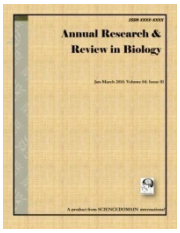


Annual Research & Review in Biology
4(13): 2252-2262, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Insecticidal and Fungicidal Activity of *Ulva lactuca Linnaeus (Chlorophyta)* Extracts and their Fractions

Moustafa A. Abbassy[1*], Mamdouh A. Marzouk[1], Entsar I. Rabea[1], and Amany D. Abd-Elnabi[1]

[1]Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author MAA designed the study, wrote the protocol, managed the literature and wrote the final draft of the manuscript. Authors MAM and EIR managed the analyses of the study and performed the statistical analysis. Author ADAE performed the laboratory work and collect the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 14th February 2014
Accepted 15th March 2014
Published 31st March 2014

ABSTRACT

The insecticidal and fungicidal activities of acetone, chloroform, ethanol, methanol and petroleum ether extracts of *Ulva lactuca* was tested against mosquito larvae, *Culex pipiens* and cotton leafworm, *Spodoptera littoralis* and three phytopathogenic fungi: *Aspergillius niger*, *Penicillium digitatum* and *Rhizoctonia solani*. The results indicated that the acetone extract was the most potent extract against *C. pipiens* however ethanol and chloroform extracts were most active as larvicides against *S. littoralis*. Methanol and ethanol extracts showed the highest pupation inhibition (%) of cotton leafworm, however, petroleum ether and methanol extracts were the most potent inhibitors for adult emergence and larval growth. Results of the antifungal activity indicated that methanol extract was the most potent against *A. niger*, *P. digitatum* and *R. solani*. The most potent methanolic extract was fractionated over silica gel column chromatography. All of the resulted fractions were tested for their antifungal activity. Fraction 7 (methanol 100%), exhibited more antifungal activity against all of the tested fungi.

**Corresponding author: Email: maabbassy@yahoo.com;*

Keywords: Ulva lactuca; Insecticidal activity; fungicidal activity; column chromatography; methanolic extract.

1. INTRODUCTION

Synthetic pesticides are widely used in the control of plant pests and disease. However, these chemicals may cause toxic residues in treated products. Also, they can cause environmental pollution owing to their low biodegradation [1]. In addition, the risk of developing the resistance by pests, microorganisms, weeds and high cost-benefit ratio are other disadvantages of synthetic pesticides application [2]. Because of these recent reports about the deleterious effects of synthetic pesticide, there is a demand for the development of new, safe, biodegradable alternatives. The marine algae are the renewable living resources which are rich source of structurally important novel and biologically active metabolites [3]. They have been shown bactericidal [4,5], fungicidal [6] and insecticidal activity [7,8]. Furthermore, seaweed extracts offer a novel approach to pest management [6,7,9]. *Ulva lactuca* is a common species of a marine macroalgae found in green tides, which grows abundantly in the coastal waters of Egypt [10]. This alga has been reported to posses antioxidant and antibacterial properties [11]. A wide range of results of *In vitro* anti-fungal activities of extracts of green algae and diatoms have also been reported [12].

In this work, the pesticidal activities of different extracts of *Ulva lactuca* on mosquito larvae of *Culex pipiens* and cotton leafworm , *Spodoptera littoralis* and three phytopathogenic fungi: *Aspergillius niger*, *Penicillium digitatum* and *Rhizoctonia solani* were investigated.

2. MATERIALS AND METHODS

2.1 Test Organisms

A susceptible cotton leafworm, *Spodoptera littoralis* (Boisd) and mosquitoes Larvae, *Culex pipiens* L. (Diptera: Culicidae) were obtained from Department of Economic Entomology, Faculty of Agriculture, Alexandria University. Three fungi, *Aspergillius niger* (Family: Trichocomaceae, class: Eurotiomycete), *Penicillium digitatum* (family: Trichocomaceae, class: Ascomycetes) and *Rhizoctonia solani* (Family: Ceratobasidiaceae, class: Agaricomycetes) were obtained from Department of Plant Pathology, Faculty of Agriculture, Damanhour University.

2.2 Collection, Extraction and Preparation of Seaweed *Ulva lactuca* Extracts

The alga. *Ulva lactuca* (Family: Chlorophyta) were collected by hand picking method from Sedi Gaber beach, Alexandria, Egypt during low tide. Taxonomic classification of the algal species was made according to the system developed by Fott, [13]. References used for the identification of the alga specie was Chapman and Gellenbeck [14]; Bold and Wynne, [15]. The collected seaweed was washed with tap water, distilled water to remove salt, sand and epiphytes and then air dried under shade for 2 weeks. The dried algal material was partially powered by using domestic blender. For extraction, 100 g of powdered algal material was extracted by soxhlet apparatus by using acetone, chloroform, ethanol, methanol and petroleum ether. The solvents were evaporated from the crude extracts under reduced pressure by rotary evaporator to yield 3.15, 4.14, 3.40, 5.10 and 3.20 g, respectively [16].

The extracts were concentrated in air tight glass vials and stored in the refrigerator for further use.

2.3 Column Chromatographic Fractionation of Crude Extracts of *U. lactuca*

Partial purification of crude extract of *U. lactuca* was carried out according to the method of Wright, [17]. The methanol extract was fractionated using glass chromatographic columns (1.2/30 cm) packed in sequence with 1g of sodium sulfate anhydrous, 8 g normal phase silica gel (200-400 mesh) and 1 g sodium sulfate. The column was tapped to achieve appropriate setting. The column was pre-washed with 25 ml of hexane and then the extract was transferred to the column. Column chromatography employing a step gradient solvent system from low to high polarity viz 1, n-hexane 100%, 2, n-hexane: Ethyl acetate 50:50%, 3, n-hexane: ethyl acetate 25:75%, 4, ethyl acetate 100% ,5, ethyl acetate: methanol 50:50%, 6, ethyl acetate: methanol 25:75%, 7, methanol 100%. These fractions were tested for their antifungal effects.

2.4 GC-MS Analysis

GC-MS analysis of the methanol 100% fraction was carried out by using Agilent 6890 gas chromatography equipped with an Agilent mass spectrometric detector, with a direct capillary column (30*0.32 mm*0.25 um thickness). Each sample was injected under the following conditions. Helium used as carrier gas at approximately 1 ml/min, pulsed split less mode. The solvent delay was 3 min and the injection size was operated in electron impact ionization mode ioning energy of 70 e.v. scanning from m/z 50/500. The ion source temperature was 230℃. The electron multiplier voltage (EM Voltage) was maintained 1050 V autotune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was start at 60℃ (3 min) then elevated to 280℃. Wiley and nist 05 mass spectral databases was used in the identification of the separated peaks.

2.5 Insecticidal Bioassay of the Extracts against *C. Pipiens*

Larvicidal activity of alga extracts was investigated against *C. pipiens* according to WHO [18] method and calculated as lethal concentration of each extractives required to produce 50% mortality (LC_{50}) for larvae in each treatment. Batches of 25 early 3rd instar larvae of *C. pipiens* in 100 ml of each concentration (0.5, 1, 2, 5 and 10 mg/ml) and distilled water (control) contained in plastic cups. Three replicates were used for each concentration. Swimming activity were observed and recorded after 24h of treatment.

2.6 Insecticidal Activity and Growth-Inhibitory Assay against *S. littoralis*

In a standardized screening toxicity test, freshly molted 3rd instar larvae of *S. littoralis* were selected. The tested extracts at concentrations of 1, 5, 10 and 25 mg/ml at treated artificial diet was divided and placed in Petri dishes. Three replicates for each treatment and control were used. Ten larvae were introduced onto each replicate. The experiments were kept in a growth chamber, at 23±2℃, 70±5% RH and a 16:8h (L: D) photoperiod. After 6 days of continuous feeding on treated diet, mortality was scored and compared with control that was exposed to a diet treated with solvent only [19]. For growth-inhibitory bioassay, the larvae were weighed before the experiment and daily weight measurements were made until the end of the experiment based on larval weight gain after 6 days of feeding on the treated diet. The growth inhibition calculated from this equation:

$$\text{Growth inhibition } (\%) = \left(\frac{\text{CL} - \text{TL}}{\text{CL}}\right) \text{x } 100$$

Where C_L is the larval weight gain in the control and T_L is the larval weight gain in the treatment.

Effects of *U. lactuca* extracts on pupal and adult development were identified by the oral feeding method. Freshly molted third -instar larvae left to feed on the diet incorporated with crude extracts at concentrations of 1, 5, 10 and 25 mg/ml until adult emergence. The pupal weight (mg), pupal mortality (%) and adult emergence (%) were recorded [19].

2.7 Fungicidal Bioassay of the Algal Extracts against *A. niger, P. digitatum and R. solani*

The mycelia radial growth inhibition technique was used to determine the antifungal activity of algae extracts and fractions of methanolic extract against *A. niger, P. digitatum* and *R. solani* as described by El-Ghaouth et al., [20]. A series of concentrations of 0.5, 1, 2, 5 and 10 mg/ml contained in PDA medium were seeded in sterile culture plates and infected with 6-mm agar plugs taken from the margin of a 7 days old culture. Three replicates were used for each fungus per concentration tested. The plates were incubated in the dark at 26 ± 2℃. When the mycelium reached to the edges, of the control plate, the radial colony growth were determined and the effective concentration causing a 50% inhibition of mycelial growth (EC_{50}) with corresponding 95% confidence limits (95% CL) was estimated by a probit analysis [21].

2.8 Statistical Analysis

Statistical analysis was done using SPSS software. The log dose–response curves allowed determination of the concentration causing a 50% of mortality (LC_{50}). The 95% CL and standard error for the range of LC_{50} values for the compound for assays on mortality were determined by least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration. Statistical significance data was determined with one-way analysis of variance (ANOVA) by comparing means using SNK method at the probability of 0.05 [22].

3. RESULTS

3.1 The larvicidal Activity of Different Crude Extracts of *U. lactuca against C. pipens*

The data of the *In vitro* larvicidal activity of different algal extracts against mosquito larvae *C. pipens* are summarized in Table 1. All tested alga extracts had moderated toxicity compared to the control. The results revealed that the acetone extract was the most potent as larvicide (LC_{50}=5.46 mg/ml), followed by ethanol extract (LC_{50}=12.82mg/ml). While, there is no significant difference between petroleum ether and methanol extracts (LC_{50}=27.55 and 27.35 mg/ml, respectively). The lowest active one was the chloroform extract.

Table 1. Larvicidal activity of different extracts of *U. lactuca* against *C. pipiens* larvae

Alga extracts	LC_{50}[a] (mg/ml)	95% Confidence limits		Slope[b]±SE	Intercept[c]±SE	(X^2)[d]	P[e]
		Lower	Upper				
Acetone	5.46	4.53	6.83	1.71±0.56	-6.39±0.56	3.39	0.33
Chloroform	67.99	27.02	518.45	0.86±0.17	-4.13±0.60	4.05	0.25
Ethanol	12.82	10.32	17.82	2.44±1.25	-10.1±1.25	4.55	0.20
Methanol	27.35	15.89	72.22	0.92±0.65	-5.12±0.65	4.02	0.25
Petroleum ether	27.55	16.85	66.74	1.32±0.84	-6.4 ±0.84	1.70	0.63

[a] LC_{50} concentration causing 50% death for the larvae [b] Slope of the concentration-mortality regression line ± standard error [c] Intercept of the regression line ± SE [d] Chi square value , [e] probability value

3.2 The Insecticidal Activity of Different Crude Extracts of *U. lactuca against S. littoralis larvae*

The results of the effects of different extracts of *U. lactuca* on larval mortality, pupation, adult emergence and growth of *S. littoralis* larvae fed on artificial diet containing 1, 5, 10 and 25 mg/ml from each extract were recorded in Table 2. Ethanol extract recorded the highest percentage of larval mortality (36.66%) followed in the descending order by chloroform with 23.33% at 25 mg/ml. On the other hand acetone extract was non-toxic to the larvae and most of the tested extracts had low effect on pupation. The highest percentages of inhibition in pupation were recorded with methanol and ethanol extracts (27.78 and 23.34%, respectively) at 25 mg/ml. On the other hand, petroleum ether and methanol extracts of *U. lactuca* caused the highest percentages of inhibition of adult emergence (37.34 and 36.11%, respectively) at 25 mg/ml as compared to the control. The insect growth inhibitory effect of *U. lactuca* extracts against *S. littoralis* is presented in Table 3. The results demonstrated that methanol extract caused the highest percentages of larval growth inhibition (36.0%) at different concentrations followed by petroleum ether extract (33.3%). However, the ethanol extract didn't achieve any growth inhibitory effect.

3.3 Fungicidal Activity of Crude Extracts of *U. lactuca against A. niger, P. digitatum and R. solani*

Result in Table 4 show the fungicidal activity of algal extracts against *A. niger*, *P. digitatum* and *R. solani*. The data indicated clearly the ethanol extract didn't cause any fungicidal activity against both *A. niger* and *R. solani* and weak fungicidal activity against *P. digitatum* with EC_{50} values of 41.39 mg/ml. On the other hand, the methanol extract was the most potent extract against *A. niger*, *P. digitatum* and *R. solani* with EC_{50} values of 2.92, 3.38 and 3.38 mg/ml, respectively. However, this extract was lower in its potency as fungicide than the synthetic fungicide carbendazim.

3.4 Chromatographic Fractionation of Crude Methanolic Extract *of U. lactuca* and Evaluations of the Resulted Fractions against the Tested Fungi

Crude methanolic extract of *U. lactuca* was fractionated on column chromatography as described previously. Obtained fractions exhibited broad spectral fungicidal activity against *A. niger, P. digitatum* and *R. solani* as shown in Table 5. Among the isolated fractions, the

methanol fraction was the most potent as fungicide with EC_{50} values of 0.21, 0.32 and 0.49 mg/ml followed by ethyl acetate: methanol 1:3 with EC_{50}=0.31, 0.40 and 0.81 mg/ml against *A. niger, P. digitatum* and *R. solani,* respectively. It can be concluded that the methanol fraction was the most active fraction against all of the tested fungi. Considering the sensitivity of the microorganisms, it was noticed that *A. niger* was more sensitive to these fractions than *P. digitatum* and *R. solani***.** However, results revealed that all of the tested fractions showed less fungicidal effects than the reference fungicide, carbendazim.

Table 2. Effect of different extracts of *U. lactuca* against *S. littorals* by feeding on artificial diet on larval mortality, pupation and adult emergence

Conc. (mg/ml)	Larval mortality (%)±SE	Pupation (%)±SE	Adult emergence (%)±SE
Acetone			
0	00.0^{c}±00.0	100.0^{b}±00.0	86.29^{b}±3.16
1	00.0^{c}±00.0	96.66^{ab}±3.33	82.96^{ab}±2.96
5	00.0^{c}±00.0	93.33^{ab}±6.66	82.59^{ab}±6.29
10	00.0^{c}±00.0	93.33^{ab}±6.66	75.20^{ab}±2.60
25	00.0^{c}±00.0	90.00^{ab}±5.77	70.38^{ab}±3.71
Ethanol			
1	10.0^{bc}±5.77	100.0^{b}±00.0	71.40^{ab}±00.0
5	16.66^{bc}±3.33	100.0^{b}±00.0	69.82^{ab}±1.57
10	16.66^{bc}±3.33	100.0^{b}±00.0	65.86^{ab}±8.72
25	36.66^{a}±8.81	76.66^{ab}±14.52	63.89^{ab}±7.34
Chloroform			
1	3.33^{c}±3.33	96.66^{ab}±3.33	77.80^{ab}±00.0
5	13.33^{bc}±3.33	92.13^{ab}±3.95	75.0^{ab}±00.0
10	16.66^{bc}±6.66	88.86^{ab}±6.43	72.60^{ab}±1.20
25	23.33^{b}±3.33	82.82^{ab}±3.95	71.66^{ab}±6.0
Methanol			
1	13.33^{bc}±3.33	91.83^{ab}±4.08	82.73^{ab}±3.90
5	16.66^{bc}±3.33	80.55^{ab}±6.94	80.94^{ab}±9.52
10	16.66^{bc}±3.33	76.39^{ab}±6.05	74.58^{ab}±8.72
25	16.66^{bc}±3.33	72.22^{a}±2.77	66.67^{ab}±00.0
Petroleum ether			
1	00.0^{c}±00.0	92.59^{ab}±3.70	72.22^{ab}±2.77
5	3.33^{c}±3.33	92.59^{ab}±3.70	64.35^{ab}±5.69
10	3.33^{c}±3.33	92.59^{ab}±3.70	62.50^{a}±00.0
25	3.33^{c}±3.33	92.59^{ab}±3.70	62.50^{a}±00.0

Data are means ±SE. of three replicates. Values followed by the same letter (s) within a column are not significantly different at P≤0.05, by Student-Newman-Keuls (SNK) Test

Table 3. Effect of different extracts of *U. lactuca* on the growth of 3rd instar larvae of *S. littoralis*

Conc. (mg/ml)	Growth inhibition (%)±SE				
	Acetone	Ethanol	Chloroform	Methanol	Petroleum ether
1	1.66^{cd}±1.6	0.0^{d}±0.0	14.50^{bcd}±3.0	32.66^{a}±6.3	24.66^{abc}±0.9
5	7.10^{cd}±2.2	0.0^{d}±0.0	15.10^{bcd}±0.4	36.20^{a}±4.9	26.66^{abc}±2.3
10	11.06^{cd}±8.4	0.0^{d}±0.0	17.56^{bcd}±1.3	36.23^{a}±5.2	29.40^{ab}±1.1
25	13.56^{cd}±1.1	0.0^{d}±0.0	17.63^{bcd}±1.7	36.30^{a}±5.3	33.30^{a}±9.5

Data are means ±SE. of three replicates. Values followed by the same letter (s) within a column are not significantly different at P≤0.05, by Student-Newman-Keuls (SNK) Test

Table 4. Fungicidal activity of different extracts of *U. lactuca* against *A. niger*, *P. digitatium* and *R. solani*

Alga extracts	EC_{50}[a] (mg/ml)	95%Confidance limits		Slope[b]±SE	Intercept[c]±SE	(X^2)[d]	P[e]
		Lower	Upper				
A. niger							
Acetone	11.41	8.33	18.07	1.34±0.16	-5.42±0.57	4.17	0.24
Chloroform	10.86	8.23	16.14	1.51±0.18	-6.12±0.63	4.40	0.22
Ethanol	-	-	-	-	-	-	-
Methanol	2.92	2.35	3.71	1.22±0.45	-4.26±0.45	0.91	0.82
Petroleum ether	7.75	5.67	12.10	0.63±0.48	-4.28±0.48	0.00	1.00
Carbendazim	0.01	0.01	0.02	2.06±0.15	-2.39±20	11.93	0.09
P. digitatium							
Acetone	12.15	10.33	16.05	3.58±2.36	14.64±2.36	0.38	0.94
Chloroform	33.41	19.34	106.51	1.66±1.22	-7.53±1.22	2.05	0.56
Ethanol	41.39	17.04	317.36	0.66±0.48	-3.06±0.48	0.00	1.00
Methanol	3.38	2.36	5.37	0.72±0.42	-2.54±0.42	0.10	0.99
Petroleum ether	18.85	13.75	39.47	2.81±2.42	12.02±2.42	0.41	0.93
Carbendazim	0.02	0.01	0.03	2.13±0.15	-2.63±0.22	13.34	0.01
R. solani							
Acetone	24.62	16.01	55.64	0.78±1.52	-9.37±1.52	6.56	0.08
Chloroform	9.63	7.26	14.31	1.38±0.16	-5.52±0.56	0.37	0.95
Ethanol	-	-	-	-	-	-	-
Methanol	3.38	2.54	4.81	0.91±0.43	-3.22±0.43	1.43	0.69
Petroleum ether	12.96	10.00	19.11	1.89±0.23	-7.78±0.87	0.54	0.91
Carbendazim	0.03	0.02	0.05	1.52±0.13	-2.23±0.20	16.50	0.002

[a]EC_{50} concentration causing 50 % mycelial growth inhibition, [b]Slope of the concentration -inhibition regression line ± standard error, [c]Intercept of the regression line ±SE, [d]Chi square value, [e]probability value

3.5 GC-MS Analysis of Methanolic Fractions of the Crude Extract of *U. lactuca*

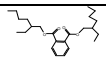
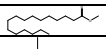
The spectra of the compounds were matched with NIST and Willey library. Their structures were identified by the (%) similarity values. They were confirmed by the study of classical fragmentation pattern, base peak and molecular ion peaks of the compounds. GC-MS analysis of methanolic fraction resulted from fractionation of crude methanolic extract of *U. lactuca* showed forty two peaks indicating the presence of forty two compounds. The major five compounds were found to be Benzene,1-ethyl 2-methyl with peak area of 1.98%, Benzene,1,2,4-trimethyl, 4.82%, Palmatic acid, 11.94%, 8-octadecanoic acid methyl ester, 3.74% and 1,2-Benzene dicarboxylic acid, bis-2-(ethylhexyl) ester, 65.42% as shown in Table 6.

Table 5. Fungicidal activity of different fractions of crude methanolic extract *U. lactuca* against *A. niger, P. digitatium and R. solani*

Fraction No.	EC_{50}[a] (mg/ml)	95 % Confidence limits		Slope[b] ±SE	intercept[c]±SE	(X^2)[d]	P[e]
		Lower	Upper				
A. niger							
1	0.59	0.44	0.88	1.03±0.13	-2.84±0.3	3.53	0.32
2	0.61	0.50	0.96	0.99±0.13	-2.76±0.3	3.39	0.35
3	0.49	0.37	0.70	1.04±0.13	-2.80±0.3	2.89	0.41
4	0.41	0.23	1.17	1.15±0.32	-3.00±0.3	7.01	0.07
5	0.47	0.26	1.75	1.14±0.33	-3.05±0.3	7.65	0.05
6	0.31	0.17	0.75	1.25±0.32	-3.10±0.3	8.44	0.04
7	0.21	0.16	0.27	1.08±0.30	-2.52±0.3	4.65	0.19
Carbendazim	0.01	0.01	0.02	2.06±0.15	-2.39±0.2	11.93	0.09
P. digitatium							
1	0.90	0.66	1.39	1.21±0.15	-3.57±0.4	1.59	0.66
2	1.03	0.72	1.81	1.05±0.14	-3.16±0.4	3.64	0.30
3	1.24	0.82	2.39	1.00±0.14	-3.11±0.4	5.03	0.17
4	0.52	0.40	0.73	1.13±0.14	-3.06±0.3	4.21	0.24
5	0.70	0.57	0.91	1.10±0.42	-4.82±0.4	0.00	1.00
6	0.40	0.31	0.55	1.05±0.13	-2.73±0.3	4.43	0.22
7	0.32	0.26	0.42	1.14±0.13	-2.86±0.3	4.09	0.25
Carbendazim	0.02	0.01	0.03	2.13±0.15	-2.63±0.2	13.34	0.01
R. solani							
1	1.64	1.14	2.92	1.24±0.49	-4.54±0.5	4.55	0.20
2	2.41	1.43	5.94	1.10±0.17	-3.72±0.4	0.05	0.99
3	3.05	1.69	8.92	1.07±0.17	-3.75±0.5	0.23	0.97
4	1.44	0.96	2.74	1.11±0.15	-3.49±0.4	5.01	0.17
5	2.11	1.25	5.25	1.00±0.38	-3.31±0.4	3.81	0.28
6	0.81	0.61	1.22	1.22±0.14	-3.56±0.4	4.33	0.23
7	0.44	0.39	0.63	1.41±0.35	-3.79±0.4	3.12	0.37
Carbendazim	0.03	0.012	0.05	1.52±0.13	-2.23±0.2	16.50	0.002

1, hexane 100%, 2, hexane : ethyl acetate 50:50%, 3, hexane : ethyl acetate 25:75%, 4, ethyl acetate 100% ,5, ethyl acetate: methanol 50:50%, 6, ethyl acetate: methanol 25:75%, 7, methanol 100% [a] EC_{50} concentration causing 50 % mycelial growth inhibition, [b] Slope of the concentration-inhibition regression line ± standard error, [c] Intercept of the regression line ± S.E, [d] Chi square value, [e] probability value

Table 6. The chemical properties of the five major compounds isolated from methanolic fraction of crude methanolic extract of *U. lactuca* using GC/MS analysis

No.	Compound	Structure	Mw[a]	Classification	Formula	Are%
1	1,2-Benzenedicarboxylic acid,bis(2-ethyl hexyl) ester		390.5	Phthalic acid	$C_{24}H_{38}O_4$	65.42
2	Palmatic acid		270.4	Fatty acid	$C_{17}H_{34}O_2$	11.94
3	Benzene,1,2,4-trimethyl		120.2	Aromatic compound	C_9H_{12}	4.82
4	8-Octadecanoic acid methyl ester		296.4	Fatty acid	$C_{19}H_{36}O_2$	3.74
5	Benzene,1-ethyl 2-methyl		120.1	Aromatic compound	C_9H_{12}	1.98

[a]Molecular weight

4. DISCUSSION

The marine algae are the renewable living resources which are rich source of structurally important novel and biologically active metabolites. A broad spectrum of algae species were screened for their effects on mosquito larvae such as *Caulerpa prolifera*, *Caulerpa serrulata*, *U. lactuca, Lobophora variegata*, *Spatoglossum asperum*, *Dictyota dichotoma*, *U. fasciata* and *Grateloupia lithophila* [23-26]. In this study, most of the different extracts of *U. lactuca* alga caused significant larvicidal effect against C*. pipiens* larvae. The acetone extract was the most potent larvicidal extract with LC_{50} value of 5.46 mg/ml. On the other hand, marine algae *U. fasciata* and *U. lactuca* have been reported to possess nymphicidal, anti-oviposetional activity, reduced fecundity, hatchability, adult longevity and relative growth rate of the red cotton bug or cotton steiner *Dysdrcus cingulatus*.

Our results revealed that ethanol and chloroform extracts of *U. lactuca* recorded the highest percentage of larval mortality of *S. littoralis* larvae (36.66 and 23.33%, respectively) at 25 mg/ml. At the same concentration, the highest inhibition (%) of pupation was obtained from methanol and ethanol extracts (27.7 and 23.4%, respectively). Also, petroleum ether and ethanol extracts were the most potent extracts as inhibitors for adult emergence (24.5 and 26.1%, respectively). In addition, methanol and petroleum ether extracts were the most potent extracts for inhibition larval growth with percentages of 36.3 and 33.3%, respectively. The insecticidal activity of a number of seaweeds has been evaluated against many insects [27].

Seaweeds considered as source of bioactive compounds and produce a great variety of secondary metabolites including antifungal substances [28,29]. The methanol extract of *U. lactuca* alga was the most potent antifungal extract against postharvest fungi *A. niger*, *P. digitatum* and soil borne fungi *R. solani* with EC_{50} values of 2.97, 3.37 and 3.38 mg/ml, respectively. However, the antifungal potency of this promising extract was lower than that of the synthetic fungicide, carbenazim. As toxicity may be enhanced by fractionating the active constituents contained in the toxic *U. lactuca* alga, attempts are made to fractionate the most potent pesticidal methanolic and acetone extracts chromatographically. Of the obtained fractions, fraction 7, the methanolic fraction, was the most potent fungicidal fraction with potency more than that the crude extract with about 14 fold against the tested fungi, *A. niger*, *P. digitatum* and *R. solani*.

GC-MS analysis of the methanolic fraction of crude methanol extractives of *U. lactuca*, revealed the percentage of 42 components. The major five components were benzene,1-ethyl-2-methyl with peak area of 1.98%, benzene,1,2,4-trimethyl (4.82%), palmatic acid (11.94%), 8-octadecanoic acid methyl ester (3.74%) and 1,2-benzene dicarboxylic acid, bis-(2-ethylhexyl) ester (65.42%). The compounds 1, 2-benzene dicarboxylic acid, Bis-(2-ethylhexyl) ester and fatty acids have been evaluated against many microbes as antimicrobial agents [30,31].

5. CONCLUSION

In conclusion, the overall results revealed that the crude methanol extract of marine alga, *U. lactuca*, caused insecticidal and fungicidal activity. GC-MS analysis of methanolic fraction of *U. lactuca* revealed the presence of many sesquiterpenes and fatty acids, which are known to have fungicidal activity. They are potential source of bioactive compounds and should be investigated for natural insecticides and fungicides.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barnard C, Padgitt M, Uri ND. “Pesticide use and its measurement”, International Pest Control. 1997;39:161-164,
2. Brent KJ, Hollomon DW. “Fungicide resistance: The risk assessment”, FRAC, Global Crop protection Federation, Brussels, Monograph. 1998;1:48,
3. N. Bhasker and K. Miyashita, “Lipid composition of padina tetratomatica (Dictyotales, Pheophyta) a brown sea weed of the west coast of India”, Indian Journal of fisheries. 2005;52:263-268,
4. Cordeiro RA, Gomes VM, Carvalho AFU, Melo VMM. “Effect of proteins from the red seaweed *Hypnea musciformis* (Wulfer) Lamouraux as the growth of humanpathogen yeasts”, Brazilian Archive of Biological Chemistry. 2006;45:2735-2739,
5. Ely R, Supriya T, Naik CG. “Antimicrobial activity of marine organisms collected off the coast of South EastIndia”, Journal of Experimental and Biological Ecology. 2004;309:121-127,
6. Rajesh S, Asha A, Kombiah P, Sahayaraj K. “Biocidal activity of algal seaweed on insect pest and fungalplant pathogen”, In: National Seminar on Harmful; 2011.
7. Sahayaraj K, Mary Y, Jeeva. “Nymphicidal andovipositional efficacy of seaweed *Sargassum tenerrimum*(J. Agardh) against *Dysdercus cingulatus* (Fab.)”; 2012.
8. Sahayaraj K, Kalidas S, “Evaluation of nymphicidaland ovicidal effect of seaweed, *Padina pavonica* (Linn.) (Phaeophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.)”, Indian Journal of Geo-Marine Science. 2011;40:(1)125-129,
9. Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi R, Panikkar MVN, “Biopotentials of seaweeds collected from southwest coast of India”, Journal of Marine Science and Technology. 2009;17:67-73,
10. Bhang YJ, Kim JH. “Patterns of interspecific interactions in the Ulvadominated intertidal community in a southern coast of Korea”, Journal of Phycology. 2000;36(s3):6-6,
11. Rouxel C, Bonnabeze E, Daniel A, Jerome M, Etienne M, Fleurence J. “Identification by SDS PAGE of green seaweeds (*Ulva* and *Enteromorpha*) used in the food industry”. Journal of applied Phycology. 2001;13:215-218,
12. Renu A, “Antibacterial activities of freshwater algae Chlorella ellipsoidea”, Journal of Basic and Applied Biology. 2010;4:(1&2)22-26.
13. Fott S. “Studies in phycology”, Academia. Prague. 1969;304.
14. Chapman DJ, Gellenbeck KW. An historical prespective of algal biotechnology. In: “Algae and cyanobacterial biotechnology” (eds: Cresswell, R.C.; Ress, T.A.V. and Shah, V.), Longman group, U.K. 1983;1-27.
15. Bold HC, Wynne MJ. Introduction to the algae: Structure and reproduction. Englewood Cliffs, New Jersey, USA. 1978;706.
16. Solomon RDJ, Kallidass S, Vimalan J. “Isolation, identification and study of antimicrobial property of a bioactive compound in an Indian medicinal plant *Acalypha indica* (Indian-nettle)”, World Journal of Microbiology and Biotechnology. 2005;21:1231-1236,
17. Wright AE. “Isolation of marine natural products. In: methods of biotechnology”, Natural product isolation, Cannell. R.P.J. (ed.). USA. Humana press inc, New Jersy. 1998;4:365-408,

18. WHO, "Instructions for determining the susceptibility of resistance mosquito larvae to insecticides", Mimeographed Document WHO/VBC/75. 1981;583.
19. Rabea EI, Badawy MEI, Rogge TM, Stevens CV, Steurbaut W, Höfte M, Smagghe G, "Enhancement of fungicidal and insecticidal activity by reductive alkylation of chitosan", Pest Management Science. 2006;62:890-897,
20. El Ghaouth A, Arul J, Grenier J, Asselin A. "Antifungal activity of chitosan on two post harvest pathogens of strawberry fruits", Phytopathology. vol. 1992;82:398-402,
21. Finney DJ. "Probit Analysis", 3rd Ed. Cambridge University Press, Cambridge. 1971;318.
22. Steel RGD, Torrie JH. "Principles and procedures of statics", Abiometrical approach 2nd Ed. MC - Graw Hill Kagakusha. Ltd. 1980;633.
23. Elbanna SM, Hegazi MM. "Screening of some seaweed species from South Sinai, Red Sea as potential bioinsecticides against mosquito larvae; *Culex pipiens*", Egyptian Journal of biological Science. 2011;4:21-30,
24. Manilal A, Thajuddin N, Selvin J, Idhayadhulla A, Kumar RS, Sujith S. "*In vitro* Mosquito Larvicidal Activity of Marine Algae Against the Human Vectors, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae)", International Journal of Zoological Research. 2011;7:272-278,
25. Poonguzhali TV, Nisha JL. "Larvicidal activity of two seaweeds, *Ulva fasciata* and *Grateloupia lithophila* against mosquito vector *Culex quinquefasciatus*", International Journal of Current Science. 2012;163-168,
26. Ravikumar S, Ali MS, Beula JM. "Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti"*, Asian Pacific Journal of Tropical Biomed. 2011;143-146,
27. Asha A, Rathi JM, Rajaand DP, Sahayaraj K. "Biocidal activity of two marine green algal extracts against third instar nymph of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)," Journal of Biopesticides. 2012;5:129-134.
28. Aruna P, Mansuya P, Sridhar S, Kumar JS, Sarangam B. "pharmacognostical and antifungal activity of selected seaweeds from gulf of mannar region", Science Technology. 2010;2:115-119,
29. Biondi NR, Piccardi M, Margheri C, Rodolfi L, Smith GD, Tredici MR. "Evaluation Nostoc Strain ATCC 53789 as a Potential Source of natural Pesticides", American Society of Microbiology. 2004;70:3313-3320.
30. Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y. "Production of bioactive metabolites by *Nocardia levis* MK-VL_113", Journal of Applied Microbiology. 2009;49:484-490,
31. Smaoui S, Mathieu F, Elleuch L, Coppel Y, Merlina G, Karray-Rebai I, Mellouli L. "Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp.TN256 strain", World Journal of Microbiology and Biotechnology. 2011;28:793-804.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=463&id=32&aid=4202