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Original article

Target and non-target effects of *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *Culex pipiens* mosquitoes



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ABSTRACT

The present study focused on extracting green larvicides from extracts of the combination of *Foeniculum vulgare* and *Matricaria chamomilla* using different solvents of increasing polarity in a Soxhlet extractor and evaluating their ovicidal, larvicidal, and cytotoxic activities. The most promising among all tested extracts was hexane extract. The ovicidal activity of the hexane PH2 extract resulted in a significant ($p < 0.05$) decrease in egg hatchability from $95.00 \pm 6.16\%$ to $15 \pm 9.04\%$ at doses ranging from 62.5 to 500 $\mu\text{g/mL}$. The larval mortality with the hexane extract ranged from $13.33 \pm 3.3\%$ to $93.33 \pm 3.3\%$ at doses ranging from 31.25 to 250 $\mu\text{g/mL}$, respectively. The LC_{50} and LC_{90} values of the larvicidal activity of the hexane extract were estimated to be 148.3 and 242.17 $\mu\text{g/mL}$, respectively, after 24 h of exposure. Similarly, the LC_{50} values after 48 and 72 h of exposure were 124.93 and 100.3 $\mu\text{g/mL}$, respectively, against the third instar of *Cx. pipiens*. PH2 treatment of larvae resulted in histopathological changes such as degenerated epithelial cells and destruction of microvilli on the epithelial cells. The PH2 extract achieved a dose-dependent decrease in the rate of cell survival. The IC_{50} value of PH2-treated HUVECs was 192.07 $\mu\text{g/mL}$ after 24 h of incubation. The cells showed changes in cellular and nuclear morphology. In conclusion, the hexane extract of PH2 could be used in mosquito management programs.

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1. Introduction

Mosquito-borne diseases are increasing in incidence and geographical distribution, emerging in new areas (Paixão et al., 2018, Stanaway et al., 2016, Bhatt et al., 2013). Around 500 million cases and 2.7 million deaths due to mosquito-borne diseases are recorded every year (Bustamante, 2020). There is currently no specific treatment for dengue, chikungunya, Japanese encephalitis,

West Nile virus, Ross river fever, St. Louis encephalitis, and Zika fever. Patients are hospitalized to receive only supportive treatment. In addition, in some diseases like malaria, the available drugs have become useless due to the development of resistance (White, 2004).

Although vector control is the best tactic to reduce disease transmission, this approach mainly uses synthetic insecticides, which cause many problems to human health and non-target organisms, as well as constituting environmental pollution, resulting in ecological disruption without solving the problem of insecticide resistance and the appearance of new species (Devine and Furlong, 2007, Sarwar et al., 2009).

Thus, researchers' attention has been directed to other alternatives to reduce the risks associated with using synthetic insecticides. Such alternatives include natural products, particularly botanical extracts, since they are rich in secondary metabolites. These have been proven to have promising different biological

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activities (JUCÁ et al., 2020, Ntie-Kang et al., 2016), including insecticidal and insect-repelling activities (Mishra et al., 2020). Natural products have attracted substantial interest as insecticidal agents because they are target-specific, less toxic, and biodegradable and can overcome insecticidal resistance (Younoussa et al., 2020, Mishra et al., 2020, Said-Al Ahl et al., 2017).

Foeniculum vulgare Mill (Apiaceae) and *Matricaria chamomilla* L (Asteraceae) are plants that are widely used in traditional healers (Diaz-Maroto et al., 2006, Miraj and Alesaeidi, 2016). The activities of these two plants have been documented in many review articles regarding their antibacterial, antifungal, antioxidant, antithrombotic, anti-inflammatory, estrogenic, hepatoprotective, antidiabetic, antihirsutism, antidepressive, antiosteoarthritis, and antidiarrheal effects (Rather et al., 2016, Badgujar et al., 2014, Miraj, and Alesaeidi, 2016).

In insect control research, the use of combinations of insecticides is recommended not only to improve the efficiency of insecticides but also to overcome insect resistance and preserve the efficacy of insecticides for many years (Nelson and Kursar, 1999, Younoussa et al., 2020). Combinations of plant extracts are widely reported in the literature, and believed to increase their effects, achieving synergistic or balanced effects (Cheesman et al., 2017) and thus potentially preventing issues regarding insect resistance (Isman et al., 2006). Synergism can result from crude plant extract or the combination of different plant extracts. Herbalists always recommend the use of the crude extract over the use of isolated compounds (Williamson et al., 2009).

In the current investigation, we evaluated the effects of the combination of two plant extracts against the third instar of *Cx. pipiens* in laboratory conditions.

2. Materials and methods

2.1. Test materials

Plants were purchased from Riyadh Herb Market, Riyadh Province, Saudi Arabia, and the voucher specimens of *Foeniculum vulgare* family Apiaceae seeds (KSU-031) and *Matricaria chamomilla* family Asteraceae fruits (KSU-032) were deposited at herbarium of Bioproduct Research Chair, King Saud University. The extracts of *F. vulgare* seeds and *M. chamomilla* fruits were ground to a powder, mixed at a 1:1 ratio, and extracted using a Soxhlet extractor. Four different solvents, namely, hexane, chloroform, ethyl acetate, and methanol, were used. Similarly, *F. vulgare* was extracted individually using the same solvents. All extracts were prepared in DMSO.

2.2. Total phenolic content (TPC)

The TPC of PH2 extract was analyzed using FC reagent (Al-Zharani et al., 2019). Different concentrations of 2 µl of standard gallic acid (5–90 g/ mL) were mixed with 20 µl of FC reagent in a 96-well plate. After mixing, the samples were incubated for 10 min (25 °C); then, 80 µl of 7.5% sodium carbonate was added and incubated in the dark for 2 h at 25 °C. Finally, plate was read at 765 nm and the concentration was calculated. The TPC was calculated as mg/g gallic acid equivalent (GAE/g).

2.3. Total flavonoid content (TFC)

The aluminum chloride method was employed to calculate TFC (Al-Zharani et al., 2019). Quercetin was used for the preparation of a standard curve by dissolving quercetin (1.0 mg) in of DMSO (1.0 mL) then, different concentrations were prepared using DMSO (5–90 µg/mL). Two microliters of standard quercetin solutions or

PH2 extract was added to methanol (60 µl), 4 µl of 10% aluminum chloride, 1 M potassium acetate (4 µl), and 112 µl of distilled water in a 96-well plate. After mixing, the plates were kept at 25 °C for 60 min and the absorbance (368 nm) was read (Thermo Scientific Multiskan, China). The concentration was calculated as mg quercetin equivalent (QE)/g. All of the experiments were performed in triplicate.

2.4. Mosquito larvicidal assays

Mosquito colonies of *Culex pipiens* were maintained as previously described (Al-Mekhlafi, 2018). For each test, 8 mL of water containing 20 larvae (third instar) was placed into a 6-well plate and aliquots of the PH2 methanol, ethyl acetate, and hexane extracts solubilized in DMSO were then added. The DMSO (negative control) was used for comparison. Mortality was reported after 24 and 48 h of treatment. The assays were carried out at 25 ± 2 °C in triplicate with different concentrations of methanol, ethyl acetate (625, 500, 375, and 250 µg/mL), and hexane (250, 125, 62.50, and 31.25 µg/mL). The data was analyzed and LC₅₀ and LC₉₀ values were calculated using SPSS 16 software.

2.5. Ovicidal bioassay

The eggs were kept in a six-well plate containing 8 mL of tap water. Different concentrations of PH2 extract (500, 250, 125, 62.5 and 0 µg/mL) were prepared for the assay. The experiment was carried out in triplicate at 25 ± 2 °C for 24 h and then each sample was transferred to a well containing tap water to assess hatching. DMSO was used as a negative control

2.6. Histopathological changes

The dead treated and control larvae were immersed in formalin (pH 7.2) and processed as reported previously (Al-Mekhlafi, 2018). In brief, each larva was processed in ethanol, embedded in paraffin, and cut into thin sections using a microtome and stained with hematoxylin and eosin and the midgut region was observed under a microscope (Olympus, Japan).

2.7. Cell lines

Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Cell Culture Collection (ATCC, USA) and grown in Dulbecco's Modified Eagle Medium (DMEM). DMEM was supplemented with L-glutamine, sodium pyruvate, 10% fetal bovine serum (Gibco, USA) and penicillin-streptomycin 1% (UFC, Saudi Arabia).

2.8. MTT assay

MTT assay was performed as reported previously (Abutaha et al., 2021). Briefly, HUVECs (5 × 10⁵ cells/well) were seeded for 24 h in a 24-well plate. The following day, the cells were treated with PH2 extract and incubated for 24 h. Later, MTT (5 mg/mL) (Merck, USA) solution was removed after incubation (2 h) and DMSO (100 µl) was added. The absorbance was read at 550 nm using a microtiter plate reader (Multiskan™ FC, USA). The percentage survival was calculated and a graph was plotted using Origin software.

2.9. Morphological assessment

Cells (1 × 10⁵ cells/ mL) were seeded for 24 h in a 24-well plate and allowed to adhere overnight. After 24 h of treatment with PH2 extract (200 µg/ mL), the morphological features were observed

and imaged under phase-contrast microscope. The cells in the same plate were fixed with ice-cold methanol and then stained with Hoechst 33,342 for 20 min. The wells were washed with PBS and imaged under a fluorescence microscope.

2.10. Fourier transform infrared spectroscopy (FTIR)

PH2 extract was mixed with potassium bromide and compacted into a disk. The samples were scanned by FTIR (NICOLET 6700, USA). The spectrum was recorded within the region of 4000–400 cm⁻¹.

2.11. GC–MS analysis

Hexane extract of PH2 was analyzed in GC–MS using Perkin Elmer Clarus 600 (PerkinElmer, USA) linked to HP-88 column (0.25 μm film × 0.25 mm i.d. × 30 m length). One microliter of the PH2 extract was injected. Helium (99.9%) at a flow rate of 1 mL/min was used. The column oven temperature was set at 40 °C, increased by 10 °C per min to reach 200 °C (held for 3 min), and then held at 300 °C. The phyto-compounds were identified using mass spectral database libraries (Adams and WILEY).

2.12. Statistical analysis

The results are expressed as mean and standard deviation using SPSS program (version 21.0 for Windows; SPSS, Chicago, IL, USA). The significance of differences (p ≤ 0.001) was evaluated using ANOVA followed by Tukey’s test. The LC₅₀ values were assessed using OriginPro 8.5.

3. Results

3.1. Total phenol and flavonoid contents

The total phenol and flavonoid contents of PH2 extract were quantified as 5.82 GAE/mg and 2.18 QE/g, respectively.

3.2. Larvicidal bioassay

The larvicidal potential of the methanol extract ranged from 3.33 ± 3.33% to 66.67 ± 6.67%. The LC₅₀ value was estimated to

be 590 μg/mL. The larval mortality of the ethyl acetate extract against the third instar of *Cx. pipiens* was 13.33 ± 3.33% and 53.33 ± 3.33% at 500 and 625 μg/mL, respectively. The LC₅₀ value was estimated to be 677 μg/mL after 24 h of exposure (Table 1). Meanwhile, the mortality of larvae treated with PH2 hexane extract ranged from 13.33 ± 3.3% to 93.33 ± 3.3% at doses ranging from 31.25 to 250 μg/mL, respectively. The LC₅₀ and LC₉₀ values were estimated to be 148.3 and 242.17 μg/mL, respectively, after 24 h of exposure. Similarly, the LC₅₀ values after 48 and 72 h of exposure were 124.93 and 100.3 μg/mL, respectively (Table 2). No mortality was observed in the negative control (0.1% DMSO). All of the extracts tested showed dose- and time-dependent activity against the third instar of *Cx. pipiens*.

3.3. Ovicidal activity

The ovicidal activity of the PH2 hexane extract resulted in a significant (p < 0.001) decrease in egg hatchability from 95.00 ± 6.16% to 15 ± 9.04% at doses ranging from 62.5 to 500 μg/mL PH2 (Fig. 1). No failure in hatching was observed in the control.

3.4. Histopathological study

The damage caused by PH2 extract to third instar *Cx. pipiens* was assessed histologically at 148.3 μg/mL. The histopathological observation revealed some changes such as edema between the degenerated epithelial cells and destruction of microvilli on the apical surface of the epithelial cells (Fig. 2C, D). Histopathological evaluation of the negative control showed no damage to the mid-gut tissues (Fig. 2A, B).

3.5. Cytotoxicity

Upon evaluation of the toxicity of PH2 extract by MTT assay using HUVECs, a concentration-dependent decrease in the rate of cell survival was identified, with an IC₅₀ value of 192.07 μg/mL for a 24 h incubation period. To assess the morphological changes, cells were observed under a microscope. As illustrated in Fig. 3, the cells treated with PH2 extract showed changes in the cellular and nuclear morphology. The cells were detached, round in shape, and revealed DNA fragmentation.

Table 1 Larvicidal activity of PH2 methanol and ethyl acetate extracts against *Culex pipiens*.

F	df	LC ₉₀ (μg/mL)	LC ₅₀ (μg/mL)	Mortality (%)				Time	Extract type
				Concentration (μg/ml)					
				625	500	375	250		
44.79	4	0.00	590.65	66.67 ± 6.67a	46.67 ± 3.33b	3.33 ± 3.33c	0.00 ± 0c	24	Methanol
52.18	4	671.98	484.45	80.00 ± 5.77 a	60.00 ± 5.77b	13.33 ± 6.67c	6.67 ± 3.33c	48	
73.33	4	627.55	301.55	93.33 ± 3.33 a	70.00 ± 5.77 ab	56.67 ± 3.33 ab	46.67 ± 8.82b	72	
236.17	4	0.00	677.72	53.33 ± 3.33 a	13.33 ± 3.33b	0.00 ± 00b	0.00 ± 0b	24	Ethyl acetate
89.33	4	665.92	442.08	80.00 ± 5.77 a	56.67 ± 3.33 a	53.33 ± 3.33 a	6.67 ± 3.33b	48	
120	4	595.79	390.34	86.67 ± 3.33 a	76.67 ± 3.33 ab	63.33 ± 3.33b	10.00 ± 00c	72	

Table 2 Larvicidal activity of PH2 hexane extract against *Culex pipiens*.

F	df	LC ₉₀ (μg/mL)	LC ₅₀ (μg/mL)	Mortality (%)				Time	Extract type
				Concentration (μg/ml)					
				250	125	62.50	31.25		
227.87	4	242.17	148.43	93.33 ± 3.33 a	40.00 ± 5.77b	13.33 ± 3.33c	0.00 ± 0c	24	Hexane
803.5	4	217.86	124.93	100.00 ± 0 a	56.67 ± 3.33b	30.00 ± 0c	0.00 ± 0 d	48	
139.20	4	201.1461	100.3904	100.00 ± 0 a	76.67 ± 3.33b	50.00 ± 5.77c	0.00 ± 0 d	72	

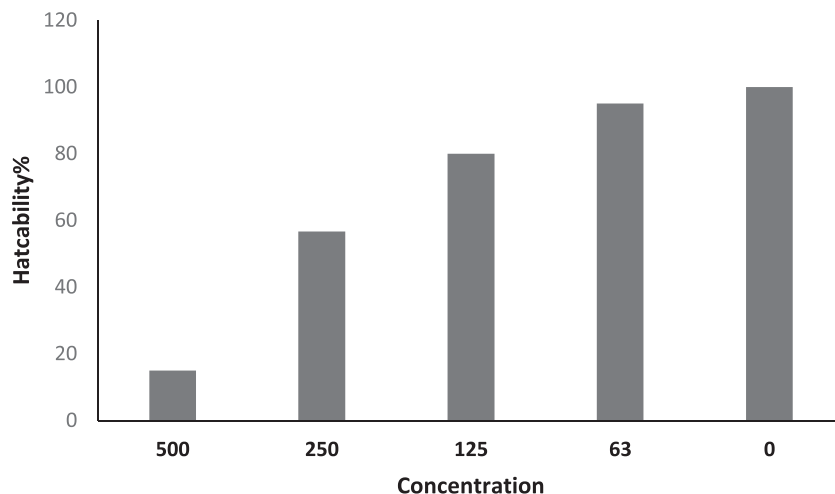


Fig. 1. Percentage hatchability of the PH2 hexane extract against *Culex pipiens*.

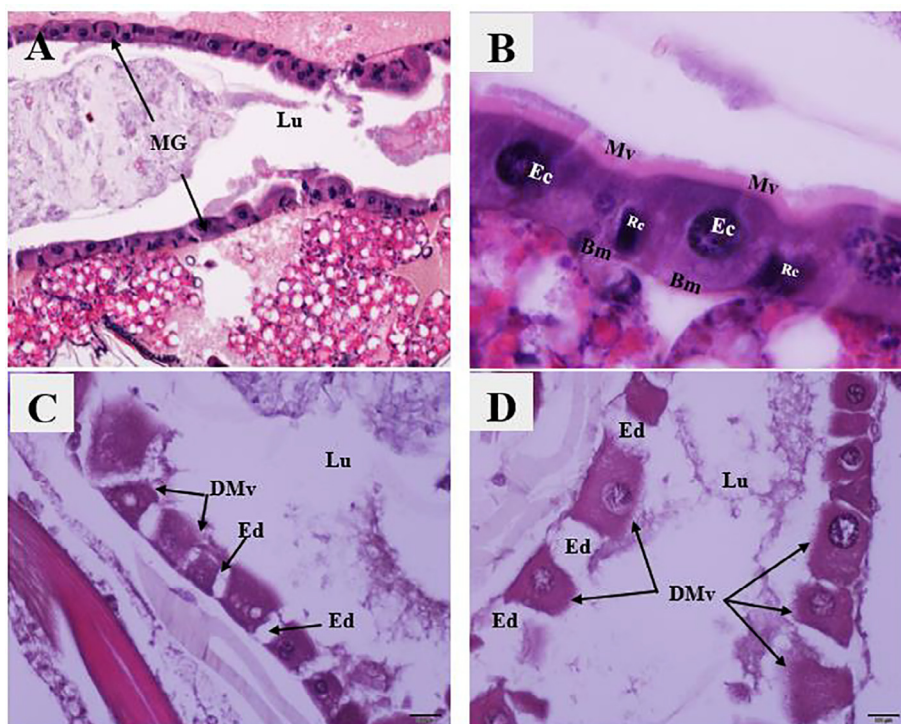


Fig. 2. Photomicrographs of midguts of *Cx. pipiens* larvae demonstrating: **{A, B}** Longitudinal sections in the midguts (MG) of **control** larvae with normal and healthy epithelial cells (Ec), microvilli (Mv), nuclei (n), and regenerative cells (Rc). Note the absence of lesions. H&E stain. **{C, D}**: Longitudinal sections in midguts of **PH2-treated** larvae, with edema (Ed) between the degenerated epithelial cells and degraded microvilli (DMv). H&E stain.

3.6. Fourier transform infrared (FTIR) spectrometry

The FTIR spectrum is shown in Fig. 4. A broad peak at 3449 cm^{-1} represents to O–H stretching phenol groups. Peaks at 2913 and 2995 cm^{-1} represent C–H of alkyne groups. The absorbance at 2361 cm^{-1} corresponds to nitro compounds (N–O). The band at 1022 cm^{-1} represents the C = C of aromatic rings. The absorbance at 1436 cm^{-1} represents carbonyl groups. The peaks at 1141 to 1436 cm^{-1} are related to the bending of O–H, C–H, and C–N–C, and stretching attributed to carboxyls, alkanes, and amines. Fig. 5.

3.7. GC–MS analysis

The major compounds of the PH2 extract were bisabolol oxide A (17.1%), 1-methoxy-4-(1-z-propenyl) benzene (10.5%), methyl ester of hexadecanoic acid (8.3%), and 10-nonadecanone (7.03%) (Table 3).

4. Discussion

Mosquitoes in the aquatic stage (eggs and larvae) are an attractive target for mosquito control because, in stagnant water, they

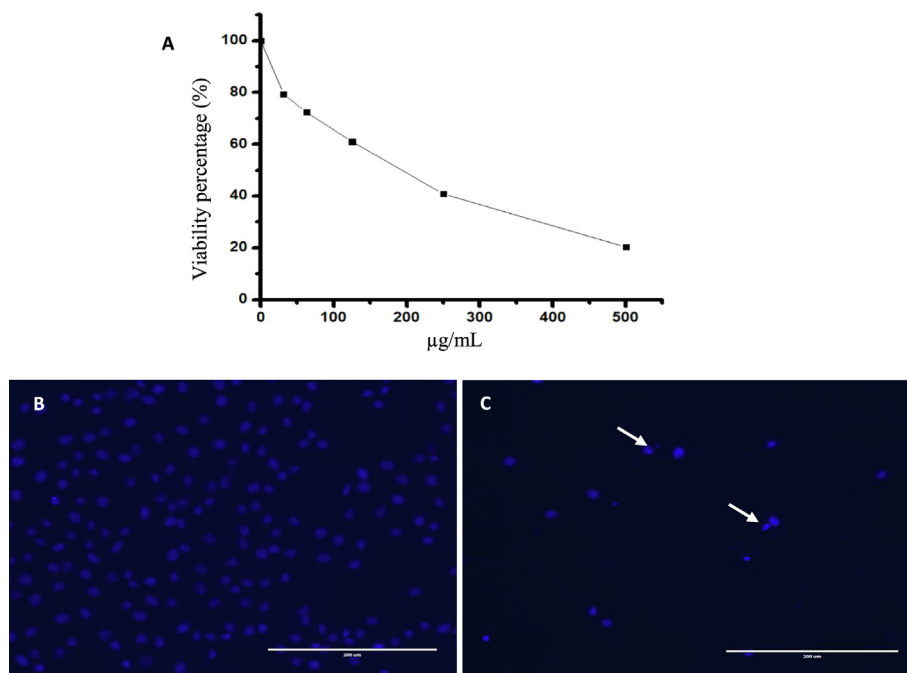


Fig. 3. MTT assay (A) for relative cell viabilities of normal HUVECs incubated with PH2 extract for 24 h. Microscopic images of HUVECs treated (B) with and without PH2 extract (C). The arrows indicate the fragmented nucleus.

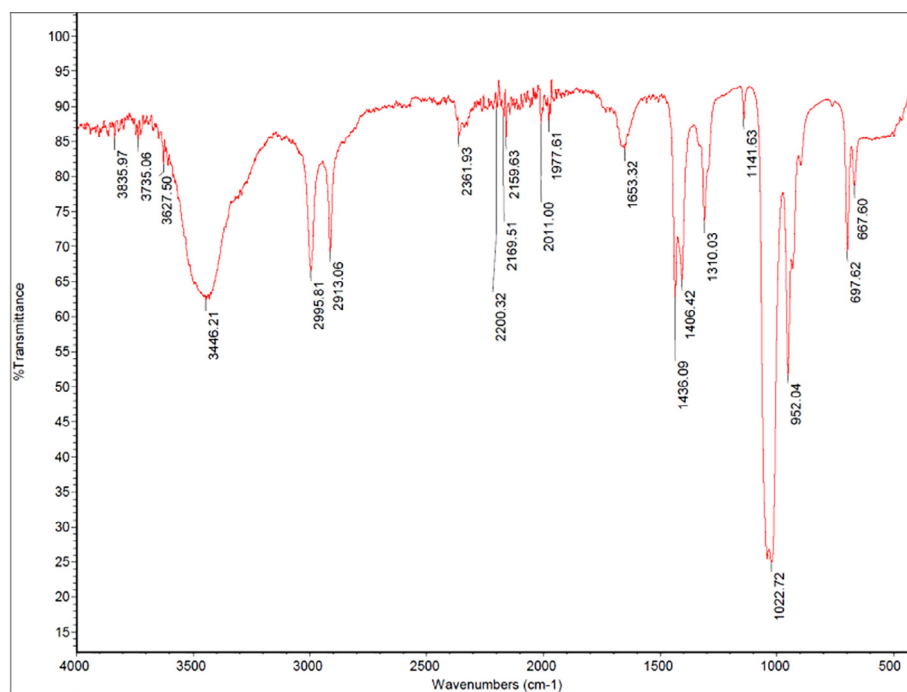


Fig. 4. FTIR spectrum from hexane extract of PH2.

are easily managed. However, introducing synthetic mosquitoicides into waterbodies may pose risks to humans, animals, and the environment (Subramanian et al., 2012, Bagavan and Rahuman, 2011). Larvicides derived from natural sources, mainly plants, are a promising alternative for managing mosquitoes (Warikoo et al., 2012, Hemalatha et al., 2015). *H. forskoolii* is used in Tanzania to control insect vector-borne diseases (Asnake et al., 2016). It was shown that *H. forskoolii* root chloroform extract was toxic to *C. quinquefasciatus*, *A. gambiae*, and *A. aegypti* with LC₅₀ values of

6.0, 2.0, and 3.8 µg/mL, respectively (Sillo et al., 2019). Similarly, in Portugal, extract of the aerial parts of *F. vulgare* resulted in LC₉₀ and LC₉₉ values of 37.1 and 52.4 µl/L, respectively, against *Ae. aegypti* larvae (Rocha et al., 2015). In our recent study (Al-Mekhlafi et al., 2020), the ethyl acetate extract of *M. chamomilla* showed an LC₅₀ value of 287 µg/mL after 24 h of exposure and reduced the hatchability of the eggs of *Cx. pipiens*. In the present investigation, the mixture of *H. forskoolii* and *M. chamomilla* showed dose- and time-dependent toxicity against *Cx. pipiens* with

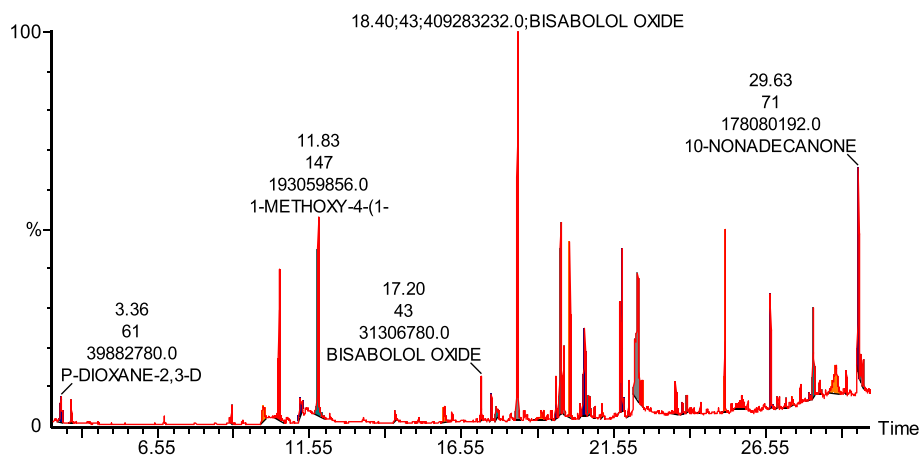


Fig. 5. GC-MS chromatogram of hexane extract of PH2.

Table 3

Compounds identified in the hexane extract of PH2 using GC-MS.

No.	Name	RT	Area %
1	HYDRAZINECARBOTHIOAMIDE	3.69	3.460
3	ESTRAGOLE	10.54	6.100
4	1-METHOXY-4-(1-Z-PROPENYL) BENZENE	11.83	10.560
5	7-METHYL-1-NAPHTHOL	14.35	0.860
6	2-NONENAL	15.98	0.610
7	BISABOLOL OXIDE B	17.20	0.900
8	BISABOLOL OXIDE A	18.40	17.110
9	2-DECEN-1-OL (CAS)	19.17	0.170
11	BISABOLOL OXIDE A (PYRAN OXIDE)	19.66	2.290
12	1,6-DIOXASPIRO[4.4]NON-3-ENE	19.81	5.860
13	METHYL ESTER OF HEXADECANOIC ACID	20.12	8.380
14	(-)-SELINA-4.ALPHA.,11-DIOL	20.46	0.330
15	HEXADECANOIC ACID	20.61	4.700
16	3,4-SECODAMMAR-4(28)-EN-3-OIC	20.74	1.400
17	METHYL ESTER OF 6-OCTADECENOIC ACID	21.84	3.190
18	METHYL ESTER OF OCTADECANOIC ACID	22.06	1.880
19	(Z,Z)-6,9-CIS-3,4-EPOXY-NONADECANE	22.35	4.270
20	OCTADECANOIC ACID	22.49	1.080
21	11-DECYL-DOCOSANE	23.60	1.810
22	METHYL ESTER OF EICOSANOIC ACID	23.82	0.640
23	TETRACOSANE	25.23	6.900
24	VITAMIN E	25.77	0.510
25	METHYL ESTER OF TETRACOSANOIC ACID	26.93	0.470
26	OCTADECANE	27.43	0.830
27	DODECANAL	27.71	0.480
28	SQUALENE	28.13	5.570
29	1-DOCOSANOL	28.34	0.520
30	2-UNDECEN-1-OL	29.21	0.580
31	10-NONADECANONE	29.63	7.030

an LC₅₀ value of 148.4 µg/mL. The toxicity of the mixed plants helped to reduce the LC₅₀ value of ethyl acetate extract of *M. chamomilla* from 287.1 µg/mL as reported in our previous study (Al-Mekhlafi et al., 2020) to 148.4 µg/mL after extracting *H. forskoalii* and *M. chamomilla* together.

Based on the LC₅₀, the activity of the plant extracts can be classified into three categories. Extracts showing LC₅₀ of < 50 µg/mL are classified as very active extracts, while those with LC₅₀ higher than 750 µg/mL are classified as inactive (Komalamisra et al., Magalhães et al., 2010, Dias et al., 2014). The LC₅₀ value reported in the present study was 148.4 µg/mL, indicating that the plant extract is considered active.

The combination of insecticidal agents is encouraged to optimize the efficacy of the insecticides, solve the problem of insect resistance, and preserve the efficiency of the insecticidal agents for years. In the current investigation, *H. forskoalii* solvent extracts tested individually were inactive against mosquito larvae, while its

combination with *M. chamomilla* extract showed synergistic properties. The combination of *Callitris glaucophylla* and *Khaya senegalensis* extract at a ratio of 1:1 exerted synergistic effects on *A. aegypti* (Shaalán et al., 2005). Synergistic efficacy was also observed in the combination of *E. camaldulensis* with *C. rigidus* extracts at a 3:1 ratio against *A. gambiae* larvae (Ríos et al., 2017). Similarly, combinations of *Canarium schweinfurthii*, *Aucoumea klaineana*, and *Dacryodes edulis* led to the improvement of their efficiency and exhibited significant activity against *A. gambiae* (Obame et al., 2016). The combination of thymol and carvacrol at a 1:4 ratio exhibited significant synergistic activity against *Cx. pipiens* larvae, compared with the compounds tested individually (Youssefi et al., 2019).

Histopathological assessment of the third instar of *Cx. pipiens* exposed to PH2 extract showed a change in the midgut architecture, mainly in the basal membrane, epithelial cells, and microvilli. Such damage was due to secondary metabolites in PH2 extract. Secondary metabolites lower the surface tension of the mucosal membrane and as a result damage the digestive system. Damage in the midgut region changes various functions, such as osmoregulation, digestion, nutrition absorption, and ion transport (Sina and Shukri, 2016). However, as in other insects, the mosquito's stomach does not function only in digestion, but also in mechanical and chemical defense against invaders (Terra, 2001). Damage to the digestive tract due to larvicidal agents generally affects the digestion and absorption of food in this region (Rohmah et al., 2020). In addition, damage to the regenerative cells impairs the development of larvae and hence metamorphosis (Procópio et al., 2015). Extracts of different parts of *A. bilimbi* including leaf, flower, and fruit were reported to damage the villi, epithelial cells, and peritrophic membrane of *Anopheles barbirostris* and *Ae. aegypti* larvae (Das et al., 2011, Suluvoy and Grace, 2017, (Rohmah et al., 2020).

A higher LC₅₀ indicates that it would take more of the extract to induce a toxic response, while a small LD₅₀ value could be deleterious to human health (Okeleye et al., 2013). The American National Cancer Institute considered that an IC₅₀ of <30 µg/mL is toxic (Hasibuan et al., 2014). When applying this information to our data, PH2 extract had some toxicity against HUVECs, as determined using MTT assay and fluorescence microscopy. However, the results cannot be generalized to human health and the risk should be assessed using different models.

The efficacy of the extract against mosquito larvae may be related to the presence of phenols, flavonoids, terpenoids, saponins, tannins, and alkaloids, which have been reported for their insecticidal activities (Shaalán et al., 2005). These toxic secondary

metabolites are consumed orally or absorbed by the cuticle and affect the physiology of insects, causing their death (Rattan, 2010). Many reports have revealed that the main constituents of the extract are responsible for bio-activity, as it contains the main fraction of the extract (Gul 1994, Vani et al., 2009, Glolade and Lockwood, 2008). Therefore, we considered four major compounds from PH2 extract. From the GC–MS results, bisabolol oxide A, 1-methoxy-4-(1-z-propenyl) benzene, methyl ester of hexadecanoic acid, and 10-nonadecanone were found to be the major compounds of PH2 extract. An oil rich in bisabolol oxide A from different plant extracts showed antimicrobial (Tolouee et al., 2010, Alireza 2012, Sashidhara et al., 2006, Roby et al., 2013) and larvicidal activities against *Aedes aegypti*, with a CL_{50} of 15.9 mg/mL (Furtado et al., 2005). It has also been reported that 1-methoxy-4-(1-z-propenyl) benzene possesses insecticidal activity (CHEBI, 2021). Estragole exhibited strong larvicidal activity with LC_{50} of 41.67 and 38.56 μ g/mL for *An. sinensis* and *An. anthropophagus*, respectively (He et al., 2018). Estragole was shown to possess contact toxicity against *Lasioderma serricornis* adults (LD_{50} : 15.58 mg/adult) (Wang et al., 2014).

5. Conclusions

PH2 hexane extract showed larvicidal activity against *Cx. pipiens* as well as structural change of the midgut region. Caution is required in the use of the PH2 extract as a larvicide against *Cx. pipiens* due to its toxic effects on normal HUVECs, as determined using MTT assay.

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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