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GC-MS analysis of bioactive compounds in alkaloid extract of leaves of *Catha edulis* and their efficacy against eggs and different larval instrae of *Culex pipienes* (Diptera :Culicidae)

Zainab Abd AL-Radaa and ESSam Kadoom Abood

Dept of Biology, Universty of Babylon, Collage of Science/ Iraq

Abstract : The bioactive alkaloid components of *Catha edulis* leaves have been evaluated using GC-MS and their activity evaluated against the eggs and different larval instrae of *Culex pipienes*, GC/MS analysis of alkaloid extract of *C.edulis* leaves revealed the existence of (33) different compounds which caused mortality was 90% against the eggs of *C.pipienes* and mortality 90% in 20 mg/ml con. against four larnvalinstrae this effect make alkaloid extract is alternative natural chemical against *Culex* mosquitous.

Keywords: bioactive compounds, Catha edulis, larval instrae, Culex pipienes, Culicidae

Introduction:

Culex pipienes is returns to order Diptera: Family Culicidae, This species is the most widespread in the world ¹ and persons who live in middle and south areas were exposed to dangerous infection with elephantiasis transmitted by culex mosquitous because of the abundance in these areas , chemical control of mosquitoes is caused to decimate aquatic microbes in addition to the health problems arising from the control so focused research on plant of what is produced from the effective compounds have defensive properties against insects ara feeding on the plants², The alkaloid compounds are one of secondary metabolic compounds produced from plant which are alkaloid crystals containing one or more atoms of carbon, nitrogen and hydrogen, but that containing oxygen atom must be liquid with bitter taste astringent³. Alkaloid compounds are widespread in Angiosperm especially in fruits, leaves and roots⁴ and have many medical uses such as the following compounds botanical origin :scopolamine, hyoscyamine and atropine ⁵that effect on neural vectors through inhibited to cholinesterase enzyme⁶. This study is aims to identification the effect of alkaloid compound of khat leaves on egg and different larval stages of culex pipienes and alkaloid compounds were analysis with used GC-MS.

Experimental section:

Plant samples

Leaves of khat plant were collected, and then were washed, cleaned and dried in dark condition at room temperature, then used electric grinder to ground dry leaves and plastic containers to kept it.

Insect culture

The culture of *Culex pipienes* were maintained in college of science, Babylon University at entomology lab with temperature of $28C^{\circ}$ and humidity of $75C^{\circ}$ for 14 hour photoperiod, the egg and larvae collected from one sewage places in the province of Babylon, then took samples of the larvae and work of her histological sections and diagnosed as *culex pipienes*.

Crude alkaloid compounds extract preparation

The alkaloid extract was prepared according to ⁷.

Bioassay of alkaloid compounds extract :

To estimate the biological activity of alkaloid compounds extract was attended the stock solution by took 2gms from dry matter and dissolving in 20 ml of ethanol 96% and completed size to 100 ml or distilled water bringing to the stock solution 20 mg/ml and from this solution attended con. (2.5,5.10,20). Either control treatment was 3 ml ethanol added to 97 ml of distilled water . the experimental tubes were 3 replications each containing 100 ml from each con. And explained 10 larvae were mortality ratio calculated after 24 h., and corrected ratios according to the equation and subjected all experiments for statistical analysis by using Least signification Differences (L.S.D.) using Abbotts formula.⁸

GC-MS method

GC-MS analysis attended according to⁹.

Results and Discussion

The alkaloid compounds extract of alkhat leaves were recorded high mortality (90%) against eggs of *Cx.pipienes* in all concentration compared with control treatment which recorded (7.68%) as in table(1). This mortality may be return to effect of alkaloid compound on outer shell activity of egg of *Cx.pipienes* or may return to hardened of egg crust and eventually led to fail egg hautch¹⁰, also this compounds effect on muscle tissue of fetus and thus prevent the embryo from hatching ¹¹ and might entered these compounds to interfere egg and embryo development process⁵.also note from the same table the mortality was 90%) at 20 mg/ml con. against four larval instra compared with control treatment which recorded (0,0,1.9,5.42)%

Table.1: Effect of alkaloid compounds of Khat leaves on mortality of egg and different larval instraof Culexpipienes

Larval instra /extract con.	Egg	1st larval instra	2nd larval Instra	3th larval instra	4thLarval instra
0	6.78	5.42	1.9	0	0
2.5	90	83.85	83.48	83.48	71.19
5	90	83.85	83.85	83.85	83.85
10	90	90	90	83.85	77.70
20	90	90	90	90	90
L.S.D.			7.723		

The reason of mortality of larval stage by alkaloid extract was returned to nerve tissue effected of larvae ¹² also the result of present study showed that the first and second larval instra more sensitive than the rest of larval instra as the sensitivity of larva reduce with age and the reason for that is due to the cuticle layer enclosed body wall , in addition to the consumption of the first and second larval stage to the large quantities of food for the purpose of growth and this presents the gut of larvae to poisoning ¹³.GC-MS analysis of alkaloid extract of alkhat leaves were demonstrated(33) compounds, as follow in table 2 which present name of compound ,molecular weight, molecular formula and retention time .

Peak	R. time	Alkaloid compound	Molecular formula	Molecular weight g/mol
				weight g/mor
1	4.074	Thiirane	C_2H_4S	60.118
2	4.074	Ammonium acetate	CH ₃ CO ₂ NH ₄	77.08
3	4.451	Acetic acid	$C_2H_4O_2$	60.05
4	6.372	N-Acetylisoxazolidine	$C_{11}H_{13}NO_4$	223.225
5	6.839	Benzaldehyde	C ₆ H ₅ CHO	106.12
6	6.839	Bis[4-acetamidophenylsulfonyl]phenyl	$C_{23}H_{22}N_2O_6S_2$	486.560
7	7 231	Acetylisovazolidine N-	CueHuzNOa	235 278
8	13 930	3-Methyl-2-butenoic acid		100.12
0	13.030	S-Wethyr-2-Outenoie acid	C.H. NO.	201 176
,	15.950	[3-(acetyloxy)-4,5-dihydro-5-isoxazolyl]methyl	C81111105	201.170
10	17.818	Nonadecane	$C_{19}H_{40}$	268.520
11	17.818	Eicosane	$C_{20}H_{42}$	282.547
12	17.818	Methoxyacetic acid, 2-tetradecyl ester	$C_{17}H_{34}O_3$	286.450
13	17.924	Dodecane, 2,6,10-trimethyl-	C ₁₅ H ₃₂	212.422
14	17.924	octadecane, 2,6,-dimethyl	$C_{20}H_{42}$	282.55
15	18.195	Isocytosine	C ₄ H ₅ N ₃ O	111.102
16	18.195	N-(4-Fluorophenyl) cyclohexanecarboxaMide	$C_{27}H_{26}FNO_4$	447.498
17	18.858	Sulfurous acid, 2-propyl tetradecyl ester	$C_{17}H_{36}O_3S$	316.499
18	19.137	Tridecanoic acid, methyl ester	$C_{15}H_{30}O_2$	228.370
19	19.137	Pentadecanoic acid methyl ester	C16H32O2	256.424
20	19.137	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.477
21	19.619	Methyl –mannoside	$C_7 H_{14} O_6$	194.182
22	19.845	cis; 2-Methyl-3-propyl-oxirane	C ₆ H ₁₂ O	100.158
23	21.254	Propyl octadecanoate	$C_{21}H_{40}O_2$	324.541
24	21.254	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_{3}O_{2}$	364.485
25	21.699	10-Methylnonadecane	$C_{20}H_{42}$	282.547
26	21.699	2-Methyloctacosane	$C_{29}H_{60}$	408.786
27	23.402	Heneicosane,11-(1-ethylpropyl)-	C ₂₆ H ₅₄	366.707
28	24.404	Glycerol 1-palmitate	CH ₃ (CH ₂) ₁₆	356.54
29	27.756	Heneicosane, 11-decyl-	$C_{31}H_{64}$	436.839
30	27.756	Tetracosane, 11-decyl-	C ₃₄ H ₇₀	478.919
31	27.756	13-undecyl-pentacosane	C ₃₆ H ₇₄	506.973
32	30.447	Decanoic acid, 2-propenyl ester	$C_{13} H_{24} O_2$	212.328
33	30.447	10-nonadecanone	$C_{19}H_{38}O$	282.504

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Fig.1:Chromatogram obtained from the GC-MS of Catha edulis leaves extract

CG-MS method used to detect compounds found in many plant extracts such as twenty six structurally different alkaloids were identified from plant *Epipremnum aureum*is highly rich in alkaloids¹⁴, also GC-MS analysis of *Ricinus communis* alkaloid leaves extract explain 9 different compounds^{15, 16}, reported 16 compounds in alkaloid leaves extract of *Rhizophora mucronata* by using GC-MS.

Conclusion:

The alkaloid compounds extract of *C.edulis* was caused compelet destraction of eggs of *C.pipines* in all used concentration also It is active against different larval stages in different concentration and therefore it may used as natural insecticide.

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