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CHARACTERIZATION OF CHEMICAL CONSTITUENTS OF *Citrullus colocynthis* (L.) EXTRACTS AND THEIR RELATION TO TOXICITY AGAINST COWPEA APHID, *Aphis Craccivora* Koch.

Heba Y. E. Ibrahim
Plant protection Research Institute, ARC, Dokki, Giza, Egypt.

ABSTRACT

Citrullus colocynthis (L.) fruits were separately extracted with different organic solvents (petroleum ether, ethyl acetate and methanol). The volatile components of each solvent extract were characterized and identified by GC/MS technique. The insecticidal properties of each extracts were tested against adults and nymphs of cowpea aphid, *Aphis craccivora* Koch. Methanol extract was the most efficient against both adults and nymphs with LC_{50} : 639.68 ppm and LC_{50}: 555.9 ppm, respectively. Ethyl acetate extract showed moderate efficacy against adults and nymphs with LC_{50} : 968.85 ppm and LC_{50}: 833.58 ppm, respectively. Petroleum ether extract showed weak toxicity against adults and nymphs with 3144.99 ppm and LC_{50}: 2768.52 ppm, respectively.

Keywords: *Aphis craccivora*, *Citrullus colocynthis*, GC/MS analysis of colothyn extract.

INTRODUCTION

Cowpea Aphid, *Aphis craccivora* Koch. is a serious pest attacking a wide spectrum of economic plants causing great loss in yield. It infests many legumes, cotton, and as well as Shephered`- purse, lambsquarters, lettuce, pepperweed, *Polygonum sp*. and *Rumex sp*. Cowpea aphid transmits nearly 30 plant viruses including cotton curliness virus (Kennedy *et al*., 1962; Blackman and Eastop, 1984). Also, it transmits Peanut stripe virus (PStV) and Peanut mottle virus (PMV) (Sreenivasulu and Demeski, 1988), Chili veinal mottle virus and pepper mottle virus (Cerkauskas, 2004). Moreover, cowpea aphid secretes toxic saliva into the host plants causing symptoms ranged from simple stippling of the plant leaves to extensive disruption of the entire plant (Summers *et al*., 1996).

The extensive use of traditional insecticides have created many problems, so, the scientists and environmental policy makers seek less toxic alternatives for controlling insect pests. Natural products of plant origin is one of the most important safer alternatives. Bitter apple, *Citrullus colocynthis* (L.) (Cucurbitaceae) or colothyn has gained deserving attention as natural insecticide because it has deterrent, antifeedant, growth regulating and fertility-reducing properties on insects (Prabuseenivasan, *et al*., 2004; Pravin *et al*., 2013 and Soam *et al*., 2013). Several active chemical constituents of this plant were isolated and characterized. These including bitter substances (colocynthin and colocynthetin); cucurbitacins A, B, C, D and E (α-elaterin) (Adam *et al*., 2001); cucurbitacin glycosides (Hatam, *et al.,* 1989 and Abbas, *et al.,* 2006); flavenoides and flavon glycosides (Maatooq, *et al*., 1997 and

Abbas *et al*., 2006) The goal of this study was to characterize the main volatile constituents of different organic solvent extracts of *C. colocynthis* fruit, then, evaluate the insecticidal effects of these extracts against *A. craccivora* in the laboratory.

MATERIALS AND METHODS

Rearing of cowpea aphid, *A. craccivora*:

The strain of *A. craccivora* was obtained from the farm of Faculty of Agriculture, Mansoura University, and had been known to be free from insecticidal contamination. Aphid strain was reared on broad bean (2-3 weeks old) planted in small pots (15 cm3) and kept under plastic greenhouse conditions of 25 ± 5^{o}C, 70 ±5 RH and 12:12 L: D. Plants were changed as needed once or twice per week. The transfer of aphids from old plants to new ones was carried out by allowing aphids to over voluntarily from detached leaves placed on new plants or by artist' s brush.

Extraction of the active fractions from tested plant:

Fruits of *C. colocynthis* were grinding into fine powder by using electric mill then it was weighted and apportioned to three equal portions. The three portions were separately soaked in Petrolleum ether, ethyl acetate and methanol for about a week then well shacked and filtered, then, washed by the same solvent three times and let solvents evaporate using rotator evaporator. The extracted crudes were kept in deep freezer until use.

Chemical study:

A sample of each *C. colocynthis* extract was analyzed by GC/MS technique for characterization and identification of its volatile components. GC/MS analysis of the volatile fractions were performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J & W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poly dimethyl-siloxane (0.5 µm. film thickness). The oven temperature was programmed from 50oC for 3 min., at isothermal, then heating by 7oC/min. to 250oC and isothermally for 10 min., at 250oC.Injector temperature was 200oC and the volume injected was 0.5 µl. Transition-line and ion source temp. were 250oC and 150oC, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to 300. Ionization energy was set at 70 eV. (Faculty of Pharmacy, Mansoura Univ.).

Bioassay study:

Plant leaves were transferred to 15 cm Petri – dishes. Each ten aphid individuals of the same age were transferred to a Petri-dish to be considered as one replicate. Each concentration had three replicates and another three replicates sprayed only with water and 0.05% aqueous Tween 80 to be considered as control. In case of testing the efficiency on nymphal stage, the adults were allowed to lay nymphs on the surface of the host leaves for a period of 24 hours, then the parents were removed and nymphs were treated when reaching the age of two days. Cowpea aphid had been treated with different concentrations of extracts by spraying methods, then the lids of Petri-dishes were sealed and placed in an incubator at 25 ± 2C^{o} ,70 ± 5 % RH., and photoperiod 12:12 hs L:D. Observations were recorded daily and the experiment continued for seven days. Broad bean leaves were replaced by fresh ones after first three days of the treatment to provide a source of nutrition.

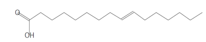
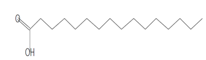
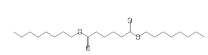
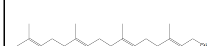
At the end of this period, the average of mortality percentages of *A. craccivora* was estimated and corrected using Abbott's formula (1925). The corrected mortality percentage of each extract was statistically calculated according to Finney (1971). The corresponding concentration probit lines (LC-p lines) were estimated in addition to determination of LC_{50} ,LC_{90} , slope values and toxicity index according to Sun`s equation.

RESULTS AND DISCUSSION

Chemical study:

The GC/MS chromatogram showed sixteen peaks corresponding to sixteen compounds. These compounds were characterized by comparing their mass spectra with those of their analogous reported by NIST library. The obtained results were reported in Table (1). Compound 12, (9,12-Octadecadienoic acid) was found to be antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary. (Maruthupandian and Mohan, 2011). Also, it was reported that compound 14, (oleic acid) has insecticidal, herbicidal and fungicida activities.

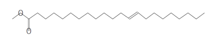
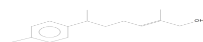
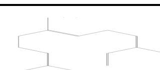
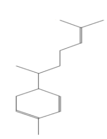
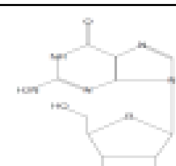
Table (1): The GC/MS an analysis of Pet. ether fraction of *C. colocynthis*

S.N	Compound name	R.T.	Area %	M.F.	Compound Structure
1	Nonane	4.39	0.85	C_9H_{20}	
2	Decane	6.41	1.44	$C_{10}H_{22}$	
3	Undecane	8.14	1.61	$C_{11}H_{24}$	
4	Dodecane.	9.67	1.23	$C_{12}H_{26}$	
5	9-Hexadecenoic acid	14.24	0.50	$C_{16}H_{30}O_2$	
6	Tridecane.	14.79	2.84	$C_{13}H_{28}$	
7	Hexadecane.	14.79	2.84	$C_{16}H_{34}$	
8	Tetradecane.	14.79	2.84	$C_{14}H_{30}$	
9	Nondecane.	16.96	3.80	$C_{19}H_{40}$	
10	Pentadecane.	17.96	1.84	$C_{15}H_{32}$	
11	n- Hexadecanoic acid.	18.63	1.44	$C_{16}H_{32}O_2$	
12	9,12-Octadecadienoic acid.	20.20	2.45	$C_{18}H_{32}O_2$	
13	Linoleic acid ethyl ester.	20.20	2.45	$C_{20}H_{36}O_2$	
14	Oleic Acid.	20.51	1.84	$C_{18}H_{34}O_2$	
15	Hexanedioic acid, dioctyl ester.	21.32	0.44	$C_{22}H_{42}O_4$	
16	Geranylgeraniol.	28.67	7.94	$C_{20}H_{34}O$	

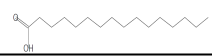
2. Study of ethyl acetate fraction:

The GC/MS chromatogram showed fifty peaks corresponding to fifty compounds. These compounds were characterized by comparing their mass spectra with those of their analogous reported by NIST library. The obtained results were reported in Table| (2). Compound 23, (9,12-octadecadienoic acid, methyl ester) was already isolated from *C. colocynthis* fruits and *Mentha microphylla* by Farghaly *et al*. (2009) who proved the toxicity of it against whitefly (*Bemisia tabaci*) and aphid (*A. craccivora*). Also, Antonious *et al*., (2007), proved the toxicity of this compound as insecticide against cabbage hooper, *Trichopulsia ni* larvae.

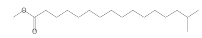
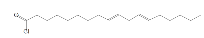
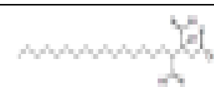
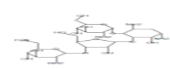
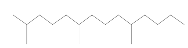
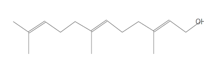
Table (2): The GC/MS analysis of ethyl acetate fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
1	2-Pentanone, 4-hydroxy-4-methyl-	3.54	35.75	$C_6H_{12}O_2$	
2	13-Docosenoic acid, methyl ester.	4.06	0.18	$C_{23}H_{44}O_2$	
3	1-Butanol, 3-methyl-, acetate	4.06	0.18	$C_7H_{14}O_2$	
4	Digitoxin	10.72	0.03	$C_{41}H_{64}O_{13}$	
5	6-(p-Tolyl)-2-methyl-2-heptenol	13.33	0.18	$C_{15}H_{22}O$	
6	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl -, (Z,E)-	13.48	0.29	$C_{15}H_{24}$	
7	Pterin-6-carboxylic acid	13.48	0.29	$C_7H_5N_5O_3$	
8	Zingiberene	14.19	0.13	$C_{15}H_{24}$	
9	Guanosine	14.64	0.15	$C_{10}H_{13}N_5O_5$	

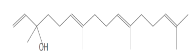
Continue Table (2): The GC/MS analysis of ethyl acetate fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
10	d-Glycero-d-galacto-heptose	14.64	0.15	$C_7H_{14}O_7$	
11	Arginine	15.78	0.07	$C_6H_{14}N_4O_2$	
12	2-Pentadecanone, 6,10,14-trimethyl-	17.30	0.05	$C_{18}H_{36}O$	
13	Folic Acid	17.85	0.09	$C_{19}H_{19}N_7O_6$	
14	Cyclopentaneundecanoic acid, methyl ester	18.11	0.6	$C_{17}H_{32}O_2$	
15	Tridecanoic acid, methyl ester	18.11	0.6	$C_{14}H_{28}O_2$	
16	Methyl tetradecanoate	18.11	0.6	$C_{15}H_{30}O_2$	
17	n-Hexadecanoic acid	18.50	1.48	$C_{16}H_{32}O_2$	
18	Undecanoic acid (35)	18.50	1.48	$C_{11}H_{22}O_2$	
20	Hexadecanoic acid, ethyl ester	18.76	0.28	$C_{18}H_{36}O_2$	
21	Dodecanoic acid, 3-hydroxy-	18.76	0.28	$C_{12}H_{24}O_3$	
22	Oleic Acid	18.76	0.28	$C_{18}H_{34}O_2$	

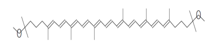
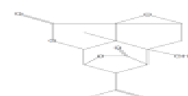
Continue Table (2): The GC/MS analysis of ethyl acetate fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
23	9,12-Octadecadienoic acid, methyl ester.	19.69	2.51	$C_{19}H_{34}O_2$	
24	Hexadecanoic acid, 15-methyl-, methyl ester.	19.97	0.31	$C_{17}H_{32}O_2$	
25	Decanoic acid, methyl ester	19.97	0.31	$C_{11}H_{22}O_2$	
26	Heneicosanoic acid, methyl ester	19.97	0.31	$C_{22}H_{44}O_2$	
27	9,12-Octadecadienal	20.09	2.66	$C_{18}H_{32}O$	
28	9,12-Octadecadienoyl chloride, (Z,Z)-	21.90	0.26	$C_{18}H_{31}ClO$	
29	Agaricic acid	23.18	0.08	$C_{22}H_{40}O_7$	
30	Scilliroside	23.21	0.07	$C_{32}H_{44}O1_{2}$	
31	Paromomycin I	24.25	0.08	$C_{23}H_{45}N_5O_{14}$	
32	Tetradecane, 2,6,10-trimethyl-	25.06	5.86	$C_{17}H_{36}$	
33	Octadecane, 1-(ethenyloxy)-	25.24	3.23	$C_{20}H_{40}O$	
34	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	25.42	2.52	$C_{15}H_{26}O$	
35	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	26.91	0.43	$C_{27}H_{44}O_3$	

Continue Table (2): The GC/MS analysis of ethyl acetate fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
36	Gibberellic acid	26.91	0.43	$C_{19}H_{22}O_6$	
37	Pregn-5-ene-3,8,11,12,14,20-hexol, (3á,11à,12á,14á)-	27.27	0.03	$C_{21}H_{34}O_6$	
38	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, -(E,E)-	28.56	4.09	$C_{20}H_{34}O$	
39	Geranylgeraniol	28.56	4.09	$C_{20}H_{34}O$	
40	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	28.83	1.84	$C_{18}H_{30}O_2$	
41	Nitro-L-arginine	30.05	0.07	$C_6H_{13}N_5O_4$	
42	Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)-	30.19	0.34	$C_{30}H_{50}O_6$	
43	Glucobrassicin	30.19	0.34	$C_{10}H_9N_2S_2O_4$	
44	Dasycarpidan-1-one	30.41	0.04	$C_{17}H_{20}N_2O$	
45	Vitamin A palmitate	30.41	0.04	$C_{36}H_{60}O_2$	
46	Cholic acid	32.04	0.69	$C_{24}H_{40}O_5$	

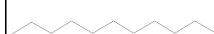
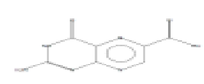
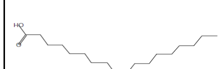
Continue Table (2): The GC/MS analysis of ethyl acetate fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
47	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	32.39	0.29	$C_{42}H_{64}O_2$	
48	Hydrocortisone hemisuccinate [anhydrous]	36.65	0.37	$C_{25}H_{34}O_8$	
49	Picrotoxinin	32.88	0.06	$C_{15}H_{16}O_6$	
50	Corynan-17-ol, 19-didehydro-10-methoxy-10-Methoxycoryn-18-en-17-ol	38.76	0.1	C_7H_8NO	

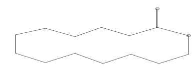
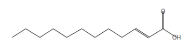
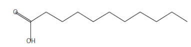
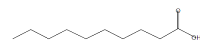
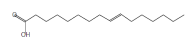
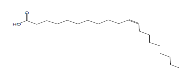
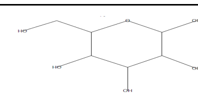
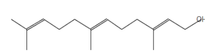
2. Study of methanol fraction:

The GC/MS chromatogram showed twenty peaks corresponding to twenty compounds. These compounds were characterized by comparing their mass spectra with those of their analogous reported by NIST library. The obtained results were reported in Table (3).

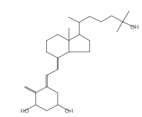
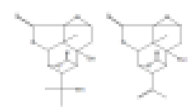
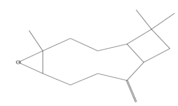
Table (3): The GC/MS analysis of Methanol fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
1	Cystine	2.70	0.86	$C_6H_{12}N_2O_4S_2$	
2	Acetaldehyde	2.70	0.86	C_2H_4O	
3	Undecane	8.00	1.34	$C_{11}H_{24}$	
4	Pterin-6- arboxylic acid	12.05	2.37	$C_7H_5N_5O_3$	
5	Oleic Acid	18.32	2.13	$C_{18}H_{34}O_2$	

Continue Table(3): The GC/MS analysis of Methanol fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
6	Oxacyclotetradecan-2-one	18.32	2.13	$C_{13}H_{24}O_2$	
7	2-Dodecenoic acid	18.32	2.13	$C_{12}H_{22}O_2$	
8	Undecanoic acid	18.50	11.06	$C_{11}H_{22}O_2$	
9	n-Decanoic aci	18.50	11.06	$C_{10}H_{20}O_2$	
10	9-Hexadecenoic acid	20.11	7.45	$C_{16}H_{30}O_2$	
11	cis-11-Eicosenoic acid	20.11	7.45	$C_{20}H_{38}O_2$	
12	L-Glucose	21.87	0.40	$C_6H_{12}O_6$	
13	Farnesol isomer a	25.41	0.88	$C_{15}H_{26}O$	
14	Squalene	25.41	0.88	$C_{30}H_{50}$	

Continue Table (3) : The GC/MS analysis of Methanol fraction of *C. colocynthis*

S.N	Compound No.	R.T.	Area%	M.F.	Compound Structure
15	Calcitriol	27.33	0.67	$C_{27}H_{44}O_3$	
16	Picrotoxin	27.33	0.67	$C_{30}H_{34}O_{13}$	
17	Aldosterone	27.79	0.25	$C_{21}H_{28}O_5$	
18	Caryophyllene oxide	28.56	6.05	$C_{15}H_{24}O$	
19	9,10- ecocholesta-5,7,10(19)-triene-3,24, 25-triol, (3á,5Z,7E)-	28.84	4.64	$C_{27}H_{44}O_3$	
20	3,4-Dimethoxycinnam-ic acid	29.88	5.04	$C_{11}H_{12}O_4$	

Bioassay Study:

Efficiency of different solvent extracts of colothyn extracts against *A. craccivora*:

Data in Tables (4&5) showed the efficiency of colothyn extracts against both of adults and nymphs of cowpea aphid. Data cleared that the mortality percent of both of adults and nymphs increased with increasing the tested extract concentrations. Methanol extract was the most effective one against both adults and nymphs, followed by ethyl acetate extract that showed moderate efficiency and then petroleum ether extract. For adults, Methanol extract showed LC_{50} of 639.68 ppm and LC_{90} of the 2472.4 ppm followed by ethyl acetate extract with LC_{50} of 968.85 ppm and LC_{90} of 5429.94 ppm, then petroleum ether extract with LC_{50} of 3144.99 ppm and LC_{90} of 32620.61 ppm. Regarding to nymphs, they showed more susceptibility to all extracts. Methanol extract was the most effective with LC_{50} of 555.9 ppm and LC_{90} of 2580.28 ppm, followed by ethyl acetate extract with LC_{50} of 833.58 ppm and LC_{90} of 4325.47 ppm, then petroleum ether extract with LC_{50} of 2768.52 ppm and LC_{90} of 27195.44 ppm.

Table 4: Efficiency of colothyn extracts against adults of *A.craccivora* under laboratory conditions of 25 ± 2 C^0 and 70 ± 5% RH.

Treatment	Slope	LC_{50}	LC_{90}	Toxicity index
Petroleum ether extract	1.26	3144.99	32620.61	20.34
Ethyl acetate extract	1.71	968.85	5429.94	66.02
Methanol extract	2.18	639.68	2472.4	100

Table 5: Efficiency of colothyn extracts against nymphs of *A.craccivora* under laboratory conditions of 25 ± 2 C^0 and 70 ± 5% RH.

Treatment	Slope	LC_{50}	LC_{90}	Toxicity index
Petroleum ether extract	1.29	2768.52	27195.44	20.08
Ethyl acetate extract	1.79	833.58	4325.47	66.69
Methanol extract	1.92	555.9	2580.28	100

The differences in chemical constituents of different solvent extracts of *C. colocynthis* fruits may explain the observed differences of efficacy of the three different extracts. Results obtained about colothyn extracts toxicity to cowpea aphid agreed with Farghaly *et al*. (2009), who revealed that the methanol extract of colothyn was the most effective with LC_{50} of 621.94 ppm. Also, agreed with Torkey *et al*. (2009) who evaluated the toxicity of different solvent extracts of *C. colocynthis* fruit against *A. craccivora*. He revealed that the highest insecticidal effect was obtained from ethanol extract.

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تعريف المكونات الكيميائية لمستخلصات نبات الحنظل و علاقتها بالسمية ضد من اللوبيا.

هبه يوسف السيد ابراهيم
معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى- الجيزة- مصر.

تم استخلاص مكونات ثمار الحنظل باستخدام المذيبات العضوية المختلفة (الايثير البترولي، أسيتات الايثيل،و الميثانول) وتم تحديد هوية المركبات المتطايرة المتواجدة بالمستخلصات المختلفة عن طريق استخدام تقنية كروماتوجرافيا الغاز/ طيف الكتلة. أيضًا تم تقييم الفاعلية الإبادية لكل من هذه المستخلصات ضد الأطوار البالغة وحوريات حشرة من اللوبيا، فكان مستخلص الميثانول هو الأعلي كفاءة يليه مستخلص أسيتات الإيثيل ثم مستخلص الإيثير البترولي. فلقد أوضحت الدراسة أن مستخلص الميثانول أظهر تركيز نصف مميت على الأطوار البالغة والحوريات: ٦٣٩.٦٨ جزء/المليون و ٥٥٥.٩ جزء/المليون، على التوالي. أيضًا ، مستخلص أسيتات الايثيل أظهر تركيز نصف مميت : ٩٦٨.٨٥ جزء/المليون و ٨٣٣.٥٨ جزء/المليون لكل من الأطوار البالغة والحوريات على التوالي. بينما مستخلص الايثيرالبترولي أظهر تركيز نصف مميت: ٣١٤٤.٩٩ جزء/المليون و ٢٧٦٨.٥٢ جزء/المليون لكل من الأطوار البالغة والحوريات على التوالي.