

## Toxicological and Phytochemical Studies of Wild Plant *Halocnemum strobilacium* Crude Extracts and Their Components Against *Aphis craccivora* Koch

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**Abstract:** Toxicological and phytochemical studies were conducted on the *Halocnemum strobilacium*. Results indicated that the ethyl acetate crude extract with LC<sub>50</sub> value 0.159 mg/cm<sup>2</sup> and LC<sub>95</sub> value 1.845 mg/cm<sup>2</sup> was the most effective against *Aphis craccivora* comparing with fraction B (LC<sub>50</sub> 1.522 mg/cm<sup>2</sup> and LC<sub>95</sub> 4.721 mg/cm<sup>2</sup>) as well as fraction A (LC<sub>50</sub> 1.299 mg/cm<sup>2</sup> and LC<sub>95</sub> 3.899 mg/cm<sup>2</sup>). Data also showed that, the crude extract was composed mainly of saturated and unsaturated fatty acids and their esters (69.34 %) followed by hydrocarbons (9.89 %). Only two terpenes were identified in this crude extract, one of them was Phytol isomer, a diterpene detected with 2.26 %. Oleic acid ester (38.18 %) and Octadecanoic acid (16.44 %) were the major constituents. The main compounds in Fraction A were hydrocarbons, fatty acids and their esters. Lumiflavine represented about 35.66 %. Five fatty acids and esters were identified and constituted 39% with Oleic acid ester (14.74%) Octadecanoic acid methyl ester (4.41 %) and Tetradecanoic acid (3.88 %) being the major representatives. In fraction B twenty three compounds were identified. Unoxxygenated compounds amount to 9.89, the main hydrocarbons being dimethyl undecane (5.20 %) and trimethyldecane (1.01%). Oxygenated compounds constitute 90.11 % of the fraction comprising fatty acids and esters, ketones as well as aldehydes (46.08 %, 17.94 % and 3.29 % of the fraction respectively). The main ester identified was octadecanoic acid, methyl ester (3.44%) while hexadecanoic acid was the main fatty acid detected (22.69 %).

**Key words:** Toxicological, Phytochemical, *Halocnemum strobilacium*, *Aphis craccivora*

### INTRODUCTION

The development of pest resistance and problems of environmental pollution have accompanied excessive reliance on pesticides. Such environmental problems have focused increased interest on pesticides occurring naturally in plants. Ideally these biochemical pest control agents should be active against limited number of species including specific target organisms. Such agents biodegradable to non-toxic products and can be suitable for use in programs of integrated pest management. Use of botanical pesticides (natural plant products) in an agro- ecosystem is now emerging as one of the primes means to product crop production free environment from pesticidal pollution, which is a global problem. Pervious screening tests were carried out in order to evaluate the toxicity of different plant extracts using different solvents against *Aphis craccivora*<sup>[3]</sup>. Comparison among the toxicity of the 29 plant extracts using different solvents showed that the ethyl acetate extracts of *Atriplex semibaccata*, *Suaeda*

*vermiculata* and *Halocnemum strobilacium* were the most toxic against *A. craccivora*. Barakat *et al.*<sup>[5]</sup> isolated and identified some of biological active compounds of *Atriplex semibaccata* against *Aphis craccivora*. Badawy *et al.*,<sup>[4]</sup> isolated some of biologically active compounds from the wild plant *Suaeda vermiculata* against *Aphis craccivora*. *Halocnemum strobilacium*, a member of the Chenopodiaceae, is a small annual herb growing wild in the Egyptian desert. It is known as Hatab Haddawy. Nothing is reported to data on the biological evaluation or the chemical composition of this plant. Therefore, the present study was conducted to investigate:

- Fractionation of the ethyl acetate crude extract of *H. strobilacium*.
- Biological activity of the promising fractions against *A. craccivora*.
- Isolation and identification of insecticidal components of the crude extract and their fractions by using GC/MS.

## MATERIALS AND METHODS

**Plant Material:** The wild plant *Halocnemon strobilacium* (Fam: Chenopodiaceae) was collected from different areas in Sinai. Sample of the collected plant was left to dry under laboratory conditions. Dried parts of plant were ground using an electric mill, sieved and kept for extraction.

The chemical constituents of *H. strobilacium* were extracted with ethyl acetate and steam distillation. The ethyl acetate extract was further fractionated by column chromatography using solvent mixtures with different polarity.

**Preparation of the Essential Oil and Crude Extract:** Samples of the plant were hydrodistilled for 3 hours using a Clevenger type apparatus. The oils were separated and dried over anhydrous sodium sulphate and kept in freezer at  $-80^{\circ}\text{C}$  until analysis. Crude extract was prepared according to the method described by Freedman *et al.*<sup>[9]</sup>.

**Fractionation of the Ethyl Acetate Crude Extracts:** Fractionation of the crude extract, *H. strobilacium* was performed on 50 cm length X 2.5cm diameter column packed with a slurry of silica gel 60 (particle size 0.063-0.2 mm, 70-230 mesh ASTM), purified in pentane/diethyl ether (95/5 v/v). The ethyl acetate crude extract was applied directly on the top of the column then eluted with the following solvents:

Phase No.	Phase	Ratio	Amounts	Fractions
1	Benzene	100	200 ml	1-20
2	Benzene / Diethyl ether	80 : 20	200 ml	21-40
3	Benzene / Diethyl ether	60 : 40	200 ml	41-60
4	Benzene / Diethyl ether	50 : 50	200 ml	61-80 A
5	Benzene / Diethyl ether	20 : 80	200 ml	81-100 B
6	Diethyl ether	100	200 ml	101-120 C
7	Acetone	100	200 ml	121-140
8	Ethanol	100	200 ml	141-160

These fractions were collected and the solvent was evaporated till dryness. Then they were examined for biological activity against *A. craccivora* using residual film technique. All percent mortalities were corrected for the natural mortality according to Abbott's formula<sup>[1]</sup> Mortality curves were drawn on probit logarithmic graph paper according to the method developed by Finney<sup>[8]</sup>.

**Identification and Determination of Essential Oil and Ethyl Acetate Crude Extract and Their Fractions by GC/MS Analysis:** The obtained essential oil, ethyl acetate crude extracts and their fractions of the wild plant, *Halocnemon strobilacium* were analyzed using GC-MS apparatus. GC-MS Finnigan mat SSQ

7000 Digital DEC 3000. Work station: Digital DEC 3000. Ionization mode Eleven 70. Column: DB-5 capillary column 30-m length, 0.32-mm i.d and 0.25  $\mu\text{m}$  thicknesses. Carrier gas: Helium at 13 psi. Temperature-programming initial column temperature was set at  $50^{\circ}\text{C}$  for 3 min then the temp. was increased by  $7^{\circ}\text{C}/\text{min}$  to reach  $250^{\circ}\text{C}$ , and held for 10 min. at  $250^{\circ}\text{C}$ . Injector temperature was  $200^{\circ}\text{C}$  and the injected volume was 1  $\mu\text{L}$ . Transition-line and ion source temperatures were  $250^{\circ}\text{C}$  and  $150^{\circ}\text{C}$ , respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from m/z 40 to m/z 350. Ionization energy was set at 70 eV. Identification was based on the comparison with the MS computer library (NIST -Software Package, Finnigan) and on the respective retention indices. The separated components were identified by matching them with the National Institute of Standards and Tech (NIST) mass spectral library data.

## RESULTS AND DISCUSSION

**Biological Studies:** Ethyl acetate extracts of *H. strobilacium* showed superior toxicity with  $\text{LC}_{50}$  0.159  $\text{mg}/\text{cm}^2$  in comparisons with petroleum ether, chloroform and ethanol extracts<sup>[3]</sup>. So, ethyl acetate crude extract of *H. strobilacium* was further fractionated by column chromatography using different solvent mixtures possess different polarity. Fractions which eluted with benzene/diethyl ether (1:1) (fraction A) and Benzene/diethyl ether (1:4) (fraction B) were the most effective against *Aphis craccivora*.

The results in Table (1) showed that the ethyl acetate crude extract with  $\text{LC}_{50}$  value 0.159  $\text{mg}/\text{cm}^2$  and  $\text{LC}_{95}$  value 1.845  $\text{mg}/\text{cm}^2$  was toxic toward *Aphis craccivora* as compared with fraction B ( $\text{LC}_{50}$  1.522  $\text{mg}/\text{cm}^2$  and  $\text{LC}_{95}$  4.721  $\text{mg}/\text{cm}^2$ ) as well as fraction A ( $\text{LC}_{50}$  value 1.299  $\text{mg}/\text{cm}^2$ , and  $\text{LC}_{95}$  3.899  $\text{mg}/\text{cm}^2$ ).

**Phytochemical Studies:** After three hours of steam distillation nothing yielded from the essential oil of *Halocnemon strobilacium*, so it is predictable to find a back of volatile in crude as well as fractions A and B.

**Identification of the Crude Extract Constituents:** The components detected, their area % and formula are compiled in Table (2). Saturated and unsaturated fatty acids and their esters were the dominant classes in this extract (69.34%), followed by hydrocarbons (9.89%). Only two terpenes were identified in this crude extract, one of them was Phytol isomer, a diterpene detected with 2.26 %. Oleic acid ester (38.18 %) and Octadecanoic acid (16.44%) were the major constituents (Table 2). Heptadecane (3.10 %),

**Table 1:** LC<sub>50</sub>, LC<sub>95</sub> and Slope values of *Halocnemon strobilacium* crude extract and its different fractions against *Aphis craccivora*.

Treatments	LC <sub>50</sub> mg/cm <sup>2</sup>	LC <sub>95</sub> mg/cm <sup>2</sup>	Slope
Crude extract	0.159	1.845	1.54
Fraction A	1.299	3.899	3.45
Fraction B	1.522	4.721	3.35

**Table 2:** Chemical constituents of the main compounds of the crude extract extracted from the wild plant *Halocnemon strobilaceum*.

Peak no.	Name of compounds	Area %
1	2-Pentanone- 4-Hydroxy-4- methyl	3.939
2	Acetic acid, pentyl ester	0.277
3	Acetic acid, octyl ester	0.394
4	Nonane, 2,6- dimethyl-	0.203
5	Benzene, 1,3,5- trimethyl	0.905
6	Decane	0.917
7	Decane ,2,4,6- trimethyl	0.326
8	2-undecane, 7- methyl-	0.328
9	Benzene, methyl (1- methylethyl)-	0.428
10	Undecane, 4,7- dimethyl-	0.543
11	Benzoic acid	1.162
12	Dodecane, 2,6,11- trimethyl-	0.797
13	2-Cyclohexen-1-ol, 3- methyl-6-(1- methylethyl)-, trans-	0.643
14	2-Decenal (E)	0.374
15	Tetradecane, 5- methyl	0.825
16	Nonanoic acid	0.111
17	Pentadecane	0.360
18	Decanoic acid	0.777
19	Hexadecane	0.925
20	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a- trimethyl (R)	0.457
21	Benzoic acid, 4-hydroxy -3-methoxy-	0.108
22	Heptadecane	3.102
23	Heptadecane, 7- methyl-	1.048
24	Tetradecanoic acid	3.705
25	Octadecane	1.314
26	2-Pentadecanone, 6,10,14 - trimethyl-	2.099
27	Pentadecanoic acid	3.153
28	Lumiflavine	4.215

**Table 2:** Continued

29	Pentadecanoic acid, 14 -methyl-, methyl ester	0.734
30	Hexadecanoic acid	1.497
31	Heptadecanoic acid	2.071
32	8- Octadecanoic acid, methyl ester, (E)-	1.997
33	Phytol	2.262
34	Oleic acid, 3-hydroxypropyl ester	38.180
35	Octadecanoic acid	16.441
36	$\alpha$ - Amyrin	3.382

**Table 3:** Chemical constituents of the main compounds of the fraction A extracted from the wild plant *Halocnemum strobilacium*.

Peak no.	Name of compounds	Area %
1	Benzene, 1,3,5- trimethyl	0.845
2	Furan, 2-propyl-	0.677
3	Decane	1.493
4	Cyclohexanol, 2-(1-methylpropyl)-	1.370
5	2-cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-, cis-	1.092
6	1-Adamantanol	6.329
7	2-cyclohexen-1-one, 4-(1-methylethyl)	0.491
8	2-cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-, trans-	1.710
9	Cyclohexanone, 2-(1-methylethylidene)-	0.953
10	Bicyclo [3.1.0] hexa-3-en-2-one, 4- methyl-1-(1-methylethyl)-	6.703
11	Thymol	3.116
12	2(4H)-Benzofuranone, 5, 6, 7, 7a-tetrahydro-4,4,7a- trimethyl, (R)	1.425
13	Heptadecane	2.814
14	Tetradecanoic acid	3.879
15	6-methyl-2-tridecanone	2.008
16	Pentadecanoic acid, 13-methyl, methyl ester	3.742
17	Lumiflavine	35.660
18	Octadecanoic acid, methyl ester (E)-	1.159
19	8-Oleic acid, 3-hydroxypropyl ester	14.744
20	Octadecanoic acid, 16-methyl-, methyl ester	4.413

Tetradecanoic acid (3.71%), Pentadecanoic acid (3.15 %)  $\alpha$ -amyrim (3.38%) and Lumiflavine (4.21) were found in appreciable amounts. Ketones constituted 6.50 % of the detected compounds.

**Identification of Fraction a Constituents:** Table (3) shows that, fraction A contained 20 main compounds, hydrocarbons, fatty acids and their esters were found as the dominant groups in this fraction. Of the identified

components, Lumiflavine represented about 35.66 % and such high percentage may explain the medicinal and domestic uses of *Halocnemum strobilacium*. Five fatty acids and esters were identified and constituted 39% with oleic acid ester (14.74%), Octadecanoic acid methyl ester (4.41 %) and Tetradecanoic acid (3.88 %) being the major representatives. Only two hydrocarbons were detected in the fraction namely decane (1.50%), and

**Table 4:** Chemical constituents of the main compounds of the fraction B extracted from the wild plant *Halocnemon strobilacium*.

Peak no.	Name of compounds	Area %
1	4-Hydroxy-4-methylpentanone	1.653
2	Acetic acid, 2-methylpropyl ester	0.502
3	1,3-Dioxolane, 2, 2, 4- trimethyl	1.575
4	2-Pentanone- 4-Hydroxy-4- methyl	5.753
5	1-Butanol, 2-methyl-, acetate	0.413
6	Hexanoic acid, 2-methyl	1.174
7	2-Pentanone, 4-methoxy-4-methyl	4.726
8	Benzene, methyl (1- methylethyl)	1.126
9	Heptanoic acid	3.824
10	Decane, 2, 4, 6- trimethyl	1.008
11	Undecane, 4, 7-dimethyl	5.202
12	3, 5-Heptadien-2-one, 6- methyl-, (E)-	2.479
13	Benzoic acid	1.553
14	Benzene acetic acid	2.615
15	Thymol	6.401
16	Decanoic acid	6.204
17	Gamma-Terpineol	2.294
18	2(4H)-Benzofuranone 5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl-(R)	1.761
19	Tyramine, N-formyl-	1.563
20	Hexadecanoic acid	22.689
21	9-Octadecanoic acid, methyl ester	3.444
22	9-Octadecanal, (Z)-	3.294
23	Octadecanoic acid	8.748

Heptadecane (2.81 %). Six monoterpene hydrocarbons were identified in the fraction (12.32 %), among them Bicyclohexenone derivative (6.70 %) which detected as the major component in this class in addition to Thymol (3.11 %), which is the main constituent of several important oils belonging to the Labiatae family e.g. thyme oil and ajowan fruit Oil.

**Identification of Fraction B Constituents:** The chemical composition of fraction B was studied by capillary gas chromatography-mass spectrometry. The components found, their formula and percentage composition are shown in Table (4). Twenty-three compounds were identified. Unxygenated compounds amount to 9.89, the main hydrocarbons being dimethyl undecane (5.20%) and trimethyldecane (1.01 %). Oxygenated compounds constitute 90.11 % of the fraction comprising fatty acids and esters, ketones as

well as aldehydes (46.08 %, 17.94 % and 3.29 % of the fraction, respectively). The main ester identified was Octadecanoic acid, methyl ester (3.44%) while Hexadecanoic acid was the main fatty acid detected (22.69 %).

Table (5) shows the area percent of the active compounds identified in the crude extract and the most potent fractions of *H. strobilacium*. Fatty acids methyl esters were the main components in the crude extract with percent area 41.582 % followed by fatty acids with area percent 27.755 %'. The other components were identified, Hydrocarbons 9.891 %,  $\alpha$ - amyryn with area percent 3.382 % and Monoterpenes 2.905 %. In fraction A, Lumiflavine with area percent 35.660 % was the main component in this fraction. Fatty acids methyl esters with area percent 24.058 %, Monoterpenes with percent area 12.621%, Hydrocarbons 6.738 % and fatty acids with the area percent 3.879 % were

**Table 5:** Percent area of active components of the ethyl acetate crude extract and their fractions of *H. strobilacium*.

Compounds	% Area of the compounds in fractions:		
	Crude extract	Fraction A	Fraction B
<b>-Monoterpenes</b>			
-Monocyclic	0.643	5.918	8.695
-Bicyclic ( $\alpha$ -Pinene)	-	6.703	-
<b>*- Diterpenes</b>			
- Phytol	2.262		
Total	2.905	12.621	8.695
<b>** -Fatty acids</b>			
-Tetradecanoic acid	3.705	3.879	-
-Pentadecanoic acid	3.153	-	-
-Hexadecanoic acid	1.497	-	22.689
-Octadecanoic acid	16.441	-	8.748
-Others	2.959	-	11.202
Total	27.755	3.879	42.639
<b>*-Fatty acids esters</b>			
<b>*- Methyl esters</b>			
-Pentadecanoic acid	0.734	-	-
-Octadecanoic acid	1.997	5.572	3.444
-Others	38.851	18.486	-
Total	41.582	24.058	3.444
Lumilflavine	-	35.660	
*-Hydrocarbons	9.891	6.738	6.210
*- $\alpha$ - Amyrin	3.382		
LC <sub>50</sub> mg/cm <sup>2</sup>	0.159	1.299	1.522

identified components in this fraction. Also, fraction B contain fatty acids with area percent 42.639 %, Monoterpenes with percent area 8.695 %, Hydrocarbons 6.210 % and fatty acids methyl esters with area percent 3.444 %. Qualitative and quantitative differences which found in the chemical constituents between the crude and the fractions and between fractions A and B, such differences may be explained the effectiveness differences among them. On the other hand, the lower efficacy of fraction B in comparison to fraction A could be explained by the lower contents of fatty acids as well as their esters.

These results were in agreement with many investigators. The potency of botanical fatty acids was reported by Messina and Renwick,<sup>[11]</sup> and Abdallah *et*

*al.*,<sup>[2]</sup> against weevil species. Tare and Sharma<sup>[13]</sup> compared the larvicidal properties of different fatty acids constituents against *Aedes aegypti* and found that the oleic acid was the most effective one. Bestmann *et al.*,<sup>[6]</sup> isolated six aphicidal constituents by steam distillation of freshly harvested leaves of *Rhus typhina* (Anacardiaceae). These compounds were phytol, linalool, tetradecanol, decosanol, tetradecanoic acid and hexadecanoic acid. They tested these compounds individually, in the same concentrations as those found in TLC fraction for insecticidal activity; none of the components exhibited the same activity as that of the whole fraction. Deshpands *et al.*,<sup>[7]</sup> reported oleic as insecticidal components from *Nigella sativa* (Ranunculaceae), which were found to be toxic to the

pulse beetle, *Callosobruchus chinensis*. Peterson *et al.*,<sup>[12]</sup> reported that, of the numerous fractions obtained from the crude plant extracts of *Chenopodium ambrosioides*, *Conyza dioscoridis* and *Convolvulus arvensis*, eleven fractions were found to be active against stored grain pests. The chemical structures of the compounds present in the eleven fractions were found, by gas chromatography/mass spectrometry, to include predominantly long-chain fatty acid esters of hexadecanoic, arachidonic and octadecanoic acids. Messeha, Samia<sup>[10]</sup> reported linoleic acid as the main constituent (37.6 %) of the saponification portion of petroleum ether extract of *Nigella sativa*, which were found to be toxic to the mosquito *Culex pipens*.

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