Pakistan J. Zool., pp 1-8, 2022. DOI: https://dx.doi.org/10.17582/journal.pjz/20210331040359

Larvicidal Potential and Phytochemical Analysis of *Garcinia mangostana* Extracts on Controlling of *Culex pipiens* Larvae

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Article Information
Received 05 September 2022
Revised 27 September 2022
Accepted 23 October 2022
Available online 23 December 2022 (early access)

Authors' Contribution
NA and FA designed the study, conducted data analyses and wote the manuscript. KI performed light microscopy experiments. MS and MA helped in writing the manuscript.

Key words
Garcinia mangostana, Culex pipiens, Larvicidal activity, Midgut, Cytotoxicity

ABSTRACT

The present study assessed the larvicidal action of *Garcinia mangostana* pericarp against *Culex pipiens* larvae. Hexane extract was prepared from the fruit. The secondary metabolites were identified using phytochemical analysis and gas chromatography-mass spectrometry (GC-MS). The cytotoxic effect was also evaluated using normal human umbilical endothelial cells (HUV-EC). The larvicidal assay was performed on the third instar stage as recommended by WHO. The phytochemical analysis showed the presence of alkaloids, sterols, and phenolic compounds. The GC-MS analysis revealed the presence of 5 compounds, of which α-Copaene constituted 61.95% of the extract. The hexane extract showed good larvicidal activity, with an $LC_{50}=33.9$ $\mu g\ mL^{-1}$ and $LC_{90} = 64.1$ µg/mL at 24 h post exposure. The midgut sections of treated larvae showed morphological alterations such as microvilli damage, loss of nuclei, and peritrophic membrane degeneration. The extract also showed high cytotoxic activity against HUV-EC cells, with an LC_{50} of 19.8 µg/mL. The results indicated that *G. mangostana* has larvicidal activity against *Cx. pipiens* larvae. Further investigation can be done to confirm the larvicidal potential of this plant against different genus and species of mosquitoes.

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INTRODUCTION

Mosquito vectors are responsible for the transmission of many vector-borne diseases (Rosenberg *et al.*, 2018). The recent global burden of the diseases showed about 229 million cases of malaria with 90 percent occurring in Sub Saharan Africa (WHO, 2020), 5.2 million cases of dengue (WHO, 2021a), 200,000 cases of yellow fever (WHO, 2021c), 117 633 cases of Chikungunya fever with majority from Brazil, India, Guatemala, Malaysia and Belize (ECDC, 2021), and 51 million people with lymphatic filariasis (WHO, 2021b).

Culex pipiens is the most widely distributed species around the globe and considered as important vector for west Nile and Usutu viruses, and also for filarial worms and avian malarial parasite (Huijben *et al.*, 2007; Martinet *et al.*, 2019). Females of *Cx. pipiens* feed on various vertebrate hosts and may therefore contribute to the spread of disease among birds, humans, and other mammals (Martinet *et al.*, 2019).

Insecticides have been used in mosquito control for decades. The excessive use of these compounds has led to insecticides resistance and affect non-target organisms (Fernandes *et al.*, 2016). The need for substitutes of new safe insecticides to the environment encourages investigation and development of new active ingredients of natural origin. Plants have been used for this purpose due to their rich diversity of safe compounds with insecticidal activities against wide range of different insect pest species. They are target-specific, readily available, affordable, non-toxic to environment, and biodegradable (Ghosh *et al.*, 2012). Tremendous efforts are made to encourage environmentally friendly new active ingredients, using different tactics to replace or to improve insecticidal control methods (Tiawsirisup *et al.*, 2007).

Garcinia mangostana L. (Clusiaceae family) is a tropical tree that grows in Southeast Asia. Its peels and seeds have been used in traditional medicine to treat gastrointestinal and urinary tract infections, and also used as an anti-scorbutic, anti-fever agent, and a laxative (Ovalle-Magallanes *et al.*, 2017). Many studies have been published showing that *G. mangostana* can induce multiple

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0030-9923/2022/0001-0001 $ 9.00/0

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biological effects such as antioxidant, anticancer (Pedraza-Chaverri *et al.*, 2008), antinociceptive (Hackel *et al.*, 2013), anti-inflammatory (Chen *et al.*, 2017), neuroprotective (Phyu and Tangpong, 2014), hypoglycemic (Taher *et al.*, 2016), anti-obesity (Liu *et al.*, 2015), analgesic (Cui *et al.*, 2010) and insecticidal activity (Larson *et al.*, 2014). The current study assessed the potential of using *G. mangostana* pericarp extract as a larvicide against the mosquito vector *Cx. pipiens*.

MATERIALS AND METHODS

Plant material

The *Garcinia mangostana* was purchased from a fruit shop in Riyadh, Saudi Arabia. The fruits were washed with distilled water, and 70 g of the fresh pericarp (rind) was grounded using a commercial blender (SF stardust, Japan). The powder was extracted using 500 mL of hexane (Honeywell, Germany) in the sonicator (Wise Clean, China) for 30 min at 40ºC. The extract was filtered using Whatman filter paper (Grade No. 1, England) and evaporated under reduced pressure (Heidolph, Germany) at 40ºC. The extract was reconstituted in Dimethyl sulfoxide (DMSO) (VDR, France) and used for both phytochemical screening and larval bioassay.

Phytochemical screening

The hexane extracts of *G. mangostana* were screened for phenolics, alkaloids, terpenoids, saponin, sterols, and anthraquinone following the standard methods (Sofowora, 1993).

Alkaloids test (Dragendorff's test)

Two milliliters of methanol containing 50 mg of the extract was mixed with 1mL of dragendorff's reagent and mixed. The result of an orange-red precipitate indicates a positive result.

Phenols test (Ferric chloride test)

The dried extract (50 mg) was dissolved in 5 mL of 5% ferric chloride solution. The development of bluish-black color reveals the presence of phenolic compounds.

Sterols test (Salkowski test)

The dried extract (50 mg) was dissolved in 2 mL of methanol. A few drops (50 µL) of concentrated H_2SO_4 were added, and the formation of reddish-brown color indicates a positive result.

Saponin test

The dried extract (50 mg) extract was mixed with water (5 mL), and shaken vigorously using a test tube. The persistent foam shows the presence of saponin.

Anthraquinones test (Borntrager's test)

The dried extract (50 mg) was dissolved in 2 mL of methanol. Then, 2.5 mL of ammonia (10%) solution was mixed and shaken. The development of rosette color indicates a positive result.

Gas chromatography-mass spectrometry (GC-MS) analysis

The phytochemical analysis of hexane extract was done on Perkin Elmer Clarus 600 gas chromatograph attached to a mass spectrometer (Turbomass, Perkin Elmer, USA). One microliter of the hexane extract was injected into the Elite-5MS column of 30 m, 0.25 µm film thickness, 0.25µm internal diameter column and processed as reported earlier (Hidayathulla *et al.*, 2018). Compounds were identified using the spectra recorded in the WILEY and National Institute of Standard and Technology (NIST) libraries (Coates, 2000; Linstrom and Mallard, 2005).

Larvicidal activity

The *Culex pipiens* larvae used in the bioassay obtained from the colony maintained in the Bio-product Research Chair Insectary, King Saud University, Saudi Arabia. The stock solution was prepared by dissolving 50 mg of the dried extract in 1mL of DMSO (VDR, France) and used for preparing the concentrations. The first test solution was prepared by adding 80 µL of stock solution (50 mg/mL) to 15.920 mL of tap water and vortexed. Four concentrations (15.63, 31.25, 62.50, and 125 µg/mL) were prepared from the stock solution (50 mg/mL) by double dilution method in tap water using a 20 mL centrifuge tube (Nest, China). Four test tubes filled with 8 mL of tap water were used for dilution. Eight milliliter was transferred from the stock solution to the first test tube to make 250 µg/mL and vortexed (first two-fold dilution.). The second two-fold dilution were carried out with the same tip and continued until the last tube (concentration). Similarly, control was diluted as above, where the DMSO concentrations were 0.25, 0.125, 0.062 and 0.031%. Each treated water (tested concentration) was introduced into the wells of a 6-well plate (Bottom width: 85.30 mm, Bottom length: 127.50 mm, and height: 20 mm) (Nest, China). A batch of 20 third instar larvae of *Cx. pipiens* were then introduced into each well containing 8 mL of test water. Three replicates (total 60 larvae) were used for each concentration. Then plates were kept at 27±1ºC and photoperiod of 12 h. No larval food was added. After 24 h, the dead larvae were counted. Larvae were considered dead if they could not reach the surface or move when touched with a wooden stick (Al-Mekhlafi *et al.*, 2021).

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Histology of larvae

The procedure was carried out following the method of Al-Mekhlafi *et al*. (2021). Briefly, the control and extract-treated larvae were fixed for 24 h in 10 % formaldehyde, dehydrated in different concentrations of ethanol, cleared with xylene, and embedded in paraffin blocks. The blocks were sectioned (5 µm) using a rotary microtome (Leica, Germany) and stained with hematoxylin and eosin (PDH, UK). The glass slides were imaged using a light microscope (Olympus, Japan).

Cytotoxicity assay

Cell viability was investigated based on the potential of the active cell to reduce a yellow tetrazolium salt (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Invitrogen, USA) to purple formazan by mitochondria. In brief, Normal Human Umbilical Endothelial cells (HUV-EC) (ATCC, USA) were grown in 24-well plates (NEST, China) at 5 × 10^5 cells/ well and treated with 0.01% DMSO or different concentrations (250-12.5 µg/mL) of hexane extract for 24 h. MTT was then added to each well and incubated for 2 h at 37°C. Crystals formed (Formazan) were solubilized using 0.01% isopropanol (WINLAB, UK) on a shaker (GFL, Germany) for 5 min. The absorbance (570 nm) was read using a plate reader (ChroMate, UK). IC_{50} (concentration needed to inhibit 50% of cell growth relative to a control) value was calculated using OriginPro 8.5 (Origin Lab Corporation, USA).

RESULTS

Phytochemical screening

The qualitative phytochemical investigation of the hexane extract of *G. mangostana* revealed the existence of different phytometabolites such as alkaloids, sterols, and phenolic compounds.

GC-MS analysis

The GC–MS analysis of hexane extract of *G. mangostana* recorded a total of 5 peaks (Fig. 1, Table I) corresponding to the phytometabolites that were recognized by comparing their mass spectral fragmentation patterns to that of the compounds described by the WILEY and NIST libraries. Overall, the five phytocompounds identified in the hexane extract are shown in Table I, along with their retention time and area percentage. The major compound detected was α-Copaene that constituted 61.95% of the extract. Other miner compounds identified were trans-α-Bergamotene, (+)-β-Costol, selin-4, 7(11)-diene, and γ-cadinene.

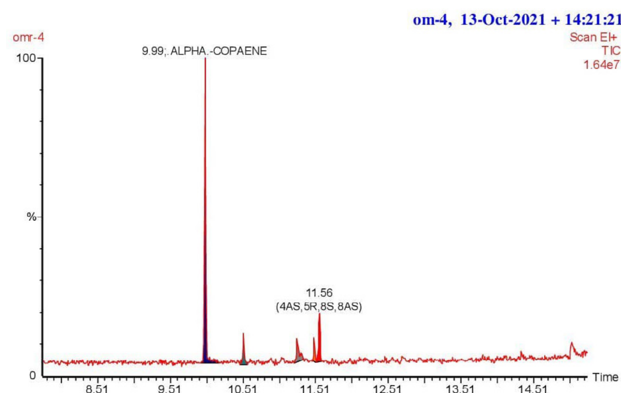

Fig. 1. Chromatogram of the hexane extract of *Garcinia mangostana* pericarp generated using gas chromatography-mass spectrometry.

Table I. Phytocompounds detected in the hexane pericarp extract of *Garcinia mangostana* using gas chromatography-mass spectrometry.

No.	Name	RT	Area %	Formula	Molecular weight
1	α-copaene	9.98	61.980	$C_{15}H_{24}$	204.35
2	Trans-α-bergamotene	10.52	13.650	$C_{15}H_{24}$	204.35
3	(+)-β-costol	11.25	7.800	$C_{15}H_{24}O$	220.35
4	Selin-4,7(11)-diene	11.49	3.530	$C_{15}H_{24}$	204.35
5	γ-cadinene	11.56	13.040	$C_{15}H_{24}$	204.35

Larvicidal activity

The larvicidal effect of the hexane extract of the *G. mangostana* was observed at 24 h, showing increased toxicity to the third instar larvae as concentration increased (Table II). The 125 µg/mL hexane extract exhibited 100% mortality after 24 of treatment. The LC_{50} and LC_{90} values correspond to 33.95 µg/mL and 64.12 µg/mL, respectively. No mortality was observed in the control test.

Table II. larvicidal potential of *G. mangostana* extract against *Culex pipiens* third instar larvae.

Concentration (µg/ml)	% mortality	LC50 (µg/mL)	LC90 (µg/mL)	df	F
Control	0.00±0.00d				
15.63	23.33±3.33c				
31.25	50.00±5.77b	33.95	64.12	4	158.30
62.5	86.67±3.33a				
125	100.00±0.00a				

Small letters indicate significant differences between concentrations. Significant differences were assessed using one-way ANOVA followed by Tukey's test, $p < 0.05$ indicates significant differences.

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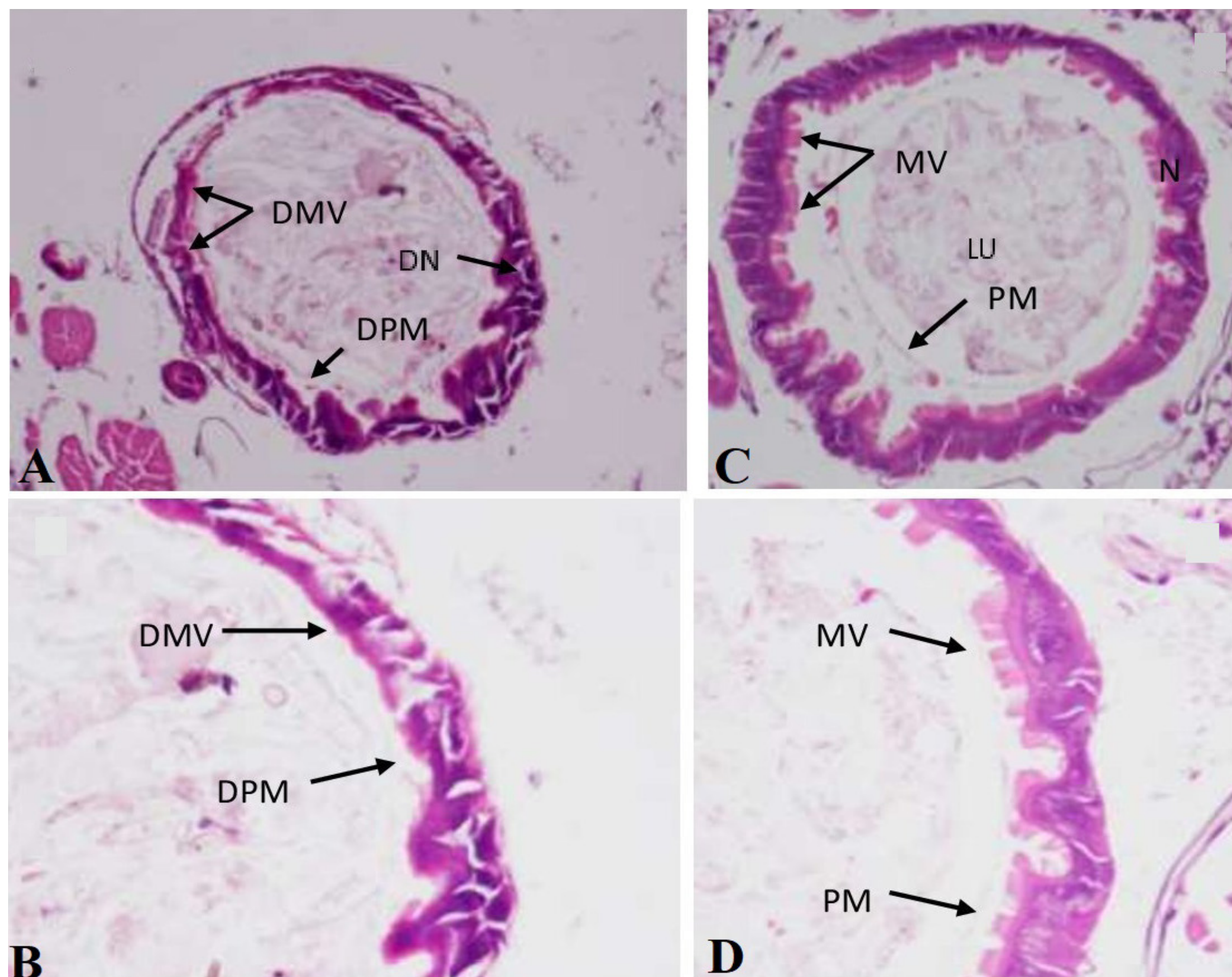


Fig. 2. Histology of the 24 h post-treatment midguts of *Culex pipiens* larvae treated with hexane extract of *Garcinia mangostana*. (A, B) Cross-sections of the midgut of hexane extract-treated larvae. (C, D) Cross-sections of the midgut of the control (0.01% DMSO) larvae. DMV, Degraded microvilli; MV, Microvilli; DPM, degenerating peritrophic membrane; PM, Peritrophic membrane; DN, degenerating nuclei; N, Nuclei; LU, lumen.

Gut-histological activity

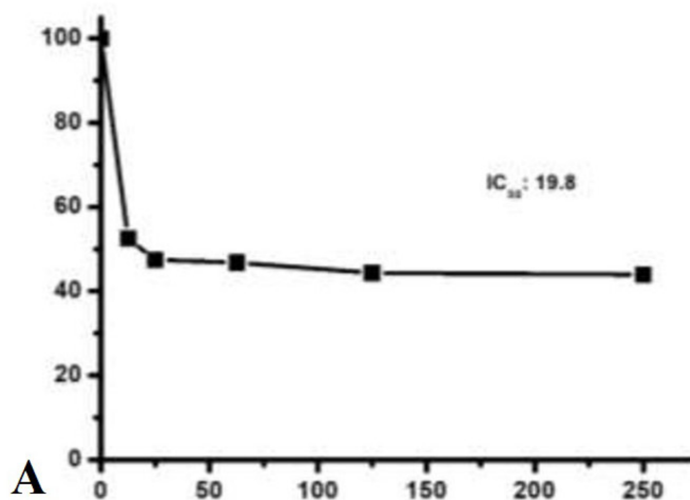
The midgut cells of *Cx. pipiens* third instar larvae treated with hexane extract (33.95 µg/mL) and the control (0.01% DMSO) are shown in Figure 2. The sections of control of the third instar larvae midgut were normal with intact peritrophic membrane (Pm), microvilli (MV), and nuclei (N). Contrary, the midgut cells of treated larvae showed morphological alterations such as microvilli damage (DMV), loss of nuclei, epithelial cells degeneration (DE), and peritrophic membrane degeneration (DPM).

Cytotoxicity assay

The cells incubated with hexane extract showed morphological changes. Images captured by a light microscope revealed cell shrinkage and floating cells. Control cells (DMSO 0.01%) showed normal cell appearance (Fig. 3). MTT results indicated an increase in cell death with the increasing concentrations of the extract (Fig. 3). The IC_{50} value was 19.8 µg/ml.

DISCUSSION

The findings of this study showed that hexane extract contains alkaloids, sterols and phenolic compounds. The composition of these phytometabolites together effectively controlled mosquito larvae of *Cx. pipiens*. Different studies have reported that presence of tannins, phenolics, flavonoids, coumarins, alkaloids, polyacetylenes, sterols



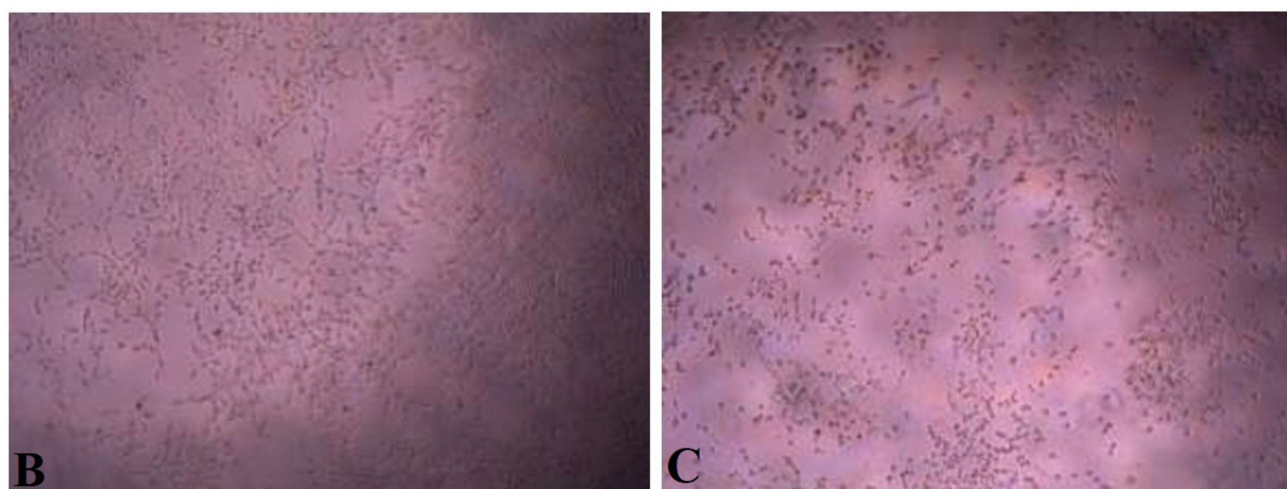


Fig. 3. Cytotoxicity effects of the *Garcinia mangostana* hexane extract against normal human umbilical endothelial cells (HUV-EC) after 24 h of treatment using MTT assay (A). Morphological changes of the HUV-EC cells after treatment with the hexane extract. (B) control cell and C, treated cells.

and terpenoids in medicinal plants with a larvicidal effect against mosquitoes (Al-Solami, 2021; Bilal and Hassan, 2012). The biological effect of these compounds originates from their ability to disturb the cholinergic system (Inhibition of acetylcholinesterase), GABA system (GABA-gated chloride channel), mitochondrial system (Inhibitor of cellular respiration), octopaminergic system (Octopaminergic receptors), and endocrine system (Hormonal balance disruption) (Rattan, 2010).

The current phytochemical and the GC-MS analysis revealed the presence of different phytometabolites included α-Copaene (61.980), trans-α-Bergamotene (13.650), and γ-cadinene (13.040) as the major compounds of hexane extract. Plants rich in α-copaene, selin-4, 7(11)-diene, and γ-cadinene have been reported to have an insecticidal activity against different mosquito species (Aguiar *et al*., 2010; Amazonas Maciel Magalhães *et al*., 2010; Costa *et al*., 2011; Mariano Fernandez *et al*., 2021). These toxic phytometabolites compounds can be absorbed by the cuticle or consumed orally and cause insects death (Rattan, 2010).

The ethanol pericarp extract of *G. mangostana* has shown larvicidal activity against *A. aegypti* larvae with LC_{50}: 4.84 µg/mL, while the hexane extract exhibited an LC_{50} value of 27.61 µg/mL (Torres *et al*., 2015). α-mangostin has been reported to be toxic against six

mosquito species, including *Cx. pipiens* (Larson *et al.*, 2014). Similarly, isolated α-mangostin showed larvicidal activity with LC_{50} of 19.4 µg/mL against larvae of *Aedes aegypti* (Ee *et al.*, 2006). In the present study, the hexane extract showed slightly higher LC_{50}. This could be attributed to the different mosquito species tested.

The histological investigation showed changes in the midgut region of *Cx pipiens* due to the toxic effect of the hexane extract of *G. mangostana*. Similar effect has been shown also with *Foeniculum vulgare* and *Matricaria chamomilla* hexane extract (Al-Mekhlafi *et al.*, 2021). The toxicity effect of *Epaltes divaricate* hexane extract on the midgut caused severe damage to gut tissues such as the peritrophic matrix, the epithelial layer, and the brush border (Amala *et al.*, 2021). Several reports stated that the primary target of phytometabolites is the midgut regions, which alters several functions, such as osmoregulation, nutrition absorption, ion transport, digestion (Rohmah *et al.*, 2020; Sina and Shukri, 2016), metamorphosis (Procopio *et al.*, 2015), and chemical defense (Terra, 2001). Although the hexane extract possessed larvicidal action against *Cx, pipiens*, the extract also showed higher toxicity to normal HUVEC cells. Therefore, precautions should be taken into consideration when used as larvicidal agents. Nevertheless, the risk should be further evaluated using different animal models.

This data highlighted the importance of screening the larvicidal potential of *G. mangostana* as a source of active ingredients that can be subjected to more biological evolution and further product development.

Funding

This research was funded by Researchers Supporting Project number (RSP2023R112), King Saud University, Riyadh, Saudi Arabia.

Statement of conflict of interest

The authors have declared no conflict of interest.

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