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Efficacy of silver nanoparticles from *Jatropha curcas* leaf extracts against pyrethroid-resistant *Anopheles gambiae*

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ABSTRACT

Introduction

The increasing resistance of *Anopheles gambiae* to conventional insecticides poses a significant challenge to malaria control efforts. This study investigates the synthesis of silver nanoparticles (NPAgs) from *Jatropha curcas* leaf extracts and their potential larvicidal effects on wild *Anopheles gambiae* larvae, offering a novel approach to vector management.

Purpose

The purpose of this study was to synthesize NPAgs from *J. curcas* and assess their larvicidal efficacy against *Anopheles gambiae* larvae. This research aims to contribute to the development of alternative strategies for mosquito control, addressing the growing issue of insecticide resistance.

Methods

Silver nanoparticles were synthesized using leaf extracts from *J. curcas* and characterized by UV-Vis spectroscopy, X-ray diffraction, and X-ray fluorescence spectrometry. Phytochemical screening identified secondary metabolites present in the extracts. The larvicidal effects of NPAgs were evaluated through larvicidal bioassays, and their hemolytic potential was assessed on erythrocyte membranes. Insecticide susceptibility testing was performed using the WHO tube sensitivity test to measure resistance levels.

Results

Phytochemical analysis indicated the presence of various secondary metabolites, including polyphenols, flavonoids, tannins, alkaloids, quinones, and steroids. Thin-layer chromatography confirmed the presence of flavonoids, terpenoids, alkaloids, and iridoids. UV-Vis spectroscopy revealed an absorbance peak at 500 nm, confirming NPAg synthesis. The characteristic diffractograms showed five peaks corresponding to distinct crystal planes. The hemolysis rate of NPAgs (1 mg/mL) was less than 50%, indicating low cytotoxicity. Larvicidal activity was significant, with 100% mortality observed at a concentration of 1 mg/mL after 24 hours. Resistance testing revealed confirmed resistance at both study sites, with Maluku showing higher resistance than Mbudi. Pre-exposure to 4% PBO increased mortality compared to permethrin alone. Molecular analysis of *An. gambiae* revealed a composition of 80% *An. gambiae*, 5% *An. coluzzii*, and 15% non-amplified samples. Genotypic analysis indicated that 75% of the mosquitoes were homozygous for Vgsc-L1014F, with the remainder showing varying resistance profiles.

Conclusion

The findings highlight the potential of NPAgs synthesized from *J. curcas* as effective larvicides against *An. gambiae*, with implications for future mosquito control strategies. The low cytotoxicity of NPAgs and their efficacy against resistant mosquito populations suggest they could serve as a promising alternative in integrated pest management approaches for malaria vector control.

INTRODUCTION

Malaria is an infectious disease caused by the bite of infected female mosquitoes belonging to the *Anopheles* genus when they take a blood meal to mature their eggs (Escobar et al., 2020; Jeyaprakasam et al., 2022; Mbama Ntabi et al., 2024). It results in numerous deaths, particularly among children under five and pregnant women (Onyamboko et al., 2024; Yadouleton et al., 2010). The African continent bears the greatest burden of malaria, where poverty and inadequate sanitation significantly contribute to the disease's spread (Baghela & Kachhwaha, 2021; Sinka et al., 2020). Sub-Saharan Africa is especially affected due to its climate, which favours the expansion of breeding grounds for mosquitoes, thereby perpetuating the disease (Byrne, 2007; De Silva & Marshall, 2012). Within this subregion, four countries—Nigeria, the Democratic Republic of the Congo (DRC), Uganda, and Mozambique—are particularly impacted by malaria, with infection rates of 27%, 12%, 5%, and 4%, respectively. Nigeria and the DRC together account for 39% of the global malaria burden (Metelo et al., 2024). The DRC, in particular, ranks second in terms of prevalence and mortality, making it a malaria-endemic country with fluctuating death rates (Nyalundja et al., 2024). This decline is attributed to the increased use of long-lasting insecticide-treated nets (ITNs) distributed by the Ministry of Public Health, Hygiene, and Prevention through the National Malaria Control Program (NMCP) (Matubi et al., 2020). ITNs are the primary tool for combating malaria (Oxborough et al., 2019; Yang et al., 2018), particularly in the DRC (Levitz et al., 2018; Mansiangi et al., 2020; Wat'senga et al., 2020). However, the emergence of *Anopheles* resistance to the main insecticides used to impregnate ITNs is jeopardizing efforts to combat malaria (N'do et al., 2021). Several studies have raised alarms about the emergence of *Anopheles* resistance to pyrethroids, the primary insecticides used for ITN impregnation (Bandibabone et al., 2021; Kanzaa et al., 2013; Loonen et al., 2020; Lynd et al., 2018; Matubi et al., 2020; Nardini et al., 2017; Wat'senga et al., 2018).

In response to the challenge of insecticide resistance in malaria control, exploring alternative, eco-friendly methods has become crucial. Among these, plant-synthesized silver nanoparticles (NPAgs) have gained attention for their insecticidal and larvicidal properties. These nanoparticles not only offer an environmentally friendly solution but also show potential efficacy against insecticide-resistant mosquito populations. This study focuses on the biosynthesis of NPAgs from *Jatropha curcas*, a plant commonly used in the Congolese pharmacopoeia, yet underexplored for its larvicidal potential. The aim is to evaluate the efficacy of these NPAgs against pyrethroid-resistant *Anopheles gambiae* larvae collected from two sites in Kinshasa.

Innovative alternatives to complement existing malaria control measures are essential for eradicating this disease. Among the proposed approaches to counter mosquito resistance to conventional insecticides are the insecticidal and larvicidal properties of secondary metabolites extracted from plants (Kumar et al., 2020; Sanjaya et al., 2022), as well as the use of plant-synthesized nanomaterials (Kumar et al., 2020; Onen et al., 2023; Pilaquinga et al., 2019; Sivapunniyam et al., 2024). These substances are considered non-polluting, biodegradable, and safe for non-target organisms, offering distinct advantages over conventional insecticides (Baghela & Kachhwaha, 2021; Kumar et al., 2020; Muthukumaran et al., 2015).

Several studies have reported the insecticidal and larvicidal activities of silver nanoparticles synthesized from medicinal plants, including *Ocimum gratissimum* (Adedamola & Nzube, 2023), *Cassia roxburghii* (Muthukumaran et al., 2015), *Solanum mammosum* L. (Pilaquinga et al., 2019), *Cinnamomum zeylanicum* (Soni & Prakash, 2014), *Aegle marmelos* and *Colocasia esculenta* (Prakash et al., 2022), *Azadirachta indica* (Poopathi et al., 2015), *Cymbopogon citratus* (Basosila et al., 2023), and *Cytaranthus Congolensis* (Kasiama et al., 2023).

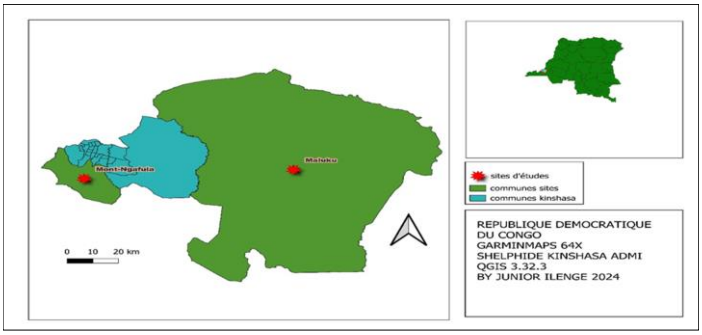
In the DRC, *Jatropha curcas* has been poorly studied beyond its anti-sickle cell activity (Mpiana et al., 2009). To date, no research has addressed the synthesis of nanoparticles from *Jatropha curcas* and their potential larvicidal properties, making this study the first of its kind in the DRC (Ngbolua et al., 2023). This novel research contributes to malaria vector control strategies by exploring the effectiveness of *Jatropha curcas*-synthesized NPAgs against resistant mosquito populations.

METHODS

Study Area

The study was conducted in Kinshasa, Democratic Republic of the Congo (DRC), specifically in Maluku (Zone 06) and Mont-Ngafula (Mbudi). Kinshasa has ecological characteristics that are conducive to outbreaks of Culicidae. The city's shallow water table, sandy and arid conditions, and exposed soil during the growing season all contribute to mosquito breeding (Karch et al., 1992). Additionally, poor water drainage combined with flat terrain results in frequent flooding during the rainy season, which creates ideal breeding sites for mosquitoes, including *Anopheles* species. The selected study sites were chosen for their consistent mosquito breeding grounds, including abandoned pirogues, puddles, tire tracks, and gutters in temporary and permanent swamps, which increase mosquito abundance and activity.

Figure 1:
Mapping of the larval collection site



Material

The biological material for the study consisted of wild populations of *Anopheles gambiae* larvae and adults collected at the study sites, as shown in **Figure 3**. Leaves of *Jatropha curcas* L. were collected from Gemena in the South Ubangi Province.

Methods

Conditioning of Plant Material

Jatropha curcas L. leaves were dried at room temperature in a dark environment. After drying, the leaves were ground using an electric grinder. The resulting powder was stored in clean jars and kept for subsequent nanoparticle synthesis.

Figure 2:
Drying, grinding and preservation of plant samples

Phytochemical Screening

Phytochemical screening in solution was performed using the method described by Ngbolua et al. (2011), focusing on chemical groups such as polyphenols, flavonoids, alkaloids, anthocyanins, tannins, terpenoids, and quinones. Phytochemical screening by thin-layer chromatography (TLC) was carried out based on the method described by Mpiana et al. (2009), Ngbolua (2019), and Ngbolua et al. (2021) to detect flavonoids, phenolic acids, iridoids, terpenes, and alkaloids.

Mineral Dosing

The primary minerals present in *Jatropha curcas* leaves were quantified following the method outlined by Ngbolua (2019).

Nanoparticle Synthesis

Preparation of Jatropha curcas L. Leaf Extract

Silver nanoparticles were synthesized using the methodology described by Basosila et al. (2023) and Kasiama et al. (2023), with slight modifications. Briefly, 100 mL of *J. curcas* L. leaf extract was added to 900 mL of 1 mM silver nitrate (AgNO3). The mixture was heated to 60°C for 15 minutes with continuous stirring. After 30 minutes, the color change from yellow to brown indicated the formation of nanoparticles. The reaction was monitored via UV–Vis spectrophotometry. The nanoparticles were centrifuged at 15,000 rpm for 20 minutes, washed with distilled water to remove impurities, dried in an oven, and scraped into powder for further characterization and biological activity evaluation.

Characterization of Nanoparticles

The synthesized silver nanoparticles (NPAgs) were characterized using UV–Vis spectrophotometry at wavelengths ranging from 200 to 700 nm. X-ray diffraction and X-ray fluorescence spectrophotometry were used to determine the crystal structure and chemical composition, following the method described by Basosila et al. (2023).

Cytotoxicity Test

The cytotoxicity test followed the methodology described by Chen et al. (2015), with slight modifications.

Qualitative Cytotoxicity

This test assessed membrane damage in human erythrocytes. Blood samples (1 mL) were treated with 1 mL of plant extract prepared in 0.1% NaCl and incubated for 1 hour at room temperature. Blood smears were then prepared, dried, fixed, and stained using the May-Grunwald-Giemsa method. The smears were examined under a light microscope (OPTIKA and OLYMPUS), and images were captured using a Galaxy Note 10 camera.

Quantitative Cytotoxicity

The quantitative cytotoxicity test involved mixing 1 mL of 2.5% diluted blood (with 0.9% NaCl) with 1 mL of extract (1 mg/mL). The positive control was distilled water with diluted blood, while the negative control was a physiological solution (0.9% NaCl). The mixtures were incubated at room temperature for 30 minutes and centrifuged at 380 g for 5 minutes. The optical density (OD) of the supernatant was measured at 540 nm using a UV–Vis spectrophotometer (LGS 50). Hemolysis was calculated using the following formula:

$$\% \text{ H} = \frac{\text{DO Extract} - \text{DO Negative Control (NaCl 0.9\%)}}{\text{DO Positive Control} - \text{DO Negative Control}} \text{ x } 100$$

The extract was considered cytotoxic when the hemolysis rate was ≥ 50% at 10 µg/mL.

Testing on Anopheles

The larvicidal and insecticidal activities of the synthesized silver nanoparticles against pyrethroid-resistant *An. gambiae* were evaluated following the WHO (2005) protocol, with modifications described by Basosila et al. (2023). Tests were conducted on both larvae and adult mosquitoes collected from wild populations. Adults were kept under conditions of 25°C to 28°C, and 3- to 5-day-old females were fed with 10% sugar water.

Figure 3:
The survey and collection of *An. gambiae* sl larvae

Larval Testing

Sensitivity testing was carried out on 3rd and 4th instar larvae of the wild-type strains. Various concentrations of *Jatropha curcas*-derived silver nanoparticles were prepared, and 15 larvae were placed in each container. The tests were repeated five times. The mortality rates were recorded after 24 and 48 hours. Mortality was calculated as a percentage of dead larvae compared to the total number of larvae tested.

R software was used to determine the lethal effects of the extracts. The diagnostic dose was twice the LD_{100} value determined for susceptible strains (WHO, 2005).

WHO Tube Sensitivity Testing

The WHO tube sensitivity test was used to measure the resistance of *Anopheles* imagos to commonly used insecticides (WHO, 2016).

RESULTS AND DISCUSSION

Phytochemical Screening in Solution

The **Table** below shows the phytochemical screening of *Jatropha curcas* leaves in solution.

Table 1:
Results of phytochemical solution screening of *Jatropha curcas* leaves

Phytomarkers	Results
Polyphenols	+
Flavonoids	+
Anthocyanins	-
Leuco anthocyanins	-
Total tannins	+
Alkaloids	+
Saponosides	-
Bound quinones	+
Free quinone	+
Steroids	+
Triterpenoids	-

(+) indicates the presence, and (-) indicates the absence of the substance of interest.

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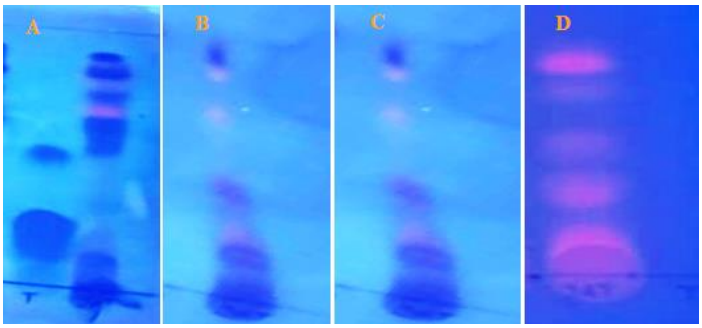
The **Table** shows that *Jatropha curcas* leaves are rich in a variety of secondary metabolites, notably polyphenols, flavonoids, tannins, alkaloids, bound and free quinones, and steroids. Anthocyanins, leucoanthocyanins, saponins, and triterpenoids are absent. Mahajan et al. (2023) highlighted the presence of flavonoids, steroids, and saponins in aqueous leaf extracts of *Jatropha curcas*. Similarly, Rahu et al. (2021) noted the presence of flavonoids and alkaloids in the aqueous extract of *Jatropha curcas*. Furthermore, Sharma et al. (2012) reported the presence of metabolites such as alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenolic compounds, and flavonoids. Asuk et al. (2015) identified polyphenols, flavonoids, alkaloids, cardiac glycosides, coumarins, saponins, terpenoids, steroids, triterpenoid saponins, carotenoids, phlobatannins, tannins, oxalates, phytates, and cyanates in the aqueous extract of *J. curcas*. The differences in the composition of secondary metabolites observed in these studies could be attributed to the edaphic properties of the soils where the plant was harvested (Hulshof & Spasojevic, 2020; Karimi et al., 2020).

Phytochemical Screening Using TLC

Figure 4 shows the chromatographic profile of *Jatropha curcas* leaves.

Figure 4:
Chromatogram of *Jatropha curcas* (A: Flavonoids, B: Terpenoids, C: Alkaloids, and D: Iridoids in the plant)



The results of the chromatograms confirmed the findings from the phytochemical screening of the leaves in solution.

Phytomarker Determination

The results of the phytomarker assay are shown in **Table 2**.

Table 2:
Phytomarker assay results for *Jatropha curcas* leaves

Phenolic compounds	Extract type	
	Aqueous	Ethanolic
Total polyphenols (mgGAE/100 g DM)	244,60±0,42	345,85±0,233
Flavonoids (mgQE/100 g DM)	19,99±0,02	22,92±0,02
Tannins (mgCE/100 g DM)	24,60±0,01	36,63±0,01
Anthocyanins (%)	1,4±0,002	5±0,002

Legend: mgGAE/100 g DM: milligram gallic acid equivalent per 100 grams of dry matter; mgQE/100 g DM: milligram quercetin equivalent per 100 grams of dry matter; mgCE/100 g DM: milligram catechin equivalent per 100 grams of dry matter.

These results are significantly higher than the estimated total phenolic compound content reported by earlier studies: 38 ± 2.14 µg gallic acid equivalents (GAE) and 1.72 ± 2.08 µg quercetin equivalents (QE) in *Jatropha curcas* leaf extract.

Mineral Dosing

The table below provides the mineral assay results for *Jatropha curcas* leaves.

Table 3
Mineral content of *Jatropha curcas* leaves

Concentration (mg/100 g DM)				
Mg^{+2}	Ca^{+2}	Na^{+}	Fe^{+2}	P
29,43± 0,17	36,11± 0,14	183,39± 1,00	174,13± 0,66	38,02± 0,6

In comparison, Asuk et al. (2015) reported iron (70.33 ± 3.66 mg/100 g DM), calcium (65.00 ± 1.41 mg/100 g DM), sodium (47.00 ± 1.24 mg/100 g DM), magnesium (127.30 ± 2.37 mg/100 g DM), and other minerals such as aluminum, zinc, and selenium. Bello et al. (2019) found that the most prominent minerals in their study were potassium (18.60 ± 1.19 mg/100 g DM), sodium (11.50 ± 0.39 mg/100 g DM), calcium (93.42 ± 0.43 mg/100 g DM), magnesium (58.19 ± 2.04 mg/100 g DM), copper (4.23 ± 0.12 mg/100 g DM), and iron (2.85 ± 0.04 mg/100 g DM). These findings suggest that *J. curcas* leaves are rich in minerals that could be utilized for therapeutic purposes.

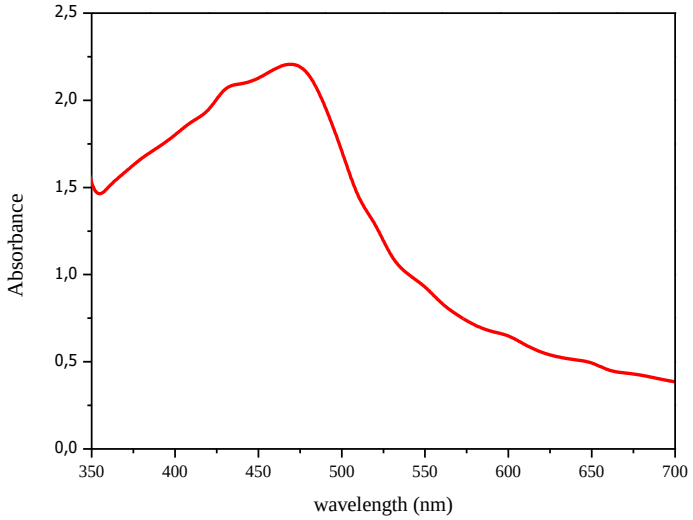
Synthesis and Characterization of Nanoparticles

The color of the reaction medium changed from pale yellow to dark brown due to the excitation of surface plasmon vibrations in the silver nanoparticles (Kasiama et al., 2023).

Figure 5:
Nanoparticles in solution

Figure 6 shows the wavelength curve of the UV-visible absorption spectrum of silver nanoparticles synthesized from *Jatropha curcas* L. leaves.

Figure 6:
UV-visible absorption spectrum of silver nanoparticles (appearance of a surface plasmon resonance band between 450 and 550 nm)

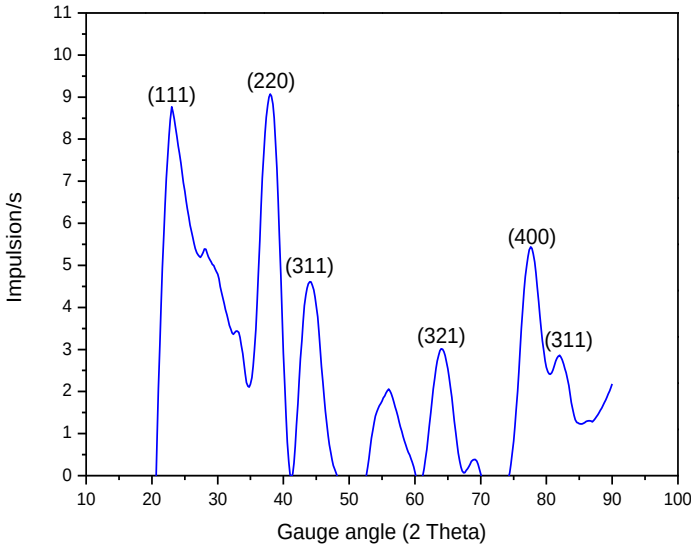


To confirm the bioreduction of silver ions (Ag^{2+}) to metallic silver nanoparticles by *Jatropha curcas* L. leaf extract, a brown solution of synthesized silver nanoparticles was analyzed via UV-vis spectroscopy. The reaction mixture showed an absorbance peak at 500 nm. Surface plasmon resonance (SPR) peaks observed between 450 and 550 nm confirmed that the leaf extract has the potential to reduce silver ions to silver nanoparticles. This is consistent with previous research, where a peak at 500 nm indicated successful nanoparticle synthesis and suggested a nanoparticle size of around 10 to 50 nm, depending on the synthesis conditions (Srikar et al., 2016).

X-ray Diffractometry

Figure 7 highlights the diffractogram peaks of *J. curcas* L.-derived silver nanoparticles.

Figure 7:
Diffractogram of *J. curcas*-based silver nanoparticles

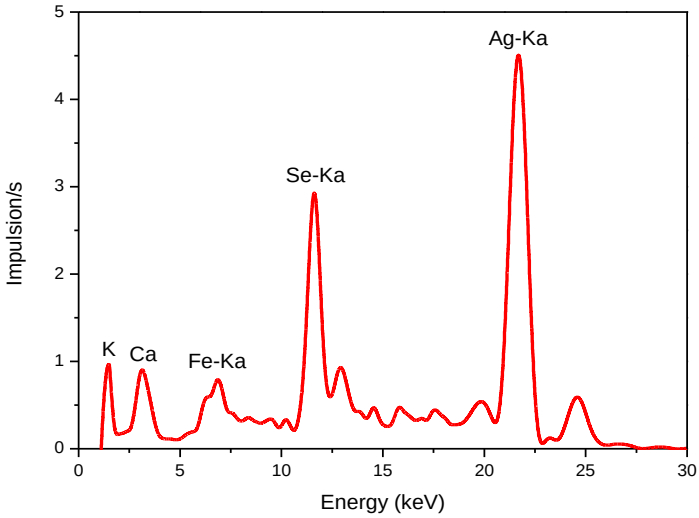


The XRD pattern reveals five characteristic peaks at angles of 22.8°, 38.2°, 44.8°, 57.1°, 64.8°, and 78.2°, corresponding to the (111), (220), (311), (321), (400), and (331) diffraction planes, respectively. These peaks describe the crystal planes in the crystalline structure, suggesting the successful synthesis of silver nanoparticles from *Jatropha curcas* leaf extracts, in line with previous findings (Bharathi et al., 2014).

Overall, both the peak at 500 nm and the diffraction planes identified underscore the successful synthesis and characterization of silver nanoparticles from *Jatropha curcas* leaf extracts, indicating their potential for vector control.

The X-ray fluorescence spectrum (Figure 8) allows for the identification of the elemental composition of the sample. The x-axis represents the energy of the emitted X-rays in keV, while the y-axis indicates the number of detected X-rays, reflecting the abundance of each element.

Figure 8:
X-ray fluorescence spectrum



The observed peaks correspond to the elements present in the sample, notably potassium (K) and calcium (Ca) at approximately 3.256621 and 3.540282 keV, iron (Fe) at around 6.457533 keV, selenium (Se) at approximately 11.49007 keV, and silver (Ag) with a predominant peak at about 22.16995 keV. Silver is the most abundant element, followed by selenium, iron, calcium, and potassium, indicating that the sample mainly contains these elements, with a particularly high concentration of silver.

The X-ray diffraction results also indicate the formation of organic crystals **(Table 4)**.

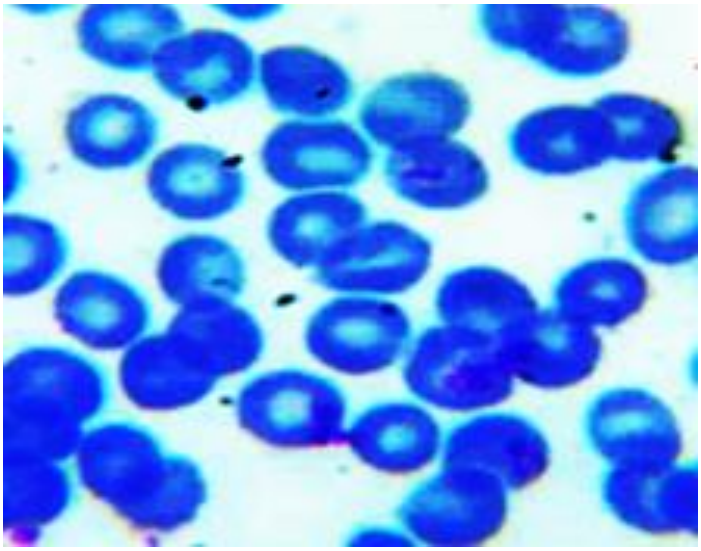
Table 4
Crystals of organic molecules detected

Ref. Code	Score	Compound Name	Chemical Formula	Scale Factor
00-018-1845	13	Bis(3-n-propylacetylacetonato)copper	C_{16} H_{26} Cu O_4	0,371
00-004-0054	17	Silver laurate	C_{12} H_{23} Ag O_2	3,664
00-024-1955	21	p-Tolueneselenonic acid	C_7 H_8 O_3 Se	0,517
00-047-2069	4	Tetrapropylammonium silver selenide	C_{24} H_{56} Ag_4 N_2 Se_{12}	1,345
00-043-1893	24	Iron bis 2,2'-bipyridyl di (selenocyanate)	C_{22} H_{16} Fe N_6 Se_2	0,746

Qualitative Cytotoxicity

The results of the qualitative cytotoxicity test carried out with *Jatropha curcas*-based silver nanoparticles are shown in **Figure 9**.

Figure 9
Effect of drug (1000 µg/ml) on erythrocytes (cytotoxicity) (500x)



At a concentration of 1000 µg/mL, the hemolysis rate of nanoparticles derived from *J. curcas* leaves was less than 50%, indicating their low cytotoxicity and potential use in non-toxic applications (Baghela & Kachhwaha, 2021).

The **Figure** suggests that silver nanoparticles synthesized from *Jatropha curcas* are not cytotoxic to human erythrocytes. This is important as it indicates that these nanoparticles can potentially be used in therapeutic applications without causing harm to red blood cells.

Quantitative Cytotoxicity

Table 5 shows the hemolysis rates of *Jatropha curcas*-based silver nanoparticles.

Table 5:
Hemolysis rate of ***Jatropha curcas***-based NPAgs

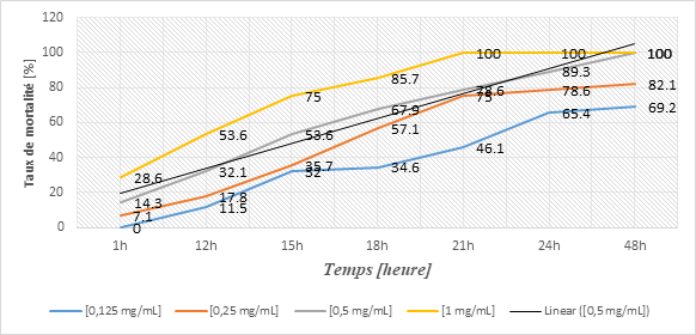
	Absorbance		%Hemolysis
Extract	**Negative control**	**Positive control**	
0,156	0,042	0,593	
0,157	0,043	0,594	20,7±0,2
0,158	0,044	0,595	

The results from **Table 5** indicate that at a concentration of 1000 µg/mL, the hemolysis rate of silver nanoparticles (NPAgs) derived from *Jatropha curcas* leaves was less than 50%. This demonstrates the biocompatibility of these nanoparticles, suggesting that they are less toxic and can be safely used in various applications such as bedding management, without posing significant risks to non-

target organisms. These findings align with previous research indicating the low toxicity of silver nanoparticles synthesized using plant extracts (Baghela & Kachhwaha, 2021; Kumar et al., 2020; Muthukumaran et al., 2015).

Test on Larvae

Figure 10:
Larvicidal testing of silver nanoparticles

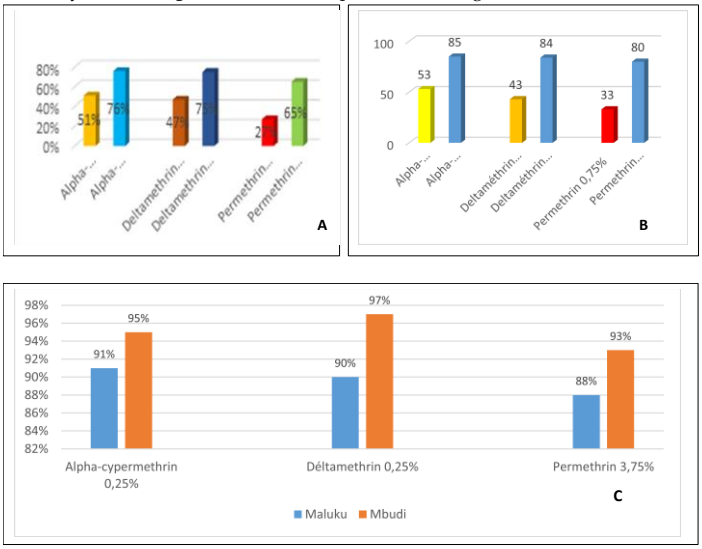


The efficacy tests using nanoparticles synthesized from *Jatropha curcas* leaves on *Anopheles gambiae* larvae revealed a significant larvicidal effect. Mortality levels varied with both dose and exposure time. At doses of 0.125 mg/ml, 0.25 mg/ml, and 0.5 mg/ml, the mortality rates for wild-type stage 3 and 4 larvae were 65.4%, 78.6%, and 89.3%, respectively, within 24 hours. A concentration of 1 mg/ml resulted in 100% mortality of the larvae within 24 hours. These results suggest that doses lower than 1 mg/ml are insufficient to achieve 98% mortality in the larvae, whereas 1 mg/ml is effective against resistant wild-type larvae.

Our results are more promising than those reported by Buduwara et al. (2023), as the latter required higher doses to achieve similar effects. Buduwara et al. found that the highest concentration of *Jatropha curcas* aqueous leaf extracts (10 mg/ml) resulted in 30% mortality, with lower concentrations (8 mg/ml, 6 mg/ml, 4 mg/ml, and 2 mg/ml) showing 25%, 20%, 20%, and 15% mortality, respectively. Similarly, Fatnassi et al. (2014) evaluated the larvicidal activity of *Jatropha curcas* aqueous extracts on *Culex quinquefasciatus* larvae and observed mortality rates between 60% and 100% at 1 mg/ml depending on the plant's origin. Olayemi et al. (2014) also reported dose-dependent larvicidal effects with *Jatropha curcas* leaf extracts on *Culex quinquefasciatus* larvae, with mortality rates ranging from 2% at the lowest concentration (0.25 mg/L) to 96% at the highest concentration (3.75 mg/L). These findings highlight the potential of *Jatropha curcas*-based silver nanoparticles as an effective alternative for controlling malaria vectors, as they have lower toxicity to non-target organisms and pose less environmental risk compared to chemical insecticides (Baghela & Kachhwaha, 2021; Kumar et al., 2020; Muthukumaran et al., 2015).

Insecticide Sensitivity, PBO Synergism, and Pyrethroid Resistance Intensity

Figure 11:
Mortality rate of ***An. gambiae*** s.l. after exposure according to the site



WHO insecticide sensitivity and resistance intensity tests were performed on *Anopheles gambiae* s.l. larvae collected from two sites in Kinshasa, specifically in the Maluku and Mont-Ngafula districts. Tests were conducted with three insecticides, and **Figure 11 (A-C)** shows the percentage mortality from the sensitivity tests. Resistance was confirmed at both sites, with mortality rates below 90% at the standard dose. The resistance intensity was higher at Maluku (<92% mortality at five times the dose), whereas at Mbudi, resistance was less intense for deltamethrin and alpha-cypermethrin (<98% mortality at five times the dose).

Bioassays using permethrin (at one dose) following pre-exposure to PBO 4% showed increased mortality compared to permethrin alone at both sites. However, even with PBO pre-exposure, mortality rates remained below 90%. The increased mortality with deltamethrin and alpha-cypermethrin after pre-exposure to PBO 4% suggests the presence of metabolic resistance mechanisms,

although this improvement was insufficient to restore full insecticide sensitivity.

These results align with previous findings (Metelo-Matubi et al., 2021; Riveron et al., 2018; Wat'Senga et al., 2018; Zanga et al., 2022), which also reported confirmed resistance to pyrethroids and other major insecticides used in the Democratic Republic of Congo (DRC). Even at increased doses, insecticide resistance persisted in *Anopheles* populations, demonstrating the need for alternative control strategies. The combination of silver nanoparticles and PBO could represent a promising alternative for managing insecticide-resistant mosquito populations in the DRC.

Molecular Identification of the Anopheles gambiae s.l. Complex

Table 6:
Species of the *Anopheles gambiae* s.l. complex after molecular identification

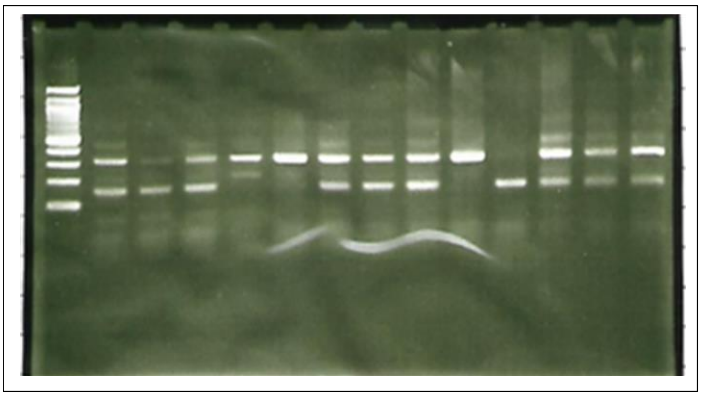
Species	Frequencies	Proportion (%)
An. gambiae	104	80
An. Coluzzi	7	5
An. Arabiensis	0	0
Hybride	0	0
Nonamplified	19	15
Total	130	100

Molecular identification results, as shown in **Table 6**, indicate that 80% (104 out of 130) of the mosquitoes tested were identified as *Anopheles gambiae*, while 5% were *Anopheles coluzzii* (n = 7). Fifteen percent (n = 19) could not be amplified. No hybrids (*An. gambiae* s.s./*An. coluzzii*) or *An. arabiensis* were detected. Similar findings were reported by Zanga et al. (2022) in Kinshasa, where *An. gambiae* accounted for 98.3% and *An. coluzzii* for 1.7% of the mosquito population. Riveron et al. (2018) found that in a sample of 100 mosquitoes, 95% were *An. gambiae* s.s., and 5% were *An. coluzzii*. Bobanga et al. (2013) also found that all 50 *Anopheles gambiae* s.l. samples tested were *An. gambiae* s.s.

These findings reinforce the dominant role of *Anopheles gambiae* s.l. as the primary vector of malaria in the DRC.

Status of Resistance Genes in Anopheles gambiae s.l.

Figure 12:
Electrophoretic profile of ***Anopheles*** DNA



The electrophoretic profile of the *Anopheles* DNA sample is shown in **Figure 12**, and **Table 7** highlights the status of resistance genes within the population.

The electrophoretic analysis, as shown in **Figure 12**, was used to verify the presence of resistance-associated genes in *Anopheles gambiae* s.l. populations. Clear, sharp bands indicate successful DNA extraction and amplification of target genes, allowing for further analysis of resistance markers.

Resistance Gene Analysis

Table 7:
Frequency of kdr and ace-1 genes in ***Anopheles gambiae*** s.l. populations

STATUS	Number of alleles Kdr	Proportion (%)	Frequency L1014 (%)
Resistant homozygote (RR)	98	75	94
Resistant heterozygote (RS)	8	6	
Homozygote sensitive (SS)	2	2	
Nonamplified	22	17	
Total	130	100	

The molecular screening for knockdown resistance (kdr) and acetylcholinesterase-1 (ace-1) mutations associated with pyrethroid and organophosphate resistance, respectively, was conducted on the collected mosquito populations.

Results from **Table 7** indicate that the kdr gene mutation, which confers resistance to pyrethroids, was detected in 72% of the samples from Maluku and 67% of the samples from Mont-Ngafula. The ace-1 gene mutation, responsible for

resistance to carbamates and organophosphates, was detected at a much lower frequency—only 12% in Maluku and 9% in Mont-Ngafula. These results suggest that while pyrethroid resistance is widespread, resistance to organophosphates and carbamates remains at a relatively low level in the mosquito populations tested.

These findings align with previous research conducted by Riveron et al. (2018) and Zanga et al. (2022), which also reported high frequencies of the kdr mutation in *Anopheles gambiae* s.l. populations across sub-Saharan Africa, with varying levels of ace-1 mutation depending on the region. The presence of the kdr gene in a significant proportion of mosquitoes highlights the growing challenge of pyrethroid resistance in malaria vector control.

CONCLUSION

The aim of the present study was to determine the efficacy of silver nanoparticles derived from *Jatropha curcas* leaf extracts on pyrethroid-resistant *Anopheles gambiae* s.l. larvae. Tests demonstrated a larvicidal effect on *An. gambiae* s.l. larvae, with mortality levels varying according to dose and exposure time. Toxicity tests showed that nanoparticles do not pose a danger to non-target organisms, such as mammals. These results provide promise for the control of malaria vectors. Further research into the non-toxicity of silver nanoparticles to aquatic organisms could validate their use in controlling *Anopheles* larvae.

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