



Research article



Exploring the larvicidal and repellent potentials of silver nanoparticles greenly synthesized using three Congolese plant extracts against *Anopheles gambiae* along with molecular docking analysis

Eunice N. Zola^{a,b,1}, Claude Josué K. Muanga^{a,b,1} , Joseph Kyana^{a,b,1}, Miryam M. Luzala^{a,b}, Sebastien N. Wendji^{a,b}, Clément M. Tshimanga^c, Justin B. Safari^{d,e} , Victoire Nsabatien^f, Josué Zanga^f, Emery M. Metelo^f, Yannick B. Nuapia^{c,g}, Patrick B. Memvanga^{a,b,*}

^a Laboratory of Pharmaceutics and Phytopharmaceutical Drug Development, Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa B.P. 212, Congo

^b Centre de Recherche et d'Innovation Technologique en Environnement et en Sciences de la Santé (CRITESS), Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa B.P. 212, Congo

^c Toxicology and Food Hygiene laboratory, Faculty of Pharmaceuticals Sciences, University of Kinshasa, Kinshasa B.P. 212, Congo

^d Department of Pharmacy, Faculty of Pharmaceutical Sciences and Public Health, Official University of Bukavu, Bukavu B.P. 570, Congo

^e Department of Chemistry, Faculty of Science, Rhodes University, P.O. Box 94, Makhanda 6140, South Africa

^f Laboratory of Bioecology and Vector Control (BIOLAV), Department of Environmental Health, Kinshasa School of Public Health, Kinshasa B.P. 212, Congo

^g Pharmacy Department, School of Healthcare Sciences, University of Limpopo, Turfloop Campus, Polokwane 0727, South Africa

ARTICLE INFO

Keywords:

Larvicidal activity
Repellent activity
Molecular docking
Anopheles gambiae
Green-synthesized nanoparticles

ABSTRACT

Chemical larvicides and repellents have long been used to combat *Anopheles gambiae*, the primary malaria vector. However, their prolonged application has raised significant concerns regarding environmental toxicity, human health risks, and the emergence of resistant mosquito populations. This study presents a sustainable alternative consisting of green-synthesized silver nanoparticles (AgNPs) derived from *Lippia multiflora*, *Ocimum gratissimum*, and *Tetradenia riparia*. These nanoparticles were characterized using several techniques, including UV–visible spectrophotometry, transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX) and Fourier transform infrared (FTIR) spectroscopy, revealing their spherical structure with diameters of 20–50 nm and stabilization by plant secondary metabolites. Aqueous suspensions of AgNPs derived from these three plants demonstrated dose-dependent larvicidal efficacy, achieving up to 95% mortality, while creams containing AgNPs from *Lippia multiflora* and *Ocimum gratissimum* exhibited an impressive 85% repellent efficiency. Molecular docking studies revealed that secondary metabolites used as capping agents enhanced larvicidal activity by inhibiting angiotensin converting enzyme of *Anopheles gambiae* (AnoACE2), further demonstrating the synergistic role of these metabolites in stabilising and boosting AgNPs efficacy. These findings highlight the potential of green-synthesized AgNPs as an eco-friendly and effective alternative to conventional chemical larvicides and repellents, addressing a pressing global need for sustainable mosquito control strategies. By leveraging the bioactivity of plant-derived compounds, this approach minimises environmental and health risks while offering high efficacy against malaria vectors. This research underscores the significant role of green nanotechnology in developing innovative solutions to vector control, paving the way for its integration into holistic malaria prevention programs and the fight against other mosquito-borne diseases.

1. Introduction

Mosquito-borne diseases (MBDs) are among the most significant

global public health challenges, contributing to over 700 million cases annually and severe disease outbreaks [10,49]. Climate change has further exacerbated this issue by altering temperatures and precipitation

* Corresponding author at: Laboratory of Pharmaceutics and Phytopharmaceutical Drug Development, Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa B.P. 212, Congo.

E-mail address: patrick.memvanga@unikin.ac.cd (P.B. Memvanga).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.nxnano.2026.100415>

Received 16 January 2025; Received in revised form 15 April 2025; Accepted 2 March 2026

Available online 6 March 2026

2949-8295/© 2026 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

patterns, thereby expanding the geographic distribution, seasonality, and prevalence of vector populations [35]. As primary vectors, mosquitoes transmit life-threatening diseases such as malaria, lymphatic filariasis and yellow fever [32]. Malaria remains endemic in tropical regions, with the highest burden concentrated in countries like Nigeria, the Democratic Republic of the Congo (DRC), Tanzania, and Niger [32]. Vulnerable populations, including children under five and pregnant women, are disproportionately affected, resulting in severe socio-economic consequences [10,53].

Among mosquito species, *Anopheles gambiae* is a key vector for malaria in tropical regions, thriving in areas with poor sanitation and stagnant water, which serve as ideal breeding grounds [3,35]. While traditional mosquito control strategies include chemical, mechanical, and biological approaches [32], the widespread reliance on chemical insecticides has caused significant ecological and health concerns [40]. These insecticides disrupt mosquito metabolic pathways, affecting their hormonal and neurological systems [28]. However, overuse has led to the emergence of resistant mosquito populations, diminishing the efficacy of these chemicals and allowing vector resurgence [28]. Additionally, the prolonged use of insecticides poses toxic risks to humans and the environment, underscoring the urgent need for effective, affordable, and environmentally sustainable alternatives [10,28].

The transmission of MBDs is primarily driven by mosquito bites, which can also trigger allergic reactions such as irritation, urticaria, and angioedema [14]. Personal protection using repellent agents is a critical preventive measure [32]. While DEET (N,N-diethyl-m-methylbenzamide) is widely used as an effective insect repellent, recent studies have raised concerns about its potential toxicity, highlighting the need for safer, plant-based alternatives [14]. In alignment with the World Health Organization's (WHO) recommendations to explore medicinal plants [52], communities in malaria-endemic regions have increasingly turned to plants with recognized mosquitocidal and larvicidal properties [26]. Notably, *Lippia multiflora* (LI-MU), *Ocimum gratissimum* (OC-GR), and *Tetradenia riparia* (TE-RI) have been traditionally used in Congolese medicine for their secondary metabolites with potent larvicidal and repellent effects [26,29]. However, achieving effective larvicidal and repellent activity with these plants often requires high concentrations of aqueous extracts, presenting practical limitations [38].

Numerous studies have also reported the insecticidal and repellent potentials of essential oils of LI-MU, OC-GR, and TE-RI. For example, monoterpenes of LI-MU like thymol and p-cymene have shown potent larvicidal activity against *Anopheles*, *Aedes*, and *Culex* species, achieving mortality rates exceeding 90% at concentrations below 100 ppm [7,24]. Similarly, OC-GR, known for its high eugenol content, has demonstrated strong repellent and larvicidal properties, often comparable to DEET [38]. On the other hand, TE-RI contains fenchone and various diterpenoids with documented larvicidal activity and neurotoxic effects on mosquitoes [12]. However, most of these investigations have primarily focused on essential oils, which are rich in volatile monoterpenes and sesquiterpenes [16]. Despite their efficacy, essential oils require energy-intensive extraction methods and large quantities of plant material, and their hydrophobic nature limits their applicability in aqueous formulations [32].

To address these challenges, green nanotechnology has emerged as a promising approach for developing safe, effective, and low-cost larvicides as well as repellents [32]. Current developments in this field focus on harnessing a variety of biological sources (e.g. plants, fungi, bacteria, and algae) as both reducing and stabilizing agents in metallic nanoparticle formation [41,54]. This approach continues to evolve toward more sustainable and efficient techniques, utilizing diverse biological systems to generate metallic nanoparticles. Unlike microbes, plants pose no risk of pathogenic contamination, making handling safer. Additionally, plants (e.g. leaves) are widely available and renewable, supporting sustainable nanoparticle production [17,23].

Metallic nanoparticles, particularly those synthesized using plant

extracts containing silver, zinc, or copper, leverage the intrinsic medicinal properties of these metals and the bioactivity of plant-derived secondary metabolites. Among metallic nanoparticles, silver nanoparticles (AgNPs) have shown the most promise due to their inherent antimicrobial properties and proven efficacy against various mosquito strains [11,32,45] [9,17]. The green synthesis of AgNPs using plant materials is particularly attractive due to its eco-friendly nature and potential for tailoring nanoparticle properties via phytochemicals such as flavonoids, polyphenols, terpenoids, and alkaloids [41,54].

Although many studies related to insecticidal AgNPs have focused on alcohol- and organic-based extracts for nanoparticle synthesis [32], aqueous extracts despite being safer, more accessible, and pharmaceutically relevant have been widely underexplored in green nanotechnology for mosquito control, representing an untapped resource in vector control and a sustainable alternative to synthetic insecticides. Indeed, aqueous extracts more representative of traditional medicinal usage are abundant in hydrophilic phytochemicals aforementioned that not only possess antioxidant and enzyme-inhibiting properties but are also environmentally benign and better suited for green synthesis applications of AgNPs [9]. The dual role of aqueous extracts as reducing/capping agents and bioactive compounds presents a compelling opportunity for eco-conscious vector control strategies.

The present study addresses this gap by utilizing aqueous extracts of LI-MU, OC-GR, and TE-RI for the green synthesis of AgNPs and evaluating their combined larvicidal and repellent efficacy against *Anopheles gambiae* mosquitoes. Molecular docking studies were conducted to elucidate the possible mechanism of action, targeting the angiotensin-converting enzyme of *Anopheles gambiae* (AnoACE2). Therefore, this study proposes a novel ligand-receptor-based approach to developing effective, sustainable, and eco-friendly larvicides and repellents by combining green synthesis methods and molecular docking insights.

2. Materials and methods

2.1. Materials

The fresh leaves of LI-MU, OC-GR and TE-RI were collected in Kinshasa, DRC. These plants were identified at the herbarium of Institut National d'Etudes et des Recherches Agronomiques (INERA) of the University of Kinshasa. The leaves were washed with distilled water, then air-dried away from light at room temperature for 10 days and crushed in fine powder using an electric grinder (Damai, Zhejiang, China). Silver nitrate ($\geq 99.7\%$) was purchased from Minema Chemicals (Johannesburg, South Africa). Glycerin, sodium lauryl sulfate, petroleum jelly and liquid paraffin were purchased from Fisher Scientific (Leicestershire, United Kingdom).

2.2. Methods

2.2.1. Preparation of leaf extracts

Five grams of LI-MU, OC-GR and TE-RI were boiled in 100 ml of distilled water for 5 min. The mixtures were then filtered using 90 mm filter paper (Boeco, Germany). The resulting filtrates were utilised as reducing and capping agents for the synthesis of AgNPs.

2.2.2. Green synthesis of AgNPs

One millilitre of aqueous extract from each plant was mixed with 5 ml (for LI-MU and OC-GR) or 9 ml (for TE-RI) of 5 mM silver nitrate solution. The mixtures were incubated at room temperature for 48 h. After incubation, the solutions were centrifuged at 2000 rpm for 10 min using an Allegra® X-15R Centrifuge (Beckman Coulter, California, USA), and the resulting pellets were collected and dried in an oven (Binder, Germany) at 50°C for 48 h.

2.2.3. Characterization of green-synthesized AgNPs

The optical properties of the green-synthesized AgNPs were analysed

using UV-Visible spectrophotometry (Agilent, California, USA). Spectra were recorded in the wavelength range of 200–800 nm with a resolution of 1 nm, using distilled water as the blank [23,45].

The morphology and size of the AgNPs were examined using transmission electron microscopy (TEM) (Zeiss Libra-120 KV TEM microscope, Carl Zeiss, Oberkochen, Germany). Diluted samples were applied onto carbon-coated copper grids, and excess liquid was removed with filter paper. The grids were air-dried at room temperature before particle visualization [17,23].

The elemental composition of the AgNP surfaces was investigated using energy-dispersive X-ray spectroscopy (EDX) with an INCAPENTA FET connected to a VAGA TESCAME system (Libusina, Czech Republic). EDX spectra were recorded at an operating voltage of 20 kV [17,23,45].

Functional groups present on the AgNPs surfaces were identified using Fourier transform infrared (FTIR) spectroscopy with a Perkin Elmer Spectrum 100 FT-IR spectrophotometer (Massachusetts, USA) employing the attenuated total reflection method. For each experiment, 16 scans were performed in the frequency range from 650 to 4000 cm^{-1} [17,23,45]. In order to assess their crystallinity, powder samples of the green synthesized AgNPs were analyzed at room temperature using an X-ray diffractometer (XRD) (Bruker D8 Discover XRD, Berlin, Germany). The instrument operated with nickel-filtered Cu K α radiation ($\lambda = 1.5404 \text{ \AA}$) at a voltage of 40 kV and a current of 30 mA [23].

2.2.4. Mosquito larvicidal assay

The larvicidal activity of the eco-friendly synthesized AgNPs was evaluated against *Anopheles gambiae* larvae. Laboratory-bred *Anopheles gambiae* (Kisumu strain) were obtained from the Laboratoire de Bio-écologie et Lutte Antivectorielle, École de Santé Publique, University of Kinshasa, DRC. Wild *Anopheles gambiae* larvae were collected during the rainy season from stagnant water sites in Selembao, Lemba, and Maluku Townships (Kinshasa, DRC) using Pasteur pipettes. The larvae were transported to the laboratory, reared in dechlorinated tap water, and fed daily with finely ground fish food. They were maintained at $25 \pm 3^\circ\text{C}$ with a relative humidity of $75 \pm 5\%$ and a 12:12-hour light/dark cycle [17].

The larvicidal activity was assessed following the World Health Organization's protocol with minor modifications [50]. Groups of 20 third- and fourth-instar larvae were exposed to 100 ml of AgNPs suspensions at concentrations ranging from 50 to 400 ppm. Temephos (Abate®) at concentrations ranging from 75 to 100 ppm and distilled water served as positive and negative controls, respectively. The larvae were kept at $25 \pm 3^\circ\text{C}$ for 24 h. Dead larvae were counted, and the percentage of mortality was calculated from the average of three replicates using Eq. (1).

$$\text{Percentage of mortality} = (\text{Number of dead larva} / \text{Number of larva introduced}) \times 100 \quad (1)$$

2.2.5. Repellence assay

The repellence assay of AgNPs incorporated in oil-in-water creams was conducted following the WHO's protocol, commonly known as the arm-in-cage method [51].

2.2.5.1. Preparation of creams loaded with AgNPs. The formulation of the repellent creams was adapted from the procedure described by Hazarika et al. [20]. The quantities of ingredients used to prepare the water-in-oil creams are detailed in Table 1. The aqueous phase, comprising glycerin, sodium lauryl sulfate, and water, was heated to 70–80°C. Simultaneously, petroleum jelly and liquid paraffin, forming the oil phase, were melted to the same temperature. After transferring the oil phase to a mortar, AgNPs were incorporated into the mixture. Preservatives (methyl paraben and propyl paraben) were added to the water phase before the two phases were combined. The aqueous and oil

Table 1

Qualitative and quantitative composition of oil-in-water creams loaded with green-synthesized AgNPs.

Ingredients	Quantity (g)							
	F1	F2	F3	F4	F5	F6	F7	F8
AgNPs from LI-MU	5	10	20	-	-	-	5	-
AgNPs from OC-GR	-	-	-	5	10	20	5	-
Petroleum jelly	8	8	8	8	8	8	8	8
Liquid paraffin	2	2	2	2	2	2	2	2
Glycerin	15	15	15	15	15	15	15	15
Sodium lauryl sulfate	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5
Methyl paraben	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
Propyl paraben	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
	100	100	100	100	100	100	100	100

phases were gradually blended while continuously stirring to achieve a homogeneous dispersion.

2.2.5.2. Evaluation of the repellent activity of creams loaded with AgNPs.

To validate the method, a preliminary test was performed to confirm the mosquitoes' viability and their interest in a blood meal. According to the WHO's protocol, the test's validity is determined by the landing of mosquitoes on the forearm of the test subjects [51]. To ensure accurate results, all test subjects abstained from using perfumes or any repellent substances for at least 12 h before the study.

Repellent activity was assessed using 3–5-day-old female *Anopheles gambiae* mosquitoes. These mosquitoes were collected from the Kindele neighbourhood (Mont Ngafula township, Kinshasa, DRC) and reared under controlled conditions in cages (40×40×40 cm) at $25 \pm 3^\circ\text{C}$ with a relative humidity of $75 \pm 5\%$ and a 12:12-hour light/dark cycle [2]. Each cage contained 100 unfed mosquitoes. Cage I was designated for testing creams containing AgNPs (F1–F7), Cage II was used to evaluate DEET as the positive control, and Cage III was assigned to test creams without AgNPs (F8) [38].

Three volunteer test subjects participated in the study. The forearm and hand of each subject were washed with distilled water and dried with paper towels before applying the test samples. Each test lasted 3 min and was repeated four times at 15-minute intervals. An attempted mosquito landing or bite during the 3-minute test marked the end of the evaluation for that sample. The protection time was recorded as the interval between the application of the repellent and the observation period immediately before a confirmed bite. The landing rate and repulsion percentage were used as key indicators of formulations' repellent activity. The mosquito's landing rate was determined by visual observation, while the repulsion percentage was calculated using Eq. 2 [2].

$$\text{Repulsion percentage} = (C - T / T) \times 100 \quad (2)$$

Where C and T represent the number of mosquitoes not repelled respectively by the negative control and the AgNPs.

The study protocol was approved by the institutional ethical committee of the University of Kinshasa (ethical clearance certificate number EPHYMED/023/CDB/2023).

2.2.6. Molecular docking study

2.2.6.1. Data collection. The molecular docking study was conducted to evaluate the interactions between active secondary metabolites

(ligands) present in the plant extracts eventually existing also in capping agents and the angiotensin-converting enzyme of *Anopheles gambiae* (AnoACE2) as the receptor. Phytochemical data for LI-MU, OC-GR, and TE-RI were collected from the scientific literature [1], which provided a list of known secondary metabolites associated with these plants. Among these, compounds such as isoverbascoside and verbascoside are recognized for their high polarity and solubility in aqueous media, making them likely to persist in the aqueous extracts and interact with silver ions as reducing and capping agents during nanoparticle synthesis. Their potential dual roles as phytochemical constituents and stabilizing molecules justify their inclusion in the docking study to evaluate mechanisms of larvicidal action.

The chemical structures of the identified compounds were retrieved from PubChem and ChEMBL databases [1,43]. Fosinoprilat, a known angiotensin-converting enzyme (ACE) inhibitor, was included as a reference or control molecule [1,17]. The chemical structures were manually drawn using ChemDraw software for ligands not available in the databases.

2.2.6.2. Ligand and receptor preparation. The collected ligands were downloaded in MDL SDF file format from the databases. For ligands drawn in ChemDraw, the chemical structures were saved in the same format. The AnoACE2 receptor structure was obtained from publicly available databases [43], ensuring compatibility with molecular docking tools.

2.2.6.3. Docking procedure. Docking simulations were performed using PyRx software, which integrates AutoDock 4.2. To define the active binding site on the AnoACE2 receptor, a grid box was configured with dimensions of $41.7 \text{ \AA} \times 38.3 \text{ \AA} \times 29 \text{ \AA}$ and a centre point at $13.8 \text{ \AA} \times -63.5 \text{ \AA} \times -21.46 \text{ \AA}$, optimizing the space for potential interactions [43]. Each ligand-receptor complex underwent docking calculations to predict binding affinities and binding energies. For each ligand, nine conformations were generated and assessed. The conformation with the lowest binding energy was selected as the optimal ligand-receptor complex [1,43].

2.2.6.4. Interaction analysis. The binding interactions between the ligands and the amino acid residues of the AnoACE2 receptor were visualized in 2D and 3D formats using BIOVIA Discovery Studio software. Specific interaction types, such as hydrogen bonding, Van der Waals forces, π - π stacking, and electrostatic interactions, were analyzed and mapped to determine the strength and stability of the ligand-receptor complexes [31,43].

2.2.7. Statistical analysis

Data were expressed as mean of percentage $\pm$ SD. All statistical analyses were performed using GraphPad Prism 8.0.2. Comparative analysis was performed by Two-way ANOVA followed by Tukey's and Sidak's tests for larvicidal and repellent data respectively. Results were considered significant when the p-value was less than or equal to 0.05. Asterisks in the figures indicate significant differences between groups

(* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ and **** $p < 0.0001$) and ns for no significant difference for each respective analysis.

3. Results and discussion

3.1. Characterization of biosynthesized AgNPs

3.1.1. Visual analysis and UV-Vis spectroscopy

The reduction of silver ions by the secondary metabolites in the plant extracts resulted in a noticeable colour change from yellow to black-brown within 15 min of incubation, as shown in Fig. 1. This color shift indicates that the secondary metabolites functioned as reducing agents, thereby facilitating the successful synthesis of AgNPs. The observed colour change is attributed to the characteristic surface plasmon resonance (SPR) phenomenon exhibited by AgNPs. Similar findings in previous studies have demonstrated that secondary metabolites such as polyphenols, tannins, alkaloids, and other bioactive compounds play a crucial role in the formation of AgNPs [17,23].

UV-visible spectra were recorded for all samples to further confirm this bioreduction process. The AgNPs synthesized using the aqueous extracts of LI-MU, OC-GR, and TE-RI exhibited maximum absorption peaks at 474 nm, 502 nm, and 510 nm, respectively. The variation in absorption wavelengths among the synthesized nanoparticles is likely due to differences in the chemical environment around the silver ions, which is influenced by the specific secondary metabolites involved in the bioreduction process. These variations in chemical composition are unique to each plant extract. As noted by Hazaa et al. [19], factors such as the concentrations of the plant extract and silver nitrate, temperature, pH, incubation time, and the chemical composition of the extract can significantly affect the maximum absorption of AgNPs.

3.1.2. Analysis of TEM images

The size and shape of the green-synthesized AgNPs were analysed using transmission electron microscopy (TEM). AgNPs synthesized from LI-MU, OC-GR, and TE-RI extracts exhibited a spherical morphology with the average particle sizes of $35.5 \pm 8 \text{ nm}$, $32.2 \pm 7.7 \text{ nm}$ and $22.4 \pm 4.7 \text{ nm}$ respectively as illustrated in Fig. 2. The TEM micrographs revealed well-dispersed individual nanoparticles, with no evidence of agglomeration. This high level of dispersion confirms that the secondary metabolites acted as reducing agents and played a crucial role in stabilizing the nanoparticles. These observations align with previous studies highlighting the significance of plant-based methods in the green synthesis of AgNPs [6,19,23].

3.1.3. Analysis of the surface of biosynthesized AgNPs

3.1.3.1. Analysis of EDX images. The chemical composition of the surface of the green-synthesized AgNPs was determined using energy-dispersive X-ray spectroscopy (EDX), and the spectra were recorded as shown in Fig. 3. The results revealed a prominent peak at 3 keV, confirming the presence of silver as the primary component [6,23]. Additionally, the EDX spectra showed peaks corresponding to carbon and

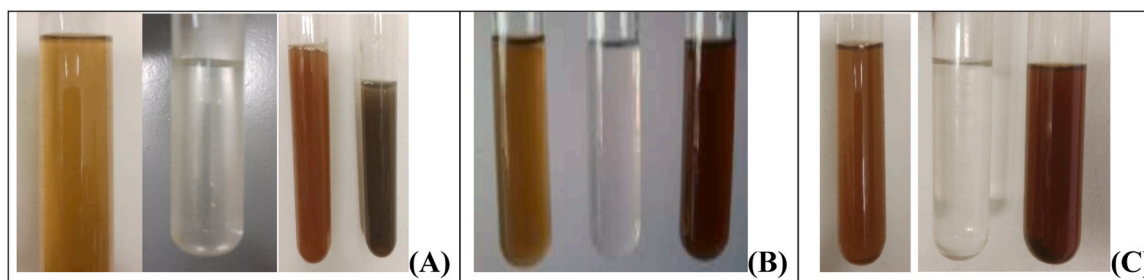


Fig. 1. Visual observation of the color change: LI-MU (A), OC-GR (B), TE-RI (C).

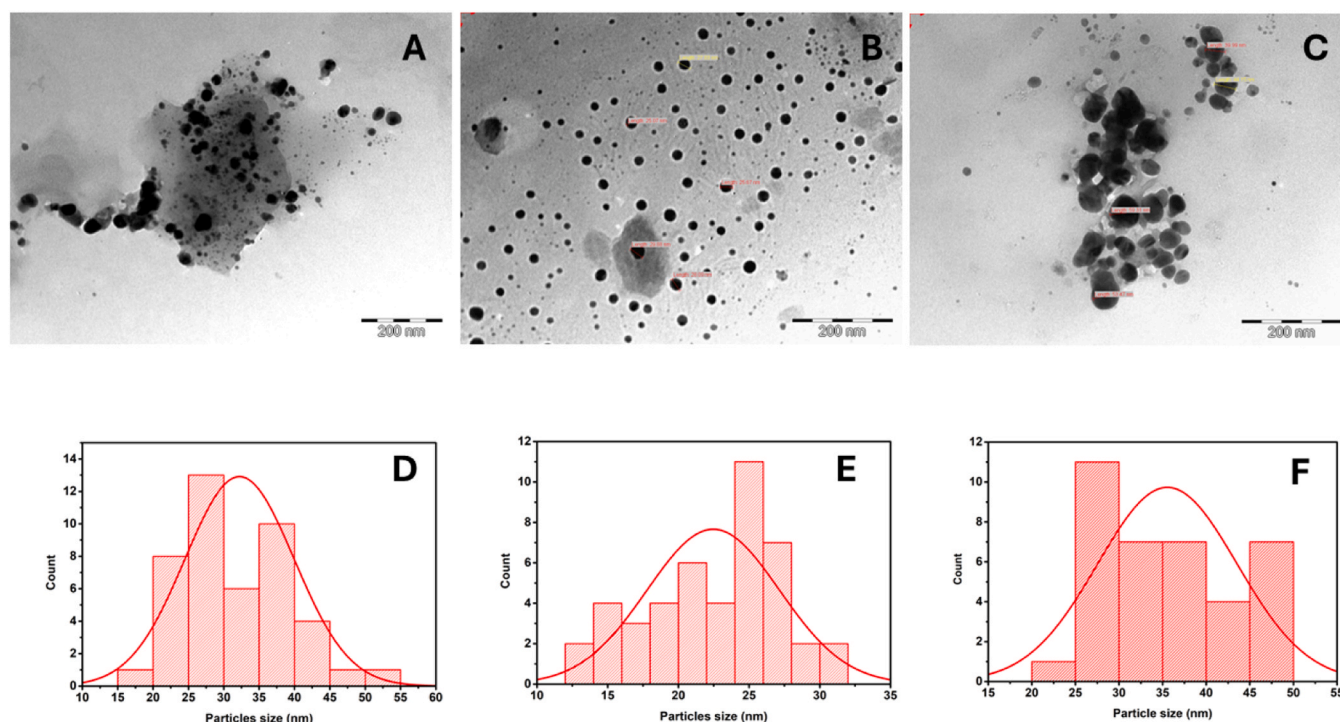


Fig. 2. TEM micrographs of green-synthesized AgNPs from LI-MU (A), OC-GR (B) and TE-RI (C). D, E and F represent particles size distribution of LI-MU, OC-GR, and TE-RI respectively.

oxygen, indicating the presence of organic compounds present on the surface of the AgNPs. These findings confirm that the secondary metabolites served as both reducing and capping agents during the synthesis process. These results are consistent with observations reported in the literature [9,17].

3.1.3.2. Analysis of FTIR spectra. The molecular interaction between silver and the secondary metabolites in the three plant extracts was evaluated using Fourier-transform infrared (FTIR) spectroscopy. This interaction was characterized by the presence of vibration bands corresponding to functional groups from the secondary metabolites capped on the surface of the AgNPs as illustrated in Fig. 4. Distinct vibration bands were observed at approximately $3275\text{--}3263\text{ cm}^{-1}$, $2915\text{--}2911\text{ cm}^{-1}$, $1352\text{--}1313\text{ cm}^{-1}$, and $1050\text{--}1330\text{ cm}^{-1}$ across all the samples. These bands correspond to alcoholic O–H stretching, CH_3/CH_2 stretching, phenolic O–H, and C–H elongation, respectively. A vibration band associated with C=O elongation was also detected at around $1606\text{--}1602\text{ cm}^{-1}$.

These findings align with the peaks observed in UV-Visible spectrophotometry and the elemental composition identified in the EDX spectra, confirming the molecular interaction between silver and the secondary metabolites. This interaction modifies the surface plasmon resonance (SPR) vibrations, further substantiating the role of secondary metabolites as reducing and capping agents in the synthesis of AgNPs, as Hazaa et al. [19] reported.

Interestingly, the characteristic IR band corresponding to N–O elongation, typically observed around 1670 cm^{-1} , was absent. This suggests the lack of nitrate residues in the green-synthesized AgNPs, corroborating the EDX results, which did not indicate the presence of nitrogen peaks [6,23].

Despite the common characteristic IR bands observed in all samples, the FTIR spectra revealed some unique bands specific to each sample. These differences likely reflect variations in the chemical composition of the secondary metabolites involved in the bioreduction of silver ions, which vary between plant extracts [6,45]. These results collectively highlight the pivotal role of plant-derived secondary metabolites in the

green synthesis of AgNPs, showcasing their dual function as reducing and capping agents.

3.1.3.3. Crystallinity of biosynthesized AgNPs. The XRD analysis shows several Bragg reflections with various 2θ values depending on batches of AgNPs (Fig. 5). All the AgNPs presented four characteristic diffraction peaks at 2θ equal to 38.19° ; 44.24° ; 64.54° and 77.36° corresponding to the reflection of (111), (200), (220) and (311) with plans of cubic lattice structure with faces centered in metallic silver (JCPDS-ICDD files No 04–0783) [23]. These findings confirm the successful biosynthesis of silver nanoparticles (AgNPs) and their crystalline nature, as evidenced by characteristic XRD diffraction peaks [6,45]. Notably, the presence of additional diffraction peaks at 2θ values of 27.87° , 32.32° , 46.31° , 54.88° , 64.54° , 77.52° , and 83.21° suggests the incorporation of phytochemicals also known as capping agents on the nanoparticle surfaces, indicating that the green-synthesized AgNPs comprise not only elemental silver but also organic components [6,23,45]. Furthermore, Bragg reflections observed between $2\theta = 24.48^\circ$ and 32.50° are typically associated with crystalline and amorphous organic phases, reinforcing the presence of plant-derived organic compounds in the nanoparticle matrix [6,17,23]. These XRD results are consistent with complementary data obtained from FTIR, EDX, and UV-Vis spectroscopy analyses, further validating the hybrid organic–inorganic nature of the synthesized nanoparticles. Comparable diffraction patterns have been reported in AgNPs synthesized using other plant extracts [6,17,23,45]. Additionally, noticeable differences in the intensity and position of XRD peaks among the three AgNP samples were observed, reflecting variations in crystallinity. These discrepancies likely stem from the distinct qualitative and quantitative compositions of the capping agents provided by the three different plant extracts LI-MU, OC-GR and TE-RI.

3.2. In vitro larvicidal activity of AgNPs

The larvicidal activity of AgNPs against *Anopheles gambiae* larvae was evaluated under laboratory conditions, and the results are illustrated in Fig. 6. All green-synthesized AgNPs exhibited interesting larvicidal

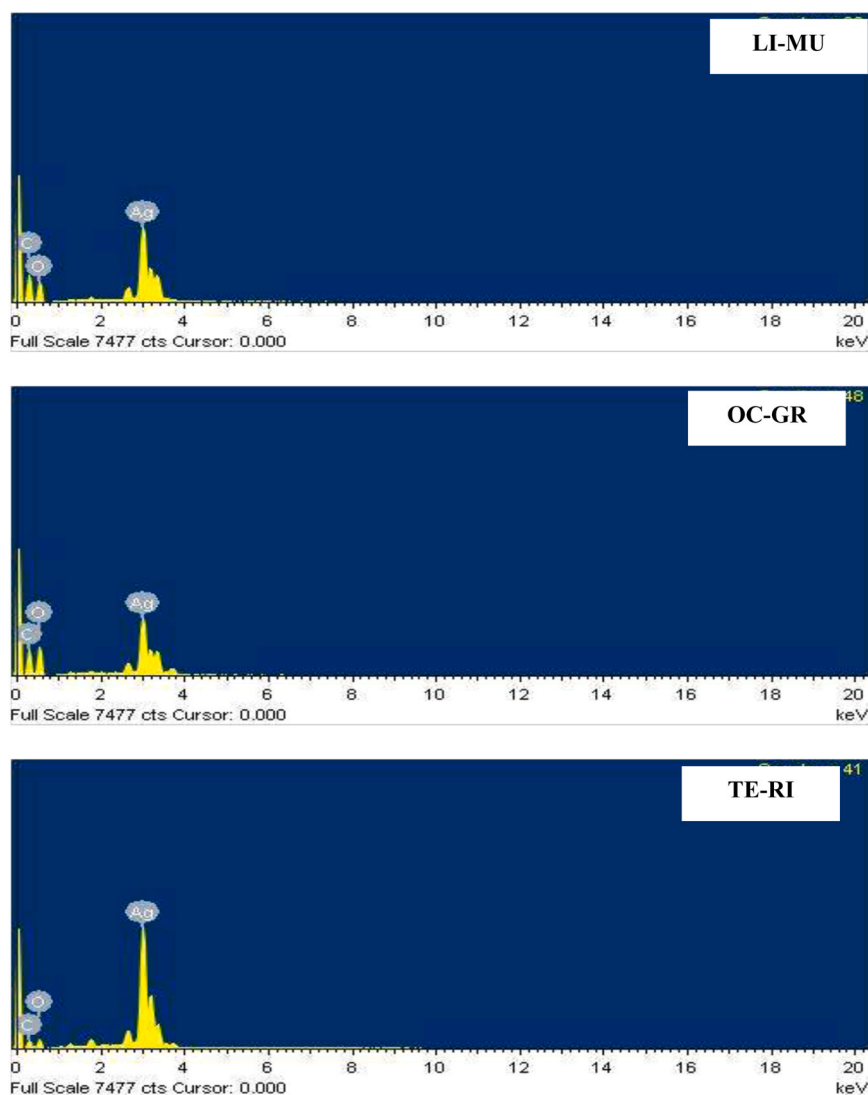


Fig. 3. EDX spectra of green synthesized AgNPs using LI-MU, OC-GR and TE-RI.

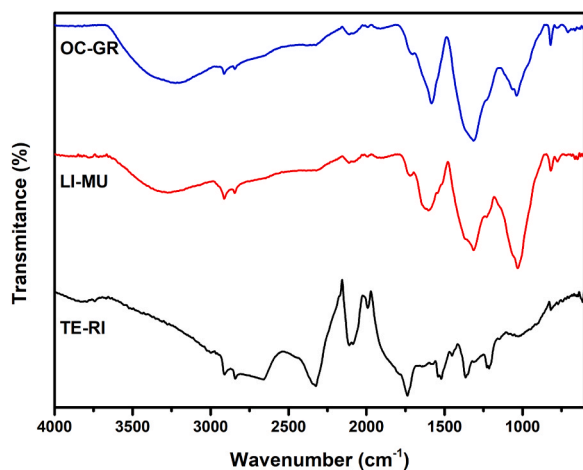


Fig. 4. FTIR spectra of the green-synthesized AgNPs using OC-GR, LI-MU and TE-RI.

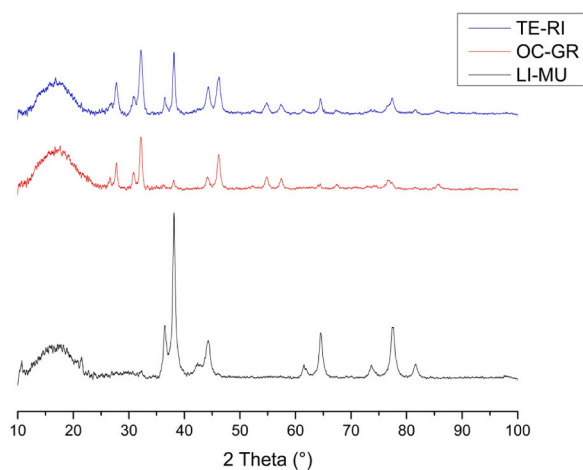


Fig. 5. XRD Spectra of biosynthesized AgNPs using TE-RI, OC-GR and LI-MU.

activity in a dose-dependent manner. Among them, dispersion of AgNPs derived from LI-MU demonstrated the highest efficacy, achieving 100%

mortality at 85 ppm and 100 ppm against *Anopheles gambiae* (Kisumu strain) and *Anopheles gambiae* (wild strain), respectively. This potent activity can be attributed to the synergistic effects of silver nanoparticles

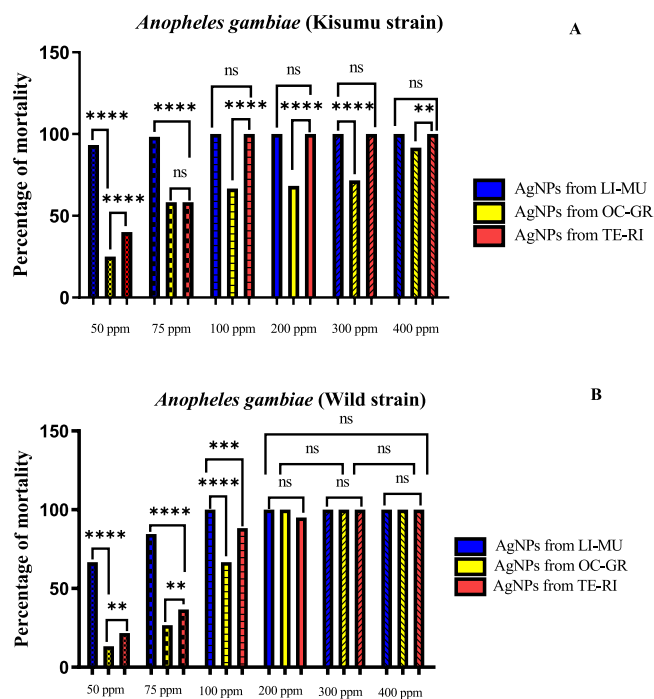


Fig. 6. Larvicidal activity of AgNPs derived from LI-MU, OC-GR, TE-RI against third and fourth instar larvae of *Anopheles gambiae* Kisumu strain (A) and Wild strain (B). Data are expressed as mean of percentage ($n = 3$). Comparative analysis was performed by Two-way ANOVA followed by Tukey's test. ** $p < 0.01$; *** $p < 0.001$ and **** $p < 0.0001$ and ns for no significant difference.

and the secondary metabolites involved in the bioreduction process of silver ions, which acted as both reducing and capping agents. Previous studies have reported that secondary metabolites, such as terpenes and flavonoids, possess intrinsic larvicidal properties, enhancing silver nanoparticle's efficacy [45].

In addition to this synergy, the larvicidal activity of AgNPs also depends on their physicochemical characteristics, including size and shape [18]. Due to their small size and large surface area, nanoparticles can penetrate the insect cuticle more efficiently, interact with organelles, and disrupt physiological processes. Hazaa et al. [19] suggested that AgNPs act by denaturing sulfur or phosphorus bridges in desoxy-ribonucleic acid (DNA), leading to the dysfunction of organelles and enzymes, ultimately resulting in larval death.

For suspension of AgNPs synthesized from OC-GR, potent larvicidal activity was observed at 400 ppm and 300 ppm against *Anopheles gambiae* (Kisumu strain) and *Anopheles gambiae* (wild strain), respectively. Interestingly, the wild strain required higher concentrations for effective control compared to the Kisumu strain. This discrepancy may be explained by the reduced sensitivity to larvicide agents following the acquisition of resistance through horizontal gene transfer among mosquito larvae cohabiting in stagnant water [27,33]. Resistance mechanisms, such as the overproduction of esterase enzymes and the acquisition of the *Kdr* gene, which reduces sensitivity to larvicidal agents, have been implicated in larval resistance to chemical products [25].

Dispersion of AgNPs synthesized from TE-RI showed improved larvicidal activity compared to the aqueous extract of TE-RI alone, achieving 100% mortality at 100 ppm and 95.0% mortality at 200 ppm against *Anopheles gambiae* (Kisumu strain) and *Anopheles gambiae* (wild strain), respectively. Comparable results have been reported by Amarasinghe et al. [6] in a study involving AgNPs biosynthesized from *Amnora glabra*. These findings highlight the potential of green-synthesized AgNPs as an effective ecological approach to

controlling mosquito larvae and reducing the transmission of MBDs.

Although green-synthesized AgNPs from these three plants showed interesting larvicidal activity, the low aqueous solubility of AgNPs may also constitute a limit to their effectiveness [39,48]. To overcome this limit, Alshehri et al., [5] reported that chitosan encapsulated AgNPs represent an alternative for improving water solubility and, therefore, larvicidal activity. In addition, others formulation strategies (e.g. sonication, etc.) can be developed for the improvement of low aqueous solubility of AgNPs and, consequently, their effectiveness.

These results demonstrate that AgNPs synthesized using green chemistry principles exhibit significant larvicidal activity against *Anopheles gambiae*. Comparative analysis reveals that these AgNPs can achieve comparable or superior efficacy to several commercially available chemical larvicides (e.g. temephos, permethrin) [6,30,44]. For instance, in our assays, AgNPs showed a mortality rate of over 90% at concentrations as low as 50 ppm within 24 h (Fig. 6A), which is consistent with or exceeds the performance of widely used chemical larvicides under similar conditions [6,30,44].

Safety is a crucial consideration where green-synthesized AgNPs offer significant advantages over traditional chemical approaches. Unlike conventional larvicides, which often contain toxic compounds harmful to non-target species and are associated with ecological disturbances, green-synthesized AgNPs are designed to minimize environmental impact [32]. Toxicity assessments on non-target aquatic organisms such as *Daphnia magna*, *Danio rerio*, and *Chironomus riparius* have consistently demonstrated minimal adverse effects at concentrations effective against mosquito larvae [13,22,37].

Although the current study focused primarily on larvicidal efficacy, a growing body of evidence underscores the environmental safety and human biocompatibility of plant mediated AgNPs [34,42]. Notably, green synthesis methods using aqueous extracts of medicinal plants avoid harmful solvents and substantially reduce ecological footprints compared to chemically synthesized insecticides [32]. Moreover, the phytochemical coatings that form during green synthesis confer biocompatibility and enhance selectivity, enabling the AgNPs to target mosquito vectors while sparing beneficial aquatic organisms [32].

Despite these promising findings, it remains essential to conduct comprehensive, large-scale environmental studies to evaluate the long-term ecological impact of green-synthesized AgNPs. Such research will be critical in validating their safety for widespread use, particularly in ecologically sensitive and human-inhabited areas. Ultimately, these efforts will support the integration of green AgNPs into sustainable, eco-friendly pest management programs that prioritize both efficacy and environmental stewardship.

3.3. Repellence assay

The landing of 10 mosquitoes on the forearm of a test subject was observed within 3 s. This observation confirmed the mosquitoes' viability and their interest in blood meals, thereby validating the method. For this test, only the AgNPs synthesized from LI-MU and OC-GR were selected. These plants were chosen because their aqueous extracts have demonstrated promising larvicidal activity against the larvae of wild *Anopheles gambiae*.

The results of the repellence assay clearly demonstrated that all creams loaded with AgNPs provided effective repellent activity against *Anopheles gambiae*. As illustrated in Fig. 7, the landing rate of samples containing AgNPs decreased as their concentrations increased. Formulations F1, F2, and F3 exhibited respectively landing rates of 50.9, 20.7 and 18.7 while the DEET and the cream without AgNPs showed no landing and a maximum of landing respectively. Similarly, formulations F4, F5 and F6 showed the landing rate of 43.4, 20.2, and 17.7 respectively. These outcomes are similar to previous studies on the use of AgNPs derived from plant extracts as mosquito repellent [2].

Regarding the repellent percentage, F1, F2, and F3 showed repulsion rates of 32%, 72%, and 75%, respectively, indicating a substantial dose-

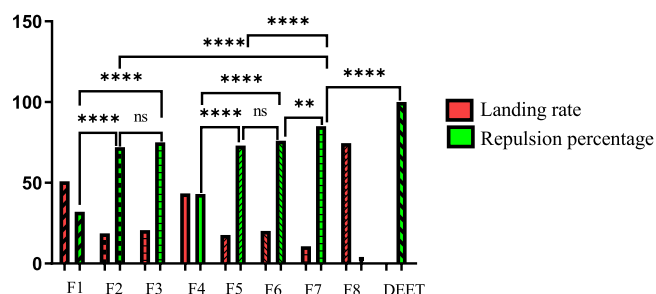


Fig. 7. Landing rate and repulsion percentage of creams containing with green-synthesized AgNPs from LI-MU and OC-GR. Data are expressed as mean of percentage (n = 4). Comparative analysis was performed by Two-way ANOVA followed by Sidak's test. **p < 0.01 and ****p < 0.0001 and ns for no significant difference.

dependent response. Similarly, creams formulated with AgNPs from OC-GR at concentrations of 5% (F4), 10% (F5), and 20% (F6) exhibited repellent activity that increase as the concentrations of AgNPs rose. This dose dependency can be attributed to the biological activity of AgNPs and the secondary metabolites capped on their surface, which are known to disrupt mosquito sensory mechanisms and deter landing or feeding [2].

The most notable result was observed with formulation F7, which combined AgNPs from LI-MU and OC-GR. This formulation achieved an 85% repellent efficiency, suggesting a significant synergistic effect between the two types of AgNPs. The enhanced repellent activity is consistent with findings from previous studies, which reported that the combination of plant-based AgNPs can amplify their bioactivity through complementary mechanisms of action [2,15]. Specifically, the secondary metabolites in LI-MU and OC-GR extracts likely work in tandem with silver ions, enhancing the stability and biological activity of the nanoparticles. This synergy has important implications for the development of more effective plant-based repellents. By leveraging the combined action of multiple plant extracts, it may be possible to reduce the concentration of silver required, thereby lowering production costs and minimizing environmental concerns associated with nanoparticle use. Moreover, such formulations can provide a dual-action mechanism: repelling mosquitoes while also exhibiting larvicidal effects, as seen in other parts of the study. This dual functionality makes these formulations particularly valuable in regions where mosquito populations have developed resistance to conventional chemical repellents and larvicides. Although F7 showed an interesting repellent activity, the difference remains significant (p < 0.0001) when comparing with the reference repellent (DEET). To overcome this difference, we hypothesized that increasing the amount of each AgNPs in F7 could be a good strategy. Nevertheless, further in-depth studies will need to be carried out to confirm this hypothesis.

The practical application of these findings is highly relevant for tropical and malaria-endemic regions, where MBDs remain a significant public health challenge. Unlike chemical repellents such as DEET, which raise concerns about toxicity and environmental persistence, these plants based AgNPs formulations offer a safer and more sustainable alternative. Additionally, the use of locally sourced plant extracts like LI-MU and OC-GR can promote cost-effective and community-driven production methods, fostering local engagement in public health initiatives. However, while the results are promising, there are still critical considerations that must be addressed before these formulations can be widely implemented.

Beyond their demonstrated environmental compatibility, the biosafety of green-synthesized AgNPs has also been increasingly supported by *in vivo* evidence from mammalian models. Studies involving biosynthesized AgNPs derived from *Psidium guajava* and *Azadirachta indica* have reported negligible histopathological alterations and low systemic toxicity at concentrations effective for mosquito control [4,46].

Moreover, dermal safety assessments including patch testing and lymph node stimulation assays have further confirmed their non-irritant and non-sensitizing properties, reinforcing their potential for use in topical repellent formulations [20]. Despite these promising findings, long-term toxicity evaluations remain essential, particularly concerning chronic exposure, dermal absorption, and potential effects on reproductive health. A comprehensive understanding of these parameters is crucial to ensure the safe integration of plant-mediated AgNPs into public health interventions.

3.4. Molecular docking-AnoACE2 protein

Molecular docking was conducted to evaluate the molecular interactions between the phytochemical compounds of LI-MU, OC-GR, and TE-RI and the AnoACE2 protein of *Anopheles gambiae*. AnoACE2 is a peptidase highly expressed in several tissues, including the reproductive tissues, midgut, and trachea of larvae and adult mosquitoes. The angiotensin-converting enzyme (ACE) in insects differs significantly from that in mammals, in substrate specificity and susceptibility to infection. These differences make ACE an attractive target for the development of larvicidal compounds. Since ACE operates effectively in soluble media and interacts specifically with plant-derived aqueous extracts, it provides an excellent framework for molecular docking studies [43].

Out of 41 identified ligands, 22 demonstrated lower binding energies to AnoACE2 compared to the reference molecule Fosinoprilat, which showed a binding energy of −8.4 kcal/mol, as summarized in Table 2 and Supplemental Table 1. Among these, Isoverbascoside from LI-MU exhibited the lowest binding energy, indicating the strongest interaction with the target protein. These results correlate well with the *in vitro* findings, which showed the highest larvicidal activity for AgNPs synthesized using LI-MU. Molecular docking interaction models of the ligand-protein complexes are illustrated in Fig. 8 and Supplemental Figure 1. Interactions such as conventional hydrogen bonding, carbon-hydrogen bonding, attractive charge interactions, Van der Waals forces, and various π -based interactions (π -Alkyl, π - π stacked, π -Cation, π -Anion, π -Sigma, and π - π shaped interactions) were observed between the ligands and the amino acid residues of AnoACE2. These interactions appear to favour the binding of the phytochemical compounds to the target protein [31].

For each ligand-protein complex, nine conformers were generated, but only the conformer with the lowest binding energy was selected as the most effective molecule. Compared to others studied, the low binding energy values observed for these ligands suggest stable interactions and significant inhibition of AnoACE2 activity. Given that AnoACE2 is known to play a critical role in the growth and development

Table 2

Binding energy in Kcal/mol and amino acid residues and of the phytochemicals of LI-MU, OC-GR, TE-RI.

Plants/ standard molecule	Phytochemicals	Binding energy Kcal/ mol	Amino acid residues
<i>Lippia multiflora</i>	Isoverbascoside	-11.4	GLN 281, ASP 284, LYS 454, PHE 379, ARG 376
	Verbascoside	-10.9	GLN 281, ASP 284, LYS 454, ASP 162, GLU 377, VAL 380, ASP 415
<i>Ocimum gratissimum</i>	Cephalin	-10.4	ASP 415, ASP 162, VAL 380, PHE 379, PHE 457, ARG 376
	Nepitrin	-10.4	SER 418, PHE 379, ASP 162, VAL 380, ALA 354, GLU 377
<i>Tetradenia riparia</i>	6,7-Dehydroroyleanone	-9.3	TYR 527, ASP 415
	Diterpendiol	-8.8	GLN 281, PHE 457, LYS 454, PHE 379, VAL 380

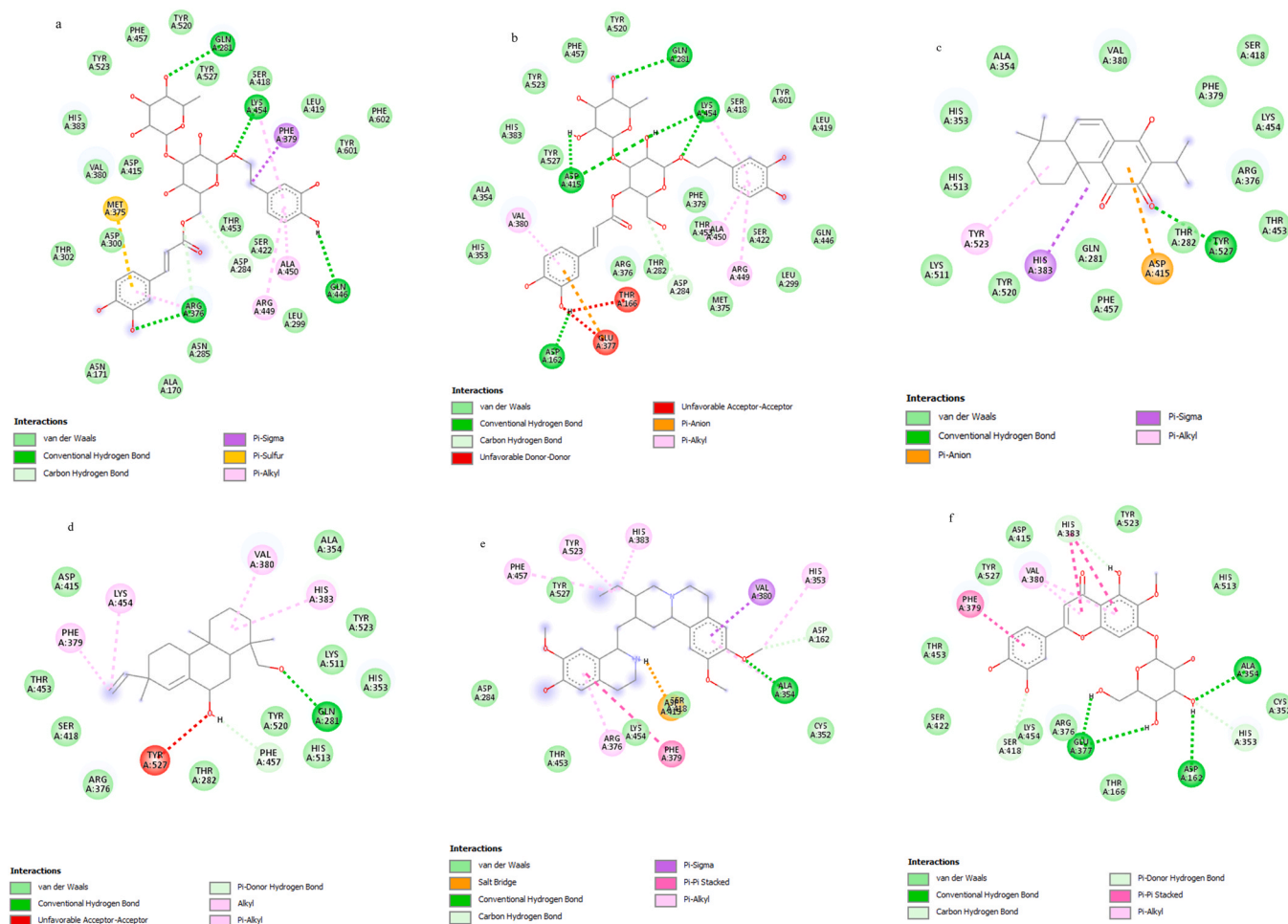


Fig. 8. Molecular interaction between phytochemical compounds from LI-MU, OC-GR, TE-RI and AnoACE2 receptor. a) Isoverbascoside b) Verbascoside c) 6,7-Dehydroroyleanone d) Diterpendiol e) Cephalin f) Nepitritin.

of *Anopheles gambiae*, its inhibition is a promising mechanism for larvicidal activity [31]. These findings confirm that green-synthesized AgNPs from the three Congolese plants exert their larvicidal effects, at least in part, by inhibiting AnoACE2. This study highlights the potential of these nanoparticles as an effective ecological tool for mosquito control.

The phytochemicals from LI-MU demonstrated the strongest binding affinities among the tested compounds, with Isoverbascoside emerging as the top-performing ligand, exhibiting a binding energy of -11.4 kcal/mol. This significantly surpasses the binding energy of Fosinoprilat (-8.4 kcal/mol), a known ACE inhibitor. Isoverbascoside formed stable interactions with key residues such as GLN 281, ASP 284, LYS 454, PHE 379, and ARG 376, suggesting a highly favorable ligand-protein complex. Other notable compounds from LI-MU, such as Verbascoside (-10.9 kcal/mol), Leucoseptoside (-10.3 kcal/mol), and Nuomioside (-9.3 kcal/mol), also exhibited strong binding affinities and interacted with crucial residues like ASP 415 and TYR 527. These interactions are essential for the inhibition of AnoACE2 activity, which is known to disrupt physiological processes critical for larval growth and survival. The presence of multiple high-affinity ligands in LI-MU aligns with its superior in vitro larvicidal activity, as these compounds collectively enhance the efficacy of LI-MU-derived AgNPs.

The phytochemicals from OC-GR displayed a broader range of binding affinities, with Nepitritin (-10.4 kcal/mol) and Cephalin (-10.4 kcal/mol) standing out as the most effective ligands. These compounds formed stable interactions with key residues such as ASP 415, PHE 379, VAL 380, and ARG 376, which are crucial for AnoACE2 inhibition. Interestingly, other compounds such as asiatic acid

(-10.2 kcal/mol) and benthamic acid (-10.0 kcal/mol) also exhibited strong binding affinities, further reinforcing the potential of OC-GR as a source of bioactive compounds. However, some phytochemicals like Hyperoside (9.5 kcal/mol) and Catechin (8.5 kcal/mol) displayed weak or positive binding energies, indicating limited or unfavorable interactions with the target protein. This variability reflects the diverse chemical composition of OC-GR and suggests that only specific compounds contribute significantly to its larvicidal activity. The moderate efficacy of OC-GR-derived AgNPs observed in vitro can be attributed to the combined effects of its high-affinity ligands, despite the presence of less effective compounds.

Phytochemicals from TE-RI, including 6,7-Dehydroroyleanone (-9.3 kcal/mol) and Diterpendiol (-8.8 kcal/mol), exhibited moderate binding affinities compared to the top-performing ligands from LI-MU and OC-GR. These compounds interacted with residues such as TYR 527, ASP 415, LYS 454, and PHE 379, which are involved in the inhibiting of AnoACE2 activity. Although their binding energies are relatively lower, these interactions still contribute to the larvicidal activity of TE-RI-derived AgNPs. The in vitro results for TE-RI align with these findings, as its AgNPs displayed moderate to strong larvicidal activity. The phytochemical composition of TE-RI appears to enhance the efficacy of its AgNPs, though its overall performance is slightly inferior to that of LI-MU-derived AgNPs.

Fosinoprilat, a known ACE inhibitor, exhibited a binding energy of -8.4 kcal/mol, interacting with residues ASN 277, VAL 380, and ARG 376. While effective, its binding energy and interaction profile were surpassed by several phytochemicals, particularly Isoverbascoside,

Verbascoside, and Nepitrin. This suggests that these phytochemicals may act as more potent inhibitors of AnoACE2 than Fosinoprilat, highlighting their potential as natural larvicidal agents.

The strong binding affinities observed for certain phytochemicals, particularly Isoverbascoside and Verbascoside, indicate their potential to inhibit AnoACE2 activity effectively. This inhibition disrupts physiological processes such as digestion and nutrient absorption in mosquito larvae, ultimately leading to their death. The interactions observed in the molecular docking study, including hydrogen bonding, Van der Waals forces, and various π -based interactions, contribute to the stability and specificity of the ligand-protein complexes. Moreover, the differences in binding energies and interaction profiles among the tested phytochemicals can be attributed to their distinct chemical structures and functional groups [8,21]. Compounds with multiple hydroxyl and phenolic groups, such as Isoverbascoside and Verbascoside, are likely to form stronger interactions with the target protein, enhancing their inhibitory potential [8,21].

The molecular docking results strongly correlate with the *in vitro* larvicidal activity observed for AgNPs synthesised using these plant extracts. The superior performance of LI-MU-derived AgNPs can be attributed to high-affinity ligands like Isoverbascoside and Verbascoside, which enhance their efficacy. Similarly, the moderate efficacy of TE-RI and OC-GR-derived AgNPs reflects the presence of lower-affinity ligands, contributing to their overall activity. These findings highlight the importance of green-synthesised AgNPs as ecological tools for mosquito control. Green nanotechnology offers a sustainable and effective alternative to chemical larvicides by leveraging the natural bioactivity of plant-derived secondary metabolites. The ability of these phytochemicals to target AnoACE2, a key protein in mosquito physiology, further underscores their potential as larvicidal agents. The molecular docking study provides valuable insights into the mechanisms underlying the larvicidal activity of green-synthesized AgNPs. The strong binding affinities observed for several phytochemicals, particularly from LI-MU, demonstrate their potential to inhibit AnoACE2 activity effectively. These findings support using green-synthesized AgNPs as a sustainable approach to controlling mosquito populations and reducing the transmission of MBDs.

4. Future perspectives

Building upon the promising findings of this study, future research will adopt a comprehensive, multi-faceted approach to elucidate the precise mechanisms of action of green-synthesized AgNPs in vector control. Detailed *in vitro* and *in vivo* comparative studies will be undertaken to distinguish the individual and synergistic contributions of metallic silver and phytochemical capping agents compared to whole plant extracts and chemically synthesized AgNPs in mediating both larvicidal and angiotensin-converting enzyme (ACE) inhibitory activities. These investigations will be essential to confirm molecular docking predictions, which have previously demonstrated strong correlations with experimental outcomes in studies of ACE inhibitors [36,47].

Additionally, advanced phytochemical profiling techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC-MS/MS), and Nuclear Magnetic Resonance (NMR) spectroscopy will be employed to isolate, identify, and quantify key bioactive constituents from both the original Congolese plant extracts and the surfaces of the resulting nanoparticles. This analytical strategy will provide deeper insight into the fate, structural transformation, and biological relevance of phytochemicals during nanoparticle synthesis. Moreover, it will account for the influence of seasonal and geographical variability on phytochemical composition of the plants, ultimately contributing to the design of more consistent and effective nanoparticle-based formulations for sustainable vector control.

5. Conclusion

This study highlights the potential of green-synthesized AgNPs derived from LI-MU, OC-GR, and TE-RI as effective larvicidal agents against *Anopheles gambiae*. The AgNPs exhibited dose-dependent larvicidal activity, with those derived from LI-MU showing the highest efficacy, achieving 100% mortality at lower concentrations than OC-GR- and TE-RI-derived AgNPs. The superior performance of LI-MU-derived AgNPs is attributed to the strong binding affinities of its phytochemicals, such as Isoverbascoside and Verbascoside, to the AnoACE2 protein, as demonstrated by molecular docking studies. These studies revealed that secondary metabolites functioned as reducing and capping agents during AgNP synthesis, significantly enhancing their larvicidal activity by inhibiting AnoACE2. Interactions between the phytochemicals and key amino acid residues, including GLN 281, ASP 284, and LYS 454, were critical in stabilizing the ligand-protein complexes and disrupting mosquito physiological processes. Additionally, oil-in-water creams loaded with AgNPs from LI-MU and TE-RI demonstrated an impressive repellent activity of 85%, suggesting a synergistic effect between AgNPs and the secondary metabolites capping them. This synergy enhanced the efficacy of the formulations and underscored the importance of the nanoparticle size and shape in improving penetration and activity against mosquito targets.

The findings provide compelling evidence that green nanotechnology, harnessing the natural bioactivity of plant-derived secondary metabolites, offers a sustainable and eco-friendly alternative to conventional chemical larvicides and repellents. By targeting specific mosquito proteins like AnoACE2, green-synthesized AgNPs present an innovative approach to mosquito control, reducing risks associated with insecticide resistance and environmental toxicity. Future research should focus on scaling up the synthesis process, evaluating the long-term environmental and ecological impacts of AgNPs, and testing their efficacy against other mosquito species and MBDs. These findings pave the way for incorporating green-synthesized AgNPs into integrated mosquito management programs, significantly contributing to global efforts to combat malaria and other MBDs.

CRedit authorship contribution statement

Eunice N. Zola: Investigation, data curation, formal analysis, methodology, Writing – original draft, **Claude Josué K. Muanga:** Investigation, data curation, formal analysis, methodology, Writing – original draft, **Joseph Kyana:** Investigation, data curation, formal analysis, methodology, Writing – original draft, **Miryam M. Luzala:** Investigation, Formal analysis, methodology, **Sébastien N. Wendji:** Investigation, Formal analysis, methodology, **Clément M. Tshimanga:** Investigation, Formal analysis, methodology, Data curation, **Justin B. Safari:** Investigation, Formal analysis, methodology, Data curation, **Bénédicte K. Baraka:** Formal analysis, methodology, Data curation, **Victoire Nsabatién:** Data curation, investigation, **Josué Zanga:** Data curation, investigation, **Emery M. Metelo:** Data curation, investigation, **Yannick B. Nuapia:** Conceptualization, Supervision, Validation, Resources, Writing – review and editing, **Patrick B. Memvanga:** Conceptualization, Supervision, Validation, Resources, Writing – original draft, Writing – review and editing

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Patrick B. Memvanga reports financial support was provided by Organisation internationale de la Francophonie. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Institut de la Francophonie pour le Développement Durable (IFDD/Canada) through the Projet de Déploiement des Technologies et Innovations Environnementales (PDTIE), funded by the Organisation Internationale de la Francophonie (OIF), the Organization of African, Caribbean and Pacific States (OACPS), and the European Union (EU) under grant FED/2020/421–370. Additionally, we acknowledge financial support from UNESCO through the TWAS SG-NAPI grant (4500454115).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.nxnano.2026.100415](https://doi.org/10.1016/j.nxnano.2026.100415).

References

- [1] Z.I. Abu Hasan, H. Williams, N.M. Ismail, H. Othman, G.E. Cozier, K.R. Acharya, R. E. Isaac, The toxicity of angiotensin converting enzyme inhibitors to larvae of the disease vectors *Aedes aegypti* and *Anopheles gambiae*, *Sci. Rep.* 7 (1) (2017) 45409, <https://doi.org/10.1038/srep45409>.
- [2] S.A. Akintelu, A.S. Folorunso, A.K. Oyebamiji, S.C. Olugbeko, Mosquito repellent and antibacterial efficiency of facile and low-cost silver nanoparticles synthesized using the leaf extract of *Morinda citrifolia*, *Plasmonics* (2021) 1–12, <https://doi.org/10.1007/s11468-021-01428-3>.
- [3] G.E. Akpan, K.A. Adepoju, O.R. Oladosu, Potential distribution of dominant malaria vector species in tropical region under climate change scenarios, *PLoS One* 14 (6) (2019) e0218523, <https://doi.org/10.1371/journal.pone.0218523>.
- [4] E.A. Albadawi, E.N.A. Musa, H.M. Ghaban, N.A. Ebrahim, M.S. Albadrani, A.I. El-Tokhy, Eco-friendly green synthesis of silver nanoparticles from guajava leaves extract for controlling organophosphorus pesticides hazards, characterization, and in-vivo toxicity assessment, *BMC Pharmacol. & Toxicol.* 25 (1) (2024) 98, <https://doi.org/10.1186/s40360-024-00826-7>.
- [5] M.A. Alshehri, A.T. Aziz, S. Trivedi, C. Panneerselvam, Efficacy of chitosan silver nanoparticles from shrimp-shell wastes against major mosquito vectors of public health importance, *Green. Process. Synth.* 9 (1) (2020) 675–684, <https://doi.org/10.1515/gps-2020-0062>.
- [6] L.D. Amarasinghe, P.A.S.R. Wickramarachchi, A.A.A.U. Aberathna, W.S. Sithara, C. R. De Silva, Comparative study on larvicidal activity of green synthesized silver nanoparticles and *Annona glabra* (Annonaceae) aqueous extract to control *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), *Heliyon* 6 (6) (2020), <https://doi.org/10.1016/j.heliyon.2020.e04322>.
- [7] M. Balboné, I. Sawadogo, D.D. Soma, S.F. Drabo, M. Namountougou, K. Bayili, O. Gnankine, Essential oils of plants and their combinations as an alternative adulticides against *Anopheles gambiae* (Diptera: Culicidae) populations, *Sci. Rep.* 12 (1) (2022) 19077, <https://doi.org/10.1038/s41598-022-23554-6>.
- [8] M.M. Baz, N.M. El-Shourbagy, A.M. Alkhaibari, H.S. Gattam, M.H. Alruhaili, A. Selim, I.T. Radwan, Larvicidal activity of *Acacia nilotica* extracts against *Culex pipiens* and their suggested mode of action by molecular simulation docking, *Sci. Rep.* 14 (1) (2024) 6248, <https://doi.org/10.1038/s41598-024-56690-2>.
- [9] K. Bhardwaj, C. Chopra, P. Bhardwaj, Biogenic Metallic Nanoparticles from Seed Extracts: Characteristics, Properties, and Applications, *J. Nanomater.* (2022) 2271278, <https://doi.org/10.1155/2022/2271278>.
- [10] B. Chala, F. Hamde, Emerging and re-emerging vector-borne infectious diseases and the challenges for control: a review, *Front. Public Health* 9 (2021) 715759, <https://doi.org/10.3389/fpubh.2021.715759>.
- [11] S. Chernousova, M. Epple, Silver as antibacterial agent: ion, nanoparticle, and metal, *Angew. Chem. Int. Ed.* 52 (6) (2013) 1636–1653, <https://doi.org/10.1002/anie.201205923>.
- [12] A.C. de Oliveira, R.C. Simões, C.P. Tavares, C.A. Lima, I.S.C. Sá, F.M. da Silva, R. A. Roque, Toxicity of the essential oil from *Tetradenia riparia* (Hochstetter.) Codd (Lamiaceae) and its principal constituent against malaria and dengue vectors and non-target animals, *Pestic. Biochem. Physiol.* 188 (2022) 105265, <https://doi.org/10.1016/j.pestbp.2022.105265>.
- [13] M. Dhanislas, S. Sampath, M. Shamy, J. Joseph, M. Yasasve, M.Z. Ahmed, A. Suresh, Green synthesis of biofabricated silver nanoparticles from *Syzygium aromaticum* seeds: spectral characterization and evaluation of its anti-mycobacterial activity, cytotoxicity assessment on zebrafish embryo and *Artemia salina*, *Mater. Technol.* 38 (1) (2023) 2269358, <https://doi.org/10.1080/10667857.2023.2269358>.
- [14] J. dos Santos, R.L. Lourenço, P. Rosa, A.I.H. Adams, Evaluation of quality and safety parameters of DEET commercial repellents: photostability, penetration/permeation and eye irritation studies: Evaluation of quality and safety parameters of DEET commercial repellents, *Drug Anal. Res.* 6 (1) (2022) 21–26, <https://doi.org/10.22456/2527-2616.124882>.
- [15] A.A. El-Sayed, A. Amr, O.M. Kamel, M.M. El-Saidi, A.E. Abdelhamid, Eco-friendly fabric modification based on AgNPs@ Moringa for mosquito repellent applications, *Cellulose* 27 (2020) 8429–8442, <https://doi.org/10.1007/s10570-020-03355-8>.
- [16] M. Ferrati, C. Baldassarri, P. Rossi, G. Favia, G. Benelli, L. De Fazi, F. Maggi, Unveiling the Larvicidal Potential of Golpar (*Heracleum persicum* Desf. ex Fisch.) Essential Oil and Its Main Constituents on *Aedes* and *Anopheles* Mosquito Vectors, *Plants* 13 (21) (2024) 2974, <https://doi.org/10.3390/plants13212974>.
- [17] L. Govindan, S. Anbazhagan, A.B. Altemimi, K. Lakshminarayanan, S. Kuppan, A. Pratap-Singh, M. Kandasamy, Efficacy of antimicrobial and larvicidal activities of green synthesized silver nanoparticles using leaf extract of *Plumbago auriculata* lam, *Plants* 9 (11) (2020) 1577, <https://doi.org/10.3390/plants9111577>.
- [18] U.M.T.M. Gunathilaka, W.A.P.P. de Silva, S.P. Dunuweera, R.M.G. Rajapakse, Effect of morphology on larvicidal activity of chemically synthesized zinc oxide nanoparticles against mosquito vectors, *RSC Adv.* 11 (15) (2021) 8857–8866, <https://doi.org/10.1039/D1RA00014D>.
- [19] M. Hazaa, M. Alm-Eldin, A.E. Ibrahim, N. Elbarky, M. Salama, R. Sayed, W. Sayed, Biosynthesis of silver nanoparticles using *Borago officinalis* leaf extract, characterization and larvicidal activity against cotton leaf worm, *Spodoptera littoralis* (Bosid), *Int. J. Trop. Insect Sci.* 41 (2021) 145–156, <https://doi.org/10.1007/s42690-020-00187-8>.
- [20] H. Hazarika, H. Krishnatreyya, V. Tyagi, J. Islam, N. Gogoi, D. Goyary, K. Zaman, The fabrication and assessment of mosquito repellent cream for outdoor protection, *Sci. Rep.* 12 (1) (2022) 2180, <https://doi.org/10.1038/s41598-022-06185-9>.
- [21] H.T. Huang, C.C. Lin, T.C. Kuo, S.J. Chen, R.N. Huang, Phytochemical composition and larvicidal activity of essential oils from herbal plants, *Planta* 250 (2019) 59–68, <https://doi.org/10.1007/s00425-019-03147-w>.
- [22] C. Kamaraj, et al., Green synthesis and biophysical characterization of silver and palladium nanoparticles using *Laureliopsis philippiana*: A potent eco-friendly larvicide with negligible impact on zebrafish (*Danio rerio*), *J. AsiaPac. Entomol.* 26 (4) (2023) 102164, <https://doi.org/10.1016/j.aspen.2023.102164>.
- [23] E.K. Kambale, C.I. Nkanga, B.P.I. Mutoonkole, A.M. Bapolisi, D.O. Tassa, J.M. I. Llesse, P.B. Memvanga, Green synthesis of antimicrobial silver nanoparticles using aqueous leaf extracts from three Congolese plant species (*Brilliantaisia patula*, *Crotophyterix febrifuga* and *Senna siamea*), *Heliyon* 6 (8) (2020), <https://doi.org/10.1016/j.heliyon.2020.e04493>.
- [24] K.C. Kobenan, G.E.C. Ochoy, I.S. Kouadio, M. Kouakou, K.K.N. Bini, R. Ceylan, O. G. Ochoy, Chemical composition, antioxidant activity, cholinesterase inhibitor and in vitro insecticidal potentiality of essential oils of *Lippia multiflora* Moldenke and *Eucalyptus globulus* Labill. on the main carophagous pests of cotton plant in Ivory Coast, *Chem. & Biodivers.* 19 (4) (2022) e202100993, <https://doi.org/10.1155/2022/8302696>.
- [25] C.Z. Koukpo, A.J.Y.H. Fassinou, R.A. Ossè, et al., The current distribution and characterization of the L1014F resistance allele of the kdr gene in three malaria vectors (*Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis*) in Benin (West Africa), *Malar. J.* 18 (175) (2019), <https://doi.org/10.1186/s12936-019-2808-9>.
- [26] J. Kyana, M.M. Luzala, C.J.K. Muanga, E.N. Zola, S.N. Wendji, B.M. Vuangi, P. B. Memvanga, Medicinal plants with antimicrobial, larvicidal, and repellent properties: An ethnopharmacological survey from the Democratic Republic of the Congo, e1128-e1128, *Orapuh J.* 5 (3) (2024), <https://doi.org/10.4314/orapi.v5i3.28>.
- [27] E.S.D. Melo, G.L. Wallau, Mosquito genomes are frequently invaded by transposable elements through horizontal transfer, *PLoS Genet.* 16 (11) (2020) e1008946, <https://doi.org/10.1371/journal.pgen.1008946>.
- [28] C. Minetti, V.A. Ingham, H. Ranson, Effects of insecticide resistance and exposure on Plasmodium development in *Anopheles* mosquitoes, *Curr. Opin. Insect Sci.* 39 (2020) 42–49, <https://doi.org/10.1016/j.cois.2019.12.001>.
- [29] G.T. Mukungulu, D.D. Tshilanda, D.S.T. Tshibange, E.M. Tshiwew, A.S. Vulama, N. N. N'sita, P.T. Mpiana, Larvicidal activity of some Congolese plant extracts against *Anopheles gambiae*, *J. Adv. Med. Life Sci.* 3 (2) (2015) 1–4, <https://doi.org/10.15297/JALS.V3I2.05>.
- [30] S. Naveenkumar, C. Kamaraj, C. Ragavendran, M. Vaithiyalingam, V. Sugumar, K. Marimuthu, *Gracilaria corticata* red seaweed mediate biosynthesis of silver nanoparticles: larvicidal, neurotoxicity, molecular docking analysis, and ecofriendly approach, *Biomass. Convers. Biorefinery* 14 (17) (2024) 20587–20609, <https://doi.org/10.1007/s13399-023-04026-x>.
- [31] B.J. Okoli, Z. Ladan, F. Mtunzi, Y.C. Hosea, Vitex negundo L. Essential oil: odorant binding protein efficiency using molecular docking approach and studies of the mosquito repellent, *Insects* 12 (12) (2021) 1061, <https://doi.org/10.3390/insects12121061>.
- [32] H. Onen, M.M. Luzala, S. Kigozi, R.M. Sikumbili, C.J.K. Muanga, E.N. Zola, P. B. Memvanga, Mosquito-borne diseases and their control strategies: an overview focused on green synthesized plant-based metallic nanoparticles, *Insects* 14 (3) (2023) 221, <https://doi.org/10.3390/insects14030221>.
- [33] P. Pescod, G. Bevivino, A. Anthoni, J. Shepherd, R. Shelton, F. Lombardo, T. Nolan, Homing gene drives can transfer rapidly between *Anopheles gambiae* strains with minimal carryover of flanking sequences, *Nat. Commun.* 15 (1) (2024) 6846, <https://doi.org/10.1038/s41467-024-51225-9>.
- [34] D.T. Rheder, N. Guilger, N. Bilecky-José, T. Germano-Costa, T. Pasquoto-Stigliani, T.B.B. Gallep, R. Grillo, C.D.S. Carvalho, L.F. Fraceto, R. Lima, Synthesis of biogenic silver nanoparticles using *Althaea officinalis* as reducing agent: evaluation of toxicity and ecotoxicity, *Sci. Rep.* 8 (1) (2018) 12397, <https://doi.org/10.1038/s41598-018-30317-9>.
- [35] J. Rocklöv, R. Dubrow, Climate change: an enduring challenge for vector-borne disease prevention and control, *Nat. Immunol.* 21 (5) (2020) 479–483, <https://doi.org/10.1038/s41590-020-0648-y>.
- [36] K. Sahin, *In silico* identification of angiotensin-1 converting enzyme inhibitors using text mining and virtual screening, *J. Biomol. Struct. & Dyn.* 40 (3) (2022) 1152–1162, <https://doi.org/10.1080/07391102.2020.1827038>.
- [37] Sakthivel, V., Narasimman, V., Ravi, K., Dhanya, G., Prasad, K., & Ramachandran, S. (2024). Biosynthesis of silver nanoparticles from the marine bacterium

- Marinobacter litoralis CARE V18 strain and evaluation of its antibacterial, antioxidant and acute toxicity properties using Zebrafish model. <https://doi.org/10.21203/rs.3.rs-4002055/v1>.
- [38] M. Sharma, A. Alexander, S. Saraf, S. Saraf, U.K. Vishwakarma, K.T. Nakhate, Mosquito repellent and larvicidal perspectives of weeds *Lantana camara* L. and *Ocimum gratissimum* L. found in central India, *Biocatal. Agric. Biotechnol.* 34 (2021) 102040, <https://doi.org/10.1016/j.bcab.2021.102040>.
- [39] A.M. Shehabeldine, M.A. Elbahnasawy, A.I. Hasaballah, Green phytosynthesis of silver nanoparticles using *Echinochloa stagnina* extract with reference to their antibacterial, cytotoxic, and larvicidal activities, *BioNanoScience* 11 (2021) 526–538, <https://doi.org/10.1007/s12668-021-00846-1>.
- [40] I.K. Shehu, H.B. Ahmad, I.K. Olayemi, D. Solomon, A.H. Ahmad, H. Salim, Insecticide susceptibility status in two medically important mosquito vectors, *Anopheles gambiae*, and *Culex quinquefasciatus* to three insecticides commonly used in Niger State, Nigeria, Saudi J. Biol. Sci. 30 (2) (2023) 103524, <https://doi.org/10.1016/j.sjbs.2022.103524>.
- [41] Y.N. Shkryl, G.N. Veremeichik, D.G. Kamenev, T.Y. Gorpenchenko, Y.A. Yugay, D. V. Mashtalyar, Y.N. Zhuravlev, Green synthesis of silver nanoparticles using transgenic *Nicotiana tabacum* callus culture expressing silicatein gene from marine sponge *Latrunculia oparinae*, *Artif. Cells Nanomed. Biotechnol.* 46 (8) (2018) 1646–1658, <https://doi.org/10.1080/21691401.2017.1388248>.
- [42] N. Shobana, P. Prakash, A.V. Samrot, S. Saigeetha, M. Sathiyasree, R. Thirugnanasambandam, R.R. Remya, Nanotoxicity studies of *Azadirachta indica* mediated silver nanoparticles against *Eudrilus eugeniae*, *Danio rerio* and its embryos, *Biocatal. Agric. Biotechnol.* 47 (2023) 102561, <https://doi.org/10.1016/j.bcab.2022.102561>.
- [43] F. Stanzione, I. Giangreco, J.C. Cole, Use of molecular docking computational tools in drug discovery, *Prog. Med. Chem.* 60 (2021) 273–343, <https://doi.org/10.1016/bs.pmch.2021.01.004>.
- [44] M. Summer, H.M. Tahir, S. Ali, S. Nawaz, R. Abaidullah, S. Mumtaz, A.H. Gormani, Nanobiopesticides as an Alternative and Sustainable Solution to Tackle Pest Outbreaks, *Biorefinery*, 14(17), 20587–20609, *J. Kans. Entomol. Soc.* 96 (4) (2024) 112–136, <https://doi.org/10.2317/0022-8567-96.4.112>.
- [45] N. Sutthanont, S. Attrapadung, S. Nuchprayoon, Larvicidal activity of synthesized silver nanoparticles from *Curcuma zedoaria* essential oil against *Culex quinquefasciatus*, *Insects* 10 (1) (2019) 27, <https://doi.org/10.3390/insects10010027>.
- [46] H. Tahir, F. Rashid, S. Ali, M. Summer, M. Afzal, Synthesis, Characterization, Phytochemistry, and Therapeutic Potential of *Azadirachta indica* Conjugated Silver Nanoparticles: A Comprehensive Study on Antidiabetic and Antioxidant Properties, *Biol. Trace Elem. Res.* 203 (4) (2025) 2170–2185, <https://doi.org/10.1007/s12011-024-04293-3>.
- [47] R.A. Tahir, A. Bashir, M.N. Yousaf, A. Ahmed, Y. Dali, S. Khan, S.A. Sehgal, In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1, *PLoS One* 15 (2) (2020) e0228265, <https://doi.org/10.1371/journal.pone.0228265>.
- [48] S. Thavamurugan, A. Annamalai, M. Narayanan, M. Devan, N. Manoharan, A. L. Prabha, Green synthesis of silver nanoparticles using *Osbeckia leschenaultiana* DC extract: Optimization of synthesis, biological activities, larvicidal activity and toxicity analysis, *Inorg. Chem. Commun.* 169 (2024) 113011, <https://doi.org/10.1016/j.inoche.2024.113011>.
- [49] World Health Organization, UNICEF, Global vector control response 2017–2030, World Health Organization, Switzerland, 2017.
- [50] World Health Organization, Guidelines for laboratory and field testing of mosquito larvicides01, World Health Organization, 2005, pp. 1–41. No. WHO/CDS/WHOPES/GCDPP/2005.13.
- [51] World Health Organization, Guidelines for efficacy testing of mosquito repellents for human skin (No. WHO/HTM/NTD/WHOPES/2009.4), World Health Organization, Switzerland, 2009. (<https://coilink.org/20.500.12592/hx5kz2>). on 08 Jan 2025. COI: 20.500.12592/hx5kz2.
- [52] World Health Organization, WHO traditional medicine strategy: 2014–2023, World Health Organization, 2013.
- [53] World Health Organization, World malaria report 2023, 2023.
- [54] Y.A. Yugay, M.R. Sorokina, V.P. Grigorchuk, T.V. Rusapetova, V.E. Silant'ev, A. E. Egorova, Y.N. Shkryl, Biosynthesis of functional silver nanoparticles using callus and hairy root cultures of *Aristolochia manshuriensis*, *J. Funct. Biomater.* 14 (9) (2023) 451, <https://doi.org/10.3390/jfb14090451>.