doi.org/10.36721/PJPS.2024.37.2.REG.297-305.1

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

Hamed A Gramah[1,2,3], Zubair Ahmad[2,4] and Essam H Ibrahim[3]
[1]Research Center for Advanced Materials Science (RCAMS), King Khalid University, Abha, Saudi Arabia
[2]Center of Bee Research and its Products, King Khalid University, Abha, Saudi Arabia
[3]Biology Department, Faculty of Science, King Khalid University, Abha, Saudi Arabia
[4]Applied College, Mahala Campus, King Khalid University, Abha, Saudi Arabia

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.*

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-Henríquez *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.

**Corresponding author:* e-mail: dzubair@gmail.com

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 ($HAuCl_4.3H_2O$; 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.

Splenic cells 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 10^6/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 CO_2 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 $P \leq 0.05$, employing the SAS software program, version 9.3 (SAS Institute, 2006). LC_{50}, and LC_{90} 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 bio-chemical compounds, including alkaloids, flavonoids and steroids etc. This change in color frequently signifies shifts in the metal oxidation state, wherein Au+ is converted to Au^0 and Ag^+ is transformed to Ag^0 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 $HAuCl_4$ to AuNPs and $AgNO_3$ 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 ($–CH_2$ stretch of alkanes), 1631.44-1646.24 (–C=C– stretch of alkanes), 517.47–591.75 (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 inter-particle 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.

Table 1: Percent normal splenic cells growth stimulation/inhibition after treatment with *AMLX* and extract prepared AMAgNPs and AMAuNPs.

(µg/mL)	% of splenic cells growth inhibition/stimulation		
	AMLX	AMAgNPs	AMAuNPs
200	88.41±0.011	92.54±0.025	58.41±0.010
100	48.73±0.014	57.62±0.012	40.16±0.013
50	47.14±0.021	41.27±0.007	33.65±0.002

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

	Urea	Creatinine	AST	ALT
AMLX	1.49489441	1.317294682	1.04954955	2.553398058
AmAgNPs	2.019592632	1.567523148	0.945945946	1.932038835
AmAuNPs	1.428894838	1.474046997	0.891891892	2.087378641

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

No.	Treatment	Absorbance at wavelength of 576 nm	RBC hemolysis (%)
1	AMLX	> 3.00	100
2	AMAuNPs	> 3.00	100
3	Control (Negative)	0.012	0
4	Control (Positive)	> 3.00	100

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

Bioinsecticide	Conc. (ppm.)	Mortality (%)	LC_{50} (ppm.) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	$\chi 2$ *d.f (n.s) =4*)	slope
AMLX	100	19.375	270.9 228.8- 313.1	1147.8 932.4- 1516.2	2.0308	2.044
	300	54.167				
	500	68.75				
	800	80.208				
	1000	91.25				
AMAgNPs	10	31.771	15.922 13.8-17.8	44.1265 38.4-52.9	5.1375	2.89
	20	55.417				
	30	77.813				
	40	85.625				
	50	96.667				
AMAuNPs	30	37.771	39.734 34.9-43.9	94.913 84.45-111.08	3.749	3.389
	50	58.417				
	70	77.813				
	90	87.625				
	110	96.667				

a: Five replicates, 20 larvae each; b: Tabulated$(\chi 2)^2$is 7.8 > calculated at 0.05 level of significance.

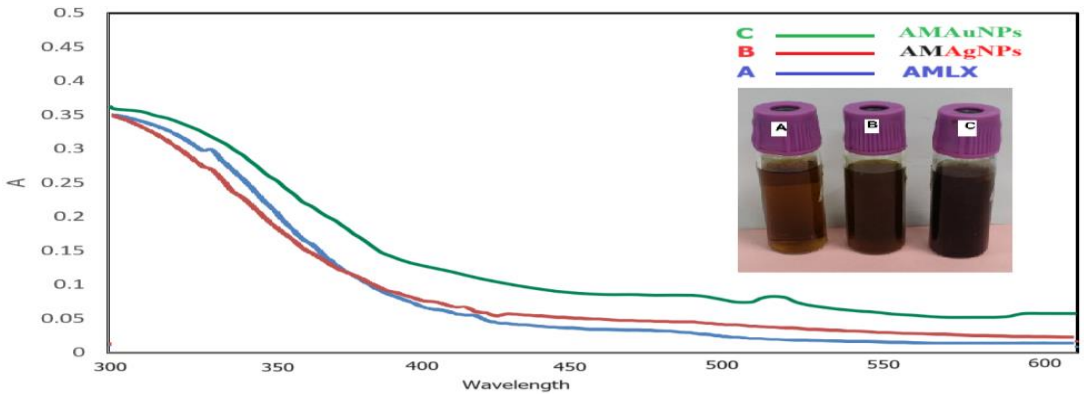

Fig. 1: UV-Vis spectra of AgNPs and AuNPs synthesized by the *AMLX*.

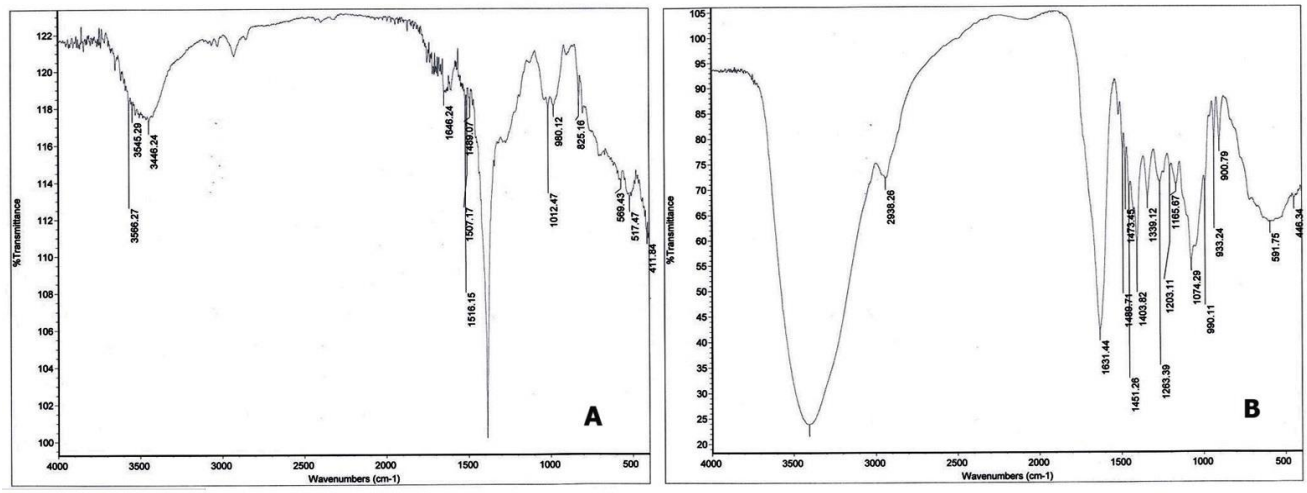

Fig. 2: FT-IR spectra of leaf extract of *A. marina* after addition of $AgNO_3$(A) and AuCL4 (B).

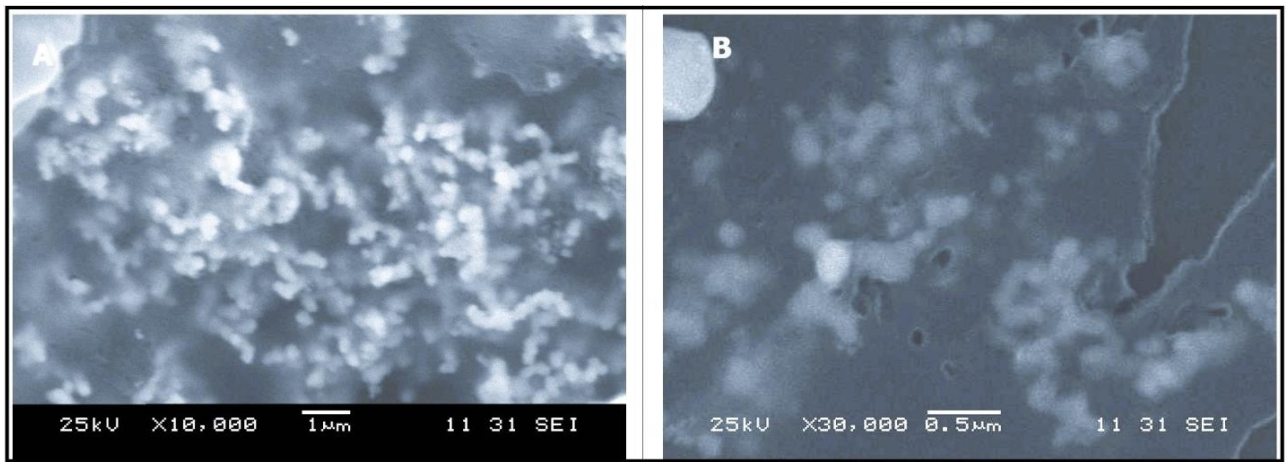

Fig. 3: The SEM image showing the crystalline silver nanoparticles as uniform and aggregates with AgNO3 (A); AuCL4 (B)

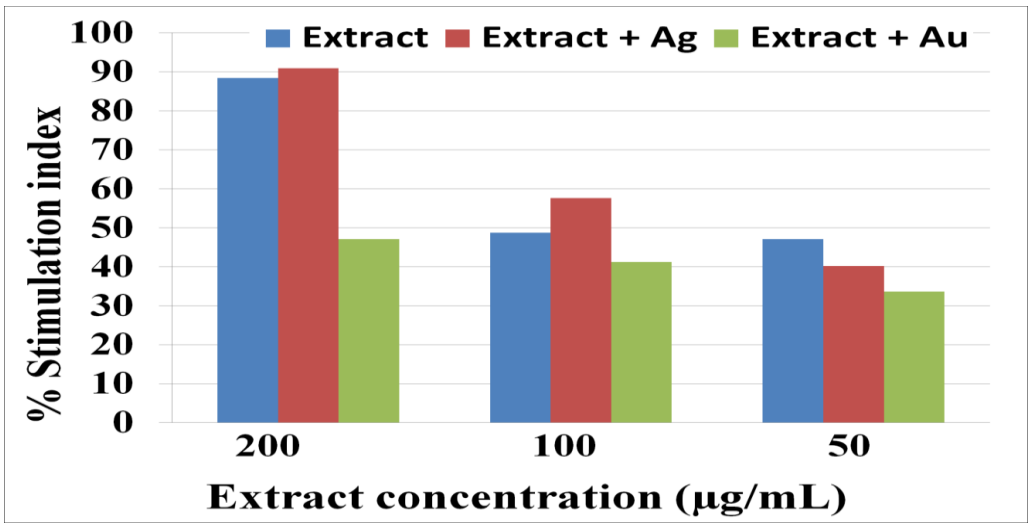

Fig. 4: Percent normal splenic cells growth stimulation/inhibition after treatment with *AMLX* and extract prepared AMAgNPs and AMAuNPs.

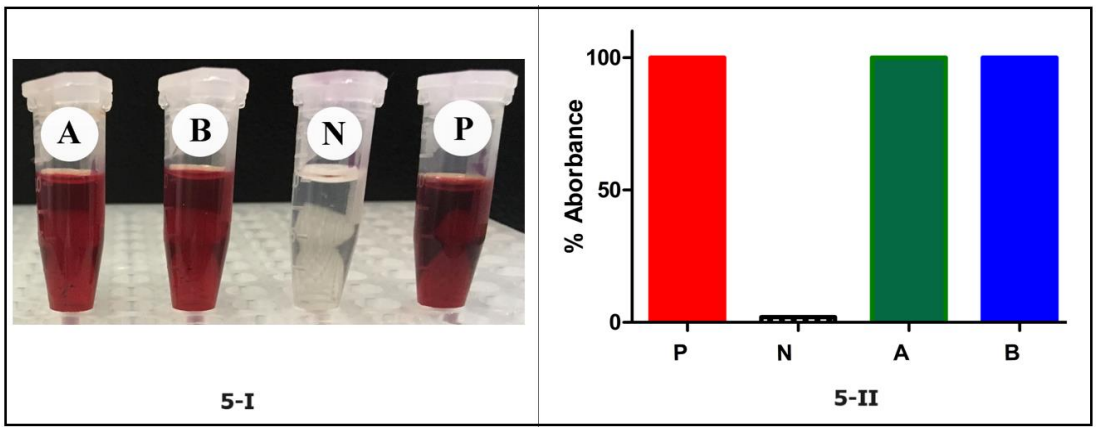

Fig. 5: (I & II)**.** The effect of (A) AMLX alone and (B) its and AMAuNPs on cow RBCs. Where, N is negative control and P is positive control.

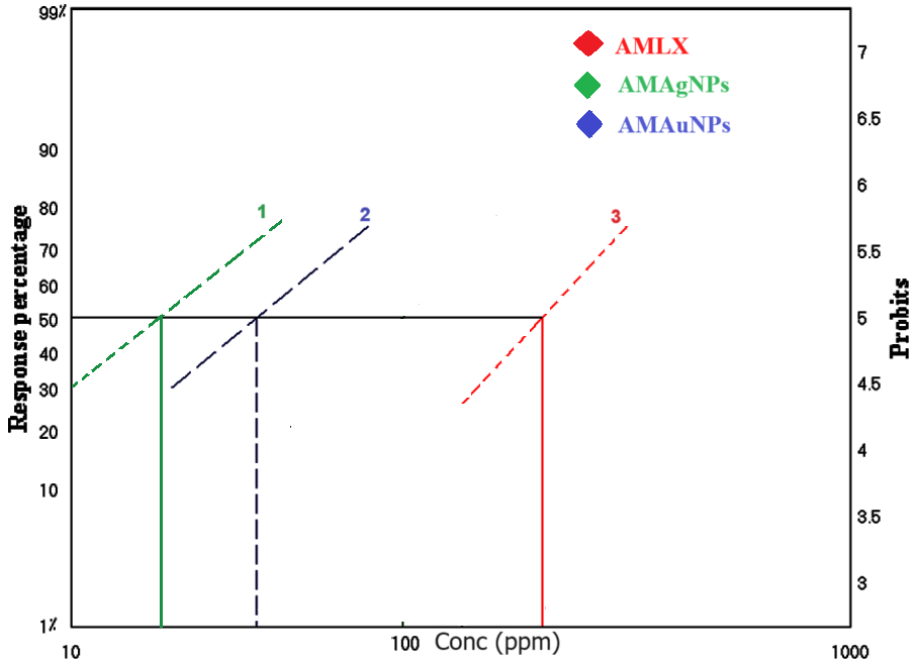

Fig. 6: The relationship between concentrations of *AMLX* and extract prepared AMAgNPs and AMAuNPs and mortality percentage of 4th instar larvae of *Aedes aegypti*, Line1: *AMLX*; Line2: AMAuNPs; Line 3: AMAuNPs

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

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 LC_{50}, LC_{90}, 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 LC_{50} and LC_{90} values into consideration, the records showed that the AMAgNPs LC_{50} (15.92 ppm) and LC_{90} (44.12ppm) proved to be a more effective than the AMAuNPs LC_{50} (39.73 ppm) and LC_{90} was (94.91 ppm) and also AMLX LC_{50} (270.90ppm) and LC_{90} (1147.80ppm), which is about 2.49, 17.01 (LC_{50}) and 2.15, 25.99 (LC_{90}) 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 chloroauric acid to the plant extract indicated time-dependent shifts from darker brown to brownish-red, potentially linked to Surface Plasmon Resonance (SPR) excitement 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 LC_{50} and LC_{90}values demonstrated that AMAgNPs were more effective (LC_{50}: 15.92 ppm, LC_{90}: 44.12 ppm) than AMAuNPs (LC_{50}: 39.73 ppm, LC_{90}: 94.91 ppm) and AMLX (LC50: 270.90 ppm, LC90: 1147.80 ppm), showing 2.49-17.01x (LC_{50}) and 2.15-25.99x (LC_{90}) 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 established physical or chemical approaches for synthesizing silver and gold nanoparticles. This approach holds promise for establishing a biological process suitable for large-scale production.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the research groups program # RGP.2/427/44.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**(2): 265-267.

Ahmed MA, Ameyaw EO, Ackah-Armah F, Acheampong DO, Gathumbi PK, Adinortey MB, Ghartey-Kwansah G, Otsyina HR and Adokoh CK (2022). *In vitro* and *In Vivo* toxicological evaluation of *Avicennia africana* P: Beauv. (Avicenniaceae) leaf extract in a rat model. *J. Toxicol*., **2022**: 11.

Al-Mur BA (2021). Biological activities of *Avicennia marina* roots and leaves regarding their chemical constituents. *Arab. J. Sci. Eng.*, **46**(6): 5407-5419.

Al-Shanfari L and Rehman T (2019). Antifungal activities of plant extracts against *Alternaria alternata* and *Botrytis cinerea*. *Agric Bio. J. North Am*., **10**(5): 219-227.

Amarasinghe LD, Wickramarachchi PA, Aberathna AA, Sithara WS and De Silva CR (2020). 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): e04322.

Andriani S, Widiastuti EL, Nurcahyani N and Busman H (2021). Cytotoxic activity of methanol extraction of *Avicennia marina* and taurin in the HeLa cancer cells. In: *J. Phys., Conference Series*, **1751**(1): 012045.

Aunjum A, Biswas R, Al Munna A, Billah M, Islam E and Islam KM (2021). *In vivo* toxicity evaluation of ethanolic extract from two mangrove plants. *Pharm. Biomed. Res*., **7**(3): 209-216

Balakrishnan S, Srinivasan M and Mohanraj J. (2016). Biosynthesis of silver nanoparticles from mangrove plant (*Avicennia marina*) extract and their potential mosquito larvicidal property. *J. Parasit. Dis*., **40**(1): 991-996.

Balasubramani G, Ramkumar R, Krishnaveni N, Pazhanimuthu A, Natarajan T, Sowmiya R and Perumal P (2015). Structural characterization, antioxidant and anticancer properties of gold nanoparticles synthesized from leaf extract (decoction) of *Antigonon leptopus* Hook. & Arn. *J. Trace Elem. Med. Biol*., **30**(2): 83-89.

Barbier EB (2016). The protective service of mangrove ecosystems: A review of valuation methods. *Mar. Pollut. Bull*. **109**(2): 676-681

Begum SJ, Pratibha S, Rawat JM, Venugopal D, Sahu P, Gowda A, Qureshi KA and Jaremko M (2022). Recent advances in green synthesis, characterization and applications of bioactive metallic nanoparticles. *Pharmaceuticals*, **15**(4): 455.

Benelli G (2016). Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: A review. *Parasitol. Res*., **115** (1): 23-34.

Castillo-Henríquez L, Alfaro-Aguilar K, Ugalde-Álvarez J, Vega-Fernández L, Montes de Oca-Vásquez G and Vega-Baudrit JR (2020). Green synthesis of gold and silver nanoparticles from plant extracts and their possible applications as antimicrobial agents in the agricultural area. *Nanomaterials*, **10**(9): 1763.

Cerri F, Giustra M, Anadol Y, Tomaino G, Galli P, Labra M, Campone L and Colombo M (2022). Natural products from mangroves: An overview of the anticancer potential of *Avicennia marina*. *Pharmaceutics*, **14**(12): 2793.

Cosgrove JB, Wood RJ, Petrić D, Evans DT and Abbott RH (1994). A convenient mosquito membrane feeding system. *J. Am. Mosq. Control Assoc*., **10**(3): 434-6.

Garba S Isa and AI Bashir NH (2018). Phytochemical composition, antibacterial and wound healing properties of *Avicennia marina* and *Bruguiera gymnorrhiza*. *Nig. J. Basic Appl. Sci*., **26**(2): 13-22.

Govindarajan M (2016). Green Synthesized Silver Nanoparticles: A Potential New Insecticide for Mosquito Control. *In*: Mehlhorn H (eds.). Nanoparticles in the Fight Against Parasites. Parasitology Research Monographs, Vol. 8. Springer, Cham. https://doi.org/10.1007/978-3-319-25292-6_7

Guleria A, Sachdeva H, Saini K, Gupta K and Mathur J (2022). Recent trends and advancements in synthesis and applications of plant-based green metal

nanoparticles: A critical review. *Appl. Organomet. Chem*., **36**(9): e6778.

Ibrahim EH, Ghramh HA, Alshehri A, Kilany M, Khalofah A, El-Mekkawy HI, Sayed MA, Alothaid H and Taha R (2021). *Lepidium sativum* and its biogenic silver nanoparticles activate immune cells and induce apoptosis and cell cycle arrest in HT-29 colon cancer cells. *J. Biomater. Tissue Eng*., **11**(2): 195-209.

Ibrahim EH, Kilany M, Ghramh HA, Khan KA and Islam S (2019). Cellular proliferation/cytotoxicity and antimicrobial potentials of green synthesized silver nanoparticles (AgNPs) using *Juniperus procera*. *Saudi J. Biol. Sci.*, **26**(7): 1689-1694.

Karthi S, Uthirarajan K, Manohar V, Venkatesan M, Chinnaperumal K, Vasantha-Srinivasan P and Krutmuang P (2020). Larvicidal enzyme inhibition and repellent activity of red mangrove *Rhizophora mucronata* (Lam.) leaf extracts and their biomolecules against three medically challenging arthropod vectors. *Molecules*, **25**(17): 3844.

Majoumouo MS, Sharma JR, Sibuyi NR, Tincho MB, Boyom FF and Meyer M (2020). Synthesis of biogenic gold nanoparticles from *Terminalia mantaly* extracts and the evaluation of their *in vitro* cytotoxic effects in cancer cells. *Molecules*. **25**(19):4469.

Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K and Kim SK (2013). Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel Nocardiopsis sp. MBRC-1. *BioMed res. int.*, **2013** (1): 287638.

Mohamed S and Mamat M (2016). Evaluation of antioxidant potential of *Avicennia marina* leaves. *Int J Res Med Sci*., **4**(2): 467-471.

Murugan K, Benelli G, Ayyappan S, Dinesh D, Panneerselvam C, Nicoletti M, Hwang JS, Kumar PM, Subramaniam J and Suresh U (2015). Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. *Parasitol. Res.*, **114**(1): 2243-53.

Oves M, Khan MS, Zaidi A, Ahmed AS, Ahmed F, Ahmad E, Sherwani A, Owais M and Azam A (2013). Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of *Stenotrophomonas maltophilia*. PloS one. **8**(3): e59140.

Pasca RD, Mocanu A, Cobzac SC, Petean I, Horovitz O and Tomoaia-Cotisel M (2014). Biogenic syntheses of gold nanoparticles using plant extracts. *Part. Sci. Technol.*, **32**(2):131-7.

Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S and Himalayan B (2000). Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia*, **71**(5):553-5.

Ranganathan R, Madanmohan S, Kesavan A, Baskar G, Krishnamoorthy YR, Santosham R, Ponraju D, Rayala SK and Venkatraman G (2012). Nanomedicine: towards development of patient-friendly drug-delivery systems for oncological applications. *Int. J. Nanomed*., **23**: 1043-60.

Reitman S and Frankel S (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *Am. J. Clin. Pathol*., **28**(1): 56-58.

Saenger P (2002). Mangrove ecology, silviculture and conservation. Springer Science & Business Media, p.360.

Şengül Demirak MŞ and Canpolat E (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. *Insects*, **13**(2): 162.

Shafey AME (2020). Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. *Green Process. Synth.*, **9**(1): 304-339.

Sheel R, Sahu K, Dutt KR and Badhe S (2017). Anti-inflammatory and analgesic properties of *Avicennia marina* and *Aegiceras corniculatum*. *Int. J. Pharm. Sci. Res*., **8**(9): 3806-3810.

Silverstein RM and Bassler GC (1962). Spectrometric identification of organic compounds. *J. Chem. Educ.*, **39**(11): 546.

Sohaib M, Al-Barakah FN, Migdadi HM and Husain FM (2022). Comparative study among *Avicennia marina*, *Phragmites australis* and *Moringa oleifera* based ethanolic-extracts for their antimicrobial, antioxidant and cytotoxic activities. *Saudi J. Biol. Sci*., **29**(1): 111-22.

Soni N and Prakash S (2014). Green nanoparticles for mosquito control. *Sci. World J*. **2014**: 496362.

Vaish S and Pathak B (2023). Mangrove synthesized bio-nanomaterial and its applications: A review. *ENMM*, 20: 100866e.

WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. WHO Publication, Geneva, Switzerland, **1**(3): 41.

Aly M, Osman K, Omar E and Mahmoud M (2021). Recent, eco-friendly approach for controlling *Culex pipiens* (L.) using novel synthesized cadmium sulphide nanoparticles of *Ocimum basillicum* extract. *Egypt. J. Aquat. Biol. Fish*., **25**(4): 359-377.