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Based on GC-MS Analysis: An Evaluation Activity of Some Algal Extracts Against *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Continual application of chemical insecticides causes a lot of environmental and health problems. Mosquitoes as vectors of diseases develop resistance toward them. Finding out safe alternatives is an urgent need. Algal extracts are a promising alternative approach as they are biodegradable and eco-friendly. The present study assessed the larvicidal efficacy of three ethanolic algal extracts (*Chaetomorpha linum*, *Ulva intestinalis* and *Sargassum dentifolium*) belonging to two different algal divisions against the 3rd larval instar of *Culex pipiens* L. (Diptera: Culicidae) under laboratory conditions. Toxicological studies showed that three species of brown and green algal extracts exhibited good larvicidal activity. Percentages of mortality increased with increasing concentrations of all algal extracts. Based on the LC_{50} values, *C. linum* (Chlorophyta) exhibited the highest larvicidal potency among the used extracts, followed by *U. intestinalis* (Chlorophyta) and *S. dentifolium* (Phaeophyta) with $LC_{50,\ s}$ of 224.45, 231.06 and 241.79 ppm, respectively, upon 48 hours exposure. GC-Mass analysis of the tested extracts revealed that the most abundant constituents in the ethanolic extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* were palmitic acid (17.42 %), linolenic acid (23.93%) and Di-n-octyl phthalate (19.03 %), respectively. The application of LC_{50} values of tested ethanolic algal extracts induced many biochemical and morphological aberrations in the treated *C. pipiens* larvae, compared to the untreated larvae. The biochemical changes were tracked through the analysis of the insect's main metabolites (carbohydrates, lipids and proteins), in addition to measuring the changes in acetylcholine esterase, GST, α- and ß- esterases after algal extracts treatment. Generally, variations were recorded in the lipid and carbohydrate after treatment with *C. linum*. Moreover, *C. linum* inhibited the activity of α and ß esterases enzymes to a great extent, compared to the untreated. Many histological abnormalities were noticed in the treated larvae. Overall, these results demonstrated that ethanolic algal extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* might be used to control *C. pipiens* mosquitoes without harming humans or the environment. Hence, they could be incorporated into integrated vector management programs.

INTRODUCTION

Mosquitoes cause millions of cases of illnesses and deaths in humans and animals each year **(Dahmana & Mediannikov, 2020)**. They are vectors of various diseases, such as

malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at both endemic and epidemic areas in many countries **(WHO, 2020; Ghouth, *et al.*, 2021)**. Among mosquito species, *Culex pipiens* L. (Diptera: Culicidae) is common and widely distributed across Egypt and has been incriminated as the main vector of bancroftian filariasis **(Selim *et al.*, 2019)**. Endless efforts have been developed to control this insect vector, but the results are still unsatisfactory **(Kassem *et al.*, 2018; Rahimi *et al.*, 2019; Aly *et al.*, 2021)**. The extensive use of chemical insecticides resulted in inducing resistance by insect vectors besides residue contamination of human food and mammalian toxicity, reducing beneficial non-target biota and environmental pollution **(James & Xu, 2012; Lozowicka *et al.*, 2014)**. These factors have created the need for environmentally safe and target-specific agents for vector control purposes. Researchers have focused their attention mainly on the usage of marine algae as a new source of insecticides, with no negative effects on the environment and insect resistance after application **(Hamed *et al.*, 2018; Rashad & El-Chaghaby, 2020; Asimakis *et al.*, 2022**). Algae are a large and diverse group of photosynthetic organisms that live in aquatic systems **(Shalaby, 2011; Ramanan *et al.*, 2016)**. They vary in their size from microalgae to seaweeds **(Pereira & Gonçalves, 2019)**. Marine algae are categorized into three major groups: green algae (Chlorophyta), brown algae (Phaeophyceae) and red algae (Rhodophyta) **(Leandro *et al.*, 2019; Hakim & Patel, 2020; Besednova *et al.*, 2022)**. Marine algae are gaining special attention because of their safety, wide acceptance by consumers and their multipurpose functional uses **(Wells *et al.*, 2017; Pangestuti *et al.*, 2018)**. They have a high content of bioactive compounds, which have been demonstrated as insecticides exactly like those found in terrestrial plants **(Salehi *et al.*, 2019)**. Examples of bioactive compounds found in brown and green marine algae are sterols, terpenoids and phenols, with effective insecticidal activity against mosquitoes **(Mekinić *et al.*, 2019; Asimakis *et al.*, 2022; Aly *et al.*, 2023)**. To enhance the efficacy of marine algae, they are extracted to be used as insecticides alternatives to the traditional insecticides in IPM programs **(Abdel-Raouf *et al.*, 2012; Taktak *et al.*, 2021)**. The advantages of using marine algal extracts are due to their potential to reproduce on a large scale and being cost-effective **(Hassaan & Nemr, 2020; Alprol *et al.*, 2023)**. The present study aimed to screen and assess the larvicidal activity of three ethanolic marine algal extracts, including *Chaetomorpha linum*: Green, *Ulva intestinalis*: Green and *Sargassum dentifolium*: Brown against 3rd larval instar of C. *pipiens* mosquito as a vector of several diseases. Besides, the study investigated the biochemical and histological changes in the treated larvae based on chromatographic screening of the chemical constituents of the three ethanolic algal extracts using GC-MS.

MATERIALS AND METHODS

Maintenance of mosquito colony

The egg rafts of *C. pipiens* were obtained from the Research and Training Center on Vectors of Diseases (RTC), Faculty of Science, Ain Shams University. The mosquitoes were reared for ten generations in an insectary in RTC under controlled conditions; 25±2˚C and RH 70±10% and photoperiod 14:10 light: dark hours.

Algae collection and extraction

The algae were collected in April 2021 from the Red Sea coast in Fayed City, Ismailia Government, Egypt (30°21'48.7"N 32°18'19.4"E). The algae were collected by hand picking and then washed with sea water followed by distilled water. The collected algae were identified according to **Zinova (1967)** and **Aleem (1993)** and examined using the binocular (BEL photonics microscope) fitted with a Canon Power shot (G12 digital camera) in the Phycology lab, Botany Department, Faculty of Science, Ain Shams University. Tested algae were shade drying at room temperature for two weeks and ground in an electric mill (Moulinex, super blender, France) to fine powder prior to extraction **(Yusuff, 2019; Alsaud & Farid, 2020)**. Powdered parts of each alga (200 gm) were successively extracted by soaking in ethanol (95%) (1kg material: 3 L solvent) inside a one-liter flask with a dark color, filtered and residues were extracted afterwards by using ethanol (95%) **(Redfern *et al.*, 2014)**. The homogenate was filtered, and the solvent was evaporated, the algal extract obtained was stored at -4˚C in the refrigerator prior to use **(Abdel Haleem *et al.*, 2022)**.

Gas Chromatography-Mass Spectrometry (GC-MS)

Algal extracts specimens were identified using Shimadzu GC-MS (model 5977 A, Japan) in the central lab, Faculty of Science, Ain Shams University. GC-MS analysis was conducted by injecting 0.5µl of each ethanolic algal extract in split mode (15:1) at 300˚C. A capillary column was also used (HP-5MS Capillary; 30 mx 0.25mm IDX 0.25 µm film). Helium was the carrier gas with a gas flow rate of 1ml/ minute. The program was as follows: the specimen was analyzed with a column held for 2 minutes at 60˚C after injection. Then, the temperature was increased to 200˚C with a 20˚C/ min heating ramp and a 2.0 minutes hold. The oven temperature was increased again to reach 300˚C, with a 20˚C/minutes heating ramp and a 2.0 minutes hold. The GC-MS scan range was 35-500 atomic mass units under electron impact ionization of 70 eV and a delay time of 4 minutes. The constituents of the algal extracts were determined by comparing the fragmentation patterns of the mass spectra with the data reported from WILEY/NIST and Tutor Libraries **(Adams, 2007; Beckley *et al.*, 2014)**.

Toxicological studies

Early 3rd instar larvae used for the toxicological tests were carried out at the same previously mentioned conditions. Larvicidal activities of the three algal extracts were studied in the range of 150 to 350 ppm extracts according to the standard **WHO (2005)** protocol. For each treatment, three replicates of twenty-five larvae were used. Each

concentration was triplicated. For the untreated samples, only distilled water with ethanol was used. Mortality was recorded at 24, 48, 72- and 96-hours post-treatment. The percentages of larval mortality were calculated for each concentration of the treatments. The mortality data were corrected according to **Abbott (1925)**.

Morphological studies

Morphological changes of the treated larvae with LC_{50} of ethanolic extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* were studied and compared to the untreated larvae at 48- hours post-treatment. The dead larvae were separated and studied under a light microscope for morphological aberrations. Treated larvae were examined using the binocular (BEL Photonics microscope) and light microscope (LABOMED) and then photographed using a Canon G12 digital camera, with magnification power 4x.

Histological studies

The specimens of untreated and treated 3rd instar larvae of *C. pipiens* with LC_{50} concentrations of ethanolic extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* 48 hours post-treatment were fixed in 5% glutaraldehyde then washed in 70% alcohol according to **Disbrey and Rack (1970)**. Samples were rinsed in 0.1 M sodium cacodylate buffer with pH 7.2 for 4 hours at room temperature and then washed twice using (0.1 M) cacodylate buffer for 15 minutes. Osmium tetroxide (1%) prepared with (0.1M) sodium cacodylate buffer was used to immerse the samples for 2 hours. Cocodylate buffer (0.1M) was used to wash them 3 times (15 minutes for each time). Finally, samples were submerged and dehydrated using graded series of ethanol and embedded into epoxy resin. Semithin sections were stained using toluidine blue for examination by light microscope (VELAB, America), model (Ve-T2) with 5.1 MP digital camera (TOUPTEK PHONETICS, China), model (LCMOS05100KPA).

Biochemical studies

Tissue samples of untreated and treated 3rd instar larvae of *C. pipiens* with LC_{50} of the ethanolic algal extracts of *C. linum, U. intestinalis* and *S. dentifolium* 48 hours post treatment were homogenized using a treatment buffer (1gm insect body/1ml) in a chilled glass Teflon tissue grinder for 3 minutes. Homogenates were centrifuged using the refrigerated centrifuge (Bioevopeak, China) at 14000 rpm for 15 minutes at -2˚C in a refrigerated centrifuge. The supernatant was stored at -5˚C (used within two weeks maximum) until use for biochemical investigation. Three replicates were carried out for each treatment **(Liu *et al.*, 2020)**. Biochemical data were expressed as mean ±SE. Data from untreated and treated groups were compared using a student t-test. Differences were significant ($P<0.05$) and highly significant ($P<0.01$). Data between treated groups were analyzed using SPSS software; the level of significance was tested using one-way analysis of variance (ANOVA), and $P<0.05$ was considered statistically significant.

Determination of total protein

Total protein was estimated based on the method of **Bradford (1976)** using bovine serum albumin to build up the standard curve.

Determination of total carbohydrate

Total carbohydrate was prepared according to the study of **Crompton and Birt (1976)**. While, it was evaluated as stated by **Dubois *et al.* (1956)** based on a phenol-sulfuric reaction.

Determination of total lipid

Total lipid in untreated and treated larvae was determined using phosphovanillin reagent and oleic acid and palmitic acid mixture standard curve **(Knight *et al.*, 1972)**.

Determination of acetylcholinesterase (AChE) activity

AChE activity was detected according to the method described in the study of **Simpson *et al.* (1964)** by using the substrate, acetylcholine bromide (AChBr).

Determination of non-specific esterase activity

The activity of α- esterases and ß-esterases were evaluated depending on the study of **Van Asperen (1962)** by using α-naphthyl acetate as substrate.

Determination of glutathione -S-transferase (GST) activity

GST activity was determined according to the work of **Habig *et al.* (1974)**, with some modifications.

Statical analysis

Mortality was analyzed using LDP line statistical software for mortality regression lines **(Bakr, 2000)**. Lethal concentration was determined at 95% confidence level using probit analysis **(Finny, 1971)**. Toxicity index and relative potency were measured according to the method described by **Sun (1950)** and **Zidan and Abdel-Mageed (1988)** to compare the potencies of the tested extracts. Biochemical data were expressed as mean ±SE. Data from untreated and treated groups were compared using student t-test. Differences were significant ($P<0.05$) and highly significant ($P<0.01$). Data between treated groups were analyzed using SPSS software; the level of significance was tested using one-way analysis of variance (ANOVA), and $P<0.05$ was considered statistically significant.

RESULTS

1. The percentage yield of ethanolic extracts from the tested algal species

The yield of ethanolic extracts of the tested algal species is shown in Table (1). The amounts of the extracts found from *C. linum*, *U. intestinalis* and S. *dentifolium* were 6.99%, 4.22% and 2.28%, respectively, of the whole algae. Remarkably, the greatest amount was obtained from *C. linum* and the least from *S. dentifolium*.

Table 1. The yield of ethanolic extracts from the tested algal species

The scientific name of the algal species	Algal order	Algal family	Algal type	Algal dry weight(gm)	Algal extract weight (gm)	Algal extract yield %
Chaetomorpha linum	Cladophorales	Cladophoraceae	Green algae	32.03	2.24	6.99
Ulva intestinalis	Ulvales	Ulvaceae	Green algae	34.15	1.44	4.22
Sargassum dentifolium	Fucales	Sargassaceae	Brown algae	60.00	1.37	2.28

2. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the tested extracts

Qualitative analysis was done to determine the constituents of ethanolic extracts of *C. linum*, *U.intestinalis* and *S. dentifolium* by using GC-MS. Tables (2, 3 & 4) show the chemical components of the algal extracts, the RT, area of the peak concentration (%) (average rate), molecular formula and molecular weight of the identified components, which led to the identification of natural chemical components.

2.1. Chemical composition of C. linum

Data in Table (2) show a chemical analysis of the ethanolic extract of *C. linum*; the main component was palmitic acid with an average rate (17.42%), and the minor component was 6-chlorohexanoic acid with an average rate of 0.01%. Most of the compounds extracted with ethanol were fatty acids (98.7%), sesquiterpene hydrocarbons (0.72%), and phenyl propanoid (0.58%)

Table 2. The main components identified in *C. linum* ethanolic extract by using GC-MS

No.	RT* (minutes)	Peak area%	Compound name	Molecular weight	Molecular formula	Chemical class
1	8.34	0.01	6-Chlorohexanoic acid, TMS derivative	222.08	$C_9H_{19}ClO_2Si$	fatty acid
2	8.66	0.11	Hydracrylic acid, 2TMS derivative	234.11	$C_9H_{22}O_3Si_2$	fatty acid
3	8.75	0.59	4-Hydroxybutanoic acid, 2TMS derivative	248.13	$C_{10}H_{24}O_3Si_2$	fatty acid
4	8.96	0.07	2-Butyne-1,4-diol, 2TMS derivative	230.12	$C_{10}H_{22}O_2Si_2$	fatty acid
5	9.21	0.12	3-Dimethyl(trimethylsilyl)silyloxytetradecane	344.29	$C_{19}H_{44}OSi_2$	fatty acid
6	9.55	0.11	Diethylene glycol, 2TMS derivative	250.14	$C_{10}H_{26}O_3Si_2$	fatty acid
7	9.71	2.98	Glycerol, 3TMS derivative	308.17	$C_{12}H_{32}O_3Si_3$	fatty acid

8	10.02	0.04	2-Ethyl-3-trimethylsilyloxy(trimethylsilyl)butyrate	276.16	$C_{12}H_{28}O_3Si_2$	fatty acid
9	10.12	0.03	Isoborneol, pentamethyldisilanyl ether	284.19	$C_{15}H_{32}OSi_2$	fatty acid
10	10.19	0.04	Glyceric acid, 3TMS derivative	322.15	$C_{12}H_{30}O_4Si_3$	fatty acid
11	10.40	0.13	2-Ketoglutaric acid, 2TMS derivative	290.10	$C_{11}H_{22}O_5Si_2$	fatty acid
12	10.48	0.05	1,3-Butanediol, 2TMS derivative	234.15	$C_{17}H_{36}O_3Si_2$	fatty acid
13	10.60	0.07	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	308.13	$C_{11}H_{28}O_4Si_3$	fatty acid
14	10.70	0.08	tert-butyl(dimethyl)silyl 2-([tert-butyl(dimethyl)silyl]oxy)-3-methylbut-2-enoate	344.22	$C_{17}H_{36}O_3Si_2$	fatty acid
15	10.76	0.05	Butanedioic acid, 2TBDMS derivative	346.20	$C_{16}H_{34}O_4Si_2$	fatty acid
16	10.87	0.09	(R,S)-3,4-DIHYDROXYBUTANOIC ACID TRITMS	336.16	$C_{13}H_{32}O_4Si_3$	fatty acid
17	11.02	0.10	Nootkatone	218.17	$C_{15}H_{22}O$	sesquiterpens hydrocarbon
18	11.14	0.13	Erythrono-1,4-lactone, (E)-, 2TMS derivative	262.11	$C_{10}H_{22}O_4Si_2$	fatty acid
19	11.23	0.03	3-Deoxyhexitol, 5TMS derivative	526.28	$C_{21}H_{54}O_5Si_5$	fatty acid
20	11.35	0.28	meso-Erythritol, 4TMS derivative	410.22	$C_{16}H_{42}O_4Si_4$	fatty acid
21	11.52	0.05	D-(-)-Erythrose, tris(trimethylsilyl) ether, pentafluorobenzyloxime (isomer 1)	531.17	$C_{20}H_{34}F_5NO_4Si_3$	fatty acid
22	11.63	0.10	2,3-Dihydroxy-2-methylpropanoic acid, 3TMS derivative	336.16	$C_{13}H_{32}O_4Si_3$	fatty acid
23	11.70	0.15	L-Threonic acid, tris(trimethylsilyl) ether, trimethylsilyl ester	424.20	$C_{16}H_{40}O_5Si_4$	fatty acid ester
24	11.90	0.16	Malic acid, 2TMS derivative	278.10	$C_{10}H_{22}O_5Si_2$	fatty acid

25	12.00	0.28	2-Pentamethyldisilanyloxypentane	218.15	$C_{10}H_{26}OSi_2$	fatty acid
26	12.20	0.55	D-(+)-Ribono-1,4-lactone, 3TMS derivative	364.16	$C_{14}H_{32}O_5Si_3$	fatty acid
27	12.50	0.14	L-(-)-Arabitol, 5TMS derivative	512.27	$C_{20}H_{52}O_5Si_5$	fatty acid
28	12.62	0.29	1,2-O-Isopropylidene-.alpha.-D-glucofuranose, 3TMS derivative	436.21	$C_{18}H_{40}O_6Si_3$	fatty acid
29	12.84	0.18	Tridecanoic acid, TMS derivative	286.23	$C_{16}H_{34}O_2Si$	fatty acid
30	12.94	0.14	Acetin, bis-1,3-trimethylsilyl ether	278.14	$C_{11}H_{26}O_4Si_2$	fatty acid
31	13.03	0.24	L-(+)-Tartaric acid, 4TMS derivative	438.18	$C_{16}H_{38}O_6Si_4$	fatty acid
32	13.46	9.05	Myristic acid, TMS derivative	300.25	$C_{17}H_{36}O_2Si$	fatty acid
33	13.79	3.37	Tetradecanal	212.21	$C_{14}H_{28}O$	fatty acid
34	14.43	1.08	Pentadecanoic acid, TMS derivative	314.26	$C_{18}H_{38}O_2Si$	fatty acid
35	15.28	17.42	Palmitic Acid, TMS derivative	328.28	$C_{19}H_{40}O_2Si$	fatty acid
36	15.96	0.26	Myo-Inositol, 6TMS derivative	612.30	$C_{24}H_{60}O_6Si_6$	fatty acid
37	16.63	7.54	Phytol, TMS derivative	368.35	$C_{23}H_{48}OSi$	fatty acid
38	17.04	7.19	9,12-Octadecadienoic acid (Z, Z)-, TMS derivative	352.28	$C_{21}H_{40}O_2Si$	fatty acid
39	17.21	5.09	13-Octadecenoic acid, (Z)-, TMS derivative	354.30	$C_{21}H_{42}O_2Si$	fatty acid
40	17.46	1.77	2-O-Glycerol-.alpha.-d-galactopyranoside, hexa-TMS	686.34	$C_{27}H_{66}O_8Si_6$	fatty acid
41	17.64	1.85	Xanthine, 3TMS derivative	368.15	$C_{14}H_{28}N_4O_2Si_3$	fatty acid
42	17.80	0.37	.beta-Gentiobiose, octakis (trimethylsilyl) ether, methyloxime (isomer 2)	947.46	$C_{37}H_{89}NO_{11}Si_8$	fatty acid
43	18.08	5.31	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 2)	947.46	$C_{37}H_{89}NO_{11}Si_8$	fatty acid
44	18.31	1.33	.beta.-D-Lactose,	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid

			(isomer 2), 8TMS derivative			
45	18.46	0.81	13-Octadecenoic acid, (E)-, TMS derivative	354.30	$C_{21}H_{42}O_2Si$	fatty acid
46	18.61	0.95	2-Buten-1-ol, (Z)-, TMS derivative	144.10	$C_7H_{16}OSi$	fatty acid
47	18.72	1.47	11,14-Eicosadienoic acid, (Z)-, TMS derivative	380.31	$C_{23}H_{44}O_2Si$	fatty acid
48	18.91	0.60	1-Nonadecanol, TMS derivative	356.35	$C_{22}H_{48}OSi$	fatty acid
49	19.06	0.41	17-Octadecynoic acid, TMS derivative	352.28	$C_{21}H_{40}O_2Si$	fatty acid
50	19.27	0.44	L-(+)-Threose, tris(trimethylsilyl) ether, ethyloxime (isomer 2)	379.20		
51	19.43	0.28	Aucubin, hexakis(trimethylsilyl) ether	778.36	$C_{33}H_{70}O_9Si_6$	fatty acid
52	19.58	0.38	5,8,11-Eicosatriynoic acid, TMS derivative	372.25	$C_{23}H_{36}O_2Si$	fatty acid
53	19.73	0.34	alpha-Ketoisovaleric acid, TMS derivative	188.09	$C_8H_{16}O_3Si$	fatty acid
54	19.95	1.70	Bis(2-ethylhexyl) phthalate	390.28	$C_{24}H_{38}O_4$	fatty acid
55	20.13	1.41	1-Monopalmitin, 2TMS derivative	474.36	$C_{25}H_{54}O_4Si_2$	fatty acid
56	20.38	1.90	beta-D-Glucopyranosiduronic acid, 4-(acetylamino) phenyl 2,3,4-tris-O-(trimethylsilyl)-, methyl ester	557.23	$C_{24}H_{43}NO_8Si_3$	fatty acid ester
57	20.59	1.59	Sarcosine, N-(4-trifluoromethylbenzoyl) -, butyl ester	317.12	$C_{15}H_{18}F_3NO_3$	fatty acid ester
58	20.74	3.36	Sucrose, 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
59	21.13	0.19	2,2,7,7-tetramethyl-4-(((5-((trimethylsilyloxy)methyl)-1,4-dioxan-2-yl) (methoxymethyl)-3,6-dioxa-2,7-disilaoctane	438.23	$C_{18}H_{42}O_6Si_3$	fatty acid
60	21.18	0.20	Aucubin, hexakis(trimethylsilyl) ether	778.36	$C_{33}H_{70}O_9Si_6$	fatty acid

61	21.37	0.81	1-Monolinolein, 2TMS derivative	498.36	$C_{27}H_{54}O_4Si_2$	fatty acid
62	21.57	0.28	Hexadecanedioic acid, 2TMS derivative	430.29	$C_{22}H_{46}O_4Si_2$	fatty acid
63	21.63	0.44	3-(1,5-Dimethyl-hexa-1,4-dienyl)-2,2-dimethyl-4-trimethylsilylcyclopentanol	294.24	$C_{18}H_{34}OSi$	fatty acid
64	21.87	1.34	Lignoceric acid, TMS derivative	440.41	$C_{27}H_{56}O_2Si$	fatty acid
65	23.16	0.27	beta-D-Galactopyranoside, methyl 2,3,6-tris-O-(trimethylsilyl)-, acetate	452.21	$C_{18}H_{40}O_7Si_3$	fatty acid
66	23.50	0.08	Hexadecane, 1-chloro-	260.23	$C_{18}H_{36}Cl_2O$	fatty acid
67	23.87	0.15	Docosa-8,14-diyn-1,22-diol, (Z)-, 2TMS derivative	478.37	$C_{28}H_{54}O_2Si_2$	fatty acid
68	24.03	1.13	beta-D-Lactose, (isomer 2), 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
69	24.09	0.57	Cholesterol, TMS derivative	458.39	$C_{30}H_{54}OSi$	fatty acid
70	24.40	0.54	3-alpha-Mannobiose, octakis(trimethylsilyl) ether (isomer 1)	918.43	$C_{37}H_{89}O_{11}Si_8$	fatty acid
71	24.53	0.95	Melibiose, octakis(trimethylsilyl)-	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
72	24.78	0.38	Campesterol, TMS derivative	472.41	$C_{31}H_{56}OSi$	fatty acid
73	24.96	0.32	D-(+)-Ribono-1,4-lactone, 3TMS derivative	364.16	$C_{14}H_{32}O_5Si_3$	fatty acid
74	25.31	0.72	Phytyltetradecanoate	506.51	$C_{34}H_{66}O_2$	fatty acid
75	25.53	4.34	Stigmast-5-ene, 3beta-(trimethylsiloxy)-, (24S)-	486.43	$C_{32}H_{58}OSi$	fatty acid
76	25.65	0.95	Fucosterol, TMS derivative	484.41	$C_{32}H_{56}OSi$	fatty acid
77	25.97	0.30	tau-Muurolol	222.20	$C_{15}H_{26}O$	sesquiterpen hydrocarbon
78	26.47	0.11	2-Dodecen-1-yl(-)succinic anhydride	266.19	$C_{16}H_{26}O_3$	fatty acid
79	26.80	0.32	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	280.31	$C_{20}H_{40}$	sesquiterpens hydrocarbons

80	26.88	0.58	1-Bromo-11-iodoundecane	360.00	$C_{11}H_{22}BrI$	phenyl propanoids
81	27.09	0.29	threo-2,5-Hexodiulose, 1,3,4,6-tetrakis-O-(trimethylsilyl)-	466.21	$C_{18}H_{42}O_6Si_4$	fatty acid
82	28.43	0.08	Trimethylsilyl-di(timethylsiloxy)-silane	280.11	$C_9H_{27}O_2Si_4$	fatty acid

*(RT) retention time

2.2 Chemical composition of U. intestinalis

Data in the Table (3) show the chemical analysis of the ethanolic extract of *U. intestinalis*; the main component was glycerol, with an average rate of 4.68%, and the minor components were alpha-D-Glucopyranoside, methyl 2-acetylamino-2-deoxy-3-O-trimethylsilyl, cyclic methylboronate and 2-oxovaleric acid, with an average rate of 0.07%. Most of the compounds extracted with ethanol were fatty acids (97.81%), sesquiterpens (2.45%), phenyl propanoid (0.52%) and amines (0.49%).

Table 3. The main components identified in *U. intestinalis* ethanolic extract using GC-MS

No.	***RT (minutes)**	**Peak area %**	**Compound name**	**Molecular weight**	**Molecular formula**	**Chemical class**
1	8.18	2.58	Lactic Acid, 2TMS derivative	234.11	$C_9H_{22}O_3Si_2$	fatty acid
2	8.88	0.60	Hydracrylic acid, 2TMS derivative	234.11	$C_9H_{22}O_3Si_2$	fatty acid
3	8.97	1.88	(+/-.)-3-Hydroxybutyric acid, 2TMS derivative	248.12	$C_{10}H_{24}O_3Si_2$	fatty acid
4	9.15	0.21	1,3-Dimethyl-5-pentamethyldisilyloxycyclohexane	258.18	$C_{13}H_{30}OSi_2$	fatty acid
5	9.32	0.21	Trisiloxane,1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	384.14	$C_{12}H_{36}O_4Si_5$	fatty acid
6	9.57	0.46	4-Hydroxybutanoic acid, 2TMS derivative	248.13	$C_{10}H_{24}O_3Si_2$	fatty acid
7	9.80	4.68	Glycerol, 3TMS derivative	308.17	$C_{12}H_{32}O_3Si_3$	fatty acid
8	9.96	0.22	Silanol, trimethyl-, phosphate (3:1)	314.10	$C_9H_{27}O_4PSi_3$	fatty acid
9	10.14	0.63	3-Methyl-4-trimethylsiloxy(trimethylsilyl)butyrate	262.14	$C_{11}H_{26}O_3Si_2$	fatty acid
10	10.23	0.30	5-Methylbenzo(b)thiophene-2-carboxylic acid	192.03	$C_{10}H_8O_2S$	fatty acid
11	10.30	0.24	3,5 (4H,8H)-Dihydro-8-thia-1,3-diaza-cyclopenta[a]inden-4-one	192.04	$C_9H_8N_2OS$	fatty acid

12	10.42	0.47	Butanedioic acid, 2TMS derivative	262.11	$C_{10}H_{22}O_4Si_2$	fatty acid
13	10.64	0.17	2-Fluoro-5-(trifluoromethyl) benzaldehyde	192.02	$C_8H_4F_4O$	phenyl propanoid
14	10.76	0.23	Dimethylglyoxime, 2TBDMS derivative	344.23	$C_{16}H_{36}N_2O_2Si_2$	fatty acid
15	10.88	0.08	Borneol, pentamethyldisilanyl ether	284.20	$C_{15}H_{32}OSi_2$	fatty acid
16	11.14	0.17	Prenol, TMS derivative	158.11	$C_8H_{18}OSi$	fatty acid
17	11.34	0.35	meso-Erythritol, 4TMS derivative	410.22	$C_{16}H_{42}O_4Si_4$	fatty acid
18	11.51	0.11	D-(-)-Erythrose, tris(trimethylsilyl) ether, ethyloxime (isomer 1)	379.20	$C_{15}H_{37}NO_4Si_3$	fatty acid
19	11.61	0.13	2-Hydroxyoctanoic acid, TMS derivative	232.15	$C_{11}H_{24}O_3Si$	fatty acid
20	11.69	0.13	L-Threonic acid, tris(trimethylsilyl) ether, trimethylsilyl ester	424.20	$C_{16}H_{40}O_5Si_4$	fatty acid
21	11.89	0.49	Benzeneacetamide, TMS derivative	207.11	$C_{11}H_{17}NOSi$	amine
22	12.18	0.21	(2-Ethoxyethoxy) acetic acid, TBDMS derivative	262.16	$C_{12}H_{26}O_4Si$	fatty acid
23	12.57	0.52	2,3,4-Trimethyl-3-hydroxyglutaric acid, O, O', O'-tris9trimethylsilyl)-	406.20	$C_{17}H_{38}O_5Si_3$	fatty acid
24	12.76	0.03	2-Pentamethyldisilanyloxypentane	218.15	$C_{10}H_{26}OSi_2$	fatty acid
25	12.84	0.06	Isoborneol, pentamethyldisilanyl ether	284.20	$C_{15}H_{32}OSi_2$	fatty acid
26	12.91	0.12	D-Allofuranose, pentakis(trimethylsilyl) ether	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
27	13.00	0.27	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl) oxy] propyl ester	460.17	$C_{16}H_{42}NO_6PSi_3$	fatty acid ester
28	13.22	0.37	D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 2)	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
29	13.28	0.16	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2)	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
30	13.38	2.32	Neophytadiene	278.30	$C_{20}H_{38}$	sesquiterpens hydrocarbon
31	13.48	1.00	Myristic acid, TMS derivative	300.25	$C_{17}H_{36}O_2Si$	fatty acid

32	13.75	0.88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.31	$C_{20}H_{40}O$	fatty acid
33	13.95	0.19	alpha-D-Allopyranose, 5TMS derivative	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
34	14.06	0.43	beta-D-(+)-Talopyranose, 5TMS derivative	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
35	14.42	0.53	Pentadecanoic acid, TMS derivative	314.26	$C_{18}H_{38}O_2Si$	fatty acid
6	14.86	0.12	beta-D-Glucopyranose, 5TMS derivative	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
37	14.96	8.62	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	316.24	$C_{21}H_{32}O_2$	fatty acid ester
38	15.31	11.33	Palmitic Acid, TMS derivative	328.28	$C_{19}H_{40}O_2Si$	fatty acid
39	16.16	0.23	Palmitelaidic acid, TMS derivative	326.26	$C_{19}H_{38}O_2Si$	fatty acid
40	16.32	0.44	1-Octadecanol, TBDMS derivative	384.38	$C_{24}H_{52}OSi$	fatty acid
41	16.52	5.30	Phytol, TMS derivative	368.35	$C_{23}H_{48}OSi$	fatty acid
42	17.10	23.93	alpha-Linolenic acid, TMS derivative	350.264	$C_{21}H_{38}O_2Si$	fatty acid
43	17.63	0.38	Petroselinic acid, TMS derivative	354.30	$C_{21}H_{42}O_2Si$	fatty acid
44	17.96	0.04	Androstan-3-ol, (3beta.,5alpha.)-, TMS derivative	348.29	$C_{22}H_{40}OSi$	fatty acid
45	18.07	1.09	Glyceryl-glycoside TMS ether	686.34	$C_{27}H_{66}O_8Si_6$	fatty acid
46	18.19	0.25	5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester	414.30	$C_{26}H_{42}O_2Si$	fatty acid
47	18.28	0.35	Molybdenum, tricarbonyl[(1,2,3,4,5, 6-.eta.)-1,4-dimethylbenzene]-	287.97	$C_{11}H_{10}MoO_3$	phenyl propanoids
48	18.38	1.94	Arachidonic acid	304.24	$C_{20}H_{32}O_2$	fatty acid
49	18.55	0.91	Eicosapentaenoic Acid, TMS derivative	374.26	$C_{23}H_{38}O_2Si$	fatty acid
50	18.63	1.16	5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative	378.30	$C_{23}H_{42}O_2Si$	fatty acid
51	19.57	0.48	3,7-dioxa-2,8-disilanonane, 5-decyl-2,2,8,8-tetramethyl-	360.29	$C_{19}H_{44}O_2Si_2$	fatty acid
52	19.85	2.17	2-Butene-1,4-diol, (E)-, 2TMS derivative	232.13	$C_{10}H_{24}O_2Si_2$	fatty acid
53	19.95	2.07	Diisooctyl phthalate	390.28	$C_{24}H_{38}O_4$	fatty acid
54	20.13	2.69	1-Monopalmitin, 2TMS derivative	474.36	$C_{25}H_{54}O_4Si_2$	fatty acid
55	20.45	0.54	Behenic acid, TMS derivative	412.37	$C_{25}H_{52}O_2Si$	fatty acid
59	20.73	2.18	Sucrose, 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
57	20.81	0.24	Maltose, octakis(trimethylsilyl)	947.46	$C_{36}H_{86}O_{11}Si$	fatty acid

			ether, methyloxime (isomer 2)		$_8$	
58	21.04	0.07	alpha-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	331.16	$C_{13}H_{26}BNO_6Si$	fatty acid
59	21.12	0.07	2-Oxovaleric acid, TBDMS derivative	230.13	$C_{11}H_{22}O_3Si$	fatty acid
60	21.18	0.28	(Methoxymethyl)trimethylsilane	118.08	$C_5H_{14}OSi$	fatty acid
61	21.29	0.28	2,2,7,7-tetramethyl-4-(((5-((trimethylsilyloxy)methyl)-1,4-dioxan-2-yl)methoxy)methyl)-3,6-dioxa-2,7-disilaoctane	438.23	$C_{18}H_{42}O_6Si_3$	fatty acid
62	21.36	0.40	1-Monolinolein, 2TMS derivative	498.36	$C_{27}H_{54}O_4Si_2$	fatty acid
63	21.44	1.13	1-Monooleoylglycerol, 2TMS derivative	500.37	$C_{27}H_{56}O_4Si_2$	fatty acid
64	21.85	0.13	Squalene	410.39	$C_{30}H_{50}$	sesquiterpens hydrocarbon
65	23.07	0.04	beta-D(-)-Lyxopyranose, 4TMS derivative	438.21	$C_{17}H_{42}O_5Si_4$	fatty acid
66	23.64	0.20	Galactopyranose, 5TMS derivative	540.26	$C_{21}H_{52}O_6Si_5$	fatty acid
67	23.74	0.10	Hexadecanedioic acid, 2TMS derivative	430.29	$C_{22}H_{46}O_4Si_2$	fatty acid
68	23.87	0.09	5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester	414.30	$C_{26}H_{42}O_2Si$	fatty acid ester
69	24.02	0.24	beta-D-Lactose, (isomer 2), 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
70	24.09	0.41	Cholesterol, TMS derivative	458.39	$C_{30}H_{54}OSi$	fatty acid
71	24.38	1.26	3-alpha-Mannobiose, octakis(trimethylsilyl) ether (isomer 2)	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
72	24.50	0.05	beta-D-Lactose, (isomer 2), 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
73	24.62	0.60	Sebacic acid, 2TMS derivative	346.20	$C_{16}H_{34}O_4Si_2$	fatty acid
74	25.33	0.37	Geranylgeraniol, TBDMS derivative	404.35	$C_{26}H_{48}OSi$	fatty acid
75	25.44	0.19	Stigmast-5-ene, 3beta-(trimethylsiloxy)-, (24S)-	486.43	$C_{32}H_{58}OSi$	fatty acid
76	25.61	5.21	Fucosterol, TMS derivative	484.41	$C_{32}H_{56}OSi$	fatty acid
77	26.63	0.08	D-Lactose, (isomer 2), 8TMS derivative	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acid
78	26.86	0.48	1-Bromo-11-iodoundecane	360.00	$C_{11}H_{22}BrI$	fatty acid
79	27.07	1.08	alpha-D-Lactose, 8TMS	918.43	$C_{36}H_{86}O_{11}Si$	fatty acid

			derivative		$_8$	
80	27.30	0.62	Melibiose, octakis(trimethylsilyl)-	918.43	$C_{36}H_{86}O_{11}Si_8$	fatty acide

*(RT) retention time

2.3. Chemical composition of S. dentifolium

The chemical constituents of the ethanolic extract of *S. dentifolium* ARE represented in Table (4); the main component was Di-n-octyl phthalate with the average rate OF 19.03%, and the minor component was 1,4-Benzenediol, 2,5- bis (1,1-dimethylethyl)- with the average rate of 0.01%. The major components detected in ethanol extract were fatty acids (62.85%), amines (26.03%), phenyl propanoid (8.19%) and oxygenated monoterpens (0.01%).

Table 4. The main components identified in *S. dentifolium* ethanolic extract using GC-MS

No.	**RT* (minutes)**	**Peak area %**	**Compound name**	**Molecular weight**	**Molecular formula**	**Chemical class**
1	12.40	0.12	3,10-Dioxa-2,11-disiladodeca-5,7-diene, 2,2,11,11-tetramethyl-	258.15	$C_{12}H_{26}O_2Si_2$	fatty acid
2	13.11	5.83	Palmitic Acid, TMS derivative	328.28	$C_{19}H_{40}O_2Si$	fatty acid
3	13.61	0.11	14-Oxopentadecanoic acid, trimethylsilyl ester	328.24	$C_{18}H_{36}O_3Si$	fatty acid ester
4	13.77	0.32	Octadecane, 1-chloro-	288.26	$C_{36}H_{74}ClOP$	fatty acid
5	14.16	2.15	11-Octadecenoic acid, (E)-, TMS derivative	354.30	$C_{21}H_{42}O_2Si$	fatty acid
6	14.44	0.66	5,8,11,14-Eicosatetraynoic acid, TMS derivative	368.22	$C_{23}H_{32}O_2Si$	fatty acid
7	14.56	2.36	Eicosane	282.33	$C_{20}H_{42}$	fatty acid
8	14.82	1.70	1-Hexadecanesulfonic acid, 3,5-dichloro-2,6-dimethyl-4-pyridyl ester	479.20	$C_{23}H_{39}Cl_2NO_3S$	fatty acid
9	14.86	0.54	Dasycarpidan-1-methanol, acetate (ester)	326.20	$C_{20}H_{26}N_2O_2$	fatty acid ester
10	14.94	3.44	1-Chloroeicosane	316.29	$C_{20}H_{41}Cl$	fatty acid
11	15.07	1.59	Oxirane, tetradecyl-	240.25	$C_{16}H_{32}O$	fatty acid
12	15.22	2.94	Demecolcine	371.17	$C_{21}H_{25}NO_5$	amine
13	15.32	4.30	1-Chloroeicosane	316.29	$C_{20}H_{41}Cl$	fatty acid
14	15.44	19.03	Di-n-octyl phthalate	390.28	$C_{24}H_{38}O_4$	fatty acid
15	15.67	12.16	Bis(2-ethylhexyl) phthalate	390.28	$C_{24}H_{38}O_4$	fatty acid
16	15.89	6.57	3,4,5-Trimethoxy-beta-methyl-.beta.-nitrostyrene	253.10	$C_{12}H_{15}NO_5$	amine
17	16.07	12.04	Antra-9,10-quinone, 1-(3-hydrohy-3-phenyl-1-triazenyl)-	343.10	$C_{20}H_{13}N_3O_3$	amine

18	16.27	2.82	Pyridine, 4-[5-(2-methoxyphenyl)-[1,3,4]oxadiazol-2-yl]-	253.09	$C_{14}H_{11}N_3O_2$	amine
19	16.35	4.60	Demecolcine	371.17	$C_{21}H_{25}NO_5$	amines
20	16.51	8.54	Lupan-3-ol	428.40	$C_{30}H_{52}O$	fatty acid
21	16.73	2.64	Stannane, (1,1-dimethylethyl) triethyl-	264.09	$C_{10}H_{24}Sn$	phenyl propanoid
22	17.00	5.55	Methyl (5-hydroxy-1H-benzimidazol-2-yl) carbamate	207.06	$C_9H_9N_3O_3$	phenyl propanoid
23	18.16	0.01	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	222.16	$C_{14}H_{22}O_2$	oxygenated monoterpens

*(RT) retention time

3. Toxicological activity

The larvicidal efficacies of three ethanolic algal extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* were evaluated against the 3rd instar larvae of *C. pipiens*, as shown in Table (5). The toxicity values varied according to the concentrations of the used algal extracts. Larval mortality increased significantly with the increase in the concentration. The results showed that *C. linum* is the most effective of them all times of exposure, and its toxicity increases gradually with time. *C. linum* and *U. intestinalis* were highly potent at 96 hours of exposure with LC50 207.02 and 224.5 ppm, respectively, in comparison with 235.99 and 237.69 ppm, respectively, at 24 hours of exposure. On the other hand, the toxicity of *S. dentifolium* extract showed variations in their activity with the time of exposure. It was noted that, *S. dentifolium* showed good activity (225.43 ppm) at 24 hours post-treatment and abruptly decreased (241.79 ppm) at 48 hours post-treatment then increased gradually with the increase of exposure time.

Table 5. The toxicological activity of the different algae ethanolic extract

Algal extracts	**Hours**	**LC_{25} (F.L at 95%)***	**LC_{50} (F.L at 95%) ***	**Slope ± SE****	***X^{2***}***	***P***	
C. linum	24	207.84 (196.33-217.28)	235.99 (226.53-245.19)	12.23 ±0.91	0.68	0.88	
	48	195.36 (183.33-205.15)	224.45 (214.58-234.12)	11.19 ±0.88	1.51	0.68	
	72	189.34 (178.46-198.27)	214.82 (205.81-223.52)	12.30 ±0.93	7.71	0.05	
	96	184.49 (174.12-192.85)	207.02 (198.56-215.22)	13.48 ±1.10	1.04	0.59	
U. intestinalis	24	213.42 (203.87-220.60)	237.69 (231.14-243.99)	14.43 ±1.23	7.20	0.07	
	48	209.04 (199.68-216.03)	231.06 (224.66-236.91)	15.50 ±1.31	6.98	0.07	
	72	207.36 (198.56-213.94)	227.17 (221.08-232.63)	17.02 ±1.41	6.59	0.08	

	96	204.77 (195.61-211.54)	224.50 (218.21-230.02)	16.88 ±1.42	7.48	0.06
S. dentifolim	24	234.70 (225.27-241.49	225.43 (249.32-260.93)	18.35 ±1.65	7.54	0.06
	48	215.18 (202.62-224.17)	241.79 (233.56-248.82)	13.32 ±1.20	3.31	0.34
	72	205.39 (191.37-215.29)	232.24 (222.87-239.81)	12.64 ±1.16	4.25	0.24
	96	191.26 (172.73-203.70)	220.85 (208.36-230.05)	10.80 ±1.10	7.68	0.05

*(F.L) fiducial limits.

**(SE) standard error, and slope of inhibition regression line.

***(X^2) chi square value.

****(p) probability

4. Morphological observations

The treated larvae with LC_{50} of ethanolic extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* exhibited morphological aberrations when compared to the untreated larvae Fig. (1a). The thorax region of the treated larvae showed darkening pigmentation, abnormally swollen, elongated neck and loss of external features as shown in Fig. (1b, c, d, e & f), compared to Fig. (1a). The anal saddle, papillae and abdomen of the treated larvae were separated from the last abdominal segment (Fig. 1b). Loss of antennae, faint abdomen and ejected alimentary canal are observed in Fig. (1c). A damaged and distorted abdomen was observed with a hardened and swollen thorax and shrinking of the first abdominal segment and collapse of abdominal segments, as shown in Fig. (1d). Abdominal setae are lost, as shown in Fig. (1f).

Fig.1. (X10) Morphological aberrations of *C. pipiens* larvae under a stereomicroscope, (a) the untreated larvae showing intact parts H: head, MB: mouth brush, An: antenna, Th: thorax, B: bristles, Ab: abdomen, Sp: siphon, S: saddle, AG: anal gills ،AC: alimentary canal. (b, c & d) the larvae treated with LC_{50} of *S. dentifolium.* (e &f) The larvae treated with LC_{50} of *C. linum*, *U. intestinalis*.

5. Histological studies

Histological examination of the untreated midgut of the 3rd instar larva of *C. pipiens* showed a normal structure of midgut epithelial cells, with obvious structure and peritrophic membrane (Fig. 2a). The larva treated with LC_{50} of *C. linum*, *U. intestinalis* (Fig. 2b, c) show many histological changes in the normal structure of the cuboid cells and peritrophic membrane. The epithelial cells were damaged with many vacuoles. In addition, the epidermal layer had enlarged nuclei, separation between the cells and de-attachment with the basement membrane.

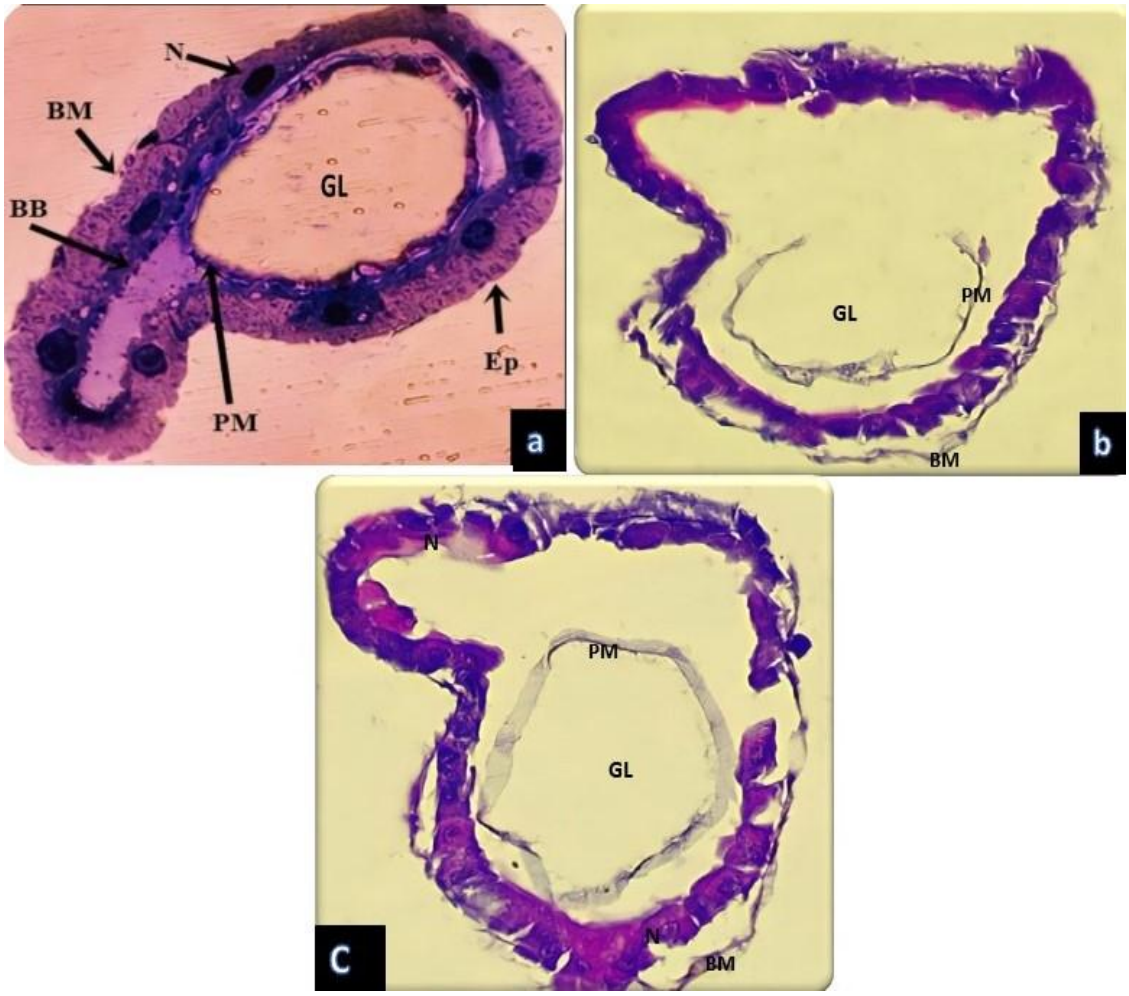

Fig. 2. (X40) Transverse section of the 3rd instar larva of *C. pipiens*; (a) The control showing intact Ep: epithelial cells, BM: basement membrane, PM: peritrophic membrane, BB: brush borders or microvilli, N: nucleus, GL: gut lumen. (b & c): treated with *C. linum* and *U. intestinalis.*

6. Biochemical studies

It was important to investigate the biochemical changes in the main body metabolites of *C. pipiens* larvae after 48 hours of treatment with $LC_{50,\ s}$ of the *C. linum*, *U. intestinalis* and *S. dentifolium*, as summarized in Table (6). *C. linum*, *U. intestinalis* and *S. dentifolium* exhibited non-significant changes in total protein amount in comparison with the untreated larvae. While, *C. linum* and *S. dentifolium* increased the amount of total carbohydrate significantly more than the untreated. In addition, total lipid was significantly enhanced by treatment with *C. linum* extract relative to untreated.

Table 6. Effect of LC_{50} values of the tested ethanolic algal extracts on total metabolites of *C. pipiens* larvae

Treatment	Total protein (mg/g. b. wt.) Mean ± SE*	Total carbohydrate (mg/g. b. wt.) Mean ± SE*	Total lipid (mg/g. b. wt.) Mean ± SE*
Untreated	24.37±0.17[a]	18.32±0.04[b]	14.36±0.18[b]
C. linum	25.19±0.11[a]	19.21±0.19[a]	15.14±0.16[a]
U.intestinalis	24.98±0.33[a]	18.62±0.38[ab]	14.90±0.26[ab]
S. dentifolium	24.91±0.34[a]	19.02±0.08[a]	14.90±0.34[ab]

The means with the same letters are not significantly different. Each value represents the mean of three replicates. *SE Standard error.

Moreover, the effects of the LC_{50} values of the tested extracts on the activity of AChE, α, ß esterases and GST in *C. pipiens* larvae 48 hours post-treatment are shown in Table (7). It was noticed that, both AChE and GST enzymes non-significantly changed in all treatments with algal extracts relative to untreated. *C. linum* extract greatly inhabited the α -esterase activity, while the *U. intestinalis* and *S. dentifolium* extracts non-significantly changed, compared to the untreated. Although, ß -esterases were remarkably inhibited to 204±2.60 and 317±7.00 (µg ß -naphthol/min/mg protein) after treatment with *C. linum* and *S. dentifolium,* respectively, *U. intestinalis* treatment non-significantly affected the enzyme amount.

Table 7. Effect of LC_{50} values of the tested ethanolic extracts on AChE, α, ß esterases and GST of *C. pipiens* larvae

Treatment	AChE±SE* (ug α-naphthol/min/mg protein)	ß -esterases ±SE* (ug α-naphthol/min/mg protein)	α -esterases ±SE* (ug α-naphthol/min/mg protein)	GST ±SE* (m.mole sub. conjugated/min/mg protein)
Untreated	517±7.00[a]	409±5.60[a]	805±7.60[a]	50±5.00[a]
C. linum	504±2.60[a]	204±2.60[c]	604±2.60[b]	41±2.60[a]
U. intestinalis	512±4.40[a]	404±3.00[a]	813±3.80[a]	47±3.00[a]
S. dentifolium	507±6.20[a]	317±7.00[b]	817±7.00[a]	45±1.00[a]

This means with the same letters are not significantly different. Each value represents the mean of three replicates. *SE Standard error.

DISCUSSION

The extensive uses of chemical insecticides have induced resistance in mosquitoes, caused contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution. These factors have created the need for

environmentally safe and target-specific agents for mosquito control purposes. Plant extracts have recently been considered environmentally safe, less hazardous to non-target biota, simple, inexpensive and can be effectively applied by using techniques more suitable for developing countries **(El-Maghraby*et al.*, 2012; Bakr *et al.*, 2018; Pateiro*et al.*, 2021)**. Previous studies proved that brown and green algae have larvicidal activity **(Ali *et al.*, 2013; Abdel Haleem *et al.*, 2022; Fouda*et al.*, 2022; Aly *et al.*, 2023)**.

Active substances extracted from algae were used as insect repellents or synergists **(Yu *et al.*, 2014)**, or effective as larvicides **(Abel Haleem *et al.*, 2022)**. Several investigators have reported a reduction in the fecundity and fertility of mosquitoes following treatment with algal extracts **(Saber *et al.*, 2018)**. In the present study, the toxicological, morphological, biochemical and histopathological impacts of three ethanolic extracts of three brown and green algae against the 3rd instar larvae of *C. pipiens* were studied. The potency of the tested extracts against mosquito larvae may be due to the different chemical components of each extract used. The chemical compositions of the three tested algal extracts revealed that all extracts were rich in fatty acids 98.7% in *C. linum* ethanolic extract, 97.81% in *U. intestinalis* extract and 62.85% in *S. dentifolium* extract; these results are in coordination with those previously reported **(Khan *et al.*, 2016; Mobin *et al.*, 2019; Malothu, 2020; Croce *et al.*, 2021).** The higher efficiency of *C. linum* ethanolic extract than the two other extracts may be due to the larger amount of fatty acids, which facilitates the penetration and increases the rate of accumulation in the larvae **(Suganya *et al.*, 2019)**. The larvicidal activity of the tested algal extracts may be ascribed to the major compounds which are mainly fatty acids, which have an insecticidal effect **(Boutjagualt *et al.*, 2022; Aly *et al.*, 2023**). The larvicide bioassay was conducted using different concentrations of the tested extracts. The results showed that the three tested extracts had a larvicidal effect against *C. pipiens*, with LC_{50} (207.02, 224.50 and 220.85 ppm) 96 hours post-treatment for *C. linum*, *U. intestinalis* and *S. dentifolium* extracts, respectively. Based on LC_{50} values, it can be concluded that, the ethanolic extract of *C. linum* was the most potent extract against 3rd instar larvae of *C. pipiens*, followed by *S. dentifolium* and *U. intestinalis*. These results agree with those of **Abdel Haleem *et al.* (2022)** who reported that, green algal extracts have insecticidal activity against 3rd instar larvae of *C. pipiens*. The toxic effect of the tested extracts on the 3rd instar larvae of *C. pipiens* was significantly increased with the increase of the concentration of the tested extract and exposure time. The morphological observations of the present study revealed that the thorax and abdominal regions were the common sites of aberrations; the aberrations in the morphology of the treated larvae induced by the three tested extracts included the deformation of the neck region between head and thorax, darkening and deformation of anal papillae. Similarly, the mosquito larvae treated with seaweed extract were observed to exhibit the same manner of aberrations as reported by **Yu *et al*. (2015)**. The deleterious effect on anal papillae interrupts the ion regulation of larvae and further causes the imbalance of homeostasis. Furthermore, the deformation of

the larval anal segment observed in the present report is suggested to cause destruction to the hydrophobic surface of the anal segment, causing the entrance of water to the tracheal trunk, which harms the respiration system of the larvae and finally contributes to the death of larvae **(Bianco *et al*., 2013; Bawin *et al*., 2016; Salvador-Neto *et al*., 2016)**. The alterations after treatment of the third larval instar of *C. pipiens* with LC_{50} of the ethanolic extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* included changes in the normal structure of midgut epithelia and the peritrophic membrane as it became unequal in thickness, the epidermal layer appeared with enlarged nuclei, and the vacuolization and masses of cellular material appeared in the lumen in all treatments **(Farag *et al.*, 2023). Yu *et al.* (2015**) studied the toxicity of seaweed extracts against *Ae. aegypti* larvae and reported various damage in the midgut epithelium of the exposed larvae.

Biochemical studies were carried out to reveal the effect of LC_{50} of the ethanolic extracts of *C. linum*, *U. intestinalis*, and *S. dentifolium* on *C. pipiens* 3rd instar larvae. The reduction in protein, lipid and carbohydrate levels was observed in the treated larvae. This reduction may be attributed to stress resulting from the application of the tested extracts or may be due to protein, lipid and carbohydrate-binding with foreign compounds such as the tested extracts **(Hamadah *et al.*, 2020; Farag *et al.*, 2023**). Similar findings were reported in the study of **Aly *et al.*, (2023)** who noticed that, protein, lipid and carbohydrate levels in *C. pipiens* larvae treated with methanolic algal extracts were reduced. Detoxification enzymes in insects are demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions **(Li & Liu, 2007)**. The detoxifying enzymes react against insecticides or compounds exhibiting insecticidal activities. They include general esterases, glutathione-S- transferase and phosphatases **(Zibaee *et al.*, 2011)**. Acetylcholinesterase is an esterase that terminates nerve impulses by breaking down the neurotransmitter acetylcholine **(Lenfant *et al.*, 2013)**. It is an important detoxifying enzyme that hydrolyzes the ester bond in synthetic chemicals. Additionally, α and ß esterases are enzymes showing the strongest reaction to environmental stimulation. Insects can use AChE to reduce the sensitivity of the target site of pesticides. When the activity of AChE is inhibited, it may directly cause insect paralysis and death **(Hemingway & Karunatne, 1998; El Hadidy *et al.*, 2022)**. In the present study, tested enzymes were inhibited in treated *C. pipiens* larvae, compared to the untreated. This finding coincides with that of **Abdel Haleem *et al.* (2022)** who found that, algal extracts inhibited acetylcholine esterase in *C. pipiens* larvae.

CONCLUSION

From the current study, based on LC_{50} values, the ethanolic extract of *C. linum* was the most promising as it showed the highest toxicity against *C. pipiens* larvae. This fact was indicated by main total metabolites, acetylcholinesterase, α, ß esterase and GST as enzymes activity monitored treatment changes. The chemical constituents of the three

tested extracts were analyzed by GC/MS technique. The GC chromatogram of *C. linum* extract showed eighty-two compounds. The main components were fatty acids. It can be concluded that, ethanolic algal extracts of *C. linum*, *U. intestinalis* and *S. dentifolium* showed a strong effect against *C. pipiens* larvae with obvious alterations in morphological and histological parameters. Thus, these extracts can contribute to integrated management programs.

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Disclosure statement
No potential conflict of interest was reported by the authors.

Ethical statement
All experiments in this research were approved by the Ethics Committee of the Faculty of Science, Ain Shams University, Cairo, Egypt (Approval code: ASU-SCI/ENTO/2023/6/1).

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