micro-dilution method as previously described by [13 and 17] with some modifications.

2.2.6. Evaluation of Antioxidant Activity

The antioxidant activity of the decoction and percolate of *C. citratus* leaves was evaluated following a method based on the degradation of the ABTS° radical (2,2'-azino-bis-3ethylBenz-Thiazoline-6-Sulfonic Acid) as previously described by [13].

2.2.7. Evaluation of Larvicidal Activity

Procedure

The larvicidal activity was performed by placing 30 mosquito larvae in 90 mL of gite water with 10 mL NPAgs in a jar. NPAgs were diluted with deionized water as a solvent to the desired (molar) concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03215 mg L^ (-1). Each test included a positive control group (larvae+ and site water) with three replicates for each individual concentration. Mortality was assessed after 24 h and 48 h to determine acute toxicity to fourth-stage Anopheles larvae.

Data Analysis

LC_{50} and LC_{90} values were calculated after 24 h treatment in a larval bioassay. LC_{50} and LC_{90} values, upper confidence limit (UCL) and lower confidence limit (LCL), degree of freedom (df) and Chi-square test were calculated using SPSS software (v16.0).

3. Results and Discussion

3.1. Phytochemistry

3.1.1. Thin Layer Chromatography

The phytochemical profile revealed the presence of chemical compounds such as phenolic acids and flavonoids (Figure 2), antocyanins and iridoids (Figure 3) in *C. citratus* leaves.

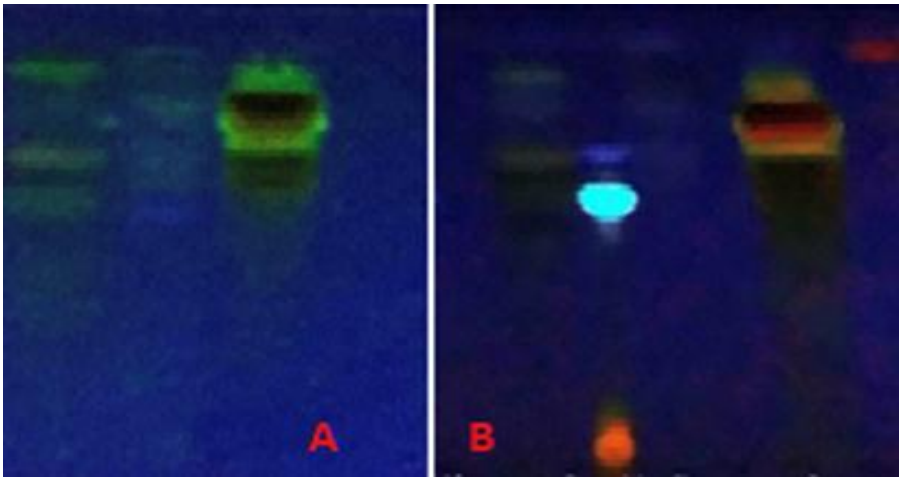

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Figure 2: TLC chromatogram of *C. citratus* methanolic extracts for phenols and flavonoids without control at 366 nm.

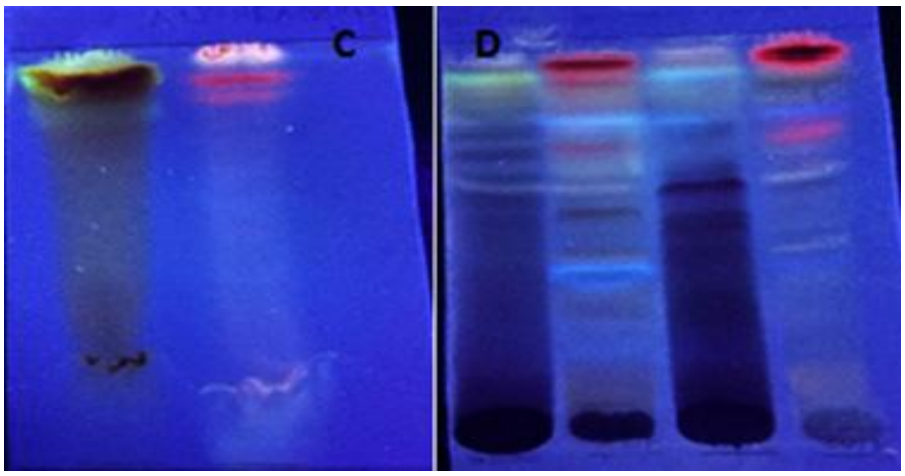

Figure 3: TLC chromatogram of methanolic extracts of *C. citratus* for anthocyanins (C) and irridoids (D) without visible light control.

3.1.2. Phytomarker Assay

Quantitative analysis showed a content of approximately 134.0210±0.0547 mgGAE/g dry extracts for total polyphenols and 15.148±0.077 mg QE/g dry extracts for flavonoids. The presence of these phytomarkers is a good indication for the choice of *C. citratus* as a source of the phytoreducers required for the green (biogenic) synthesis of Silver nanoparticles. Comparing the results of this study with those of Gazwi [18], it can be seen that *C. citratus* harvested in DR Congo is richer in total polyphenols (134.0210±0.0547 mgGAE/g Vs 65.20 ±0.134 mgGAE/g). This difference in content can be explained by the different bioclimatic, geological, and other conditions prevailing in this environment [19, 20].

3.2. Synthesis and Characterization of NPAgs

It was observed that after the aqueous extract of *C. citratus* leaves was added to the flask containing the $AgNO_3$ solution exposed to a heat source, the color of the solution changed to brown within two hours, indicating the synthesis of silver nanoparticles. The color change of the solution is due to the excitation of the surface Plasmon vibrations of the NPAgs, i.e. the phenolic compounds contained in our extract have reduced the silver. Visual observation of the color change of the $AgNO_3$ solution has been reported by other authors [21]. Figure 4 shows the formation process of silver nanoparticles (NPAgs) in aqueous media using phytoreducers. This is therefore a variant of the Tollens reaction in which reducing sugars are replaced by phytoreducers.

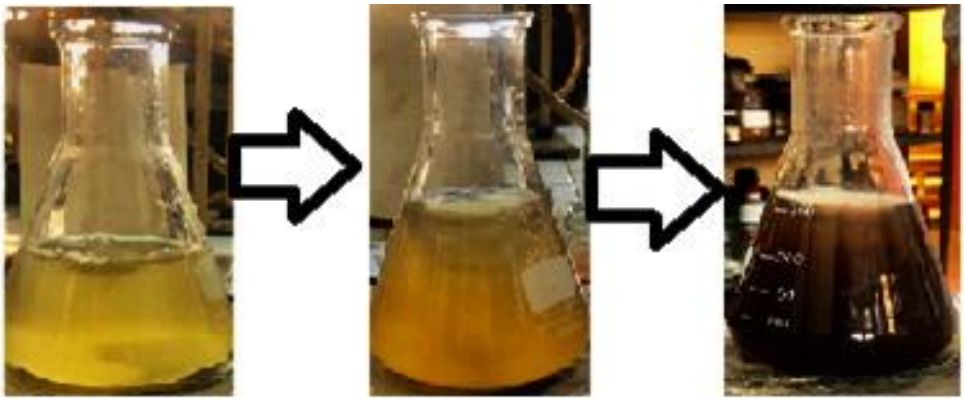

Figure 4: Formation of Silver nanoparticles based on *C. citratus* 10% (w/v); Silver nitrate (1mM).

3.3. Characterization of NPAgs

3.3.1. Characterization by Ultraviolet-visible Spectroscopy

To confirm the bioreduction of silver ions (Ag+) into metallic silver nanoparticles by the aqueous extract of Citronella leaves, the brown solution of synthesized silver nanoparticles was analysed by UV-Vis spectroscopy. Figure 5 shows the UV-visible spectrum of the synthesized NPAgs.

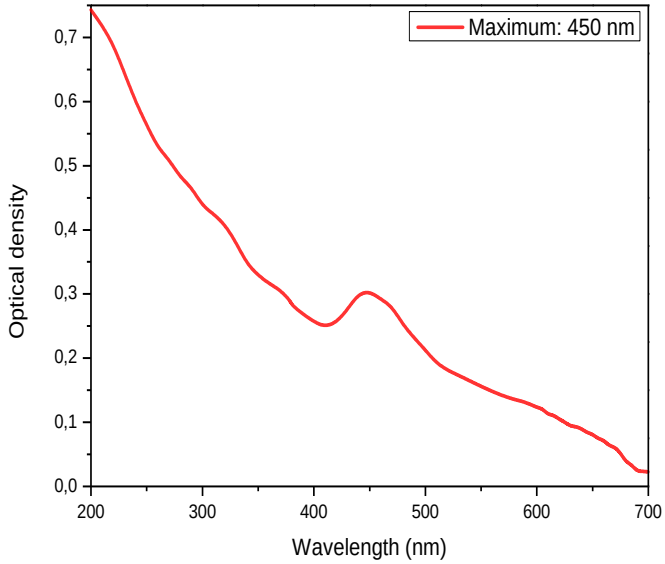

Figure 5: UV-visible absorption spectrum of silver nanoparticles (appearance of a surface plasmon resonance band between 400 and 500 nm).

Typically, a surface plasmon resonance (SPR) peak observed in the 400-470nm wavelength range indicates that the aqueous leaf extract possesses the potential to reduce Ag ions to Ag nanoparticles. Qayyum *et al.* [22] suggested that the visualization of additional absorption peaks may be due to the presence of numerous participating organic compounds that may interact to reduce silver ions. In UV-Vis spectral analysis, the maximum absorbance peak is observed at 450 nm in the visible region. This colour change is due to the excitation of surface plasmon vibrations by NPAgs [23, 24]. Characterization by X-ray diffractometry and X-ray Fluorescence X-ray diffraction enables the study of the crystalline structure of a compound in a solid state. This technique was used to study the crystallinity of silver nanoparticles electrodeposited on the anodized aluminium wafer. Characterization of the NPAgs was carried out using a PHYWE X-ray 4.0 diffractometer using X-radiation from the Kα emission of copper, wavelength λ=1.5406 Å. Figure 6 shows the spectrum that gives the X-ray diffraction peak values of the NPAgs.

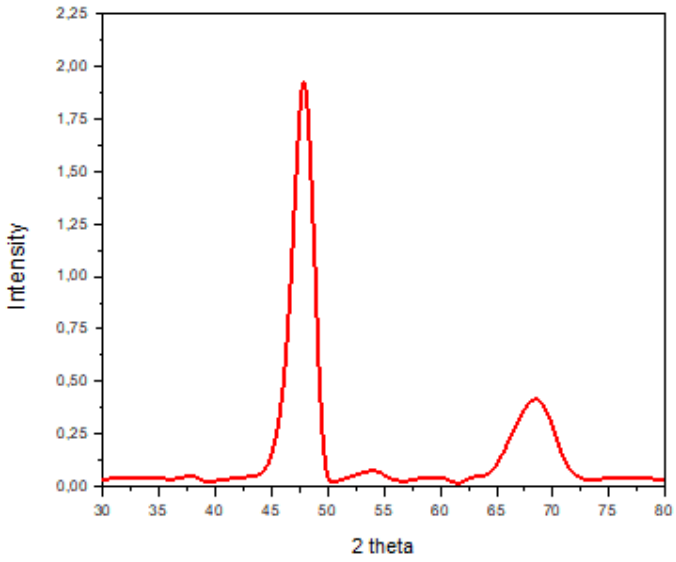

Figure 61: XRD pattern of NPAg.

Diffraction peaks appeared at 2θ at values of 47.83 (111) and 68.73 (211). The presence of (111) and (211) peaks in the DRX spectrum indicates the formation of the high crystallization purity of Silver nanoparticles [25]. The average crystallite size of AgO nanoparticles was estimated at 29.49 nm. Typically, NPAgs have a variant size between 25-35 [26]. After calculations, the results showed that the Silver nanoparticles have a size of 29.49 nm. To further confirm the presence of the Ag-Kα and Ag-Kβ elements, X-ray fluorescence spectra were recorded.

3.4. The anti-free radical activity of *C. citratus*. Figure 7 shows the evolution of ABTS radical inhibition by *C. citratus* aqueous extract and percolate.

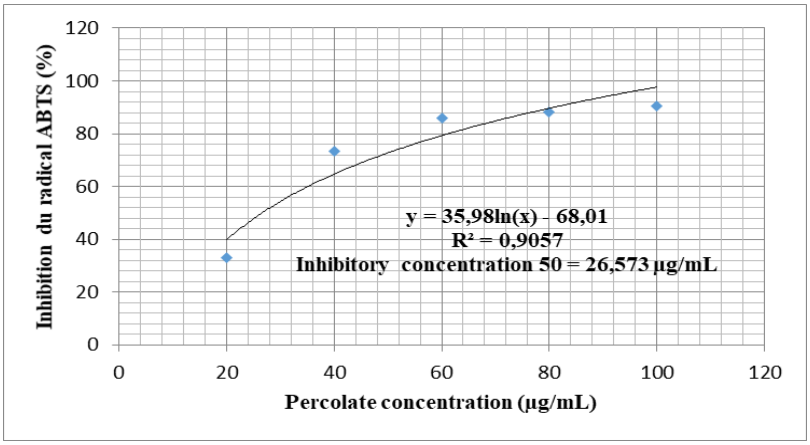

Figure 7: ABTS radical inhibition rate as a function of *C. citratus* percolate concentration. Table 1 gives the antibacterial activity of NPAgs derived from *C. citratus* extracts.

Table 1: Antibacterial activity of NPAgs derived from *C. citratus* extracts.

Bacterial strains	Concentration (µg/mL)									
	MIC	2000	1000	500	250	125	62.5	31.25	15.25	7.8125
E. coli ATCC 25922	31,25	-	-	-	-	-	-	-	+	+
S. aureus ATCC 25923	31,25	-	-	-	-	-	-	-	+	+
P. aeruginosa ATCC 27853	31,25	-	-	-	-	-	-	-	+	+

Legend: (+) Bacterial growth, (-) Bacterial growth inhibition.

This table shows that the nanoparticles are active against the three types of bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aerugunosa* ATCC 27853) at the minimum inhibitory concentration (MIC=31.25 µg/mL). Nanoparticles have been reported to have antibacterial properties [27, 28].

3.5. Anti-larval Activity of *C. citratus*-based Silver Nanoparticles

Figure 8 shows the mortality rate of *Anopheles gambiae* SI larvae (stages 3 and 4) after exposure to the drug:

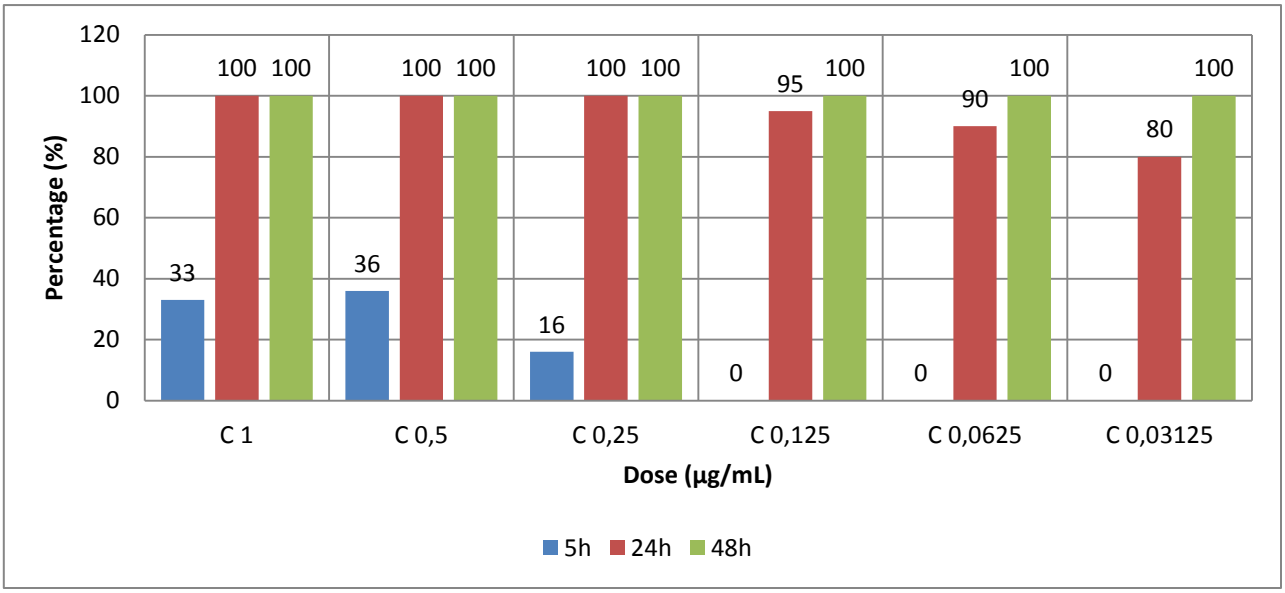

Figure 8: Mortality rate of Anopheles gambiae SI larvae (Stages 3 and 4) after drug exposure.

This figure shows that, whatever the drug dose, the rate of inhibition of larvae by NPAgs is maximal (100%) after 48 hours of exposure. However, we can also see that larval inhibition is dose-dependent for a 24-hour exposure period. From this graph we can deduce that the mortality rate of stage 3 and 4 larvae of *Anopheles gambiae* is exponential with time. Maximum mortality was also observed by Kalimuthu et *al.* [26] and Sareen et *al.* [29] after 48 hours. This anti-larval activity is not selective, since stage 3 and 4 larvae are all sensitive to NPAgs synthesized from *C. citratus*. The ability of NPAgs to eliminate Anopheles larvae is therefore of interest for medical applications, particularly in vector control [30].

4. Conclusion

The aim of the present study was to synthesize silver nanoparticles based on the aqueous extract of *C. citratus* leaves, characterize them and evaluate their antibacterial and anti-larval activity. *C. citratus* contains phytoreducers, notably phenolic compounds such as flavonoids and anthocyanins. The presence of these compounds was confirmed by their ability to reduce ABTS radicals *in vitro*. The NPAgs synthesized have high antibacterial and anti-larval activity. These NPAgs exhibit a characteristic UV-Visible spectrum and a high degree of crystallization purity as indicated by diffractogram and X-ray fluorescence. It is therefore desirable to use scanning electron microscopy to determine the shape of these NPAgs. Infrared spectroscopy (FTIR) will also help identify the chemical functions involved in stabilizing these nanoparticles. The results of this research provide valuable information for the development of new AgNPs-based pharmaceutical for the control of infectious diseases and vectors of medical importance.

Conflict of Interest

All authors declare that they have no conflict of interest in this study.

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