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Research Paper

Furocoumarin and Quinolone Alkaloid with Larvicidal and Antifeedant Activities Isolated from *Ruta chalepensis* Leaves

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ABSTRACT

As an alternative to synthetic ones, plants have received much attention as a source of pesticidal active secondary metabolite such as phenolics, terpenoids, coumarins and alkaloids. The aqueous ethanolic extract (80%) of *Ruta chalepensis* leaves (Rutacea) showed larvicidal and antifeedant activities against the larvae *Spodoptera littoralis* (Boised). Bioassy-guided isolation of this aqueous extract yielded two compounds, which were characterized as furocoumarin alkaloid- 3(2",2"dimethyl butenyl) 3′- hydroxy dihydrofuropsoralen and quinolone alkaloid- rutamine. Both compounds showed larvicidal activity against the *Spodoptera littoralis* with LC_{50} of 1.598, 1.215 mg / ml, together with deterrent activity with SC_{50} of 0.15, 0.67 mg / ml, respectively.

Keywords: *Ruta chalepensis*, Rutacea, Furocoumarin, Rutamine, larvicidal activity, antifeedant activity, *Spodoptera littoralis*.

INTRODUCTION

Plants constitute a rich source of bioactive compounds such as phenolics, terpenoids, coumarins and alkaloids (Harborne, 1993; Ahn, et al., 1998). Since these compounds which are often active against a limited number of species including specific target insects, are biodegradable to non toxic products and potentially suitable for use in integrated pest management programs, they could lead to the development of new classes of safer insect control agents (Park, et al., 2002; Mansour, et al., 2004).

Ruta chalepensis L. (Family-Rutaceae) is a small shrub, native to the Mediterranean Basin (Polunin and Huxley, 1987). This plant is widely used in folk medicine as an antirhematic, an antispasmodic, and a treatment for snake bites, headaches and wounds (Ghazanfar, 1994). Also has many biological activities such as molluscicidal activity (Hmamouchi, et al., 2000), larvicidal activity (Mookey, et al., 2002), and repellent activity (Hadis, et al., 2003). Previous phytochemical research on this plant has resulted in the isolation of alkaloids, coumarins and flavonoids (Ulubelen, et al., 1986, 1994; El-sayed, et al., 2000; Farag, et al., 2005; Emam and Mahmoud, 2005).

The present paper deals the isolation and structural elucidation of compounds responsible for larvicidal and antifeedant activities of the aqueous ethanolic extract (80%) of *Ruta chalepensis* leaves against the larvae *Spodoptera littoralis* (Boised).

MATERIALS AND METHODS

Plant Collection: Leaves of *Ruta chalepensis* L., were collected at the flowering stage in 2004 from plants growing on the experimental farm of the Faculty of Agriculture, Cairo University, Giza. Plant taxonomists in the Botany Department, Faculty of Science, Cairo University confirmed the taxonomic identification of the plant species. A voucher specimen {R.C. 30} was deposited in the herbarium of the Biochemistry Department, Faculty of Agriculture, Fayoum University.

Plant Extraction: Ground air-dried leaves (350 g) were extracted three times with 80% ethanol (each 700 ml) at room temperature (25 ± 2 ºC). The extract were filtered and evaporated under reduced pressure to afford 55.2 g of residue extract and tested for larvicidal activity. A portion of the aqueous ethanolic extract (40 g) was dissolved in water (150 ml), and extracted with $CHCl_3$ (3×50 ml) to give $CHCl_3$ soluble components (designated as fraction A (Fr. A), 6.5 g).

The aqueous layer was freeze-dried (33.5 g) and then extracted with $CHCl_3$ - MeOH-H_2O (70:30:5; 150 ml). After centrifugation, both the supernatant and the precipitate were dried under reduced pressure to afford 4.6 g (Fr. B) and 28.8 g (Fr. C), respectively. The three fractions i.e. A, B and C were tested for larvicidal activity.

Insecticidal Activity

Insects: Susceptible strain of cotton leaf worm *Spodoptera littoralis* (Lepidoptera) was used for bioassay. This strain has been colonized for many years in laboratory of Econ. Entomol. and Pesticides Department., Faculty of Agriculture, Cairo University, without exposure to any pesticides and reared on castor bean leafs. All experiments and culture were carried out at 28 ± 2°C, 65 % relative humidity, with a 14:10 light: dark cycle under conditioning rearing room by using thermometer, hygrometer and light controlled by timer adjusted to 14 hours on and 10 hours off.

Insecticides: Two commercial formulations of insecticides used in bioassays were representing, as carbamate [Methomyl, S-methyl N-(methylcarbamoylox) thioacetimidate (Lannate 90% SP), purchased from Scientific Office DuPont in Egypt] and organophosphates classes of insecticides [Profenofos, O-4-bromo-2-chlorophenyl O-ethyl O-propyl phosphorothioate (Selecron 72% EC) purchased from Scientific Office Syngenta in Egypt].

Toxicity Line: Five serial concentrations (mg/ml) of the 80% ethanol extract, chloroform fraction (Fr. A), and the isolated sample tested of compounds I & Π were prepared in

acetone. Five discs (5 cm diameter) of castor bean leaves were used for each concentration and treated using dipping technique for 10 sec., then allowed to air drying. One hundred, one-day-old second instar larvae of *Spodoptera littoralis* were exposed to the treated leaves in five replicates, 20 larvae each. Each replicate was hold in a glass jar covered with musline. The mortality percent was recorded after 24 h. exposure, corrected by the formula of Abbott, (1925), and data were analyzed by the log-probit method of Finney, (1971) using the EPA probit analysis program version 1.5, to calculate slope of the toxicity lines, $LC_{25,}$ LC_{50} and LC_{90} values.

Binary Combination of the Tested Fractions: The joint action of different binary combination of the 80% ethanol extract, chloroform fraction (Fr. A), or the compounds I and Π and insecticides (Profenofos and Methomyl) was assessed according to the method described by Swelam and Sayed, (2006).

The combined action of the different mixtures was expressed as co-toxicity factor which was estimated by the equation of Mansour, et al., (1966)

Assay for Antifeedant Activity: Different concentrations of the of $CHCl_3$ fraction (Fr. A) and the isolated compounds (I and Π) in acetone were applied (by a pipette) on the two surfaces of castor bean leaves discs (7 cm diameter), and then placed in convenient small glass jars. Ten 4^{th} instar larvae were weighed and then placed on each treated disc. Four replicates were used for each concentration. Control (untreated check) discs were treated with pure acetone only. Forty larvae were left without feeding during the whole period of the experiment, and then their weights were recorded. The weight of larvae was recorded 24 hrs post treatment. Percent starvation for each treatment was calculated by the equation of Abdel-Mageed, et al., (1975) and Kandil, et al., (1985).

Isolation of the Bioactive Compounds: Analytical and preparative TLC were carried out on Merck precoated silica gel plates (F245 thickness-0.25 mm and 2.0 mm, respectively) using the following solvent systems-

1. n-Butanol - Acetic acid - Water (4:1:5) upper layer
2. Ethylacetate - Acetic acid - Formic acid - Water (100:11:11:27)
3. Chloroform - Methanol -Water (100:0:0 ; 90:10:0 ; 85:15:0 ; 70:30:5 ; 60:40:5)
4. Chloroform - Benzene (90:10 and 85:15)

Spots on TLC were detected under UV light (254 and 365 nm) and by spraying with concentrated H_2SO_4 followed by heating at 105 ºC for 5 min. and by spraying with modified Dragendroff's reagent to detect alkaloids.

The bioactive fraction (Fr. A) was subjected to the isolation of the insecticidal component as follows:-Six gram of this fraction were fractionated on silica gel column (230-400 mesh,150 g Merck) and eluted with a gradient of $CHCl_3$: MeOH : H_2O (100:0:0 ; 90:10:0 ; 85:15:0 ; 70:30:5 ; 60:40:5 and 0:100:0 , 200 ml for each eluent).Ten fractions of each eluent were collected. According to differences in composition monitored by TLC using system as the same eluent, 17 fractions were obtained and then tested for insecticidal activity. Maximum bioactive fraction No.5 (which have maximum mortality percent), eluted with $CHCl_3$: MeOH (90:10) between (140-180 ml; 870 mg residue) was further purified on silica (15 g) with Chloroform-Methanol (100:0 and 95:5; 100 ml for each) as eluent. Ten fractions of 10 ml each were collected. The eluents were combined on the basis of similar TLC (using the same eluent) to afford 5 fractions designated as (A, B,…..E). The fraction B (284.2 mg) eluted with $CHCl_3$ (100 %) between 20-70 ml and fraction C (456.8 mg) eluted with $CHCl_3$: MeOH (95:5) between

10-80 ml which containing the major compounds, were further purified several times on silica gel column (15 g) with $CHCl_3$-Benzene (85:15) as an eluent followed by preparative TLC with Chloroform : Benzene (90:10) to give two active compounds I (168.8 mg; 2.81%) and Π (214.6 mg; 3.57%). The purity of the two isolated compounds was established by the resolution of each one as a single spot on the four different TLC solvent systems as described above.

Identification of the bioactive compounds: The purified compounds were characterized by detection tests (Farnsworth, 1966) and spectroscopic methods (^{1}H, ^{13}C-NMR and MS). ^{1}H, ^{13}C-NMR spectra were recorded in DMSO- d6 on a Varian Mercury VXR 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). The chemical shifts (ppm) were related to that of the solvent. Mass spectra were recorded on GC/MSQP 100 Ex Shimadzu Mass spectrometer at 70e.v. Spectroscopic analysis (^{1}H, ^{13}C-NMR and MS) were performed at central laboratory in Faculty of Science, Cairo University.

RESULTS

The aqueous ethanolic extract (80%) of the air dried leaves of *Ruta chalepensis* L. showed larvicidal activity against the second instar larvae of *Spodoptera littoralis*. This aqueous extract was fractionated into three fractions A, B, C, and tested for insecticidal Activity**.** Among the three tested fractions (A, B, C), only the chloroform soluble component (Fr. A) exhibited larvicidal activity. Bioactivity-guided separation of this fraction (Fr. A), by using chromatographic methods as described above, resulted into isolation of two chromatographically pure compounds I & Π.

***Compound* I:** It was obtained as colorless amorphous powder. The EI mass spectrum of this compound showed a molecular ion peak [M^+] at m/z 286 indicating the molecular formula $\mathbf{C_{17}H_{18}O_4}$ which was supported by 17 carbon signals in the ^{13}C-NMR spectrum.

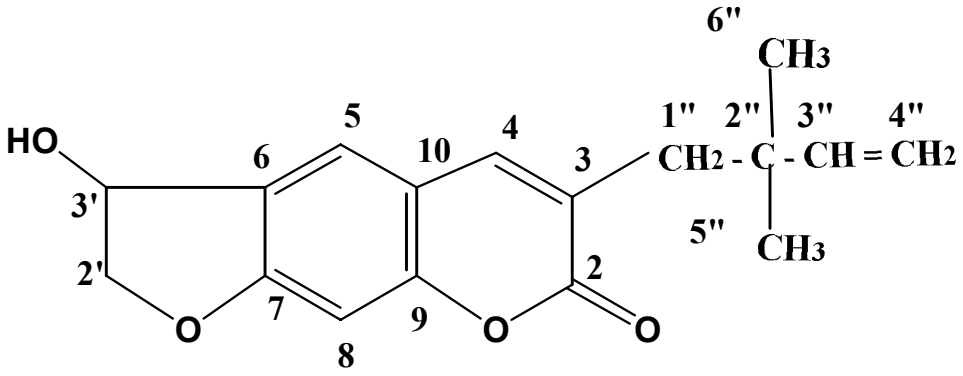
The 3'- hydroxy dihydrofuropsoralen was established from the appearance of eleven signal peaks corresponding to 6 aromatic carbon atoms (between δ 95.84 to 162.51 ppm). Two olefinic carbon atoms (δ 129.01 and 138.52 ppm), carbonyl group (δ 159.18 ppm), hydroxy methine (δ 69.93 ppm) and oxymethylene (δ 90.71 ppm) in the ^{13}C-NMR spectrum (Table 1) and from the appearance of β-coumarin proton signals at δ 7.71 ppm (1H, s), two aromatic proton signals at δ 6.71 ppm (1H, s ; H-8) and δ 7.45 ppm (1H , s ; H-5), two proton signals at δ 4.66 ppm (dd, J = 8.1, 11.7 Hz ; H -3') and one proton signals at δ 3.1 ppm (t ; H-2′) in the ^{1}H –NMR spectrum.

The presence of 2″,2″-dimethyl butenyl group was confirmed from the ^{1}H –NMR spectrum (Table-1) due to the signal peaks corresponding to a methylene group at δ 1.38 ppm (2H, s), a geminal dimethyl at δ 1.12 ppm (3 H, s) and δ 1.13 ppm (3H, s) and a vinylic group at δ 5.01 ppm (2H, dd, J = 5.1,10.5 Hz) and δ 6.12 (H, dd, J = 10.5, 17.7 Hz) as well as from the ^{13}C-NMR spectrum due to the presence of six signal peaks assigned to the carbon atoms of this group (Table-1). The presence of only β- coumarin proton signal indicated that α proton was substituted by 2″,2″-dimethyl butenyl group. From these data the structure of the furocoumarin was deduced to be 3 (2", 2"dimethyl butenyl) 3′- hydroxy dihydrofuropsoralen (I).

The ^{13}C-NMR data of this compound was similar as previously reported by Duddeck and Kaiser, (1982).

Table-1: ^{1}H, ^{13}C - NMR spectra of 3 (2", 2"dimethyl butenyl) 3′-hydroxy dihydrofuropsoralen (Compound I) in DMSO – d6.

Atom NO	^{13}C-NMR spectrum	^{1}H –NMR spectrum
1	-	-----
2	159.18	----
3	129.01	------
4	138.52	7.71(s)
5	123.72	7.45 (s)
6	125.12	-----
7	162.51	-----
8	95.84	6.71(s)
9	153.92	--------
10	112.13	-------
′1	----	----
′2	90.71	4.66 (dd, J=8.1, 11.7 Hz)
′3	69.93	3.1 (t)
″1	28.74	1.38 (s)
″2	24.73	--------
″3	111.65	6.12 (dd, J=10.5,17.7Hz)
″4	145.47	5.01 (dd, J = 5.1,10.5 Hz)
″5	25.95	1.13 (s)
″6	25.82	1.12 (s)



Compound (I)

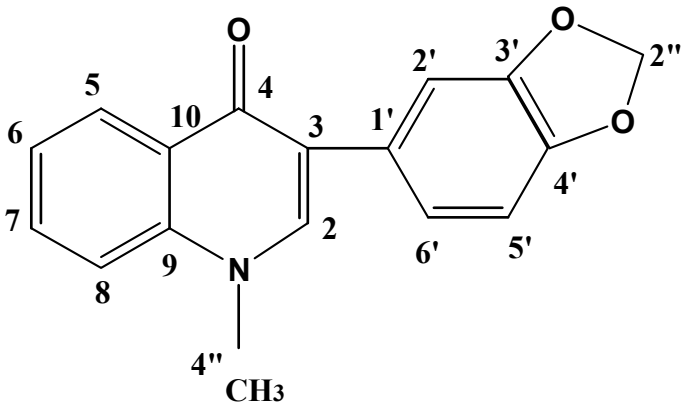
***Compound* II:** This compound was obtained as colorless amorphous powder and gave positive colour with modified Dragendorff 's reagent on TLC, suggesting it is an alkaloid compound.

Its molecular formula was established to be **$C_{17}H_{13}O_3N$** by the combination of EI-MS ([M^+], m/z 279) and ^1H, ^{13}C–NMR data (13 proton signals and 17 carbon signals).

The NMR spectra of this compound (Table-2) showed the presence of 3-substituted 1-methyl-quinolone moiety, due to the appearance of four adjacent aromatic proton signals at δ 8.21 (1H, d, J = 7.8 Hz) , δ 7.78 (2H, d, J = 3.0 Hz) and δ 7.43 (1H, pent, J = 3.9 Hz), N-methyl group at δ 3.59 (3H, s) and olefinic proton singlet at δ 5.98 (1H,s) in the ^1H- NMR spectrum and six aromatic carbon signals between δ 117.32 to 154.25 ppm , carbonyl group (δ 175.59 ppm), olefinic group (δ 111.17 and 141.69 ppm) and N-methyl group at δ 37.21 ppm in the ^{13}C –NMR spectrum. Also the NMR spectra revealed the presence of 3,4-methylene dioxyphenyl moiety (Somanathan and Smith, 1981) due to the appearance of three aromatic proton signals; two coupled with each other at δ 7.02 (1H, dd, J=8.1,1.5 Hz) and δ 7.15 (1H, d, J = 1.5 Hz) and one singlet at 7.06 (1H,s) and the dioxymethylene group signal at δ 6.13 (2H,s) in the ^1H- NMR spectrum and six aromatic carbon signals (between δ 108.4 to 148.17 ppm) and dioxymethylene group at δ 101.58 ppm in the ^{13}C – NMR spectrum.

Table - 2: ^1H, ^{13}C - NMR Spectra of 1-methyl-3-[3', 4'-(methylenedioxy) phenyl] -4-quinolone (Compound II) in DMSO- d6.

Atom NO	^{13}C-NMR spectrum	^1H –NMR spectrum
1	-------	-------
2	141.69	5.98 (s)
3	111.17	-------
4	175.59	------
5	125.22	8.21(d, J=7.8 Hz)
6	123.32	7.43 (pent, J= 3.9 Hz)
7	132.22	7.78 (d, J = 3.0 Hz)
8	117.32	7.78 (d, J = 3.0 Hz)
9	154.25	------
10	126.24	------
′1	129.24	------
′2	109.06	7.08 (s)
′3	148.17	------
′4	147.32	------
′5	108.40	7.02 (dd, J = 8.1, 1.5 Hz)
′6	122.72	7.15 (d, J = 1.5 Hz
″1	-------	-----
″2	101.58	6.13 (s)
″3	-------	-------
″4	37.21	3.59 (s)



Compound (II)

The NMR characteristics of this compound were similar to those for 1-methyl-3-[3', 4'-(methylenedioxy) phenyl]-4- quinolone (trivial name is Rutamine) previously isolated from *Ruta graveolens* (Gonzalez, et al., 1974).

A detailed chemical abstracting (Faculty of Pharmacy library, Cairo University from 1970 up to 2006) shows that compounds I and II were isolated for the first time from this plant

The toxicity effect: The toxicity effect of aqueous ethanolic extract (80%), chloroform fraction (Fr. A), isolated compounds I & II and synthetic insecticides (Profenofos and Methomyl) are given in Table-3. The data indicated that the chloroform fraction shows more toxicity than 80% ethanol crude extract. Their LC_{50} were recorded as 0.89 and 2.42 mg / ml respectively. The isolated compounds I & II show moderate effect between both chloroform fraction (Fr. A) and 80% ethanol crude extract. They have LC_{50} value 1.598 and 1.215 mg/ml, respectively. The LC_{90} of all tested material show that no crossing in their toxicity effect, where they take the same arrangement. They could be arranged in descending order as follow ; 7.70, 4.401, 3.251 and 2.99 mg / ml for 80% ethanol extract, compound I, compound II and chloroform fraction (Fr. A), respectively .

The LC_{50} of the 80% ethanol crude extract was used to estimate the relative potency where as the LC_{50} value for Profenofos (LC_{50} 0.0181) was used as standard insecticide to calculate the toxicity index according to the equation of Sun, (1950).

The relative potency could be arranged in descending order as follow, Profenofos, Methomyl, chloroform fraction (Fr. A), compound II, compound I and 80% ethanol crude extract with a value of 133.7, 18.33, 2.72, 1.99, 1.52 and 1, respectively (Table-3). The chloroform fraction (Fr. A) showed the higher effect than compound II, compound I and 80% ethanol crude extract. The effectiveness of the chloroform fraction (Fr. A) expressed as the toxicity index (2.03%) when compared with Profenofos, 1.59 % for compound II, 1.13 % for compound I and 0.75 % for 80 % ethanol crude extract.

These data show that the 80% ethanol extract contains some constituents which have antagonism effect to each other. The non polar part of this extract, which is

separated through the chloroform, was more effective than its mixture with more polar part.

Table-3: Lethal Concentration (LC_{25}, LC_{50}, LC_{90}), slope and toxicity index of 80% ethanol extract, chloroform fraction (Fr. A), isolated compounds (I & II) and synthetic insecticides (Profenofos and Methomyl) against second instar larvae of *S. littoralis*.

Tested material	LC_{25} (mg/ml)	LC_{50} (mg/ml)	Chi-Square		LC_{90} (mg/ml)	Slope	Relative potency	Toxicity index
			Cal.	Tab.				
Ethanol 80%	----	2.42	1.61	9.488	7.70	2.48	1.00	0.75
Chloroform (Fr. A)	----	0.89	3.088	9.488	2.99	2.32	2.72	2.03
compound I	0.77	1.598	4.26	9.488	4.401	2.849	1.52	1.13
compound II	0.71	1.215	4.094	7.815	3.251	1.701	1.99	1.59
Profenofos	0.0049	0.0181	3.924	9.488	0.112	2.13	133.70	100
Methomyl	0.056	0.132	2.33	7.815	0.451	2.56	18.33	13.7

- Cal. : calculated Chi-square for heterogeneity; Tab. : tabular Chi-square for heterogeneity at 0.05 level
- Relative potency : obtained by comparing the potency of the lowest effective material at the level of the LC_{50} to the tested material
- Toxicity index : obtained by comparing the efficiency of the tested material at the level of LC_{50} to their most effective material

***Joint action**:* The joint action of binary mixtures of the ethanolic crude extract (80%), chloroform fraction (Fr. A), compound I and compound II with Profenofos or Methomyl insecticides against the second instar larvae of the cotton leaf worm *S. littoralis* are presented in Table-4. Five mixtures exhibited a potentiation, 15 additive and 7 inhibition affect on the tested larvae. The mixtures with the potentiation effect can be arranged in descending order as chloroform / Methomyl (1 : 1), chloroform / Profenofos (1:1), compound II / Profenofos (1:2), chloroform / Methomyl (1:2) and compound I / Profenofos (1:2) with a co-toxicity factor 80, 70, 25, 22.7 and 21.3, respectively .

An antagonistic effect was recorded using the mixture between compound I and Methomyl at the ratios of 1:1 and 2:1, with co-toxicity -22.2 and -41.3, respectively. Also the mixtures between compounds II with Methomyl at the ratio of 2:1 with co-toxicity -42.3 and between compounds I with Profenofos (2:1) by a factor of – 21.2 show inhibition effect (Table-4).

The mixture of compound I and II gave an antagonism at the ratios of 1:1 and 1:2. On the other hand, this mixture exhibited an additive effect against the *S. littoralis* at the ratio of 2:1. The highest additive effect was recorded for the mixture between 80 % ethanol crude extract and Methomyl (1:2) with the co-toxicity factor of 18.1 and the lowest was recorded for the binary mixture of compound II and Methomyl (1: 2) by a co-toxicity factor -20.0 (Table-4).

Table-4: The effect of binary mixtures of 80 % ethanol extract, chloroform fraction (Fr. A), isolated compounds I & II and insecticides on the second instar larvae of *S. littoralis*.

Combination	Mixing ratio	% Expected mortality	% Observed mortality	Co-toxicity factor	Joint action category
chloroform fraction (Fr. A)+ profenofos	1:1	50	85.0±4.9	70	Potentiation
	2:1	75	65.0±4.6	13	Additive
	1:2	75	80.0±3.8	6.7	Additive
chloroform fraction (Fr. A) + methomyl	1:1	50	90.0±5.6	80	Potentiation
	2:1	75	85.0±2.0	13.3	Additive
	1:2	75	92.0±3.4	22.7	Potentiation
80% ethanol crude ext.+ profenofos	1:1	50	60.0±4.4	20	Additive
	2:1	75	55.0±2.3	- 26.7	Inhibtion
	1:2	75	76.0±3.5	1.35	Additive
80% ethanol crude ext. + methomyl	1:1	50	46.6±1.9	2.6	Additive
	2:1	75	81.2±2.0	8.3	Additive
	1:2	75	88.6±4.8	18.1	Additive
compound II + Profenofos	1:1	50	42.2±2.0	-15.6	Additive
	2:1	75	66.0±5.1	-12.0	Additive
	1:2	75	94.0±2.4	25.0	Potentiation
compound II +Methomyl	1:1	50	52.0±2.3	4.0	Additive
	2:1	75	43.3±2.2	-42.3	Inhibition
	1:2	75	60.0±4.4	-20.0	Additive
compound I + Profenofos	1:1	50	55.6±2.2	11.2	Additive
	2:1	75	59.1±3.2	-21.2	Inhibition
	1:2	75	91.0±5.4	21.3	Potentiation
compound I + Methomyl	1:1	50	38.9±4.5	-22.2	Inhibition
	2:1	75	44.0±3.4	-41.3	Inhibition
	1:2	75	67.8±2.1	-9.6	Additive
compound I + II	1:1	50	38.3±2.0	-23.4	Inhibition
	2:1	75	75.8±4.3	1.1	Additive
	1:2	75	54.6±2.4	-27.2	Inhibition

Insect antifeeding activity: The phenomenon of anti-feeding effect of some chemicals or plant extracts has attracted the attention of several investigators for the possibility of using it in the field of plant protection, in general, and in integrated control program in special. The present piece of work is a contribution in the direction against the *Spodoptera littoralis*.

In the present experiments, the 4th instar larvae were fed on the two surfaces of castor bean leaves discs (7 cm. diameter), either untreated or treated with different concentrations of the of $CHCl_3$ fraction (Fr. A) and the isolated compounds (I and Π) in acetone. Differences in larval weights after 24h were recorded and the percent starvation was calculated (Table-5). The starvation curves were statistically analyzed according to Finney, (1971).

The SC_{50} for the chloroform fraction (Fr. A), compounds II and I were 1.60, 0.67 and 0.15 mg/ml respectively, while the SC_{90} were 6.02, 2.44 and 0.51 mg/ml. The chloroform fraction (Fr. A) and the two compounds I and II isolated from *Ruta chalepensis* L. *exhibited* positive anti-feeding effect against the 4th instar larvae of *S. littoralis*.

Table-5: Starvation concentration (SC_{50}, SC_{90}), slope and toxicity index of chloroform fraction (Fr. A) and isolated compounds I and II against the 4th instar larvae of *S. littoralis*.

Tested material	SC_{50} (mg/ml)	Chi-Square		SC_{90} (mg/ml)	Slope	Relative potency	Toxicity index
		Cal.	Tab.				
Chloroform fraction (Fr.A)	1. 60	1.162	7.815	6.02	2.178	1.00	10.127
Compound I	0.15	1.297	11.07	0.51	2.583	10.67	100
Compound II	0.67	4.242	11.07	2.44	2.282	2.39	23.881

- SC_{50} : Starvation concentration needed to starve 50% of exposed larvae
- SC_{90} : Starvation concentration needed to starve 90% of exposed larvae
- Cal. : calculated Chi-square for heterogeneity; Tab. : tabular Chi-square for heterogeneity at 0.05 level
- Relative potency : obtained by comparing the potency of the lowest effective material at the level of the SC_{50} to the tested material
- Toxicity index : obtained by comparing the efficiency of the tested material at the level of SC_{50} to their most effective material

DISCUSSION

Despite the literature referring to synergism in insects, the physiological mechanism by which one insecticide or any substance which is toxic or non-toxic improved the effect of another insecticide, remains unclear in some cases. The above results demonstrate that the chloroform fraction (Fr. A), synergise the effect of the Profenofos (1:1) and Methomyl (1:1 and 2:1). The compound I and II synergise the effect of the Profenofos only at the ratio of 1:2 for both compound. These results indicate that both compound I and II have the same mode of action. The crude ethanolic extract (80%) exhibited additive effect with both insecticides with exeption to the mixture with Profenofos at the ratio of 2:1 (Table- 4).

This result may be due to the effect of Profenofos insecticide in inhibition of the cytochrome P450 monooxygenase, and this corresponds with previously reported by Neal and Wu, (1994) and Scott, (1996).

The results in the present study agree with those, which reported by Diawra, et al., (1993). They found that the combination of furocoumarin; psoralen with bergapten, xanthotoxin, or both resulted in significantly antagonistic effects on insect mortality. However the combination of bergapten and xanthotoxin, produced additive effects on the *Spodoptera exigua* (Hubner).

Coumarin surangin B compound displays particularly potent activity against mosquito larvae, houseflies, crickets and high toxicity toward a variety of arthropod species. The insecticidal activity is associated predominantly with coumarins containig 4-(1-acetoxypropyl) side chain. Insecticidal coumarins (including surangin B) and non insecticidal coumarins isolated from *Mammea* species act as mitochondrial uncouplers at low (0.5 mg/ml) concentrations (Zheng, et al., 1998).

According to Enriz, et al., (2000), the presence of the furan ring in the side chain is mandatory for an acceptable antifeedant effect. In fact, evidence obtained previously suggests that they can play a role as feeding deterrent, in agreement with previous observation that furanocoumarins isolated from the fruits of *Tetradium daniellii* were potent feeding deterrents to larvae of *Spodoptera littoralis* and *Heliothis virescens* (Stevenson, et al., 2003). Moreover, furanocoumarins were evaluated as inhibitors of *Manduca sexta* midgut cytochrome P450, while the absence of furan showed a reversible inhibitor with an I_{50} value two orders of magnitude higher than psoralen. All of the tested inhibitory furanocoumarins were mechanism-based irreversible inhibitors. The mechanism of inhibition proposed that furanocoumarins is oxidized by cytochrome P450 at the double bond of the furan ring forming an unstable epoxide that can react with cytochrome P450 (Neal and Wu, 1994).

CONCLUSION

On the basis of the present study we concluded that isolated two compounds furocoumarin; 3(2",2"dimethyl butenyl) 3′- hydroxy dihydrofuropsoralen and quinolone alkaloid; rutamine were in part responsible for the larvicidal and antifeedent activities of the aqueous ethanolic extract of *Ruta chalepensis* leaves and these two compounds could be used to control the larvae of *Spodoptera littoralis*.

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