Facile synthesis of silver and gold nanoparticles using mangrove (Avicennia marina) leaves extract and its cytotoxicity and larvicidal activity

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INTRODUCTION

Incorporating biological entities, like plant extracts into metallic NPs synthesis not only introduces an environmentally friendly aspect but also holds promise for applications across various fields (Begum et al., 2022). The eco-friendly synthesis of metallic nanoparticles utilizing plant extracts, driven by their exceptional properties, has emerged as the most sought-after route in the field of Nanoscience (Castillo-Henriquez et al., 2020; Guleria et al., 2022). Due to their diminutive nanoscale dimensions, these nanomaterials enhance bioactivities and offer unique attributes that render them indispensable across various fields, such as biomedicine, sensors, environmental remediation and sustainable energy production (Pasca et al., 2014). Several investigations have documented the effective creation of biogenic nanoparticles utilizing various plant species (Majoumouo et al., 2020).

A. marina, a mangrove recognized as a salt-tolerant plant species, is extensively distributed along the coastlines of tropical and subtropical regions, including areas of the Middle East, Australia, Asia and Africa (Saenger, 2002). This hardy plant is characterized by its unique adaptations to saline and brackish environments and is highly valued for its ecological significance as well as its potential medicinal properties (Barbier, 2016). A. marina has a history of use in traditional medicine systems in many coastal regions to treat a range of ailments (Al-Mur., 2021). Extracts from the leaves and bark have been studied for their potential anti-inflammatory and analgesic properties (Garba et al., 2018). They have been used in traditional remedies to alleviate pain and reduce inflammation (Sheel et al., 2017). The plant is known to contain antioxidants and Anti-Diabetic Potential, which can help to protect cells from oxidative stress and related damage (Mohamed & Mamat, 2016; Nayak et al., 2013). The Anti-Malarial and Anti-Fungal Properties of A. marina plant extracts are also tested in traditional medicine practices, where it has been used to combat malaria and various fungal species (Al-Shanfari & Rehman, 2019; Amarasinghe et al., 2021). Recently, Karthi et al., 2020 evaluated the potency of acetonic extracts of A. marina against three medically important mosquitoes i.e. Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus.

In the present study, screening of mangrove plant viz., A. marina leaf extracts (AMLX) and synthesis of its silver (AMAgNPs) and gold (AMAuNPs) nanoparticles have been investigated for biological properties, including cytotoxic and proliferative effects, along with toxicity against fourth instar larvae of Culex pipiens. UV/Visible spectrophotometry.

Abstract: The field of bio-fabricated noble metallic nanoparticles (NPs) has gained significant attention in applied research due to their eco-friendly and biocompatible nature. This study focuses on employing a green synthesis method to produce silver and gold nanoparticles (bio-fabricated) using a Mangrove plant extract and assessing their insecticidal and growth-inhibitory effects for environmentally friendly pest control. The resulting NPs underwent comprehensive characterization through various spectroscopy techniques. The morphology of both silver and gold mediated nanoparticles of Avicennia marina leaf extract displayed a spherical shape, with average sizes measuring around 70-80 nm and 95-100 nm, respectively. Regarding cytotoxicity, the inhibitory effects of silver nanoparticles were less than that observed by the extract alone while gold nanoparticles showed stronger cell growth inhibitory effects on splenic cells. The hepatic toxicity of silver and gold nanoparticles showed significant toxic effects as compared to A. marina extract alone. Notably, as prepared silver nanoparticles exhibited substantial larvicidal toxicity as compared to gold nanoparticles, when tested against fourth instar Culex pipiens larvae. These biocompatible silver and gold nanoparticles prepared from A. marina leaf extract hold promise for future applications as larvicides to effectively control mosquito species.

Keywords: Gold nanoparticles, silver nanoparticles, cytotoxicity, larvicidal activity, Avicennia marina, Culex pipiens.
Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) spectrometry were employed to validate the biosynthesis of gold (AuNPs) and silver (AgNPs) nanoparticles. To our knowledge, this research represents the initial documentation of the green synthesis of Ag and Au NPs utilizing AMLX from Saudi Arabia and explores their respective mosquitocidal activities.

MATERIALS AND METHODS

Preparation of A. marina leave extract (AMLX)
A. marina leaves were collected from costal region of southwestern Saudi Arabia and identified. The leaves were washed with tap water, then gently air-dried in the shade at room temperature (20-23°C) and finally, they were blended to achieve a fine powder. The powder underwent an extraction process using 70% ethanol at room temperature. Subsequently, 100 grams of the resulting powder was combined with 300 mL of ethanol (70%). The blend was stirred for 40 hours at ambient temperature and subsequently filtered and then processed using a rotary evaporator (150 RPM) and dried at 40 °C for three hours. The final dried product amounted to approximately 4 grams, forming a semi-solid crude extract. Stock solutions were prepared from this dried material, including a 1% acetone (Sigma–Aldrich ®) solution and a 0.5% dimethyl sulfoxide (DMSO; Sigma) solution.

Synthesis of AMAgNPs and AMAuNPs using AMLX
In the synthesis of metallic nanoparticles, the leaf extract of A. marina (AMLX) served as both a capping and reducing agent. To produce silver nanoparticles, 1 mL of the AMLX stock solution was blended with 99 mL of a 1 mM silver nitrate (AgNO3; Sigma–Aldrich ®) solution and continuously stirred for 24 hours at room temperature, until a noticeable change in color to brown signified the successful formation of AgNPs. For the gold nanoparticles (AuNPs), 12 mL of AMLX was introduced into 88 mL of a 1 mM solution of chloroauric acid (HAuCl4·3H2O; Sigma-Aldrich ®) in a flask at room temperature. Changing color of solution transformed from yellowish to ruby red within 1 hour, indicating the successful formation of gold nanoparticles.

Characterization of AMLX produced AMAgNPs and AMAuNPs
The examination of AgNPs and AuNPs production and their characteristics involved various techniques. The resulting solution was scanned at wavelength 300-600 nm using a spectrophotometer (UV-Visible Spectrophotometers, GENESYS 10S Series; Thermo Scientific™- United States) 1nm resolution to detect the silver and gold nanoparticles formation. The size and shape of the synthesized AMAgNPs and AMAuNPs analysed by Scanning electron microscope (SEM; JEOL, Japan). The identification of functional groups presents in the AMAgNPs and AMAuNPs was performed using Fourier Transform infrared spectroscopy (FT-IR, JASCO 460 plus). The FT-IR analysis covered a range of 400-4000 cm⁻¹, sampled at a resolution of 16x with a clarity spectral of 4 cm⁻¹.

Cytotoxic/proliferative in vitro effects of AMLX and extract prepared AMAgNPs and AMAuNPs on normal splenic cells proliferation.
Splenocells culture formulation
For the preparation of splenic cell culture, single-cell suspensions were obtained from the spleen of a rat, weighing approximately 280 grams. These cells were suspended at a density of 0.05 X 10⁶/ml in a complete Roswell Park Memorial Institute medium (RPMI-1640; Gibco), which consisted of 10% fetal calf serum, 2 mM L-glutamine, 2 mM sodium pyruvate and bicarbonate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer all from Gibco. Cell culture was performed in a 96-well tissue culture-plate (TPP, Merk) by introducing 100 µL of the cell suspension (equivalent to 5000 cells per well) along with 100 µL of AMLX or AMAgNPs or AMAuNPs (Ibrahim et al., 2019).

Study of cytotoxic/proliferative effects
The assessment of cytotoxic or proliferative effects of AMLX and its prepared nanoparticles on splenic cells was conducted by introducing various concentrations of AMLX, AMAgNPs and AMAuNPs. These concentrations included final concentrations of 50-200 µg/ml in culture media, each added separately to wells containing cells in triplicates. A control culture was set up by seeding 5000 splenic cells per well in 200 µL of culture medium. Subsequently, all plates were incubated in a CO2 incubator at around 37 °C for about 72 hours and the cell viability in each plate was evaluated as per the method of Ibrahim et al., 2021.

Acute cytotoxicity study of AMLX leaf acetone extract and AMAgNPs and AMAuNPs
To evaluate hepatic toxicity potentially associated with AMLX produced AMAgNPs and AMAuNPs, a one 1 mL dose régime (100 µg/mL) of extracts was administered to five rats (200-250 gm). After a 24-hour period, the rats were dissected, and serum were collected from the blood. LF (Liver function) was assessed by calorimetrically evaluating the amount of sera aspartate amino-transferase (AST) using the Randox Kit (UK) based on the method outlined by Reitman and Frankel (1957).

Mosquitocidal activity
Collection of C. pipiens Larvae
C. pipiens larvae were collected from different breeding sites in Abha, Saudi Arabia. These larvae were placed in separate plastic containers filled with tap water. They were reared in the laboratory until they reached the mid-fourth instar stage, during which they were fed a blend of
dog feed and yeast in a ratio of 3:1 for their nutritional intake, as described by Rahuman et al. in 2008. The fourth instar larvae were collected approximately six days after hatching, when they had grown to a length of about half an inch.

**Larval bioassay AMAgNPs and AMAuNPs**

The larval susceptibility test followed the WHO (2005) protocol. Groups of *C. pipiens* fourth instar larvae (20 larvae per group) were subjected to different concentrations of AMLX produced AMAgNPs and AMAuNPs for 24 hours in five separate replicates. A control group, consisting of twenty larvae, was treated with tap water only. All groups were kept at 27 °C with a light/dark cycle of sixteen hours of light and eight hours of darkness. The larvae were provided with their usual diet as mentioned previously during the experiments (Cosgrove et al., 1994). After 24 hours of initial treatment, the mortalities of the larvae in all groups were recorded (WHO, 2005).

**Hemolytic activity of AMLX and AMAuNPs**

Hemolytic activity of the AMLX and AMAuNPs was assessed using the following materials and methods. To determine cytotoxicity, the hemolytic activity of the AMLX and AMAuNPs was individually tested at final concentrations of 200 μg/ml, following the procedure of Oves et al., (2013) with some modifications. Fifty milliliters of cow blood were divided into several sterile 15 mL Falcon tubes and centrifuged for 10 minutes at 1,500rpm. Following centrifugation, the supernatants were gathered and the absorbance was assessed at 576 nm. The percentage of hemolysis was then calculated.

**STATISTICAL ANALYSIS**

All gathered data underwent statistical analysis, means were compared utilizing LSD at a significance level of P ≤ 0.05, employing the SAS software program, version 9.3 (SAS Institute, 2006). LC350, and LC90 values were determined using probit analysis program as per Finney's method (1972). The mortality percentage was adjusted for control mortality using Abbott's formula (Abbott, 1987).

**RESULTS**

**Characterization of NPs**

*Change color.*

The alteration in the color of the extract (AMLX) mixed with silver nitrate and Chloroauric acid was monitored visually (fig. 1). Observations revealed a color shift in the solution from light yellow to brown, eventually transitioning to a Dark brown (AMAgNPs) dark brownish-red shade (AMAuNPs). The progression towards darker brown to brownish red was time dependent and is characterized by the intensification of color, potentially linked to the excitement of Surface Plasmon Response (SPR) involving metal NPs, as explained by Ranganathan et al. (2012). The color modifications in the solutions can be ascribed to biochemical compounds, including alkaloids, flavonoids and steroids etc. This change in color frequently signifies shifts in the metal oxidation state, wherein Au+ is converted to Au0 and Ag+ is transformed to Ag0 by biomolecules found in the plant extract, including proteins and other components (Manivasagan et al., 2013).

**UV-Vis analysis of AMLX – mediated AMAgNPs and AMAuNPs**

The occurrence of gold/silver nanoparticles in AMAgNPs and AMAuNPs were detected by UV– visible spectroscopy and the visible absorbance of both was noted between 200 and 700 nm (fig. 1). The reduction of HAuCl4 to AuNPs and AgNO3 to AgNPs was found at 596 nm and 483 nm respectively.

**Fourier transform infrared (FTIR) spectroscopy**

FTIR spectroscopy was used to identify potential bioactive molecules within the leaves that contribute to the synthesis of gold and silver nanoparticles. Fig. 2 (A and B) indicates the FTIR spectra of AMAgNPs and AMAuNPs respectively. The major absorption bands appeared in the range of 3566.27–3446.24 (–OH stretch of carboxylic acids), 2800.00–2938.26 (–CH2 stretch of alkanes), 1631.44-1646.24 (–C=C– stretch of alkanes), 517.47–591.75 cm–1 (C–Br stretch of alkyl halides) and 446.34 cm–1 (Silverstein, 1981). After the biological reduction, the peaks were formed at 3470.91, 2938.26, 1631.44, 1451.26, 1403.82, 1339.12, 1263.39, 1165.67, 1074-26, 933.24, 900.79, 591.75 cm–1 for gold nanoparticle, whereas for silver nanoparticles the peaks were at 3566.27, 3545.29, 3446.24, 1646.24, 1516.15, 1012.47, 980.12, 825.16, 569.43, 517.47 cm–1.

**Morpho-characterization using SEM**

Scanning electron micrographs (SEM) were instrumental in characterizing both the size/shape of the Silver and gold NPs (fig. 3 A and B). The SEM analysis of the synthesized AgNPs distinctly revealed clustered and irregular shapes, predominantly aggregated, with an average size ranging from 40 to 60 nm and observable interparticle distance, as magnified at ×10,000 times. Similarly, the AuNPs also exhibited random shapes (aggregated) with an average size of 95-100 nm and interparticle distance, as observed at 10,000x magnification.

**Cytotoxic/proliferative in vitro effects of AMLX and extract prepared AMAgNPs and AMAuNPs on normal splenic cells proliferation**

The cytotoxic or stimulatory characteristics present in the AMLX were examined at various concentrations. The findings indicated inhibitory effects of AMLX at all investigated concentrations (200-50 μg/mL). The inhibitory effect heightened as the extract concentration decreased.
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Table 1: Percent normal splenic cells growth stimulation/inhibition after treatment with AMLX and extract prepared AMAgNPs and AMAuNPs.

<table>
<thead>
<tr>
<th>(µg/mL)</th>
<th>% of splenic cells growth inhibition/stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMLX</td>
</tr>
<tr>
<td>200</td>
<td>88.41±0.011</td>
</tr>
<tr>
<td>100</td>
<td>48.73±0.014</td>
</tr>
<tr>
<td>50</td>
<td>47.14±0.021</td>
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</table>

Table 2: Biochemistry of AMLX and extract prepared AMAgNPs and AMAuNPs.

<table>
<thead>
<tr>
<th>Urea</th>
<th>Creatinine</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMLX</td>
<td>1.49489441</td>
<td>1.317294682</td>
<td>1.04954955</td>
</tr>
<tr>
<td>AmAgNPs</td>
<td>2.019592632</td>
<td>1.567523148</td>
<td>0.945945946</td>
</tr>
<tr>
<td>AmAuNPs</td>
<td>1.428894838</td>
<td>1.4704997</td>
<td>0.891891892</td>
</tr>
</tbody>
</table>

Table 3: Absorbance of AMLX and AMAuNPs with their red blood cells (RBCs) lysis percentage.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Absorbance at wavelength of 576 nm</th>
<th>RBC hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AMLX</td>
<td>&gt; 3.00</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>AMAuNPs</td>
<td>&gt; 3.00</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Control (Negative)</td>
<td>0.012</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Control (Positive)</td>
<td>&gt; 3.00</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Susceptibility of C. pipiens larvae to bioinsecticides AMLX, AMAgNPs and AMAuNPs following continuous exposure for 48 h.

<table>
<thead>
<tr>
<th>Bioinsecticide</th>
<th>Conc. (ppm.)</th>
<th>Mortality (%)</th>
<th>LC₅₀ (ppm.) (LCL-UCL)</th>
<th>LC₉₀ (ppm) (LCL-UCL)</th>
<th>χ² d.f (n.s) = 4</th>
<th>slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMLX</td>
<td>100</td>
<td>19.375</td>
<td>270.9</td>
<td>1147.8</td>
<td>2.0308</td>
<td>2.044</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>54.167</td>
<td>228.8-313.1</td>
<td>932.4-1516.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>68.75</td>
<td>80.208</td>
<td>313.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>91.25</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMAgNPs</td>
<td>10</td>
<td>31.771</td>
<td>15.922</td>
<td>44.1265</td>
<td>5.1375</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>55.417</td>
<td>38.4-52.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>77.813</td>
<td>13.8-17.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>85.625</td>
<td>34.9-43.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>96.667</td>
<td>84.45-111.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMAuNPs</td>
<td>30</td>
<td>37.771</td>
<td>37.734</td>
<td>94.913</td>
<td>3.749</td>
<td>3.389</td>
</tr>
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<td>50</td>
<td>58.417</td>
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<td>70</td>
<td>77.813</td>
<td>84.45-111.08</td>
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<tr>
<td></td>
<td>110</td>
<td>96.667</td>
<td>34.9-43.9</td>
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</table>

a: Five replicates, 20 larvae each; b: Tabulated(χ²)is 7.8 > calculated at 0.05 level of significance.

Fig. 1: UV-Vis spectra of AgNPs and AuNPs synthesized by the AMLX.
FT-IR spectra of leaf extract of *A. marina* after addition of AgNO$_3$ (A); AuCl$_4$ (B).

**Fig. 2:** FT-IR spectra of leaf extract of *A. marina* after addition of AgNO$_3$ (A); AuCl$_4$ (B).

The SEM image showing the crystalline silver nanoparticles as uniform and aggregates with AgNO$_3$ (A); AuCl$_4$ (B).

**Fig. 3:** The SEM image showing the crystalline silver nanoparticles as uniform and aggregates with AgNO$_3$ (A); AuCl$_4$ (B).

Percent normal splenic cells growth stimulation/inhibition after treatment with *AMLX* and extract prepared AMAgNPs and AMAuNPs.

**Fig. 4:** Percent normal splenic cells growth stimulation/inhibition after treatment with *AMLX* and extract prepared AMAgNPs and AMAuNPs.
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The rise in inhibitory potential corresponded to the decrease in the extract concentration may be due to the balance between inhibitory and stimulatory biomolecules found in the extract (fig. 4; table 1). The same trend is shown regarding the extract containing the silver and gold nanoparticles. Regarding the extract containing the silver nanoparticles AMAgNPs, the inhibitory effects were less than that observed by the AMLX alone. The extract containing AuNPs (AMAuNPs) showed stronger cell growth inhibitory effects than those in case of AMLX and the AMAgNPs.

Acute cytotoxicity study of AMLX leaf acetone extract and and AMAgNPs and AMAuNPs
The potential hepatic toxicity in AMLX was assessed in rats. The results indicated non-significant changes in aspartate aminotransferase (AST) compared to the control group, while alanine aminotransferase (ALT) demonstrated a twofold increase over the control group (table 2). In the AMAgNPs and AMAuNPs treated group, levels of AST showed a non-significant decrease and a significant increase in ALT. Regarding the testing of kidney functions, all preparations caused the increase in both urea and creatinine.

Hemolytic activity of AMLX and AMAuNPs
The percentage (%) of RBC lysis was determined by contrasting the absorbance of the sample with the positive/negative controls (table 3). The positive control (1.5 % Triton X=100) exhibited complete lysis (100%), whereas the negative control (P.B.S) demonstrated no lysis effects on the RBCs. Both AMAuNPs and the

Fig. 5: (I & II). The effect of (A) AMLX alone and (B) its and AMAuNPs on cow RBCs. Where, N is negative control and P is positive control.

Fig. 6: The relationship between concentrations of AMLX and extract prepared AMAgNPs and AMAuNPs and mortality percentage of 4th instar larvae of Aedes aegypti, Line1: AMLX; Line2: AMAgNPs; Line 3: AMAuNPs
AMLX extract alone exhibited 100% RBC lysis (fig. 5 I&II).

**Larvicidal effects of AMAgNPs and AMAuNPs using AMLX on C. pipiens**

The susceptibility level of *C. pipiens* larvae, treated with various concentrations of AMLX extract alone and extract with AMAgNPs and AMAuNPs against fourth larval instars, are detailed in Table 4 and Figure 6, including the corresponding mortality rates at various concentration of LC50, LC90, regression equations and chi-square data. Various concentrations of AMLX (50, 100, 150, 200 and 250ppm); synthesized AMAgNPs (30, 50, 100, 150 and 200ppm) and synthesized AMAuNPs (10, 30, 50,70 and 100ppm) were tested against the 4th instar of *C. pipiens*. Consequently, 19.37-91.25% (AMLX), 31.77-96.66 % (AMAgNPs) and 37.77-96.66 % (AMAuNPs) larval mortality were obtained when treated with the above-mentioned concentrations. By decreasing the concentration of AMAgNPs and AMAuNPs, the mortality rate in fourth instars larvae decreased (table 3: fig. 6), demonstrating a significant positive correlation, the tested concentrations show a connection with the percentage of larval mortality. Corresponding to the tested concentrations of AMLX, AMAgNPs and AMAuNPs and by taking LC50 and LC90 values into consideration, the records showed that the AMAgNPs LC50 (15.92 ppm) and LC90 (44.12 ppm) proved to be a more effective than the AMAuNPs LC50 (39.73 ppm) and LC90 was (94.91 ppm) and also AMLX LC50 (270.90ppm) and LC90 (1147.80ppm), which is about 2.49, 17.01 (LC50) and 2.15, 25.99 (LC90) folds, respectively. The x2 value was significant at p <0.05.

**DISCUSSION**

The green synthesis approach employing botanical extracts as both reducing and capping agents in nanoparticle synthesis has been explored (Shafey et al., 2020). Visual observation of color changes after adding silver nitrate and chloroaucic acid to the plant extract indicated time-dependent shifts from darker brown to brownish-red, potentially linked to Surface Plasmon Resonance (SPR) excitation involving metal nanoparticles, as explained by Manivasagan et al., 2013; Ranganathan et al., 2012. The UV-visible spectroscopy characterization confirmed the synthesis of gold and silver nanoparticles, with peaks at 596 nm and 483 nm, respectively, consistent with studies such as Balasubramani et al. (2015). Additional evidence from the UV-visible spectrum at 543 nm and 445 nm supported nanoparticle synthesis, as observed by Haiss et al., 2007. FTIR identified bioactive molecules in *A. marina* leaves, upon comparing the spectra, it was observed that the presence of phenol and flavonoid compounds in mangrove plants might play a role in the formation of gold/silver nanoparticles (Vaish et al., 2023).

The FTIR spectra revealed shifts in bands related to C=O, O–H stretching modes and vibrations of the C–OH group, indicating bioorganic molecules as capping or reducing agents for Au and Ag nanoparticles (Wang et al., 2000). SEM scanning electron micrographs demonstrated clustered and irregular shapes for Silver NPs (40-60 nm) and random shapes for Gold NPs (95-100 nm). These comprehensive characterizations contribute to a deeper understanding of the interactions between bioorganic molecules and metallic nanoparticles in synthesized colloidal solutions.

The *in vitro* cytotoxic or proliferative effects of *A. marina* plant extract, silver nanoparticles (AMAgNPs) and gold nanoparticles (AMAuNPs) on normal splenic cell proliferation were investigated, shedding light on the complex interactions and potential cytotoxic effects of these substances (Andriani et al., 2021; Cerri et al., 2022; Sohaib et al., 2022; Ahmed et al., 2022). Subsequent acute cytotoxicity studies on AMLX leaf acetone extract and nanoparticles in rats revealed potential hepatic toxicity, with alterations in AST and ALT levels (Aunjum et al., 2021; Ahmed et al., 2022). Notably, AMAgNPs and AMAuNPs demonstrated significant toxic effects on the liver *in-vivo*, potentially influenced by bio-active compounds in AMLX. Additionally, the hemolytic activity of AMLX and AMAuNPs resulted in 100% RBC lysis, emphasizing potential adverse effects on blood cells (Andriani et al., 2021). These comprehensive findings contribute valuable insights into the potential cytotoxicity and biological interactions associated with *A. marina* extracts and synthesized nanoparticles.

The larvicidal effects of silver nanoparticles (AMAgNPs) and gold nanoparticles (AMAuNPs) synthesized using *A. marina* leaf extract (AM LX) on Culex pipiens mosquitoes were assessed, revealing significant efficacy compared to AsExt (AM LX extract alone). The calculated LC50 and LC90 Values demonstrated that AMAgNPs were more effective (LC50: 15.92 ppm, LC90: 44.12 ppm) than AMAuNPs (LC50: 39.73 ppm, LC90: 94.91 ppm) and AMLX (LC50: 270.90 ppm, LC90: 1147.80 ppm), showing 2.49-17.01x (LC50) and 2.15-25.99x (LC90) fold greater potency, respectively. Plant-based nanoparticles are increasingly recognized for their effectiveness in mosquito vector control programs due to their specificity and eco-friendly nature (Benelli, 2016; Govindarajan et al., 2016; Soni & Prakash, 2014). This aligns with numerous studies highlighting the mosquitocidal potential of plant-mediated nanoparticles (Murugan et al., 2015; Karthi et al., 2020; Sengul et al., 2022; Balakrishnan et al., 2016; Amarasinghe et al., 2020; ZY, 2021). The study represents the initial documentation of mosquito-larvicidal activity for AMAgNPs and AMAuNPs synthesized from AMLX, consistent with earlier reports suggesting the involvement of biomolecules present in AMLX, such as phenols.
terpenoids and flavonoids, in the observed larvicidal activity. The study highlights the potential of these nanoparticles in environmentally friendly mosquito control strategies, marking a significant contribution in Saudi Arabia.

CONCLUSION

The utilization of A. marina leaves extract in the biosynthesis of silver and gold nanoparticles demonstrated remarkable mosquito-killing activity and cytotoxic/proliferative effects. The inhibitory effects of silver nanoparticles were less than that observed by the extract alone while gold nanoparticles showed stronger cell growth inhibitory effects on splenic cells. The hepatic toxicity of AMAgNPs and AMAuNPs showed significant toxic effects on liver in vivo. Consequently, the green approach seems to be a economical substitution to toxic effects on liver in vivo. Consequently, the green approach holds promise for establishing a biological process suitable for large-scale production.

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REFERENCES


