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Phytochemical and insecticidal activity of *Euphorbia pulcherrima* willed extracts for controlling cotton leaf worm, *Spodoptera littoralis* Boisd

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Abstract
Chemical constituents of *Euphorbia pulcherrima* Willed. Aerial parts were extracted and tested for their insecticidal activity against different stages (eggs, 4th instar larvae and pupae) of *Spodoptera littoralis* Boisd. Under laboratory conditions. The most effective fraction against all stages was petroleum ether fraction with LC_{50}s of: 591.14, 859.90 and 3004.44 ppm for eggs, 4th instar larvae and pupae, respectively. Also, the volatile constituents of each extract fraction were qualitatively and quantitatively characterized and identified by Gas Chromatography-Mass Spectrometry technique. *E. pulcherrima* extracts showed promising activity as green alternatives of traditional insecticides.

Keywords: *Euphorbia pulcherrima*, extracts, phytochemical, insecticidal activity and *Spodoptera littoralis*

Introduction
The cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera; Noctidae) is one of the most destructive polyphagous agricultural pests. It attacks many economically crucial crops such as cotton, tomato, maize and vegetables as well as many kinds of fruits [1, 2, 3]. It harm the plants directly by extensive feeding of the larvae on plant leaves or bear into the buds for consumption [4]. Also, the presence of the larvae or their faeces reduces the marketability of vegetables and ornamentals [5]. The extensive and intensive use of broad-spectrum synthetic insecticides against this serious pest led to the development of resistance to many registered insecticides [6]. Moreover, problems such as residuals toxicity and harmful effects on beneficial insects were raised. So, the global public concern of looking for environmental friendly alternatives for insect control was increased. Phytoinsecticides are considered promising agents in this trend.

Many plants belonging to various families were studied for searching for novel insecticidal components [7, 8, 9]. Euphorbiaceae family, is well known for its diverse characteristic content of phenolic compounds (phenolic acids, tannins, coumarins, flavonoids, lignans, phenanthrenes, quinones, etc…), triterpenoids and associated compounds (alcohols, sterols and hydrocarbons), alkaloids, cyanogenic glucosides and Glucosinolates [10]. *E. pulcherrima* contains numerous chemical groups such as terpenoids, alkaloids, flavonoids, steroids, saponins, glycosides, reducing sugars [11, 12].

The objective of this study was to investigate the phytochemistry and the insecticidal activity of *E. pulcherrima* aerial parts extracts against different stages of the cotton leaf worm, *S.littoralis.*

Materials and Methods
Plant Material
E. pullcherima aerial parts were collected in November 2019 from garden of Mansoura University, Egypt. Plant identification was confirmed by department of Botany, Faculty of science, Mansoura Univ., Egypt.

Extraction and phytochemical screening of *E. pulcherima*
Aerial parts of the plant were dried at room temperature and grounded to a powder, then, soaked in methanol three times, each time for three days. Methanolic extract of *E. pulcherrima* was filtered, evaporated to approximately 1/3 of its volume, then diluted with distilled water and successively extracted by different organic solvents of ascending polarity (petroleum ether, methylene chloride and ethyl acetate) by using separating funnel. Anhydrous sodium sulphate was used for drying all fractions, then, they were evaporated to dryness. Each fraction was formulated in serial concentrations to evaluate its insecticidal activity against different stages of *S. littoralis*.

Phytochemical screening
Volatile components of *E. pulcherrima* extracts were characterized by GC/MS technique using a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermos-mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i. d., 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 C for 1min; rising at 4.0 C/min to 240 C and held for 1min. The injector and detector were held at 210ºC. Diluted samples (1:10 hexane, v/v) of 1µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Identification of the chemical components were de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices, mass spectrum matching

to Wiley spectral library collection and NSIT library database.

The tested insect pest

Susceptible strain of *S. littoralis* was obtained from Plant protection Research Institute, Agricultural Research Center, Giza, Egypt. It was reared under laboratory controlled condition (27± 2ºC, 65±5% R.H). Larvae were maintained on fresh castor leaves till pupation. Pupa were collected and kept in clean jars till emergence of adults. The emerged adults were fed on 10% honey solution on cotton wick.

Bioassay

Assessment of ovicidal activity of different fractions of *E. pulcherrima* extract

Newly egg masses of *S. littoralis* were sprayed by the formulated emulsions of *E. pulcherrima* fractions containing 0.3% Tween-80. Each treatment was replicated three times. Another three replicates were sprayed only with water and 0.3% Tween80 to act as control.

Assessment of insecticidal activity of different fractions of *E. pulcherrima* against *S. littoralis* 4th instar larvae

Fresh castor leaves were cleaned and sprayed with different concentrations of each extract fraction and given to the larvae. Each concentration was represented by three replicates, each of them contain 10 larvae. In addition to the three replicates of control (water + Tween 80).

Assessment of insecticidal activity of different fractions of *E. pulcherrima* against *S. littoralis* Pupae

Newly formed pupae of *S. littoralis* were sprayed with different fractions concentrations. After 10 days, the dead pupae or that failed in emerging adults were counted.

Statistical analysis

Mortality percentages of the tested instar were estimated and corrected by using Abbott`s formula [13]. The LC_{50}, LC_{90} values and slope were determined according to Finney, (1971) [14]. Also, toxicity index of each tested fraction was estimated using Suns' equation [15].

Results and Discussion

Phytochemical screening

The volatile components of different fractions of *E. pulcherrima* aerial parts were characterized by GC/MS technique "Table 1". It was found that pet ether fraction revealed 6 compounds represented by 6 peaks, while methylene chloride fraction revealed 22 compounds. Also, the major volatile components of ethyl acetate fraction were seven compounds corresponding to seven peaks.

Table 1: The GC/MS analysis of different fractions of *E. pulcherrima* aerial parts

Pet. ether fraction of *E. pulcherrima* aerial parts				
Compound Name	**R_t, min**	**Area%**	**M. F.**	**M. wt.**
Anethole (1)	13.92	8.08	$C_{10}H_{12}O$	148
Neophytadiene (2)	27.10	7.07	$C_{20}H_{38}$	278
Hexadecanoic acid, methyl ester (3)	28.88	1.41	$C_{17}H_{34}O_2$	270
n-Hexadecanoic acid (4)	29.61	1.51	$C_{16}H_{32}O_2$	256
Phytol (5)	32.47	80.03	$C_{20}H_{40}O$	296
Squalene (6)	43.50	1.05	$C_{30}H_{50}$	410
Methylene chloride fraction of *E. pulcherrima* aerial parts				
Hexanoic acid (7)	6.21	3.40	$C_6H_{12}O_2$	116
3,5,5-trimethyl-2-cyclohexen-1-one= α-Isophorone (8)	9.40	1.59	$C_9H_{14}O$	138
Benzeneethanamine, N,à-dimethyl- =Metamfetamine (9)	11.20	1.56	$C_{10}H_{15}N$	149
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- (10)	12.55	4.36	$C_7H_9NO_2$	139
5,9-Undecadien-2-one, 6,10-dimethyl-, (E) =Geranylacetone (11)	18.27	0.61	$C_{13}H_{22}O$	194
2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (12)	20.07	4.94	$C_{11}H_{16}O_2$	180
Aspidospermidin-17-ol, 1-acetyl-16-methoxy- (13)	20.46	0.60	$C_{22}H_{30}N_2O_3$	370
Dodecanoic acid, 1-methylethyl ester (14)	22.51	0.28	$C_{15}H_{30}O_2$	242
2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5 trimethyl- =9-hydroxy-4,7-megastigmadiene-3-one (15)	22.87	0.69	$C_{13}H_{20}O_2$	208
1,1,4,7-tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol= Himbaccol (16)	22.92	0.91	$C_{15}H_{26}O$	222
Retinal (17)	23.28	0.37	$C_{20}H_{28}O$	284
2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl- (18)	24.12	2.90	$C_{13}H_{22}O_2$	210
2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl 3cyclohexen-1-yl)-,[3S-[3à, 6à(R*)]]-= α-Bisabolol oxide A (19)	25.10	0.82	$C_{15}H_{26}O_2$	238
2(4h)-benzofuranone,5,6,7,7a -tetrahydro-6-hydroxy-4,4,7a-trimethyl-,(6s-cis)- (20)	25.50	9.53	$C_{11}H_{16}O_3$	196
Caryophyllene oxide (21)	26.40	0.37	$C_{15}H_{24}O$	220
Neophytadiene (2)	27.11	0.79	$C_{20}H_{38}$	278
Ethyl (9z,12z)-9,12-octadecadienoate (22)	27.98	0.50	$C_{20}H_{36}O_2$	308
Hexadecanoic acid, methyl ester (3)	28.88	2.76	$C_{17}H_{34}O_2$	270
8,11,14-Eicosatrienoic acid, (Z,Z,Z)- = hydomogammalinoleinc acid (23)	32.21	0.55	$C_{20}H_{34}O_2$	306
Phytol (5)	32.45	16.95	$C_{20}H_{40}O$	296
1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10 apentamethyl-,[3R-(3à,4aá,6aà, 10aá,10bà)]- = manoyl oxide (24)	33.89	4.70	$C_{20}H_{34}O$	290
Lup-20(29)-ene-3, 28-diol, (3á)- = Betuline (25).	43.50	1.02	$C_{30}H_{50}O_2$	442
Ethyl acetate fraction of *E. pulcherrima* aerial parts				
2-propenoic acid, 2-methyl-,Dodecyl ester= Lauryl methacrylate (26)	14.64	4.59	$C_{16}H_{30}O_2$	254
2-Furanmethanol,tetrahydro- à,à,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-,[2S-[2à,5á(R*)]]- = German chamomile (27)	23.10	4.22	$C_{15}H_{26}O_2$	238
2H-Pyran-3-ol,tetrahydro- 2,2,6-trimethyl-6-(4-methyl-3 cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]- (28)	25.10	5.42	$C_{15}H_{26}O_2$	238
2(4h)-benzofuranone,5,6,7,7a -tetrahydro-6-hydroxy-4,4,7a-trimethyl-,(6S-CIS)- (29)	25.50	18.13	$C_{11}H_{16}O_3$	196
Cylopentanetridecanoic acid, methyl ester (30)	28.88	2.14	$C_{19}H_{36}O_2$	296

9-octadecenoic acid (z)- = oleic acid (31)	29.61	2.71	$C_{18}H_{34}O_2$	282
2-hexadecen-1-ol,3,7,11,15- tetramethyl-,[r-[r*,r*-(e)]]- (32)	32.45	5.83	$C_{20}H_{40}O$	296

Insecticidal activity

Ovicidal activity of *E. pulcherrima* extracts on *S. littoralis* eggs

Data in "Table 2" showed that the hatchability of *S. littoralis* eggs was significantly suppressed by all fractions of *E. pulcherrima* extracts. Petroleum ether fraction was the most efficient showing the highest ovicidal activity followed by methylene chloride fraction then ethyl acetate fraction with LC_{50}: 591.14, 686.18 and 1678.76 ppm, respectively. The absolute reduction of the eggs hatching was recorded at 10000 ppm of both of petroleum ether and methylene chloride fractions and at 20000 ppm of ethyl acetate fraction. Egg masses failed to hatch without any morphological changes. So, the ovicidal activity of the tested fractions may be occured by disrupting or inhibiting embryogenesis obstructing their hatch [16].

Table 2: Ovicidal effects of *E. pulcherrima* extracts against *S. littoralis* eggs.

Treatment	Conc.	% of Reduction of egg hatching	LC_{50} (ppm) and confidence limits at 95%		LC_{90} (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
Pet. ether fraction	1250	64.53	591.14		6685.72		1.22 ± 0.34	0.23	100.00
	2500	79.33							
	5000	86.32	111.6	1009.43	4244.85	26057.56			
	10000	100.00							
Methylene chloride fraction	1250	60.05	686.18		9417.67		1.13 ± 0.32	0.649	86.15
	2500	76.54							
	5000	82.13	131.38	1142.87	5346.35	61489.68			
	10000	100.00							
Ethyl acetate fraction	1250	47.21	1678.76		24181.66		1.11 ± 0.199	2.81	35.21
	2500	56.15							
	5000	64.53	1029.53	2262.41	13677.20	75364.67			
	10000	84.35							
	20000	100.00							

Insecticidal activity of different *E. pulcherrima* extracts against *S. littoralis* 4th instar larvae Data in "Table 3" showed that the most effective fraction against *S. littoralis* 4th instar larvae was petroleum ether fraction followed by ethyl acetate then methylene chloride with LC_{50}: 859.91, 1291.97 and 2515.79 ppm, respectively. All tested fractions exhibited shrinkage of the larvae bodies, loss of body weight and abnormal pupation before the normal time. Ethyl acetate extract turned the color of larvae to black and the body takes out its liquid contents. The present data was consistent with Almeida *et al*., (2017) [17] who reported growth inhibition and poor weight of *S. frugiperda* 2nd instar larvae treated with the ethanolic extract of *E. pulcherrima*. It was argued that the loss or poor weight of the larvae attributed to feeding and metabolism reduction [18].

Table 3: Insecticidal activity of different fractions of E. pulcherrima extract against S. littoralis 4th instar larvae

Treatment	Conc.	Larval Mortality %	LC_{50} (ppm) and confidence limits at 95%		LC_{90} (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
Pet. ether fraction	500	36.67	859.90		5612.68		1.57 ± 0.38	0.11	100
	1000	53.33							
	2000	70.00	480.79	1222.68	3214.03	22808.32			
	4000	86.67							
Methylene chloride fraction	1000	30.00	2515.79		21195.83		1.38 ± 0.36	0.06	34.18
	2000	43.33							
	4000	60.00	1535.87	3836.72	9910.35	195175.41			
	8000	76.67							
Ethyl acetate fraction	1000	43.33	1291.97		7150.27		1.72 ± 0.403	0.39	66.56
	2000	63.33							
	4000	76.67	657.78	1839.57	4556.58	20280.11			
	8000	93.33							

Insecticidal activity of different *E. pulcherrima* extracts against *S. littoralis* pupae

Data in "Table 4" showed that *S. littoralis* pupae were less susceptible to the all tested fractions of *E. pulcherrima* extract than larvae. Petroleum ether fraction kept the first rank showing the highest efficacy against *S. littoralis* pupae, followed by ethyl acetate fraction then, methylene chloride fraction with LC_{50}s: 3004.44, 3984.94 and 5904.67 ppm, respectively. Also, there was a great reduction of pupae weights comparing to the control to the extent that it looks as if it were empty especially when treated with petroleum ether fraction. Ethyl acetate extract turned the color of pupae to a dark black with solidified secretions at the pointed end of the pupae. The current data emphasized that all fraction of *E. pulcherrima* extract have a contact effect and having the ability to penetrate the cuticle of pupae hindering the emergence of adults.

Table 4: Insecticidal activity of different fractions of *E. pulcherrima* extract against *S. littoralis* pupae

Treatment	Conc.	Mortality%	LC_{50} (ppm) and confidence limits at 95%		LC_{90} (ppm) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
Pet. ether fraction	1000	20.00	3004.44		14919.04		1.84 ± 0.38	0.15	100
	2000	36.67							
	4000	56.67	2180.68	4212.98	8688.99	49128.21			
	8000	80.00							
Methylene chloride fraction	2500	26.67	5904.67		22510.78		2.21 ± 0.4	2.64	50.88
	5000	36.67							
	1000	63.33	4382.38	7689.76	15176.75	47936.571			
	20000	93.33							
Ethyl acetate fraction	1250	16.67	3984.94		8398.31		1.93 ± 0.39	0.34	75.4
	2500	36.67							
	5000	53.33	2949.73	5554.31	10953.25	55728.20			
	10000	80.00							

The insecticidal properties of any plant extract depends upon its chemical constituents. Accordingly, the high insecticidal activity of the petroleum ether fraction is related to its chemical components such as diterpene componds (Neophytadiene and Phytol), and triterpene (squalene) which has been previously proven its miticidal activity against plant mites [19], causing systemic toxicity on *Tetranychus cinnabarinus* [20]. Terpenes compounds have high insecticidal properties because they interfere with the insects` octopaminergic system, which responsible for neurotransmition and neuromodulation [21]. Also, presence of anethole may support the toxicity of petroleum ether fraction whereas it was noticed that several essential oils contain high amount of anethole have insecticidal activity against the mosquito *Ochlerotatus caspius* [22] and *Aedes aegypti* [23, 24] larvae. Also, Phytol had deterrence properties for controlling sumac flea beetles [25].

Along the same lines, methylene chloride fraction activity may be due to presence of phytol and alkaloidal compounds (metamfetamine). Also, the insecticidal activity of ethyl acetate fraction may be as due to its chemical constituents such as oleic acid which recorded high insecticidal potency against *Aedes aegypti* [26]. We can`t confirm the exact ingredient responsible for the insecticidal property of the tested fractions due to the large number of its components and the synergistic effect of them to reveal their toxicity.

Conclusion

The present study characterized the volatile components of different fractions of *E. pulcherrima* extract by using GC/MS technique. Also, the insecticidal activity of these different fractions against different stages (eggs, 4th instar larvae and pupae) of *S. littoralis* were studied. The most active fraction against all tested stages was petroleum ether fraction. Our results emphasized that *E. pulcherrima* is not just an ornamental tree but, it can be a source of extracts or compounds having insecticidal activity, which can be used as environmental friendly insecticides.

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