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Original article

Insecticidal effects of a novel polyherbal formulation (HF7) against *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Plant secondary metabolites represent the most efficient and convenient method to control and overcome environmental pollution and insecticidal resistance. This study explored the mosquitocidal activity of the combined extract of seven plants, (HF7) extracted using a Soxhlet extractor against *Culex pipiens* under laboratory conditions. Exposure of the 3rd instars of *Cx. pipiens* to HF7 hexane extract resulted in LC_{50}:114.5 μg/mL and LC_{90}:117.0 μg/mL values after 24 h. The ovicidal activities of hexane extract against *Cx. pipiens* eggs were 21.6%, 48.3%, and 71.6% at 187.5, 93.7, and 46.88 μg/mL, respectively. HF7-treated larvae showed the formation of irregular blebbing of epithelial cells toward the lumen and sloughing into the gut lumen. HF7 extract resulted in 100% adulticidal mortality at the concentration of 3.7 mg/test tube after 30 min of exposure. The IC_{50} of HF7 extract was 97.03 μg/ml against larvae, at which nuclear and morphological changes were observed. The spectroscopy spectrum of HF7 hexane extract disclosed the presence of 57 different secondary metabolites, among which the dominant compound was eugenol (32.3%). HF7 hexane extract could serve as a botanical insecticide for controlling *Cx. pipiens* and potentially other mosquito species.

1. Introduction

Mosquitoes are a source of nuisance and transmission of protozoan and viral diseases to humans and animals. Their risk to transmit diseases through the bite of female mosquitoes increases during their blood meal search before oviposition. The diseases transmitted by female mosquitoes include chikungunya, yellow fever, malaria, filariasis, Zika virus disease, dengue fever, and Japanese encephalitis. These diseases cause risk to millions of people, particularly in tropical and subtropical regions of the world (Korgaonkar et al., 2012). Several arboviral diseases are transmitted by *Culex pipiens* that is found in several regions of the world. Cases of West Nile virus have been reported worldwide, whereas Rift Valley fever and Japanese encephalitis have been found to be prevalent in East Asian countries and Africa, respectively (Vinogradova, 2000).

Mosquito management is accomplished using chemical insecticidal agents that have been applied with success, irrespective of their toxicity to human health and nontarget organisms, environmental threats (Lee et al., 2001a), development of resistance in the mosquitoes, and the nondegradability that leads to biomagnification (Yousuf et al., 2014). The current situation prevailing in mosquito management necessitates exploration of new insecticides that can overcome the abovementioned problems. Such characteristics are found in plant secondary metabolites that have been reported for their mosquitocidal activity (Aydin et al., 2017). Numerous plant secondary metabolites such as essential oils, alkaloids, steroids, phenolics, and terpenoids extracted from plants have been reported as insecticides (Aydin et al., 2017). The secondary metabolites produced by plants protect them from herbivores. They target several cellular components such as nucleic acids, proteins, and biomembranes, which in turn affects the insect physiology through several mechanisms, primarily by causing disruptions in nervous system, cellular respiration, and hormonal balance; mitotic poisoning; disruption of the molecular events of morphogenesis; and alterations in behavior (Rattan, 2010).

The activity of the combinations of plant extracts against mosquito species has been reported in several studies. An earlier study reported that a combination of *Vitex negundo* and *Pongamia glabra* (1:1) demonstrated effective larvicidal activities, with an LC_{50}

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value of 191.73 μg/mL, against *Aedes aegypti* (Yankanchi et al., 2014). Likewise, a combination of *Tecoma stans*, *Nerium oleander*, *Lantana camara*, and *Hyptis suaveolens* extracts resulted in LC_{50} values of 7.19 and 4.32 μg/mL against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively (Hari and Mathew, 2018). All the plant extracts used in that study are widely used by herbalists and have been reported to possess various properties, including antidepressant, antianxiety, anti-HIV, antidiabetic, anticancer, anti-inflammatory, antimicrobial and antioxidant (Singh et al., 2021), antiulcer, fertility-enhancing (Abdalla and Abdallah, 2018) herbicidal, nematicidal, (Kaur and Kaushal, 2019), hypolipidemic, and antihypertensive (Delaviz et al., 2017) activities. Moreover, they have been used in the management of gastrointestinal illnesses, pain, metabolic syndrome, (Salehi et al., 2018), memory improvement, stress, cardiovascular diseases, anxiety, Alzheimer's disease, and depression (Miraj and Kiani, 2016), and also as hypolipidemic and immunomudulator agents (Huseini et al., 2010) (. In the present study, we evaluated the larvicidal and ovicidal activities of the secondary metabolites of HF7 extract and the histological changes in the midgut region of *Cx. pipiens*.

2. Materials and methods

2.1. Plant extracts

The bark of *Cinnamomum zeylanicum*, rhizome of *Zingiber officinale*, seeds of *Syzygium aromaticum*, kernel of *Juglans regia*, fruit of *Capsicum annuum*, leaves of *Salvia officinalis*, and rhizome of *Curcuma longa* were first washed with tap water and then with distilled water. An equal amount of dried plant powders was grounded using an electrical grinder, and 15 g of powder was extracted for 24 h in 450 mL of a solvent of increasing polarity (hexane, chloroform, ethyl acetate, and methanol) using a Soxhlet apparatus. The obtained extracts were vaporized using a rotary evaporator and then stored in in dark airtight bottles at −4 °C.

2.2. Estimation of total phenols

HF7 extract (2 μL), Folin–Ciocalteu reagent (20 μL), 7.5% Na_2CO_3 (80 μL), and water (100 μL) were mixed in a 96-well plate and kept in the dark for 90 min at 25 °C. Then, the absorbance was measured at 740 nm. Different concentrations of gallic acid (5–100 μg/mL) were prepared and treated as in the extract. Results are reported as mg/g gallic acid equivalent (GAE/g) (Abutaha et al., 2021).

2.3. Estimation of total flavonoids

HF7 extract (2 μL) was added to methanol (60 μL), 10% $AlCl_3$ (4 μL), 5% $NaNO_2$ (60 μL), 1 M potassium acetate (4 μL), and water (112 μL). The HF7extract was incubated at 25 °C for 60 min. Then, the absorbance was measured at 368 nm. Different concentrations of quercetin (5–100 μg/mL) were prepared and treated as in the extract. Results are expressed in mg quercetin equivalent (QE/g) of dried plant material (Abutaha et al., 2021).

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

The phytochemical evaluation of HF7 extract was conducted using a GC–MS equipment (Turbomass, PerkinElmer, Inc., Waltham, MA, USA). Experimental conditions were as follows: HP-88 capillary standard column; dimension, 0.25 mm; film thickness, 0.20 μm; flow rate of the carrier gas (He), 1.0 mL/min. For gas chromatography, the oven temperature (temperature program) was 40 °C, which was increased to 200 °C at 5 °C/min, and the injection volume was 1 μL. Results were compared using the spectral library search program Wiley (McLafferty and Stauffer, 1989).

2.5. IR analyses of bio active principle

Dried sample was subjected to infrared (IR) spectroscopy analysis as previously reported (Abutaha et al., 2021).

2.6. Rearing of Cx. Pipiens

Cx. pipiens larvae were obtained from the Bio-product Research Chair, King Saud University, Riyadh. The larvae were placed in plastic trays filled with tap water at 28 °C ± 2 °C. Emerged larvae were fed on fish food. The trays were shifted to cages after the formation of pupae. After 2 days, the emerged adults from the pupae were provided with a beaker containing cotton soaked in sugar solution (10%). For female mosquitoes to feed on blood, an albino mouse was kept overnight in the rearing cage.

2.6.1. Mortality bioassay test

Five concentrations of HF7 extract, ranging from 23.44 to 187.5 μg/mL, were prepared to conduct the bioassay (Al-Mekhlafi et al., 2020). A total of 20 *Cx. pipiens* larvae were treated with different concentrations of each extract. A negative control group (0.01% methanol) and a positive control group were treated with permethrin (250 μg/mL) in water. Three replicates were performed for each test, and the mortality percentage was calculated at 24, 48, and 72 h after treatment. Immovable larvae were considered as dead and kept in buffered formaldehyde (10%) in 1-mL Eppendorf tubes to further evaluate the histological changes.

2.6.2. Ovicidal assay

A total of 25 freshly laid eggs of *Cx. pipiens* were exposed to four different concentrations, 23.4, 46.8, 93.75, and 187.5 μg/mL, of HF7 extract. Moreover, a negative control (0.01% DMSO) was maintained. Egg viability was evaluated using a stereomicroscope. The percentage ovicidal activity was calculated at 24 h after treatment. Testing was repeated thrice (Su and Mulla, 1999).

2.6.3. Adulticidal bioassay

Whatman No. 1 filter papers (7 × 2 cm) were treated with different amounts of HF7 extracts (3750, 2500, 1250, 625, 312.5, 156.2, and 78.1 μg), allowed to dry, and then inserted into sterile centrifuge tubes (Nest, China). A piece of blank filter paper treated with 100% methanol was considered as a control. Unfed mosquitoes (n = 20) were released into each tube using a battery-operated aspirator. Mosquitoes were considered as dead if they were incapable of flying or immobile at 24 h after exposure. The assay was conducted at 28 °C ± 1 °C. Immobile mosquitoes were shifted to a recovery tube containing sucrose solution (10%) to evaluate recovery in a 24-h period. The LD50 values were calculated using OriginPro 8.5.

2.6.4. Histological analysis

Histological procedures were conducted according to previously reported procedures (Al-Doaiss et al., 2021). Briefly, the 3rd instars were fixed for 24 h in formaldehyde (10%), dehydrated, and then cleared with xylene. Then, the embedded blocks using an embedding station (Sakura, Japan), were sectioned (4–6 μm thick sections) and then stained with hematoxylin and eosin using an autostainer (Leica Biosystems, Wetzlar, Germany). Slides were examined and photographed using an Olympus microscope (BX53 equipped with a digital camera, Japan).

2.7. Cell culture

HUVECs (Human umbilical vein endothelial cells) cells were purchased from ATCC (Manassas, VA, USA) and cultured in DMEM supplemented with FBS (10%), penicillin, and streptomycin (100 U/mL) in a humidified atmosphere (5% CO_2) at 37 °C.

2.7.1. Cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Abutaha et al., 2021) was used to evaluate cell viability. Cells were trypsinized (0.25%) and seeded (5×10^5 cells/well) for 24 h in 24-well plates. The test extracts were dissolved in DMSO, diluted with DMEM, and pipetted into 24-well plates at concentrations of 1000 µL/well. Control wells were maintained in DMEM containing 0.1% DMSO. After 24 h, 100 µL of a 5% MTT solution was added to each well and incubated for 2 h. The formazan crystals were solubilized in 1000 µL of DMSO, after which the plate was placed on a shaker for 5 min. Optical density (570 nm) was measured using a plate reader (Biochem, England).

2.7.2. Morphological and nuclear observation

HUVECs were seeded as described in the previous section and then incubated with 100 µg/mL of HF7 extract or control (DMSO < 0.1%). After 24 h of treatment, the morphology of HUVECs was observed and imaged using a phase-contrast microscope (Leica, Germany). Cells were fixed (ice cold ethanol) for 30 min and then stained with Hoechst 33342 for another 30 min. Wells were washed twice with 1000 µL of PBS. The cells were observed under a fluorescence microscope (EVOS, USA).

3. Results

3.1. Extraction yield and total phenolic and flavonoid contents

The yields of different solvent extracts were 1.6, 0.92, 0.24, and 2.8 g for hexane, chloroform, ethyl acetate, and methanol, respectively, indicating that methanol extract had the maximum yield, whereas ethyl acetate extract had the minimum yield. The total phenolic and flavonoid contents of HF7 extract were 61.3 ± 0.02 mg of GAE/g and 2.3 ± 0.05 mg of QE/g for the hexane extract, respectively.

3.2. GC–MS of HF7 hexane extract

A total of 57 different secondary metabolites were recorded in the HF7 hexane extract (Table 1), and the dominant compounds were 3-allylguaiacol (12.810%), eugenol (34.23%), and phenol, 2-methoxy-4-(2-propenyl)-, acetate (12.5%). The compounds 3-allylguaiacol and phenol, 2-methoxy-4-(2-propenyl)-, acetate are considered as derivatives of eugenol (4-allyl-2-methoxy phenol). All these metabolites belong to the category of phenolic compounds (Fig. 1).

3.3. FT-IR of HF7 hexane extract

The spectral features of HF7 hexane extract are depicted in Fig. 2. The band observed at 1669 cm^{-1} represented C-C stretch of phenyl. The band at 1406 cm^{-1} was due to CH_3 asymmetric deformation. The peak observed at 1435 cm^{-1} was due to the CO stretching vibration. The band at 952 cm^{-1} was related to ring CH deformation, which could reflect the structural information about polyphenols. For lower frequencies, the peak at 3447 cm^{-1} was due to the OH-stretching of phenol, and the peak at 2913 cm^{-1} was due to the CH_2 stretch of lipid methyl groups.

3.4. Mortality bioassay test

Dose- and time-dependent larvicidal activity was observed for the extract (Table 2), with the activity being positively correlated with the extract concentration and exposure period. Exposure of the 3rd instars of *Cx. pipiens* to HF7 hexane extract resulted in LC_{50} and LC_{90} values of 114.5 and 177.09 µg/mL, respectively, after 24 h (Table 1). In contrast, there was no larvicidal activity in the negative control, whereas 100% mortality was recorded for permethrin treatment at 250 µg/mL.

3.4.1. Ovicidal assay

Among the different solvent HF7 extracts HF7tested in this study, the hexane extract demonstrated ovicidal activities of 21.6%, 48.3%, and 71.6% at concentrations of 187.5, 93.7, and 46.88 µg/mL against *Cx. pipiens* eggs (Fig. 3). The ovicidal activity was dose-dependent, and the rate of ovicidal activity correlated positively with increasing concentrations of the extract.

3.4.2. Adulticidal bioassay

The HF7 extract demonstrated significant dose-dependent adulticidal potential against adult *Cx. pipiens* compared with the control. HF7It resulted in 100% adulticidal mortality at the concentration of 3.7 mg/test tube after 30 min of exposure, and no recovery was observed when the mosquitoes were shifted to recovery tubes. HF7The LD50 value was 1.55 mg/test tube (Fig. 4).

3.4.3. Histological analysis

Histological observations were conducted on the early 3rd midgut sections of the instars of *Cx. pipiens*. In the control assays (Fig. 5, the tissue of the midgut section of the 3rd instars of *Cx. pipiens* was composed of an epithelial layer with two types of cells, regenerative and digestive, attached to a basement membrane. The peritrophic membrane (PM) was regular in shape and attached to the epithelium. Nuclei were spherical and located basally with prominent decondensed chromatin. The larvae exposed to HF7 extract at 114.5 µg/mL showed induced partial destruction of PM. The basement membrane was displaced is some areas from the epithelial layer. The PM was damaged and broken at several places. The HF7-treated larvae demonstrated formation of irregular blebbing of epithelial cells toward the lumen and sloughing into the gut lumen (Fig. 5).

3.5. Cytotoxic activity

The cytotoxic activity and IC_{50} values of the HF7 extract were investigated using MTT assay in HUVECs treated with HF7 hexane extract (500–31.2 µg/ml). Cell survival analyses showed that the HF7 extract caused dose-dependent growth inhibition of HUVECs (Fig. 6). After 24 h of incubation, the IC_{50} value of HF7 extract was calculated as 97.03 µg/ml. The cells treated with HF7 extract demonstrated nuclear and morphological changes. Light microscopic observation revealed cell shrinkage and floating of cells. The cells incubated with Hoechst 33,342 dye showed reduced number of cells, chromatin condensation, and membrane integrity loss. Control cells (untreated) displayed normal cell morphology.

4. Discussion

Over the past decades, synthetic insecticides have been extensively used against vector mosquitoes, which has consequently resulted in toxic hazards to nontarget organisms and environmental pollution at variable levels. Therefore, there exists a need to identify target-specific and ecofriendly mosquitocides (Nivsarkar

Table 1
Compounds identified in HF7 hexane extract *HF7*by GC–MS.

	Name						RT	Area %
1	CAMPHOR					13.84	0.470	
3	BICYCLO[2.2.1]HEPTAN-2-ONE			14.75	0.660			
4	BORNEOL					15.53	0.700	
5	3-CYCLOHEXENE-1-METHANOL		16.18	0.320				
6	CYCLODECANOL				16.43	0.320		
7	3-PHENYL- 2-PROPENAL			17.22	1.640			
8	3-ALLYLGUAIACOL				19.43	12.810		
9	1-ACETOXY-P-MENTH-4(8)-ENE		20.28	0.230				
10	EUGENOL					20.72	34.230	
11	ALPHA.-COPAENE				21.12	0.410		
12	CARYOPHYLLENE				22.28	2.320		
13	ALPHA.-HUMULENE				23.16	1.190		
14	(-)-AR-CURCUMENE				23.78	2.460		
15	BERGAMOTENE				24.37	0.480		
16	(+)-ENDO-6-METHYL-2-METHYLENE-		24.51	0.620				
17	Phenol, 2-methoxy-4-(2-propenyl)-, acetate		24.80	12.580				
18	BETA.-SESQUIPHELLANDRENE		24.98	1.500				
20	CARYOPHYLLENE OXIDE			26.59	0.620			
21	1-OCTADECANOL				26.73	0.160		
22	3-CYCLOHEXEN-1-CARBOXALDEHYDE	27.26	0.130					
23	TRANS-.ALPHA.-BERGAMOTENE		27.87	0.070				
24	GAMMA.-CIS-SESQUICYCLOGERANIOL	27.98	0.440					
26	3-PHENYL-1,2,3,4-TETRAHYDROISO		28.55	2.370				
27	(. + -.)-AR-TURMERONE				28.55	4.060		
28	(Z,Z)-.ALPHA.-FARNESENE			28.79	0.090			
30	CURLONE					29.44	2.050	
31	(+)-.ALPHA.-ATLANTONE			31.08	0.170			
32	1-OCTADECANOL				31.49	0.220		
34	2,6,10,15,19,23-HEXAMETHYL-1,6,10,14,18,22-TETRACOSAHEXAEN-3-OL	33.17	0.480					
35	SELINEN-11-EN-4-OL				33.77	0.410		
36	METHYL ESTER OF HEXADECANOIC ACID	34.25	1.500					
37	HEXADECANOIC ACID			35.17	0.620			
41	METHYL ESTER OF OCTADECANOIC ACID	38.09	0.680					
42	(Z)6,(Z)9-PENTADECADIEN-1-OL		38.47	0.310				
43	OCTADECANOIC ACID			38.88	0.100			
45	CIS-6-SHOGAOL				39.77	1.450		
46	TRANS-6-SHOGAOL				41.03	1.860		
47	12-CHLOROMERCURIOTOTARA-8,11,13-TRIEN-13-OL	41.61	0.240					
48	BARBATUSOL					42.31	1.340	
49	1-DOCOSANOL					42.68	0.560	
50	EMODIN 1,8-DIMETHYL ETHER		43.40	0.260				
51	TRANS-8-SHOGAOL				44.38	0.840		
52	2,3-DIHYDRO-HEXADECANOIC ACID		44.54	0.150				
53	TRANS-10-SHOGAOL				47.51	1.250		
54	TETRACOSANE				50.04	0.610		
55	VITAMIN E					52.97	0.320	
56	9-OCTADECENOIC ACID			53.17	0.090			
57	2-UNDECEN-1-OL				54.18	0.110		

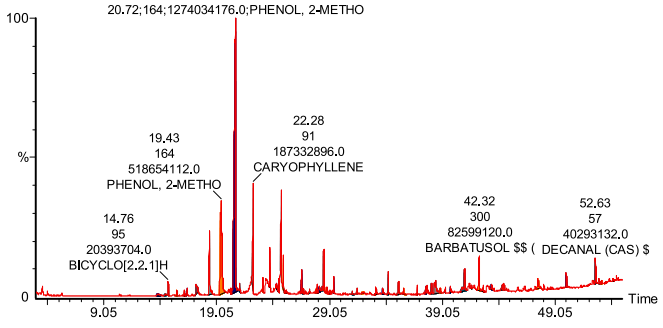


Fig. 1. GC–MS chromatogram of HF7 hexane extractHF7.

et al., 2001). Several studies have documented the promising, eco-friendly, target-specific, and high efficacy of natural extracts for controlling vector mosquitoes (Cavalcanti et al., 2004).

The present study demonstrated that the phytochemical constituents extracted from HF7 extract might be promising candidates to be developed as an alternative to synthetic insecticides.

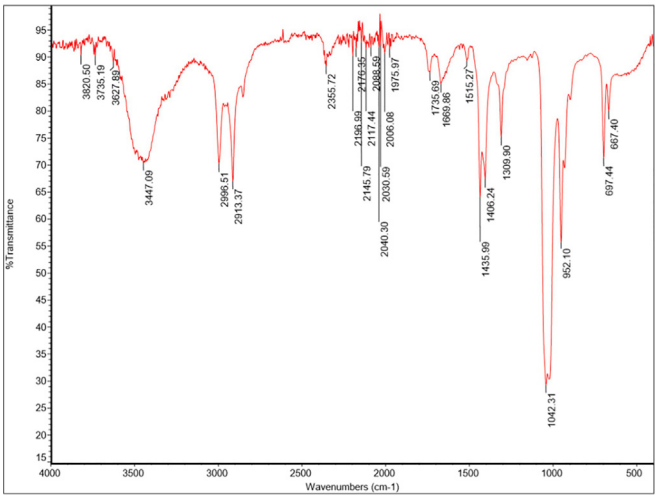


Fig. 2. FTIR spectrum of HF7 extract.

Plants contain different secondary metabolites that exert different mechanism of action against eggs and larvae. Therefore, combination of plant secondary metabolites might be more promising compared with using a single compound due to the synergism that can be effective in controlling mosquitoes that are resistant to insecticidal agents.

The results of the present study showed that HF7 extract has promising larvicidal and ovicidal activities against Cx. pipiens. Our results are similar to those of a previous study (Intirach et al., 2012), which reported the larvicidal activity of binary mixtures of the oils of *Piper sarmentosum* and other plants extract against *Anopheles cracens*. The binary mixtures of *Zanthoxylum piperitum*, *P. sarmentosum*, *C. longa*, *Foeniculum vulgare*, and *Myristica fragrans* at ratios of 25%:75% led to significant reduction in LC_{50} values as well as synergistic activity, with the values being 18.32, 16.81, 18.18, and 17.99 ppm, respectively.

Furthermore, researchers have reported that the combination of *H. suaveolens* and *L. camara* exerted a significant larvicidal activity against *Ae aegypti* (LC50 = 14.04%) compared with individual extracts (Pisan, 2005). Likewise, the activity of the mixture of *Annona squamosa* and *P. glabra* extracts against *Cx. quinquefasciatus*, *A. stephensi*, and *Ae. aegypti* was found to be more promising compared with that of the biopesticide neem extract (George and Vincent, 2005).

Volatile organic compounds emitted from plants into the atmosphere may act as a defense mechanism against insects, similar to chemical signals in plant–animal interactions (Penuelas and Llusià, 2001). The HF7 extract is aromatic with a pleasant smell. The adulticidal activity (LC50) was 156.25 µg/mL. The efficacy of the HF7 extract in causing immobility and 100% mortality still requires further study.

Our findings corroborate with those of several reports in that botanical insecticides are less effective compared with synthetic ones (Mohan et al., 2010). This is probably accredited to the complex mixture of inactive or active components in plant-derived insecticides, whereas synthetic insecticides constitute only a single compound. The complexity of plant extracts with different mechanisms of action could enhance the bioactivity or prevent the evolution of resistant mosquito populations (Tak and Isman, 2015).

Phenolic compounds comprise a large number of compounds that are widely found in the plant kingdom (Pereira et al., 2009), which have several useful properties for human health such as antimicrobial, cytotoxic, anti-inflammatory, and antiallergic activities; however, the most important action of phenolic compounds is their antioxidant potential (Podsędek, 2007). The larvicidal activity of the HF7 extract was probably attributed to the phenolic compounds present in the extract, which can significantly reduce the survival and growth of mosquitoes by inactivating enzymes and forming phenol–protein complexes that are difficult to digest (Mello and Silva-Filho, 2002). More than 100 plants have been screened for TPC and antioxidant and larvicidal potential against *Ae. aegypti* (El-Hela et al., 2013).

Several researchers believe that plant extracts and their bioactive secondary metabolites affect the midgut region of mosquitoes (Pavananundt et al., 2013). The midgut of mosquito larvae helps in osmoregulation, digestion, ion transport, and absorption (Bernick et al., 2007). The midgut region is considered as the major component of cellular responses to toxicants (David et al., 2000). It has been suggested that toxicants can disrupt the PM, which has harmful effects on the midgut structure, resulting in disintegration of columnar cells, osmotic imbalance, cytoplasmic vacuolization, apoptosis (Lehane and Billingsley, 1996), vacuolated epithelial layer, inflamed cells (Hamouda et al., 1996), cell hypertrophy, microvillus damage, and enlargement of gut cells (Kaewnang-O et al., 2011). The present study demonstrated histopathological changes in the midgut region of *Cx. pipiens* 3rd instars when exposed to the HF7 extract. These changes include enlargement of intercellular spaces, cytoplasmic vacuolization, destructed or deformed epithelial layer, and disintegrated nuclei. Furthermore, detachment of PM and destruction of microvilli lead to the complete destruction and malfunctioning of the midgut. These histopathological changes are consistent with previous reports (Elumalai et al., 2016).

Several metabolites present in HF7 extract have been reported previously for their mosquitocidal activity either as individual compounds or as a major compound in the crude extract, such as eugenol (Medeiros et al., 2013), caryophyllene (Govindarajan et al., 2016), camphor (Nerio et al., 2010), AR-curcumene (Lee et al., 2001b; AlShebly et al., 2017), α-humulene and farnesene (Govindarajan and Benelli, 2016), copaene (Amazonas et al., 2010), atlantone (Chaudhary et al., 2011), tetracosane (Mohammed et al., 2017), and 9-octadecenoic acid (Kannathasan et al., 2008). HF7 extract contains eugenol (32.3%) as its major con-

Table 2
Larvicidal activity of *HF7* hexane extract against *Culex pipiens*.

F	df	LC_{90} (µg/mL)	LC_{50} (µg/mL)	Mortality%					Time	Extract Type
				Concentration (µg/mL)						
				187.5	93.75	46.88	23.44	co		
98.00	4	177.09	114.59	93.33 ± 3.33 Aa	46.67 ± 8.82 Ab	0 ± 0 Bc	0 ± 0 Ac	0 ± 0 Ac	24	Hexane
79.60	4	173.02	101.43	93.33 ± 3.33 Aa	60 ± 10 Ab	10 ± 0 Bc	0 ± 0 Ac	0 ± 0 Ac	48	
98.00	4	153.67	53.99	100 ± 0 Aa	76.67 ± 6.67 Ab	40 ± 5.77 Ac	0 ± 0 Ad	0 ± 0 Ad	72	
				2	2	2	2	2	df	
				39.00	3.05	2.00	0	0	F	

Similar capital letters in columns indicate insignificant differences at $P \leq 0.001$.
Similar small letters in rows indicate nonsignificant differences at $P \leq 0.001$.

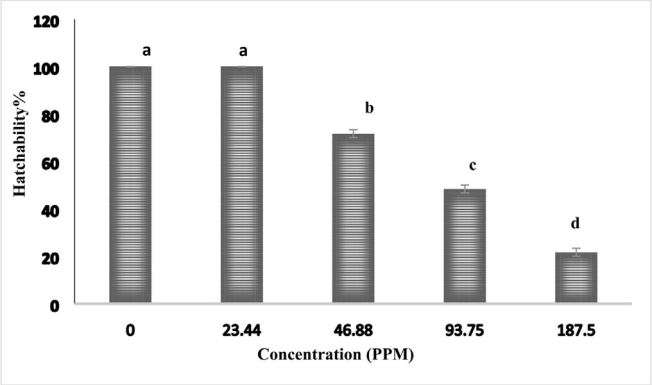


Fig. 3. Percentage hatchability and activity of HF7 hexane extract HF7against *Culex pipiens*.

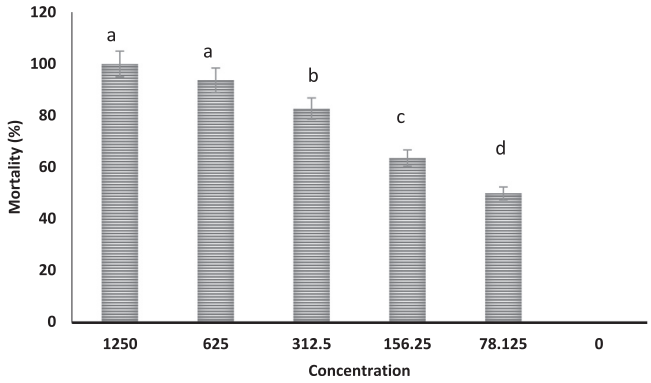


Fig. 4. Adulticidal activity of HF7 extract against *Culex pipiens* at different concentrations after 24 h of exposure.

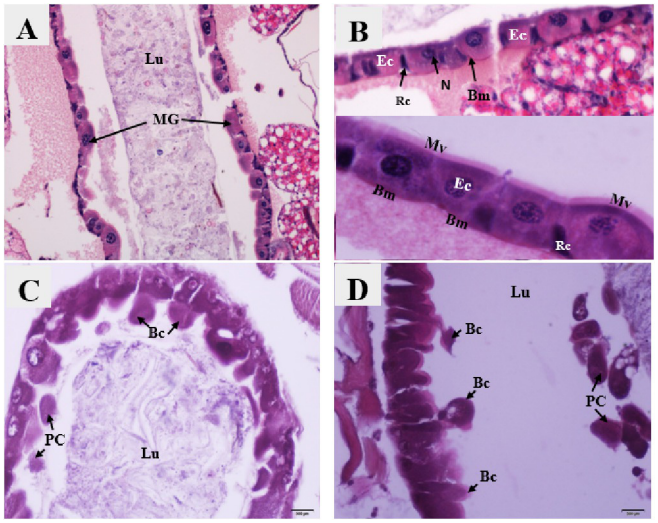
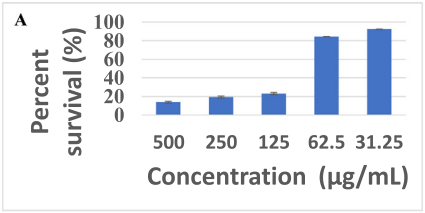


Fig. 5. A and B: Histology of the midgut of *Culex pipiens* larvae in the control group. C and D: Histology of the midgut of *Cx. pipiens* larvae treated with HF7 hexane extract at 114.49 μg/mL. Larvae exposed to HF7 extract showed partial destruction of peritrophic membrane, displacement of epithelial layer, damage to peritrophic membrane, formation of irregular blebbing, and sloughing into the gut lumen. (Ec), microvilli (Mv), nuclei (n), and regenerative cells (Rc), blebbing (Bc) and protruding (Pc), edema (Ed), loss of some epithelial cells (Lc), and degraded microvilli (DMv).

stituent, which has been reported to possess high antimicrobial and insecticidal activities and has been used in several formulations to control pathogens and insects (Yoo et al., 2005). Eugenol



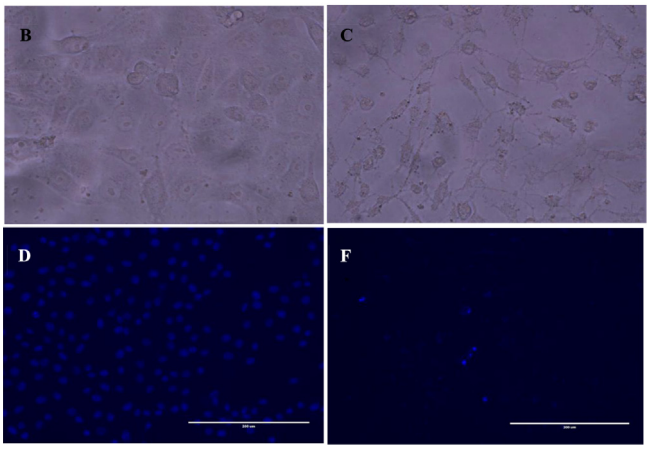


Fig. 6. A: Cytotoxicity of HF7 extract on human umbilical vein endothelial cells. Effect of HF7 extract on HUVEC morphology B (control) and C (treated) and nuclear features D (control) and F (treated) after 24 h of exposure. Images were captured under an inverted microscope (200 × magnification). The arrows indicate fragmented DNA.

(4-allyl-2-methoxy phenol) is a major essential oil of clove that was registered under the United States Environmental Protection Agency as a pesticide (CAS # 8000-34-8). Moreover, clove oil has been classified as a minimum-risk pesticide as its ingredients are safe for human use (Shahavi et al., 2019).

The results of the present study showed that HF7 extract has promising larvicidal and ovicidal activities against *Cx. pipiens* (114.5 μg/mL) and lower toxicity (IC50 97.03 μg/mL) against HUVECs. However, the selectivity index of HF7 extract was 0.08, which is < 1, is a sign of cytotoxicity. This is consistent with the result of *Eugenia calycina* leaf extract that was reported to exhibit low toxicity against the 3rd instars of *Ae. aegypti* (199.3 ± 1.2 μg/mL) compared with that against Vero cells (167.2 ± 24.5 μg/mL), with the selectivity index being 0.8 (Silva et al., 2021). The selectivity index for HUVECs was 0.08, indicating toxicity to normal human cells. The cell morphology was also changed, and cells were detached. Likewise, Hoechst staining showed DNA fragmentation in HUVECs (Fig. 6). In other words, at high concentration, the extract was toxic to normal human cell lines, and thus precaution is needed when considering to use this extract.

5. Conclusions

This study has demonstrated the effectiveness of HF7 extracts against the mosquito *Cx. pipiens* in different stages of development. The findings showed that HF7 hexane extract is effective and can be developed as an ecofriendly larvicide to control the spread of mosquitoes. We suggest further investigation of this extract in small-scale field trials for the development of a green insecticide.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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