INSECTICIAL ACTIVITY OF *ACOKANTHERA SPECTABILIS* FRUITS EXTRACTS AGAINST *CULEX PIPIENS* L. (DIPTERA: CULICIDAE)

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ABSTRACT

Fresh fruits of Bushman's Poison, Winter sweet, Acokanthera spectabilis, Apocynaceae shrub, a common species in Egypt were washed and divided to seven fractions using different solvent systems. These fractions were evaluated for their mosquitocidal effects against Culex pipiens 4th larval instar. They showed different larval lethal effects and inhibition of both pupation and adult emergence. Fraction I (extracted with aqueous acetone) was the most effective with complete death of the treated larvae at 500µg/ml after 48 hours with LC_{50} values of 53.8 and 26.5µg/ml after 24 and 48 hours, respectively. It exhibited complete suppressing the alive larvae pupation and the adult emergence at 200 and 100 µg/ml, successively. Further chromatographic separation of the most active fraction on Silica gel lead to eight subfractions. Subfractions 2 and 4 were more toxic with complete killing of the treated larval population at 500 and 10 µg/ml. Their LC_{50} values were 54 and 22.5 µg/ml compared with <10 and <10 µg/ml after 4 and 5 days exposure, respectively. Their LT_{50} values were 5.41, 4.74 and 3.73 compared with 1.93, 1.18 and < 1.0 days at 10, 50 and 100 µg/ml. At 50 µg/ml, subfraction 2 reduced pupation to 11.1%, while subfraction 4 completely suppressed it with complete stop of adult emergence. Both subfractions harshly suppressed the treated eggs hatchability. Subfraction 4 appeared to be the most effective on larval mortality, pupation and adult emergence. Its GC-MS analysis proved it as a cardenolide glycosides rich component.

Keywords: *Acokanthera spectabilis, Culex pipiens , Insecticide, Extraction*

INTRODUCTION

The house mosquito *Culex pipiens* L. (Diptera: Culicidae) is one of the most harmful insects affecting humans and farm animals, transferring several pathogens as Cache-Valley (CV) and West Nile Virus that cause infertility and congenital malformations in ruminants (Edwards *et al.*, 1998 and (Smartt and Erickson, 2008)). This insect showed great resistance to the used synthetic insecticides by generating resistance genes (Raymond *et al.*, 2001) or producing high esterases levels (Dary *et al.*, 1990). It also appeared to be sensitive to several plant species. Myrrh (oleo-gum-resin) obtained from the stem of *Commiphora molmol* proved to have insecticidal activity affecting fat, muscles, gut and nervous tissues of the larvae (Massoud and Labib, 2000). Methanolic extracts of aerial parts of the medicinal plant Argel, *Solenostemma argel* (Del.) Hayne, incorporated into rearing media of *Culex pipiens* L. reduced oviposition, hatchability and larval viability (Al-Doghairi *et al.*, 2004). Cetin *et al.* (2006) reported high larvicidal activity of *Teucrium divaricatum* Sieber, *Mentha longifolia* (L.) Huds., *M. pulegium* L., *Melissa officinalis* L. and *Salvia sclarea* L. oils more than temephos in 24 h exposure tests. Zhu *et al.* (2006) added that oils of thyme, catnip, amyris, eucalyptus, and cinnamon revealed larvicidal activity besides repellent effect on this pest. Several plant species extracts killed and altered developmental periods, pupation rates and adult emergences of this insect (Khater and Shalaby, 2008). Several pure active potential sources of larvicidal substances against mosquito were isolated as beta-thujaplicin from *Chamaecyparis obtusa* leaves (Jang *et al.*, 2005), 7-hydroxycoumarin from *Stellera chamaejasme* root powder (Xiaorong and Taiping, 2008) and pure synthetic natural naphthoquinones (alkannin, shikonin, and shikalkin) and three acetylated derivatives of shikonin (Michaelakis *et al.*, 2009). Among the plant families, Apocynaceae is a large family of considerable economic and medicinal importance. *Acokanthera oppositifolia* Lam is attractive of white flowers and red berries. Its latex, fruit and decoctions of wood were widely used as arrow poisoning (Van Wyk *et al.*, 2002). Poisoning of animals by this plant is surprisingly rare (Kellerman *et al.*, 1988). The antiviral activities of the 80% methanolic extracts of *A. schimperi* and *A. oppositifolia* against coxsackievirus B3 (CVB3), influenza A virus and herpes simplex virus type1 Kupka (HSV-1) were exhibited (Adedapo *et al.*, 2008). Several parts of *A. schimperi* are used for the treatment of scabies, leprosy, *Tinea capitis*, wound, eczema, acne, warts, rheumatic pain and elephantiasis (Gelahun, 1989). Its hydroalcoholic extracts showed antimicrobial activity against different strains of bacteria and fungi.

This study aimed to study the insecticidal potency of *Acokanthera spectabilis* fruits constituents as a common species in Egypt against *Culex pipiens* 4th larval instar regarding the insecticidally active constituent as an alternative of synthetic insecticides avoiding their resistance and environmental hazardous..

MATERIALS AND METHODS

I- Extraction and separation of plant sample:

Fresh fruits (1100 gm) of *A. spectabilis*, Apocynaceae were collected from the campus of Faculty of Agriculture, Alexandria University. The collected sample was washed from dust and freshly blended with acetone (1.0 L) two times for a month in the dark at room temperature. The acetone extract was filtered in a light green solution that was concentrated at < 60 °C under reduced pressure. Some solid materials which appeared under freezing were filtered

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and dried over phosphorus pentoxide (P_2O_5) to fraction I (4.015 gm of brown powder). The resulted filtrate was further concentrated to 0.25 L and its solid contents were determined to be 40.2 gm (fraction II). The plant sample was further re-extracted respectively with 1.7 L. of the mixture (Hydrochloric acid: glacial acetic acid: water) (1:1:15 in volume) for 15 days four times with methanol (1.0 L.). The acidic extract was filtered to creamy latex, which was washed from acids and dried to fraction III (0.870 gm) of crystalline powder. The acidic filtrate was discoloured by passing through a charcoal column.

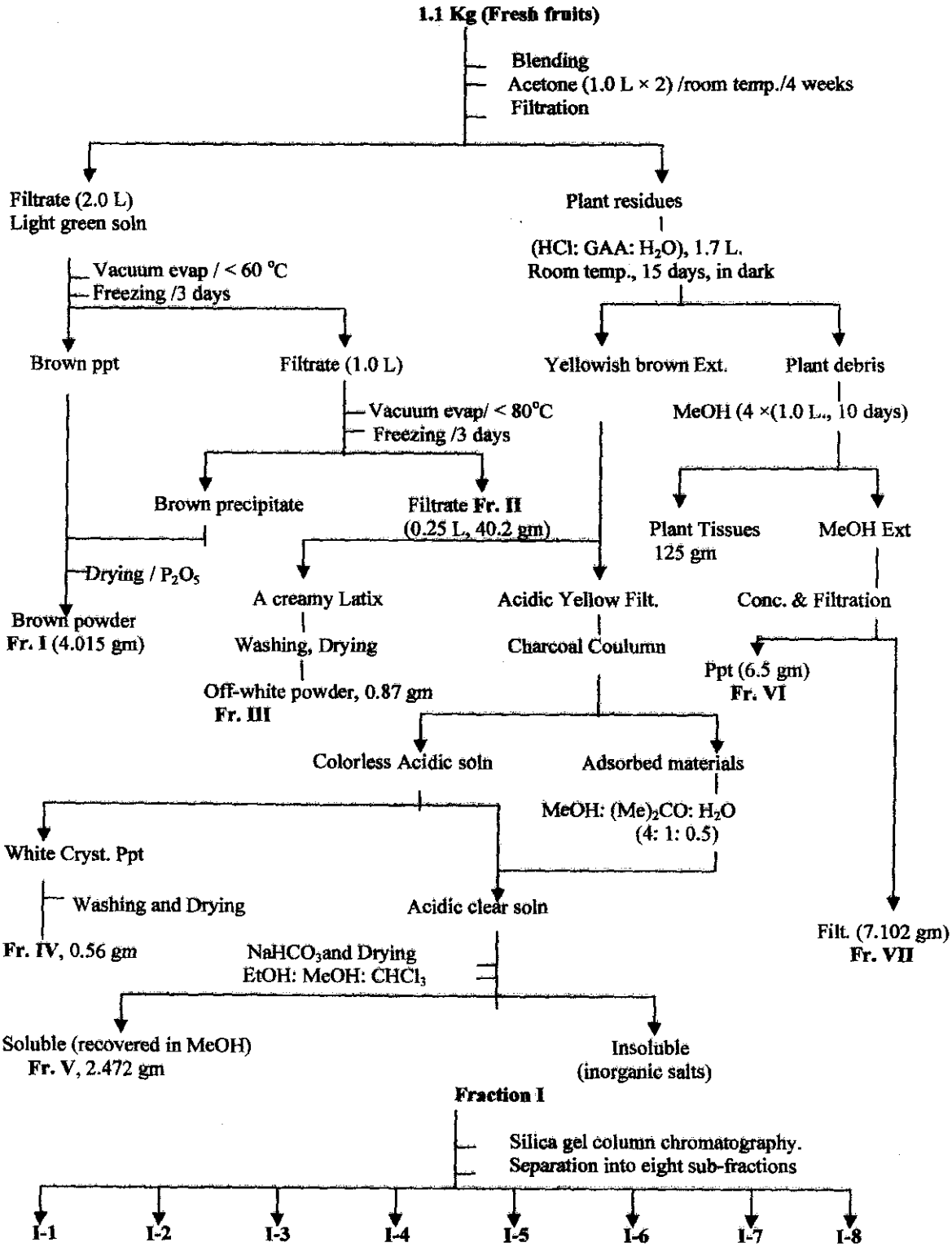


Fig. (1): Extraction and Isolation of *A. spectabilis* fruits constituents

Fraction IV (0.56 gm, white crystals) was separated from the resulted cleaned filtrate that was neutralized with sodium bicarbonate and re-extracted with a mixture of (ethanol, methanol and chloroform in a volume ratio of (1:1:1). The organic layer was evaporated and the soluble residue was recovered in methanol as fraction V (2.472 gm), while the insoluble materials were assumed to be inorganic salts. Regarding the resulted brownish yellow methanol extract, it was concentrated under vacuum to a brown precipitate, which was dried over P_2O_5 to fraction VI (6.59 gm). The remained filtrate's content was determined and recovered in water as fraction VII (7.102 gm). In general, the obtained acetone, acidic and methanol extracts were 44.215, 3.90 and 13.692 gm with 4.02%, 0.355% and 1.245% of the un-extracted fresh sample, respectively. The most active fraction (I) was further separated on Silica gel 60-120 mesh, (50 cm ×1.0 cm id) column chromatography (CC). The column was constructed in ethanol. Two grams of the sample were applied in 25 ml of 70% aqueous acetone. Elution was successively carried out with 1:1 volume mixture of benzene and petroleum ether 40-60 and chloroform (100 ml of each), followed by 40% (125 ml) and 70% (115 ml) of methanol in chloroform. The column was rinsed with 70 % aqueous acetone (200 ml). Seven resulted sub-fractions were collected. Sub-fraction 1 (turbid, 50 ml, 0.1gm); 2 (bright green, 40 ml, 1.07 gm); 3 (colorless, 80 ml, 0.03 gm); 4 (light green, 90 ml, 0.27 gm); 5 (bright green, 100 ml, 0.30 gm); 6 (yellow, 150 ml, 0.10 gm); 7 (yellow, 150 ml, 0.08 gm). The column was destructed in a warm equivalent volume of chloroform-methanol-acetone mixture and filtered. The filtrate was considered as sub-fraction 8 (0.05 gm). Separated fractions were concentrated under reduced pressure at < 50 ^{o}C. The total recovered amount was 1.90 gm with 95 % recovery. Extraction process is summarized in Figure (1).

II- GC–MS analysis

GC-EIMS analysis of the most active sub-fraction was performed on a GCMS-QP 2010, GC Hewlett-Packard HP 6890A, Column HP5 (coated with 5% diphenyl/ 95% dimethyl-poly-siloxane (30 m ×0.25µm film thickness × 0.32mm i.d.) was used as the stationary phase. Helium served as mobile phase with a constant pressure of 187.1 kPai. Direct injection of the sample in acetone (1µl of the standard solution, concentration 0.5 mg/ml) was performed in a splitless mode with an inlet temperature of 220 ^{o}C. Column Oven Temp.: 80.0 ^{o}C. Column Flow: 3.00 ml/min with linear velocity: 63.8 cm/sec. The applied oven temperature program included an initial step for 1 min at 80 ^{o}C; temperature shifted up to 250 ^{o}C with 10 ^{o}C/min, followed by 70 min at 250 ^{o}C. Mass conditions continued to 88 min with m/z range of 45.00- 950.00.

III- Insecticidal activity:

The tested mosquito larvae were firstly collected from a water bond in Abbis area, Alexandria Governorate, Egypt since August, 2001. The obtained larvae were reared under laboratory conditions of 27 ± 1.0 ^{o}C and 70 ± 5% R.H. with (14:10 L:D). The WHO standard test method for mosquito larvae (WHO, 2001 Protocol) was used to test the insecticidal activities of the obtained *A. spectabilis* extracts against the 4th instar larvae of mosquito (*Culex pipiens* L.) at the concentrations of 10, 50, 100, 200, 500, and 1000 µg/ml. Thirty larvae were used in each replicate and three replicates were considered as one treatment. Control was concurrently conducted under the same conditions. Subfractions 1-7 (originated from fraction I) were tested at 10, 50, 100, 200 and 500 µg/ml at ca 20±2.0^{o}C. The larval lethality was determined daily for 7 days and the effect of time was measured. Mortality percents were determined (Topps and Wain, 1957). LC_{50} (Lethal concentration which caused 50% mortality) and LT_{50} (Lethal time which caused 50% mortality) were also calculated (Finney, 1971). Inhibition of pupation and adult emergence were also studied. Pupation and adult emergence percentages were calculated from the alive larval numbers by the end of exposure time. The two active subfractions were tested for their ovicidal effects against the hatchability. Three sets of eggs (53 ± 8) were used for each treatment and subjected to the tested concentrations series (1, 2, 10, 20, 40 and 50 µg/ml). The newly emerged larvae were counted and their mortality percentages were calculated at different exposure times in comparison to control.

RESULTS AND DISCUSSION

I- Insecticidal effects:

A- Preliminary screening:

As presented in Table (1), the separated fractions affected the treated larval population differently as fractions separated from the acidic extract exhibited weak effects. Fraction III caused 2.2-12.2 % and 12.2–34.4% of larval mortality after 24 and 48 hours, respectively. It exhibited no inhibition on pupation and decreased the adult emergence to 90% of control. Fraction IV was nearly similar in its mortal effect reducing both the pupation and adult emergence percents systematically with the tested concentration. Fraction V exhibited only slight inhibition in adult emergence. Fractions VI and VII (methanol originated fractions) differed in their effects as the first exhibited 11.1 and 22.2% mortality after 24 and 48 hours and lowering the adult emergence only to 91.7 of control with no effect on the pupation of the treated larvae. Fraction VII showed high lethal effect of 52.2 and 70.0 % after 24 and 48 hours with LC_{50} values of 777.5 and 232.3µg/ml. It decreased the pupation of the treated larvae to 60% with complete transformation to the adult stage of the produced pupae. Fractions of the

aqueous acetone extract were more effective. Fraction II exhibited lethal effect arranged systematically with the tested concentration with LC_{50} values of 445.4 and 135.3µg/ml, respectively after 24 and 48 hours. It diminished pupation and adult emergence to 59.1 and 11.57 % of the control. Fraction I was the most effective among the tested fractions with complete death of the treated population at 500µg/ml after 48 hours with LC_{50} values of 53.8 and 26.5µg/ml after 24 and 48 hours, respectively. It also completely prevented the pupation and the adult emergence at 200 and 100µg/ml with effective concentrations which inhibited 50% of both populations 54.9 and 18.8 µg/ml, respectively

B- Effect of the active fraction constituents (subfractions):

As presented in Tables (2a & b), subfractions originated from the active fraction (I) caused their larval mortality in both concentration and exposure time. Subfractions 5, 6 and 7 were less effective with mortality percents ranged from 0 to < 50% at concentrations up to 500µg/ml. Meanwhile, subfraction 6 showed low effect till seven days exposure with 471 µg/ml LC_{50} value and 5.84 days LT_{50} value at 500 µg/ml. Subfractions 5 and 7 gave LC_{50} values of 466 and 297 in comparison to 300 and 215 µg/ml after six and seven days exposure with LT_{50} values of 5.03 and 4.75 exposure days at 500 µg/ml, respectively. Subfractions 1 and 3 appeared to be more toxic against the treated larvae showing systematic lethal effect. Mortality was affected with time of exposure in case of subfraction 1 more than subfraction 3 as their LC_{50} values were 430, 131, 49 and 23.8 µg/ml in comparison to 290, 190, 119 and 76 µg/ml after four, five, six and seven days exposure with LT_{50} values of 6.17, 4.28 and 2.34 comparing with 5.66, 4.80 and 3.61 days at 100, 200 and 500 µg/ml, respectively. Subfraction 8 exceeded the mentioned subfractions in its effect with mortality percents ranging from 5.9 to 88.1% exhibiting LC_{50} values of 190, 39, 23 and 15 µg/ml, after four, five, six and seven days exposure and with LT_{50} (values of) 5.81, 3.92, 3.38 and 2.83 days at 50, 100, 200 and 500 µg/ml, respectively. Subfractions 2 and 4 proved to be more toxic with complete killing of the treated population at 500 and 10 µg/ml emphasizing that subfraction 4 is the most active subfraction. Their LC_{50} values were > 500, > 500, 347, 54, 22.5, 12.6 and 5.2 µg/ml compared with 41.2, 13.8, 7.3, 6.4, 4.9, < 4.9 and < 4.9 µg/ml after 1, 2, 3, 4, 5, 6 and 7 days exposure, respectively. Their LT_{50} values were also decreased with increasing the tested concentration. It is important that using the most active subfraction lead to complete killing of the treated population respectively at 10, 50, 100, 200 and 500 µg/ml for 7, 5, 4, 3 and 2 days i.e 70, 250, 400, 600 and 1000 CT units ensuring the importance of treatment with low concentrations for long time than at high concentrations for short time.

Regarding pupation and adult emergence, it was found that at 50µg/ml, pupation percents in all treatments were less than in the control. Pupation percents increased systematically with time to 25%, 36.7%, 37.5%, 31.1%, 53.3% and 40% of the treated population in case of subfractions 1, 3, 5, 6, 7 and 8, respectively compared with 67% in the untreated population after 10 days continuous exposure. Subfraction 2 increased pupation% dramatically with exposure time to 11.1%, while subfraction 4 completely prevented pupation of the treated larvae within the exposure time (Figure 2). Adult emergence was increased systematically in control and subfractions 3, 5, 6 and 7 to 60%, 21.3%, 42.9%, 14.3% and 10 % of the treated population. Meanwhile subfractions 1, 2, 4 and 8 completely stopped adult emergence at the tested concentrations.

Regarding the effect on eggs, subfraction 2 harshly reduced hatchability from 94.3 to 53.3 at 1 and 10µg/ml, respectively, followed by complete suppressing at 20µg/ml. However, subfraction 4 reduced it to 94.6 and 61.3 after the same exposure period compared with the control with effective concentration on 50% of egg- hatchability of 6.52 and 9.17 µg/ml, respectively. These two subfractions showed lethal effects against the newly hatched larvae in concentration and time dependant effect (Table 3). On the other hand 2.5X magnified photos proved some malformations in the treated larvae in comparison to the untreated larvae as some of them produced un-melaninized pupae (albino pupae), larval pupal intermediates with attached organs like attaching of larval siphon with pupal paddles region, eighth abdominal segment or trumpets (Figure 3).

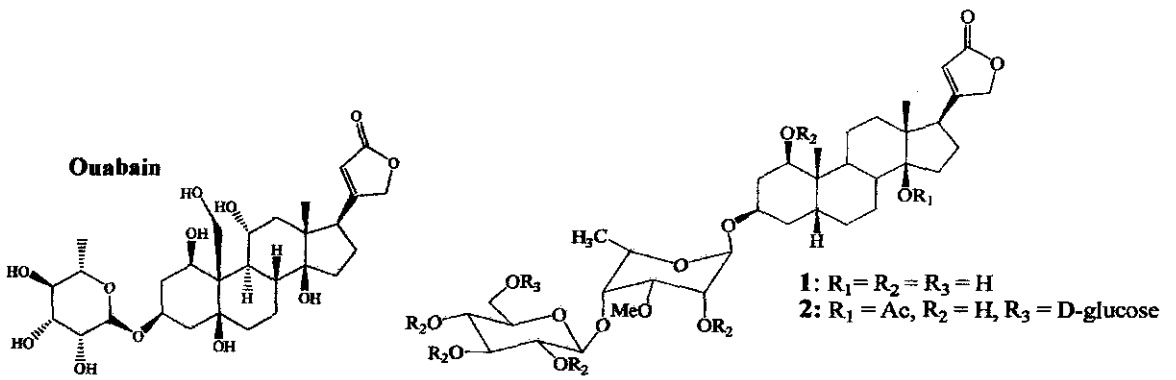
From the previous data, subfraction 4 appeared to be the most effective on larval mortality, pupation and adult emergence showing strong inhibition of eggs hatchability and high lethal effect on the newly produced larvae.

II- GC-MS analysis:

GC-MS spectral analysis of the most active fraction proved that it contains cardenolide derivatives. The aglycone (steroid nucleus) was fragmented at m/z 393 (8.01), 372 (0.3), 342 (2.16), 315 (16.99), 297 (7.46), 279 (0.13), 262 (0.24), 218 (76.12), 207 (74.55), 203 (45.67), 189 (78.94) and 95 (100). These data agree with those reviewed by Melero *et al.* (2000) for Acobioside A cardenolide glycoside. The sugar moiety of this compound is 3-*O*-[1-(3-methylrhamnosyl)-(4-1')-*O*-glucopyranose)]. Fragments at m/z 355 (0.98), 427 (16.95), 426 (48.92) and 411 (21.33) are produced through breaking the ether bond between the two sugar moieties followed by demethylation of the substituted methyl group on rhamnose ring. However fragments at m/z 207 (74.55)

147 (7.78), 163 (4.24), 167 (0.26) and 109 (15.89) are due to fragmentation of sugar ring through diels-alder ring fission or elimination of water molecules. Moreover, fragments appearing at m/z 440 (0.23), 453 (4.74) and 468 (11.84) proved presence of an acetylated steroid aglycone as explained by Pa'duaa *et al* (2008). From previous data it could be said that the active fraction contained Acobioside A and 14-*O*-acetylacovenioside C that were eluted at retention time of 24.06 and 31.48 minutes with91.96 and 4.80% of area besides ouabain at retention time of 39.7 min.

In conclusion, the insecticidal effects could be referred to the cardenolide glycosides content as ouabain acovenoside, a cardiac glycoside which is the major toxic component in the genus *Acokanthera*. These compounds exhibited strong biological activity as insecticidal, insect repellant molluscicidal, acaricidal, antifilarial and antibacterial compounds (Al-Rajhy *et al.* (2003), Haussein *et al.* (1999) and Huq *et al.* (1999)). These cardenolides may affect insects through inhibition of Na^+-K^+ ATPase enzyme as proved in butterflies (Mebs *et al*, 2000). On the other hand Abe *et al.* (1995) reported that they affect both esterase and glucosidase in the digestive system in other small phytophagus animals as snails.



Structure of ouabain, Acobioside A (**1**) and 14-*O*-acetylacovenioside C (**2**)

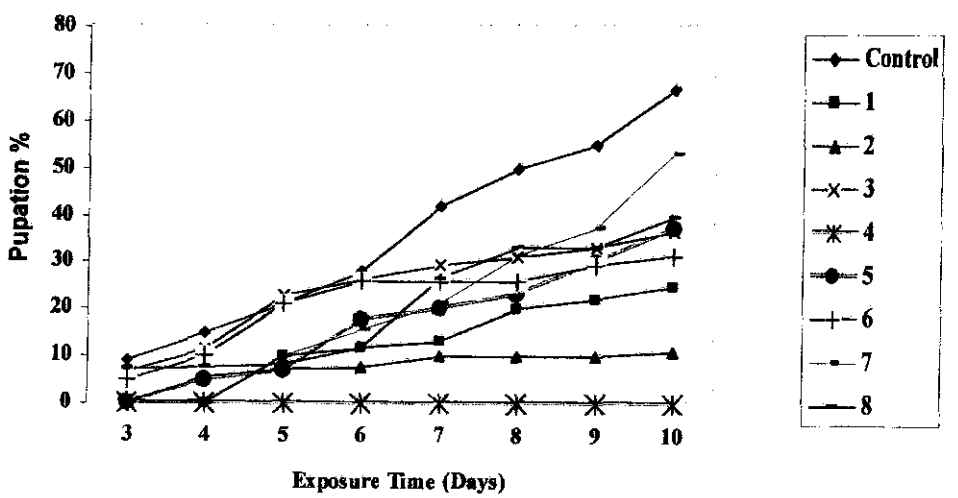


Fig. 2: Effect of the separated subfractions on *Culex pipiens* larvae pupation

Table 1: Preliminary screening of *A. spectabilis* extracts for insecticidal activity against 4th larval instar of *C. pipiens*

Fract.	Effect on	Time (hr)	Mortality % at different concentration (µg/ml) 0	10	50	100	200	500	1000	LC_{50} /EC_{50} (95% CL)
I	Larval Mortality%	24	0	24.5± 1.92	43.3± 3.34	60.2± 1.92	72.2± 3.85	87.8± 1.93	96.7± 3.33	53.8 (42-68.8)
		48	0	27.8± 6.94	56.7± 3.33	85.6± 1.93	98.9± 1.92	100 0	100 0	26.5 (21.5-32.5)
	Pupation %		100	85	69.3	42.3	0	-	-	54.9 (46.6-64.5)
	Adult emerg. %		100	60	46.2	0	-	-	-	18.8 (14.3-24.6)
II	larval Mortality%	24	0	14.4± 1.93	18.9± 3.33	20± 1.91	41.1± 1.91	52.2± 1.92	65.6± 1.93	445.4 (307-650)
		48	0	23.3± 3.34	43.3± 3.34	44.4± 1.93	51.1± 1.92	63.3± 3.33	73.3± 3.34	135.3 (92.9-197)
	Pupation %		100	88.2	87.5	83.3	78.6	64.7	59.1	> 1000
	Adult emerg. %		100	64.3	62.5	58.3	52	50	11.67	282.4 (157.7-513.4)
III	Larval Mortality%	24	0	2.2 ± 1.91	4.4 ± 1.89	7.8 ± 1.94	11.1± 1.91	12.2± 1.91	- -	> 1000
		48	0	12.2± 1.91	20.0± 3.32	24.4± 1.94	25.6± 1.93	34.4± 1.94	- -	> 1000
	Pupation %		100	100	100	100	100	100	-	> 1000
	Adult emerg. %		100	100	95.8	95.7	90.9	90	-	> 1000
IV	Larval Mortality%	24	0	0	0	0	4.5 ± 1.97	10.5± 3.33	18.9± 1.92	> 1000
		48	0	0	2.2± 1.91	11.1± 3.85	22.2± 1.93	31.1± 1.92	40.0± 3.34	> 1000
	Pupation %		100	100	88.9	61.1	27.8	22	0	156.7 (136.9-179.4)
	Adult emerg. %		100	100	83.3	44.4	16.7	11.1	0	119.7 (105-136.4)
V	Larval Mortality%	24	0	0	0	0	0	0	0	> 1000
		48	0	0	0	0	0	0	0	> 1000
	Pupation %		100	100	100	100	100	100	100	> 1000
	Adult emerg. %		100	100	96.7	96.7	93.3	90	86.7	> 1000
VI	Larval Mortality%	24	0	1.1 ± 1.90	2.2 ± 1.91	4.4 ± 1.93	6.7 ± 0.02	7.8 ± 1.92	11.1± 1.92	> 1000
		48	0	8.9± 1.93	11.1± 1.90	15.5± 1.94	18.9± 1.92	21.1± 1.92	22.2± 1.91	> 1000
	Pupation %		100	100	100	100	100	100	100	> 1000
	Adult emerg. %		100	100	96.3	96	96	95.8	91.7	> 1000
VII	Larval Mortality%	24	0	0	7.8± 1.93	13.3± 3.34	25.6± 1.94	42.2± 1.93	52.2± 1.92	777 (568-1067)
		48	0	3.3± 3.35	12.2± 1.92	33.3± 0.02	44.5± 1.94	54.4± 1.95	70.0± 3.34	232 (260-424)
	Pupation %		100	100	100	100	100	85	60	> 1000
	Adult emerg. %		100	100	100	100	100	100	100	> 1000

Pupation and adult emergence percents are calculated in relation to the remained alive larval numbers.
LC_{50}, lethal concentration of 50% of the treated larval population in µg/ml
EC_{50}, effective concentration of 50% of pupation or adult emergence in µg/ml

Table 2-a: Lethal effects of separated sub-fractions against 4th larval instar of *C. pipiens;* shown as LC_{50} (µg/ml) with 95% confidence limits

Sub-Fract.	LC_{50} (µg/ml) at different exposure time (Days)						
	1	2	3	4	5	6	7
1	>500	>500	>500	430 (210-906)	131 (88-195)	49 (27-88.8)	23.8 (13.2-42)
2	>500	>500	347 (187-657)	54 (30-97)	22.5 (15-34)	12.6 (8-19.6)	5.2 (2.5-10.2)
3	>500	>500	>500	290 (194-436)	190 (128-484)	119 (82.5-171)	76 (54-107)
4	41.2 (29-59)	13.8 9.7-19.5	7.13 (4.8-10.5)	6.4 (4.6-8.8)	4.9 (3.0-8.01)	< 4.9	< 4.9
5	>500	>500	>500	>500	>500	466 (355-615)	300 (249-362)
6	>500	>500	>500	>500	>500	>500	471 (380-586)
7	>500	>500	>500	>500	>500	297 (245-359)	215 (182-253)
8	>500	>500	>500	190 (100-371)	39 (24-63)	23 (14-38)	15 (8.4-26.1)

Tested concentrations: 10, 50, 100, 200 and 500 µg/ml

Table 2-b: Lethal effects of separated sub-fractions against 4th larval instar of *C. pipiens;* shown as LT_{50} (days) with 95% confidence limits

Sub-Fract.	LT_{50} (days) at different concentrations (µg/ml)				
	10	50	100	200	500
1	>7	>7	6.17 (5.12-7.52)	4.28 (3.82-4.8)	3.47 (3.14-3.83)
2	5.41 (4.77-6.15)	4.74 (4.21-5.34)	3.73 (3.4-4.1)	2.52 (2.28-2.78)	1.93 (1.75-2.12)
3	>7	>7	5.66 (4.88-6.61)	4.80 (4.13-5.63)	3.61 (3.14-4.14)
4	1.93 (1.69-2.2)	1.18 (1.03-1.34)	0.86 (0.71-1.01)	0.68 (0.5-0.84)	< 0.68
5	>7	>7	>7	>7	5.03 (4.62-5.48)
6	>7	>7	>7	>7	5.84 (4.88-7.06)
7	>7	>7	>7	>7	4.75 (4.42-5.10)
8	>7	5.81 (5.07-6.67)	3.92 (3.55-4.32)	3.38 (3.08-3.71)	2.83 (2.55-3.13)

Mortality was followed till 7 days exposure

Table 3: Effect of subfractions 2 and 4 on *C. pipiens* eggs

Sub-fr.	Effect on	Time Days	Concentration (µg/ml)							EC_{50}/ LC_{50} (95% CL)
			0	1	2	10	20	40	50	
2	Hatch. %	3	100	94.3± 1.81	91.4± 4.06	53.5 ± 3.12	0	-	-	6.52 (5.64-7.51)
	Produced larval mortality %	7	0	44.1± 2.6	52.6± 1.8	65± 2.1	100	-	-	1.64 (1.13-2.28)
		9	0	61.1± 2.3	73.7± 2.8	83.3± 1.9	100	-	-	< 1.0
		11	0	81.4± 3.1	100	100	100	-	-	< 1.0
4	Hatch.%	3	100	94.6 ± 2.32	82.7 ± 4.04	61.3± 4.04	31.3 ± 2.52	13.8 ± 3.36	0	9.17 (7.78-10.79)
	Produced larval mortality %	7	0	24.3± 1.1	52.86± 3.21	77.6± 2.41	95.3± 3.23	100	100	2.02 (1.59-2.53)
		9	0	39.05± 2.1	64.3± 2.34	85.05± 3.21	100	100	100	1.16 (0.86-1.50)
		11	0	68.6± 2.12	100	100	100	100	100	< 1.0

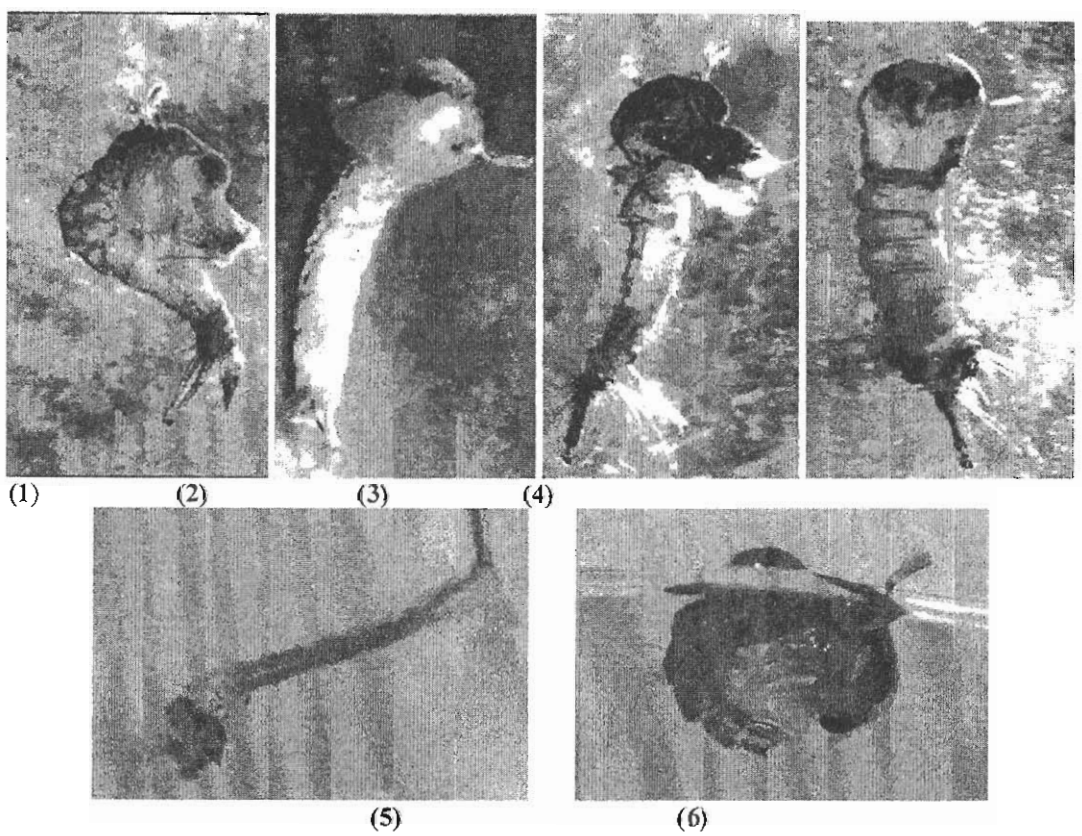


Fig.3: Some morphological effects of the tested extracts on *C. pipiens* stages

1- Pupa showing larval exuvium attached to cephalothorax region and larval siphon attached at the eighth abdominal segment.
2- Unmelaninized pupa (albino pupa).
3- Larval-pupal intermediate showing larval siphon and pupal trumpets.
4- Pupa showing larval siphon attached to pupal paddles region.
5- Untreated larva
6- Pupa developed from untreated larva

REFERENCES

Abe, F.; T. Yamauchi and K. Minato (1995). Presence of cardenolides and ursolic acid from oleander leaves in larvae and frass of *Daphnis nerh. Phytochemistry,* 42 (1): 45-49.

Adedapo, A. A.; F. O. Jimoh; A. J. Afolayan and P. J. Masika (2008). Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera. BMC Complementary and Alternative Medicine,* 8: 54

Al-Deghairi, M.; A. El-Nadi; E. Elhag and H. Al-Ayedh (2004). Effect of *Solenostemma argel* on oviposition, egg hatchability and viability of *Culex pipiens* L. larvae. *Phytother Res.*, 18 (4): 335-8.

Al-Rajhy, D. H.; A. M. Alahmed; H. I. Hussein and S. M. Kheir (2003). Acaricidal effects of cardiac glycosides, azadirachtin and neem oil against the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae). *Pest Manage Sci.*, 59: 1250–4.

Cetin, H.; I. Cinbilgel; A. Yanikoglu and M. Gokceoglu (2006). Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. *Phytother. Res.*, 20 (12): 1088-90.

Dary, O.; G. P. Georghiou; E. Parsons and N. Pasteur (1990). Microplate adaptation of Gomori's assay for quantitative determination of general esterase activity in single insects. *J. Econ Entomol.*, 83 (6): 2187-92.

Edwards, J. F.; S. Higgs and B. J. Beaty (1998). Mosquito feeding-induced enhancement of Cache Valley Virus (Bunyaviridae) infection in mice. *J. Med. Entomol.*, 35 (3): 261-5.

Finney, D. J. (1971). Probit analysis. 3rd edition Cambridge University Press, London; pp. 138.

Gelahun, A. (1989). Ethiopian Traditional Medicine. In: Sebsebe, D. (Ed.), Etse Debdabe, Biology Department, Science Faculty, Addis Ababa University, pp. 64, 123.

Huq, M. M.; A. Jabbar; M. A. Rashid and C. M. Hasan (1999). A novel antibacterial and cardiac steroid from the roots of *Nerium oleander. Fitoterapia,* 70: 5–9.

Hussein, H. I.; D. Al-Rajhy; F. I. El-Shahawi and S. M. Hashem (1999). Mollusci-cidal activity of *Pergularia tomentosa* (L.), methomyl and methiocarb against land snails. *Int. J. Pest Manag.*, 45: 211–3.

Jang, Y. S.; J. H. Jeon and H. S. Lee (2005). Mosquito larvicidal activity of active constituent derived from *Chamaecyparis obtusa* leaves against 3 mosquito species. *J. Am. Mosq. Control Assoc.*, 21(4): 400-3.

Kellerman, T. S.; J. A. W. Coetzer and T. W. Naude (1988). Plant poisonings and mycotoxicoses of livestock in Southern Africa. Oxford University Press, Cape Town, 25-26.

Khater, H. F. and A. A. Shalaby (2008). Potential of biologically active plant oils to control mosquito larvae (*Culex pipiens*, Diptera: Culicidae) from an Egyptian locality. *Rev Inst Med Trop Sao Paulo.*, 50 (2): 107-12.

Massoud, A. M and I. M. Labib (2002). Larvicidal activity of *Commiphora molmol* against *Culex pipiens* and *Aedes caspius* larvae. *Journal-of-the-Egyptian-Society-of-Parasitology.*, 30 (1): 101-115.

Mebs, D.; R. Zehner and M. Schneider (2000). Molecular studies on the ouabain binding site of the NaC, KC-ATPase in milkweed butterflies. *Chemoecology*, 10: 201–203.

Melero, C. P.; M. Medarde and A. S. Feliciano (2000). A short review on cardiotonic steroids and their aminoguanidine analogues. *Molecules*, 5: 51-81.

Michaelakis, A.; A. T. Strongilos; E. A. Bouzas; G. Koliopoulos and E. A. Couladouros (2009). Larvicidal activity of naturally occurring naphthoquino-nes and derivatives against the West Nile virus vector *Culex pipiens. Parasitol Res.*, 104 (3): 657-62.

Pa'duaa, R. M.; R. Waibel; S. P. Kuate; P. K. Schebitz; S. Hahn; P. Gmeiner and W. Kreis (2008). A simple chemical method for synthesizing malonyl hemiesters of 21-hydroxypregnanes, potential intermediates in cardenolide biosynthesis. *Steroids*, 7 3: 458–465.

Raymond, M.; C. Berticat; M. Weill; N. Pasteur and C. Chevillon (2001). Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? *Genetica.*, 112-113: 287-96.

Smartt, C. T. and J. S. Erickson (2008). CNAct-1 gene is differentially expressed in the subtropical mosquito *Culex nigripalpus* (Diptera: Culicidae), the primary West Nile Virus vector in Florida. *J. Med. Entomol.*, 45 (5): 877-84.

Topps, J. H. and R. L. Wain (1957). Investigation on fungicides. III. The fungitoxicity of 1- and 5-alkyl salicylanilide and p-chloroanilines. *Ann. Appl. Biol.*, 45 (3): 506 -511.

Van Wyk, B.; F. Van Heerden and B. Van Oudtshoon (2002). Poisonous plants of South Africa. *Briza Publications, Pretoria*: 46-49.

World Health Organization, 2001. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland, p. 1.

Xiaorong, T. and H. Taiping (2008). Separation and identification of botanical insecticide 7-hydroxycoumarin and its biological activity against *Aphis craccivora* and *Culex pipiens pallens. Nat Prod Res.*, 22 (4): 365-70.

Zhu, J.; X. Zeng; Yanma; T. Liu; K. Qian; Y. Han; S. Xue; B. Tucker; G. Schultz; J. Coats; W. Rowley and A. Zhang (2006). Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J. Am. Mosq. Control Assoc.*, 22 (3): 515-22.

الملخص العربي

النشاط الإبادي لمستخلصات ثمار نبات الآكوكانثيرا سبكتابيليس ضد بعوضة الكيولكس بيبينز.

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تم تجميع ثمار نبات الآكوكانثيرا سبكتابيليس من حدائق كلية الزراعة – جامعة الأسكندرية. تم غسيل الثمار من الأتربة وإستخلاصها بمذيبات مختلفة تباعا حيث تم فصل عدد ٧ مستخلصات و بتقييم هذه المستخلصات لنشاطها الإبادي على بعوضة الكيولكس بيبينز وجد أن المستخلص الأول و الذي تم فصله بإستخلاص الثمار بمذيب الأسيتون المائي هو الأكثر فعالية حيث أعطى نسبة موت تناسبت طرديا مع التركيز المستخدم بتركيز قاتل لـ٥٠ % قدره ٥٣,٨ ميكروجرام / مل بعد ٢٤ ساعة، ٢٦,٥ ميكروجرام / مل بعد ٤٨ ساعة من بدء المعاملة. أوقف هذا المستخلص تعذير اليرقات المعاملة تماما و كذلك خروج الحشرات الكاملة من العذارى على الترتيب على تركيز ٢٠٠ ، ١٠٠ ميكرو جرام/ مل في حين أنه أدى إلى قتل عشيرة اليرقات المعاملة بالكامل على تركيز ٥٠٠ ميكروجرام/مل.

إعادة الفصل الكروماتوجرافي لهذا المستخلص الأول (الأكثر فعالية) على عمود السيليكاجيل بإستخدام مذيبات إزاحة Eluents مختلفة أدى إلى فصل ٨ مكونات تم إختبارها على نفس الحشرة. وجد أن المكون الثاني (المفصول مع الكلوروفورم) و المكون الرابع (المفصول مع ٧٠% ميثانول في الكلوروفورم) هما الأكثر سمية حيث سببا ١٠٠% موت لعشيرة اليرقات على ٥٠٠، ١٠ ميكروجرام / مل بتركيز قاتل لـ ٥٠% من العشيرة المعاملة قدره ٥٤، ٢٢,٥ مقارنة بـ ٦,٤، ٤,٩ ميكروجرام / مل بعد ٤، ٥ أيام من المعاملة على الترتيب، و بزمن قاتل لـ ٥٠% من العشيرة المعاملة قدره ٥,٤١ و ٤,٧٤ و ٣,٧٣ يوم مقابلة لـ ١,٩٣ و ١,١٨ و< ١,٠ يوم بنفس الترتيب. أخمد المكونان كلا من التعذير و ظهور الحشرات الكاملة وكذلك فقس البيض مع التسبب فى موت اليرقات الناتجة بصورة معنوية . بعمل فصل والتعرف على محتويات المكون الأكثر فاعلية ووجد أنه يحتوى على Cardenolide glycosides و بالأخص مشتقات الـ Acovenioside النشطة بيولوجيا و التي قد يعزى لها التأثيرات الإبادية السابقة.