



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2017; 5(5): 1980-1985

© 2017 JEZS

Received: 13-07-2017

Accepted: 14-08-2017

**Hanan S Gaballa**

Agricultural Biochemistry,  
Fac. of Agric., Cairo University,  
Egypt

**Mona N Wahba**

Plant Protection Research  
Institute, Agricultural Research  
Center, Egypt

**Magda H Naroz**

Economic Entomology and  
Pesticides, Fac. of Agric., Cairo  
University, Egypt

## Antioxidant and insecticidal effect of squirting cucumber, *Ecballium elaterium* extracts against *Aphis craccivora* and *Phthorimaea operculella*

Hanan S Gaballa, Mona N Wahba and Magda H Naroz

### Abstract

The aim of this study was to test the antioxidant and insecticidal activity of these extracts against, *Aphis craccivora* (Homoptera: Aphididae) and *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Results showed that the antioxidant activity of different extracts ranged from 10 to 1000 µl/ml. Where found that ethanolic and acetone extracts were having higher activity than other extracts and standard by radical DPPH scavenging and Hydroxyl radical scavenging activity. Identification of phenolic compounds of ethanolic extract was carried out by GC / MS / MS analysis. It has been shown that, *E. elaterium* may contain compounds that can act as botanical pesticides. Three concentrations (100, 500 and 1000 µg/ml) of each dry extract were obtained by dissolving the dry extract in 0.01% solution of dimethyl sulfoxide (DMSO). The Results showed that the high concentration (1000µg/ml) of the extracts caused high mortality of *A. craccivora* adults after 72h from exposure. Both the acetone and ethanol extracts showed higher mortality (68 and 71% respectively) than the hexane, chloroform and ethyl acetate extracts (42, 58 and 63%, respectively). All extracts reduced larval penetration deterrent of *P. operculella* at concentration 1000 µg/ml. The data demonstrated that ethanol, chloroform and acetone extracts were highest reduction percentage of larval penetration of *P. operculella* 70.5%, 72.1% and 73.3%, respectively, at the same concentration.

**Keywords:** *Ecballium elaterium*, *Aphis craccivora*, *Phthorimaea operculella*, phenolic compounds, antioxidant activity and insecticidal activity

### Introduction

Aphids (Homoptera: Aphididae) are a large group of the insects. They are serious pests of a wide range of agricultural crops that they can cause severe damage directly, by depriving the plant of its essential nutrients and indirectly, by transmitting viruses most aphid species are host-specific and known to feed on a restricted range of host plants [1]. Black legume aphid, *Aphis craccivora* is from important aphids that infested Fabaceae family (bean, peas, etc).

The potato tuber moth, *Phthorimaea operculella* (Zeller) is (Lepidoptera: Gelechiidae), is an oligophagous and serious pest of the solanaceous plants such as potato, tomato, tobacco and egg-plant worldwide. It is widely spread in Egypt especially in the northern areas of Lower Egypt. It attacks several cultivated solanaceous plants. The preferred hosts are potato and tobacco plants, but potato is considered the main host to this insect in different parts of the world. It causes serious damage to potato crops in both field and storage [2].

Intensive use of insecticides could be developing to resist insects, also may leave hazardous residues in the fruits and pollute the agro ecosystem. So the scientists began to search about alternative methods of insecticides. Allahverdizadeh and Mohammadi [3] found that *Marrubium vulgare* and *Achillea millefolium* leaf extracts caused great reductions in the number of eggs deposited of *P. operculella* moth. Among the tested extracts, except hexane extract of *M. vulgare* and ethyl acetate extract of *A. millefolium* remains induced the greatest antioviposition deterring effect, with no eggs oviposited. The fumigant toxicity of the *M. vulgare* and *A. millefolium* crude extract against 1<sup>st</sup> larval instar and adults of *P. operculella* was different. So plant extract may be better substitutes of synthetic ones. *Ecballium elaterium* (L.) (Cucurbitaceae) is a medicinal plant found abundantly in the wild South-West Europe and North Africa in stony ground, in the rubble and slope [4]. The plant is known as grass officinal herb and has a long tradition of uses in the Mediterranean basin. It is often used in dropsy (edema), especially pulmonary edema and also as a repulsive in brain diseases [5].

### Correspondence

**Hanan S Gaballa**

Agricultural Biochemistry,  
Fac. of Agric., Cairo University,  
Egypt

The fresh raw juice is frequently used in the treatment of sinusitis and jaundice by nasal aspirates [6]. Many biological activities of this species have been attributed to cucurbitacins and their glycosylated derivatives such as antiproliferative activity on various types of cancer cells [7, 8]. But his most interesting potential activity can be antiviral [9]. The plant is perennial, fleshy, rough hairy with stems 30 - 100 cm long. The flowers are greenish-yellow and the fruit are large juicy berry, 3 - 4 cm, ovate-oblong, detaching itself explosively at maturity scattering seeds and juice (Fig. 1). The mature seeds are the oil-bearing part of the fruit [10] and the watery juice inside the fruit yields a powerful drug called “elaterium”.



Fig 1: The flowers of *E. elaterium*

## Materials and Methods

### Extraction of the Plant Material

Seeds of *E. elaterium* (L.) were collected from the natural habitat of the faculty of Agriculture, Cairo University in September in 2015 and identified by Flora & Plant Classification Research Department, Agricultural Research Centre in Egypt. Extracts obtained by successive extraction of *E. elaterium* seeds (after defatted by chloroform/methanol, 2:1) using solvents different in their polarity (hexane (60-80 °C), chloroform, ethyl acetate, acetone and ethanol). The extracts obtained were evaporated to dryness. Under vacuum at 40 °C using rotary evaporator and dried at 50 °C under vacuum oven for 5-6 hours.

### Drugs and chemicals

Ascorbic acid, 2, 2 diphenyl-1-picryl hydrazyl hydrate (DPPH), ammonium molybdate, beta carotene, xanthine oxidase were purchased from Himedia, Mumbai. Hypoxanthine, 2-deoxy-2-ribose, quercetin and butyl hydroxyl toluene (BHT) were procured from SRL, Mumbai. Thiobarbituric acid and trichloroacetic acid were purchased from SD Fine ltd., Mumbai. All other chemicals employed in the study were of analytical grade which was purchased from respective suppliers.

### Free radical scavenging assay

The free radical scavenging effect of different extracts was assessed by the decoloration of a methanolic solution of DPPH radical (violet color) according to the method of [15]. The quenching of free radicals by extracts was evaluated spectrophotometrically at 517nm against the absorbance of the DPPH radical. A freshly prepared DPPH Solution was used for the assay. Samples were dissolved in methanol and methanolic solution of DPPH served as a control. A mixture of 5 ml from DPPH solution and 10, 30, 50, 100, 500 or 1000 µg/ml of plant extracts was used. After 30 min, the absorbance was measured at 517 nm using a spectrophotometer (Jen way 6305 UV/Vis Spectrophotometer). BHT was used as a reference free radical

The fruit juice of the plant contains several bioactive ingredients such as proteins, lipids, cucurbitacins (B, D, E, I and L) and cucurbitacin derivatives such as glycosyl cucurbitacins and triterpenoids glycosides [11, 12, 13,4]. These tetracyclic triterpenoids compounds such as A, E, D, I are of interest medicinally because of their cytotoxic, antitumor and antijaundice properties [14]. Still these bioactive compounds have been reported to possess other pharmacological. The main objectives of this work are the large scale production of the bioactive compounds of *E. elaterium* and determination of the antioxidant activities of different extracts against, *Aphis craccivora* and *Phthorimaea operculella*.

scavenger; all reactions were carried out in hexaplicates.

The percentage of DPPH decoloration was calculated as following;

$$\text{Decolouration \% (I)} = \frac{A_t - A_c}{A_c} \times 100$$

Where  $A_t$  was the absorbance of the tested extracts  
 $A_c$  was the absorbance of control DPPH solution only.

### Deoxyribose degradation assay (Hydroxyl radical scavenging activity)

Hydroxyl radicals were decomposed by Enhancing the Employment of Vulnerable Groups (EEVG), which was determined by the assay of malondialdehyde chromogenic formation due to 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1 ml: 100 µl of 28 mM 2-deoxy 2-ribose dissolved in phosphate buffer, pH 7.4, 500 µl of the EEVZ of various concentrations (5-80 µg) in buffer, 200 micro l of 20 mM ferric chloride (1:1 v/v) and 1.04 µM EDTA and 100 µl of 1.0 µM hydrogen peroxide and 100µl of 1.0 µM ascorbic acid. After incubation of the test sample at 37 °C for 1 h the extent of free radical damage imposed on the substrate deoxyribose was measured during thiobarbituric acid (TBA) test. For the TBA test, about 1 ml of thiobarbituric acid in 50 mM sodium hydroxide and 1ml of 2.8% W/V trichloroacetic acid was added to the test tubes and heated at 100°C for 20 min. after cooling absorbance was measured at 532 nm against a blank containing deoxyribose and buffer only. Percentage inhibition of deoxyribose degradation was calculated. Quercetin was used as standard [16].

### GC / MS / MS analysis

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min.

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature [17].

### Bioassay

One hundred fifty grams of the *E. elaterium* were extracted exhaustively in a Soxhlet extractor with hexane (purity 99.5%), chloroform (purity 99.5%) to extract phenolic aglycones, Ethyl acetate (purity 99.5%), acetone (purity 99.5%) and ethanol (purity 99.8%) to extract phenolic glycosides, respectively [18]. The extracts obtained were evaporated to dryness. The extraction yields were approximately 150–200 mg. Solvents were purchased from Fluka (Buchs, Switzerland). Each extract was kept in a small screw-capped glass tube over silica gel until used for insect bioassays. Bioassays were carried out within 2 weeks of extraction. Three concentrations (100, 500 and 1000 µg/ml) of each dry extract were prepared by dissolving the dry extract in 0.01% (v/v) dimethyl sulfoxide (DMSO) solution.

### *Aphis craccivora*

Lab-strain of black legume aphid, *A. craccivora* was obtained from the Pests & Plant Protection Department, Egypt. This strain was reared for several generations in the laboratory. Slid-dipping technique was used to evaluate the toxicity of the tested extracts against adult stage of *A. craccivora*. Serial concentrations of each extract were prepared. By means of fine brush, ten adults were affixed to double face scotch tape and stuck tightly to slide on the dorsal part. The slides were then dipped in the prepared solutions for ten seconds. Each extract tested at three different concentrations. Three replicates of ten adults were used for each concentration. Observations of mortality were carried out after 24 h and 72 h, using a magnifying lens (×4). The numbers of dead and live aphids were counted along with the numbers left in the plastic vial attached to that plant. The mortality percentage of *A. craccivora* was calculated by taking the number of dead adults from the total number of aphids.

### Insecticidal activity assay against *P. operculella*

#### Oviposition deterrence experiment

To evaluate of the five extracts of *E. elaterium* seeds on the oviposition deterrence, each extract was three concentrations, 100, 500 and 1000 µg/ml, each experiment was five replicates, the adults of males and females were new emerge to introduce into plastic box covered with mesh screen treated by each extract and kept at a temperature of 26±2 °C. Eggs in each box were counted three days after starting the

experiment. Total number of eggs in each box was used to calculate an oviposition deterrent index (ODI) as follows: For the oviposition-preference activity, the number of eggs was determined under a binocular microscope.

$$ODI = 100 \times (C-T) / (C+T)$$

Where: C=represents the total number of eggs in the control box

T= the total number of eggs in the treated box.

### The percentage of larval penetration of *P. operculella*

To examine the percentage of larval penetration of *P. operculella*, the first larval instar was used because it searches and mines into the host. At first, each potato tuber was dipped in 100, 500 and 1000 µg/ml. of each extract for 10 min. The organic solvents were eliminated by evaporation at room temperature for 24h. After solvent was evaporated from potato tubers, potato tubers were transferred into plastic boxes with ventilated lids under 25±2°C and 65 ± 10% RH. Infested tubers were introduced on each box (five potato tubers per treatment). Therefore, larval penetration was recorded with the number of individuals moving into potatoes. The number of larvae penetrated into each potato tuber was counted and the larval penetration deterrent was determined by a larval penetration deterrent index (LPDI) as follows:

$$LPDI = 100 \times (C-T) / (C+T)$$

Where C= represents the total number of larvae in the control T = the total number of larvae in the treated tubers.

### Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at  $P < 0.001$ ) and Duncan test (SPSS 20.0).

### Results

The antioxidant activity of *E. elaterium* (L.) of five different extracts (hexane, chloroform, ethyl acetate, acetone and ethanolic) at different concentrations (10, 30, 50, 100, 500 and 1000 µg/ml) were evaluated as free radical DPPH scavenging and their results are presented in Table (1) that the ethanol extract (85.68% & 97.93% at 500 and 1000 µg/ml respectively) and acetone extract (85.15% & 92.37% at 500 and 1000 µg/ml respect.) has the highest antioxidant activity, followed by hexane extract (24.03% & 42.64% at 500 and 1000 µg/ml respect.), chloroform extract (45.20% & 872.43% at 500 and 1000 µg/ml respect.), ethyl acetate extract (66.30% & 79.37% at 500 and 1000 µg/ml respect.) and BHT standard (64.86% & 74.69% at 500 and 1000 µg/ml respect.). It is clear that ethanolic extract has higher activity than other extracts and standard (BHT). Also, acetone extract has the highest activity when compared with other extracts and BHT.

**Table 1:** Free radical scavenging activity (Decolouration %) Squirting cucumber extracts (µg/ml).

Extract	% Decolouration of concentrations					
	10 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
Standard (BHT)	34.04 <sup>c</sup> ±0.08	34.27 <sup>c</sup> ±3.52	43.42 <sup>c</sup> ±0.41	63.96 <sup>b</sup> ±1.62	64.86 <sup>b</sup> ±4.01	74.69 <sup>a</sup> ±0.55
Hexane	3.88 <sup>d</sup> ±1.15	5.14 <sup>d</sup> ±1.47	6.43 <sup>d</sup> ±0.94	6.81 <sup>d</sup> ±1.07	24.03 <sup>c</sup> ±1.05	42.64 <sup>c</sup> ±2.26
Chloroform	5.40 <sup>d</sup> ±0.18	12.43 <sup>d</sup> ±1.62	13.88 <sup>d</sup> ±2.37	14.67 <sup>d</sup> ±2.63	45.20 <sup>c</sup> ±0.67	72.43 <sup>b</sup> ±1.04
Ethyl acetate	12.29 <sup>d</sup> ±0.24	18.61 <sup>d</sup> ±0.44	22.28 <sup>c</sup> ±3.67	38.69 <sup>c</sup> ±1.16	66.30 <sup>b</sup> ±2.04	79.37 <sup>a</sup> ±5.24
Acetone	32.01 <sup>c</sup> ±2.38	35.14 <sup>c</sup> ±3.17	36.60 <sup>c</sup> ±1.43	80.50 <sup>a</sup> ±0.08	85.15 <sup>a</sup> ±0.41	92.37 <sup>a</sup> ±1.06
Ethanol	43.41 <sup>c</sup> ±0.78	63.86 <sup>b</sup> ±2.52	65.25 <sup>b</sup> ±0.77	81.50 <sup>a</sup> ±2.31	85.68 <sup>a</sup> ±3.11	97.93 <sup>a</sup> ±0.07

In Table (2), dioxyribose degradation assay recorded ethanolic extract has higher activity than other extracts and BHT. The results obtained show that the antioxidant activity

of ethanolic extract depends on which phenolic compounds contained this extract.

**Table 2:** Dioxiribose degradation assay.

Extract	% Inhibition of concentrations					
	10 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
Standard (BHT)	33.45 <sup>c</sup> ±2.51	43.13 <sup>b</sup> ±1.07	53.22 <sup>b</sup> ±0.15	53.83 <sup>b</sup> ±1.34	74.28 <sup>a</sup> ±3.24	86.34 <sup>a</sup> ±4.31
Hexane	1.32 <sup>d</sup> ±0.01	2.11 <sup>d</sup> ±2.05	5.21 <sup>d</sup> ±0.36	5.80 <sup>d</sup> ±0.01	16.34 <sup>c</sup> ±2.03	22.14 <sup>c</sup> ±2.33
Chloroform	3.14 <sup>d</sup> ±1.34	5.48 <sup>d</sup> ±1.18	10.52 <sup>c</sup> ±1.05	12.45 <sup>c</sup> ±0.03	20.35 <sup>c</sup> ±0.07	23.28 <sup>c</sup> ±0.14
Ethyl acetate	6.18 <sup>d</sup> ±2.11	10.91 <sup>c</sup> ±1.41	12.87 <sup>c</sup> ±0.61	24.16 <sup>c</sup> ±3.41	47.27 <sup>b</sup> ±0.16	48.21 <sup>b</sup> ±0.35
Acetone	24.37 <sup>c</sup> ±0.32	28.66 <sup>c</sup> ±3.68	44.49 <sup>b</sup> ±3.01	60.12 <sup>b</sup> ±0.04	65.03 <sup>a</sup> ±0.22	70.62 <sup>a</sup> ±3.42
Ethanol	37.21 <sup>b</sup> ±1.06	51.76 <sup>b</sup> ±0.33	55.08 <sup>b</sup> ±4.22	67.88 <sup>a</sup> ±1.13	73.54 <sup>a</sup> ±1.30	87.71 <sup>a</sup> ±0.01

Phenolic compounds contents of *E. elaterium* ethanolic extract are shown in Table (3), The results demonstrated that the relative percentage of hexanoic acid (2.94), p-allylphenol (5.02), sinapyl alcohol (0.62), phytol (0.38), β-hydroxyisovaleric acid (1.08), methylmalonic acid (1.7), 3,5-dimethoxycinnamic acid (0.35), β-eudesmol (0.34), nabilone (0.56), terpineol (1.72), genistin (0.39), 7,8-dihydro-α-ionone (1.65), phenol (0.45), humulane-1,6-dien-3-ol (0.77), santalol E-cis, epi-β-(0.15), patchoulane (0.6), α-melibiose (0.36), homomyrtenol (0.21), Phenol, 2,3,5-trimethyl- (2.52), (-)-(E)-Pinane-1,10-diol (0.45), 3,3',4',7-Tetramethoxyflavone (3.34), D-verbenone (1.81), α isomethyl ionone (1.09), p-cymen-7-ol (1.31), epiglobulol (1.02), 4-methylcatechol (1.18), cubedol (0.38), isolongifolol (0.79), ferulic acid (2.05), (+)-α-tocopherol (0.55), cis-vaccenic acid (0.35), γ-selinene (0.57), geranylisovalerate (0.38), 3',4',7-trimethylquercetin (0.17), patchoulol (1.95), D-saccharic acid (1.37), 6,8-Di-C-glucosylluteolin (0.38), β-citronellol (1.13), zearalenone (0.76), terpinyl acetate (2.57), farnesol (1.3), fisetin (6.45), kaempferol (3.86), squalane (2.41), resorcinol (2.47), shyobunone (12.72), arachidonic acid (6.85), apigenin 7-methyl ether (8.25), cyanidincation (3.18), glycitein (1.0), flavidin- diacetate (4.1), trans-isohumulone (0.84) and diosmetin 7-O-glucoside (0.98). The results show that the

plant contains many phenolic compounds that have effects as antioxidants and insecticides.

#### *E. elaterium* extracts against, *A. craccivora*

The results in Table (4) demonstrated that the hexane, chloroform, ethyl acetate, acetone and ethanol extracts of *E. elaterium*, at a concentration of 100 µg/ml during 24h and 72 h weren't significant difference of mortality percentage of *A. craccivora* adults, while the chloroform extract caused mortality percentage of *A. craccivora* greater than hexane, ethyl acetate, acetone and ethanol extracts at a concentration of 500 µg/ml, during 24h, but not significant difference among extracts at same a concentration after 72h. Data presented in the same table showed that mortality percentage of *A. craccivora* adults at concentration of 1000 µg/ml during 24 h wasn't significant difference among five extracts, but mortality percentage of *A. craccivora* adults at same concentration after 72h was 63.0%, 67.7% and 71.0% of ethyl acetate, acetone and ethanol extracts, respectively, also mortality percentage of *A. craccivora* was 42.0% and 57.7% of hexane and chloroform, respectively. The DMSO 0.01% solution showed no significant mortality effect against the *A. craccivora* as control.

**Table 3:** Phenolic compounds of Squirting cucumber of ethanolic extract identified by GC / MS / MS analysis.

Peak number	RT	Name of compound	Area %	Peak number	RT	Name of compound	Area %
1	3.8	Hexanoic acid	2.94	28	12.178	Isolongifolol	0.79
2	4.5	p-Allylphenol	5.02	29	12.55	Ferulic acid	2.05
3	4.85	Sinapyl alcohol	0.62	30	12.64	(+)-α-Tocopherol	0.55
4	5.058	Phytol	0.38	31	13.18	cis-Vaccenic acid	0.35
5	5.47	β-Hydroxyisovaleric acid	1.08	32	13.47	γ-Selinene	0.57
6	6.12	Methylmalonic acid	1.7	33	13.98	Geranylisovalerate	0.38
7	7.138	3,5-Dimethoxycinnamic acid	0.53	34	14.05	3',4',7-Trimethylquercetin	0.17
8	7.15	β-Eudesmol	0.34	35	14.157	Patchoulol	1.95
9	7.715	Nabilone	0.56	36	14.6	D-Saccharic acid	1.37
10	8.67	Terpineol	1.72	37	14.76	6,8-Di-C-glucosylluteolin	0.38
11	8.9	Genistin	0.39	38	15.14	β-Citronellol	1.13
12	9.2	7,8-Dihydro-α-ionone	1.65	39	15.3	Zearalenone	0.76
13	9.59	Phenol	0.45	40	15.46	Terpinyl acetate	2.57
14	9.64	Humulane-1,6-dien-3-ol	0.77	41	15.53	Farnesol	1.3
15	9.76	Santalol, E-cis, epi-β-	0.15	42	15.691	Fisetin	6.45
16	9.82	Patchoulane	0.6	43	15.85	Kaempferol	3.86
17	9.96	α-Melibiose	0.36	44	16	Squalane	2.41
18	10.19	Homomyrtenol	0.21	45	16.5	Resorcinol	2.47
19	10.238	Phenol, 2,3,5-trimethyl-	2.52	46	16.88	Shyobunone	12.72
20	10.44	(-)-(E)-Pinane-1,10-diol	0.45	47	17.05	Arachidonic acid	6.85
21	10.95	3,3',4',7-Tetramethoxyflavone	3.34	48	17.11	Apigenin 7-methyl ether	8.25
22	11.154	D-Verbenone	1.81	49	17.3	Cyanidincation	3.18
23	11.3	α Isomethyl ionone	1.09	50	19.14	Glycitein	1.0
24	11.38	p-Cymen-7-ol	1.31	51	21.3	Flavidin- diacetate	4.1
25	11.49	Epiglobulol	1.02	52	21.5	trans-Isohumulone	0.84
26	11.66	4-Methylcatechol	1.18	53	21.76	Diosmetin 7-O-glucoside	0.98
27	12.04	cubedol	0.38				

**Table 4:** Mortality percentage of *A. craccivora* adults was effected with different extracts of *E. elaterium* concentrations.

Extracts	100 µg/ml		500 µg/ml		1000 µg/ml	
	24 h	72 h	24 h	72 h	24 h	72 h
Hexane	19.7 <sup>a</sup> ± 4.7	36.3 <sup>a</sup> ± 3.8	29.7 <sup>b</sup> ± 4.6	41.0 <sup>a</sup> ± 5.9	36.7 <sup>a</sup> ± 5.8	42.0 <sup>b</sup> ± 7.8
Chloroform	20.0 <sup>a</sup> ± 4.3	33.7 <sup>a</sup> ± 4.4	34.0 <sup>a</sup> ± 2.3	46.3 <sup>a</sup> ± 4.7	43.0 <sup>a</sup> ± 9.0	57.7 <sup>b</sup> ± 5.9
Ethyl acetate	19.3 <sup>a</sup> ± 2.1	43.8 <sup>a</sup> ± 3.5	27.2 <sup>b</sup> ± 1.5	45.1 <sup>a</sup> ± 1.5	34.0 <sup>a</sup> ± 5.4	63.0 <sup>a</sup> ± 4.9
Acetone	20.0 <sup>a</sup> ± 4.0	36.0 <sup>a</sup> ± 5.9	20.0 <sup>b</sup> ± 4.0	44.3 <sup>a</sup> ± 11.3	39.7 <sup>a</sup> ± 5.9	67.7 <sup>a</sup> ± 8.3
Ethanol	23.3 <sup>a</sup> ± 2.4	43.3 <sup>a</sup> ± 5.9	20.0 <sup>b</sup> ± 4.0	33.7 <sup>a</sup> ± 5.9	53.3 <sup>a</sup> ± 4.5	71.0 <sup>a</sup> ± 5.9
Water + DMSO	0.0 <sup>c</sup> ± 0.0	8.0 <sup>b</sup> ± 0.0	0.0 <sup>c</sup> ± 0.0	8.0 <sup>b</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	8.0 <sup>c</sup> ± 0.0

Data were arcsine-transformed before subject to ANOVA. Means within the same column that have the same letters are not.

Significantly different ( $P < 0.001$ ) using Least Significant Differences LSD.

#### *E. elaterium* extracts against, *P. operculella* oviposition

The oviposition deterrent activity for the tested extracts of *E. elaterium* at different concentrations is reported in Table 4. The extracts (hexane, chloroform, ethyl acetate, acetone and ethanol) reduced significantly the number of eggs laid compared to control. The highest level of deterrent effect was recorded to ethanol extract with 25.3%, 69.3% and 94.2% at

concentrations, 100, 500 and 1000µg/ml, respectively. But acetone extract caused reduction of oviposition with 29.9%, 71.5% and 92.3% at concentrations, 100, 500 and 1000µg/ml, respectively. The results in table (5) showed that the control (coragein) didn't differ significant about acetone and ethanol extracts at concentration 1000 µg/ml. In the same way, all extracts reduced larval penetration deterrent of *P. operculella* at concentration 1000 µg/ml. The data demonstrated that ethanol, chloroform and acetone extracts were highest reduction percentage of larval penetration of *P. operculella* 70.5%, 72.1% and 73.3%, respectively, at the same concentration.

**Table 5:** Effect of different concentrations of *E. elaterium* extracts on the oviposition and larval penetration of *P. operculella*.

Treatment	Oviposition deterrent index%			Larval penetration deterrent index%		
	100 µg/ml	500 µg/ml	1000 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
Hexane	28.1±7.7 <sup>b</sup>	65.7±6.3 <sup>ab</sup>	83.3±2.2 <sup>ab</sup>	6.2±2.9 <sup>a</sup>	25.6±6.2 <sup>ab</sup>	51.6±10.3 <sup>a</sup>
Chloroform	14.3±4.2 <sup>a</sup>	54.5±7.6 <sup>a</sup>	75.2±2.1 <sup>d</sup>	23.5±5.8 <sup>b</sup>	36.2±7.3 <sup>b</sup>	72.1±10.5 <sup>b</sup>
Ethyl acetate	20.7±2.3 <sup>ab</sup>	63.2±6.4 <sup>ab</sup>	71.1±2.5 <sup>d</sup>	23.9±6.7 <sup>b</sup>	19.5±4.4 <sup>a</sup>	41.6±9.0 <sup>a</sup>
Acetone	29.9±6.1 <sup>b</sup>	71.5±9.4 <sup>b</sup>	92.3±2.4 <sup>b</sup>	16.3±3.7 <sup>ab</sup>	30.6±9.0 <sup>b</sup>	73.3±6.4 <sup>b</sup>
Ethanol	25.3±6.5 <sup>ab</sup>	69.3±9.2 <sup>ab</sup>	94.2±3.1 <sup>b</sup>	13.5±5.6 <sup>a</sup>	34.8±6.4 <sup>b</sup>	70.5±6.0 <sup>b</sup>
Coragein	90.4±7.6 <sup>c</sup>	91.5±6.5 <sup>c</sup>	97.3±2.1 <sup>b</sup>	91.4±2.4 <sup>c</sup>	93.3±3.6 <sup>c</sup>	100±0 <sup>c</sup>

Data were arcsine-transformed before subject to ANOVA. Means within the same column that have the same letters are not.

Significantly different ( $P < 0.001$ ) using Least Significant Differences LSD.

#### Discussion

The antioxidant activity of *E. elaterium* (*L.*) of five different extracts (hexane, chloroform, ethyl acetate, acetone and ethanolic) at different concentrations was evaluated as free radical DPPH scavenging. These results are in a good agreement with El-Haci and Bekkara [19] and Felhi, *et al.* [20]. Dioxiribose degradation assay recorded ethanolic extract has higher activity than other extracts and BHT. The results previously reported to enhance antioxidant activity [21, 22]. Many researchers have shown that an increase in DPPH inhibition of radicals maybe explained by several factors, such as the higher antioxidant power of polyphenols at an intermediate state of oxidation, the increase in reducing sugar and formation of Maillard reaction products, known to have a great antioxidant activity, which isothen exerted in a chain-breaking and DPPH type mechanism [23, 24, 25].

The mortality percentage of *A. craccivora* adults at concentration, 1000µg/ml after 72h was 63.0%, 67.7% and 71.0% of ethyl acetate, acetone and ethanol extracts, respectively; also mortality percentage of *A. craccivora* was 42.0% and 57.7% of hexane and chloroform, respectively. The DMSO 0.01% solution showed no significant mortality effect against the *A. craccivora* as control.

Ben Hamouda' results revealed that acetone and methanol extracts caused high mortality percentage of *Myzus persicae*

[26], also, study on *P. operculella* generated a very pronounced deterrence of oviposition and larval penetration. Ben Hamouda *et al.* [27] showed that methanolic extracts from leaves and seeds of *Solanum elaeagnifolium* have insecticidal and repellent properties against three pest species (*M. persicae*, *P. operculella* and *Tribolium castaneum*). [3] demonstrated that All tested concentrations of *Marrubium vulgare* and *Achillea millefolium* crude extracts caused great reductions in the number of eggs deposited of *P. operculella* moth [28]. revealed that the toxicity of the essential oils of *Rosmarinus officinalis* on adult longevity is enlarged with the concentration and the exposure time. Concerning the hatchability of eggs and the survivorship of neonate larvae, the results [29] showed that some of the repellent oils were tested for their effect on certain biological aspects of *P. operculella*. Eugenol and peppermint oils, each at the 0.01% conc., caused a significant depression in the fecundity of moth and decreased the percentage of egg hatchability. Eugenol oil was much more effective than peppermint oil at 1%. These results are in agreement with recent study of [18] reporting the antioxidant properties of *E. elaterium* stems and leaves. Conclusion, the plant contains many phenolic compounds that have effects as antioxidants and insecticides, the antioxidant activity of *E. elaterium* (*L.*) of different extracts at different concentrations caused mortality of *A. craccivora* adults after 72h from exposure. Also, all extracts reduced larval penetration deterrent of *P. operculella* at high conc. The data demonstrated that ethanol, chloroform and acetone extracts were highest reduction percentage of larval penetration of *P. operculella*, the same concentration.

## References

- Blackman RL, Eastop VFE. Aphids on the world's crops: an identification and information guide. Willy Interscience Publ. 1984, 466.
- Cunningham IC. Alternative host plants of tobacco leaf miners, *Phthorimaea operculella* (Zell.) Queens hand. Journal of Agriculture and Animal Science. 1969; 26:107-111.
- Allahverdizadeh NM, Mohammadi D. Bioactivity of *Marrubium vulgare* and *Achillea millefolium* leaf extracts on potato tuber moth *Phthorimaea operculella* Zeller. Munis Entomology & Zoology, 2016; 11(1):114-122.
- Greige-Gerges H, Abou Khalil R, Abou Mansour E, Magdalou J, Chahine R, Ouaini N. Cucurbitacins from *Ecballium elaterium* juice increase the binding of bilirubin and ibuprofen to albumin in human plasma. *Chemo-Biological Interactions*, 2007; 169:53-62.
- Büyükkokuroğlu ME, Gülçin İ, Oktay M, Küfrevioğlu Öİ. *In vitro* antioxidant properties of dantrolene sodium. *Pharmacology Research*. 2001, 44491495.
- Kloutsos G, Balatsouras DG, Kaberos AC, Kandiloros D, Ferekidis E, Economou C. Upper airway edema resulting from use of *Ecballium elaterium*. *Laryngoscope*. 2001; 111(9):1652-1655.
- Blaskovich MA, Sun J, Cantor A, Turkson J, Jove R, Sebti SM. Discovery of JSI-124 (Cucurbitacin I), a Selective Janus Kinase/Signal Transducer and Activator of Transcription 3 Signaling Pathway Inhibitor with Potent Antitumor Activity against Human and Murine Cancer Cells in Mice. *Cancer Research*. 2003; 63:1270-1279.
- Sun J, Blaskovich MA, Jove R, Livingston SK, Coppola D, Sebti SM *et al.* a selective STAT3 activation inhibitor with potent antitumor activity. *Oncogene*. 2005; 24:3236-3245.
- Bernard B. *Plantesmédicinales du monde réalistesetcroyances*. Paris: Estem. 2001, 395.
- Alapetite GP. *Flore de la Tunisie'* (Angiospermes Dicotylédones Gamopétales Imprimerie Officielle de la République Tunisienne. 1979.
- Lavie D, Szinai S. The constituents of *Ecballium elaterium* II a-Elaterin. *Journal of the American Chemical Society*, 1958; 80:707.
- Erclyes AT, Karaosmanoglu F, Clvelekoglu H. Fruit oils of four plant species of Turkish origin. *Journal of the American Oil Chemists' Society*. 1989; 66:1459-146.
- Heitz A, Chiche L, Le-Nguyen D, Castro B. H2D NMR and distance geometry study of the folding of *Ecballium elaterium* trypsin inhibitor, a member of the squash inhibitors family. *Biochemistry*, 1989; 28:2392-2398.
- Salhab AS. Human Exposure to *Ecballium elaterium* Fruit Juice: Fatal Toxicity and Possible Remedy. *Pharmacology and Pharmacy*, 2013; 4:447-450.
- Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technology*. 1995; 28:25-30.
- Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, 1987; 165:215-219.
- Santanal PM, Miranda M, Payrol JA, Silva M, Hernández V, Peralta E. Gas Chromatography-Mass Spectrometry Study from the Leaves Fractions Obtained of *Vernonanthura patens* (Kunth) H. Rob. *International Journal of Organic Chemistry*. 2013; 3:105-109.
- Sullins DW, Denton TT, Eisenbraune J. Soxhlet apparatus and chromatography columns combined. *Journal of Chemical Education*. 1996; 73:380-381.
- El-Haci IA, Bekkara FA. Antioxidant activity of stems and leaves organic fractions of *Ecballium elaterium* L. *Annals of Biological Research*. 2011; 2:327-332.
- Felhi S, Hajlaoui H, Ncir M, Bakari S, Ktari N, Saoudi M *et al.* Nutritional, phytochemical and antioxidant evaluation and FT-IR analysis of freeze dried extracts of *Ecballium elaterium* fruit juice from three localities. *Food Science and Technology (Campinas)*. 2016; 36(4):646-655.
- Attard E, Attard H. Antitrypsin activity of extracts from *Ecballium elaterium* seeds. *Fitoterapia*. 2008; 79(3):226-228.
- Bernard SA, Olayinka OA. Search for a novel antioxidantanti-inflammatory/analgesic or anti-proliferative drug: Cucurbitacinshold the ace. *Journal of Medicinal Plants Research*. 2010; 4(25):2821-2826.
- Madrau MA, Pisicopo A, Sanguinetti AM, Del Caro A, Poiana M, Romeo FV *et al.* Effect of drying temperature on polyphenolic content and antioxidant activity of apricots. *European Food Research and Technology*. 2009; 228:441-448.
- Herch W, Kallel H, Boukhchina S. Physicochemical properties and antioxidant activity of Tunisian date palm (*Phoenix dactylifera* L.) oil as affected by different extraction methods. *Food Science and Technology (Campinas)*, 2014; 34(3):464-470.
- İncedayi B, Tamer CE, Sinir GO, Suna S, Çopur OU. Impact of different drying parameters on color,  $\beta$ -caroteneantioxidant activity and minerals of apricot (*Prunus armeniaca* L). *Food Science and Technology (Campinas)*, 2016; 36(1):171-178.
- Ben Hamouda A, Boussadia O, Khaoula B, Laarif A, Braham M. Studies on insecticidal and deterrent effects of olive leaf extracts on *Myzus persicae* and *Phthorimaea operculella*. *Journal of Entomology and Zoology Studies*. 2015; 3(6):294-297.
- Ben Hamouda A, Zarrad K, Laarif A, Chaieb I. Insecticidal effect of *Solanum elaeagnifolium* extracts under laboratory conditions. *Journal of Entomology and Zoology Studies*. 2015; 3(3):187-190.
- Hannour K, Boughdad A, Maataoui A, Bouchelta A. Chemical composition and toxicity of Moroccan *Rosmarinus officinalis* (Lamiaceae) essential oils against the potato tuber moth, *Phthorimaea operculella* (Zeller, 1873) Zeller (Lepidoptera, Gelechiidae). *Journal of Materials and Environmental Sciences*. 2017; 8(2):758-769.
- Sharaby A, Abdel-Rahman H, Moawad S. Biological effects of some natural and chemical compounds on the potato tuber moth, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae). *Saudi Journal of Biological Sciences*. 2009; 16:1-9.