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MOLLUSCICIDAL SESQUITERPENE LACTONES OF CYPERUS ARTICULATUS AGAINST THE LAND SNAIL MONACHA CARTUSIANA

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ABSTRACT

In a survey of five plant extracts to Molluscicidal activity against the land snail *Monacha cartusiana*, only that of *Cyperus articulatus* L. (as a crude defatted extract) showed a high activity (23.3%, 56.6% and 76.6% mortality for concentrations of 0.25, 0.50 and 1.0%, respectively). The LC_{50} was 0.47%. The extract of *Cyperus alopecuroides* Rottb. showed low activity, while those of *Cynanchum acutum* L., *Malva parviflora* L. and *Bombax malybricum* L. were inactive.

Fractionation of the active extract of *Cyperus articulatus* into four successive fractions by solvent extraction (pet. ether, CH_2Cl_2, $CHCl_3$, MeOH extracts), followed by further separation of the pet. ether extract into a volatile and a non-volatile fractions by steam distillation, and subsequent examination of the molluscicidal activity revealed no activity of the different fractions.

Chromatographic separation of the different fractions followed by GC/MS analysis resulted in the identification of thirty seven compounds, including eight monoterpenoids, twenty one sesquiterpenoids, two sesquiterpene lactones and six fatty acids.

Reexamination of the fraction containing the sesquiterpene lactones to the molluscicidal activity proved its high activity. It gave 100.0, 86.6 and 80.0% mortality for concentrations of 0.50, 0.25 and 0.125%, respectively. The LC_{50} value was 0.056%. The molluscicidal activity was attributed to the sesquiterpene lactones hence no correlation between the fatty acids (the other constituents of the fraction) in other fractions and the molluscicidal activity. The two molluscicidal sesquiterpene lactones of *C. articulatus* are 1(10),4-germacradien-12,6-olide and 4-germacren-12,6-olide.

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INTRODUCTION

Cyperus articulatus L. (Cyperaceae) is a plant commonly used in traditional medicine in Africa and Latin America to treat a wide variety of human diseases. Rakotonirina *et al.*[5] found that the rhizome of *C. articulatus* has pharmacological properties similar to those of sedatives. The sedative actions probably explain at least part of the therapeutic efficiency claimed for this plant in traditional medicine [**Rakotonirina *et al.*, (2001)**]. The mechanism of action of *Cyperus articulatus* extracts as sedative and anticonvulsant was studied [**Bum *et al.*, (2004)**]. They reported that *Cyperus articulatus* extracts possessed components that could decrease excitation and increase inhibition in the central nervous system. The methanolic extract [**Bum *et al.*, (2001)**] as well as the water extract [**Bum *et al.*, (2003)**] of rhizomes of *Cyperus articulatus* L. possesses anticonvulsant properties in animals that might explain its use as a traditional medicine for epilepsy in Africa.

Typical compounds of the family Cyperaceae are flavonoids [**Abdel-Mogib *et al.*, (2000) and Dawidar *et al.*, (1994)**], sesquiterpenes [**Rakotonirina *et al.*, (2001); Bum *et al.*, (2001); Bum *et al.*, (2003) and Bum *et al.*, (2004)**]. benzoquinones [**Allan *et al.*, (1978)**], stilbenes [**Dawidar *et al.*, (1994)**], coumarins [**Awaad & Zain (1999)**]. furocoumarins [**Awaad & Zain (1999)**], phenolics [**Allan *et al.*, (1970)**], triterpenes [**Fedeli & Cortesi (1970) and Nassar *et al.*, (2000)**] and sterols [**Fedeli & Cortesi (1970)**]. The literature survey indicated that *Cyperus articulatus* L. only contains monoterpenoids and sesquiterpenoids.

Couchman *et al.*, (1964) reported the monoterpenoids myrtenal and myrtenol as well as the sesquiterpenoids copaene, an unidentified hydrocarbon isomer with copaene, a sesquiterpenoid ketone articulone. and the corresponding alcohol articulol in essential oil of *Cyperus articulatus* L. Additionally, the sesquiterpenoids cyperotundone [**Hikino *et al.*, (1967)**], a monocyclic sesquiterpenic diketone, named mandassidione. mustakone and isopatchoul-4(5) en-3-one were isolated from the rhizomes of *Cyperus articulates* [**Nyasse *et al.*, (1988b)**]. **Nyasse *et al.*, (1988a)** reported the isolation of the sesquiterpenoids α-corymbolol and corymbolone from the rhizomes of *Cyperus articulatus*.

Corresponding molluscicidal activity of *Cyperus articulatus* L., nothing was reported. In this article we report, in addition to the composition of the essential oil. the molluscicidal activity of sesquiterpene lactones of *Cyperus articulatus* against the land snail *Monacha cartusiana*.

RESULTS AND DISCUSSION

The extract of *Cyperus articulatus* is highly aromatic with a permanent lovely smell that resembles "Oad" smell. Repeated chromatographic separation trials failed to separate its components. Monitoring of separation processes by ^1H NMR measurement always revealed the presence of complicated mixtures of terpenoids and fats. Steam distillation of the extract didn't completely separate the aromatic matter from the extract. The CH_2Cl_2-extract of the remained matter after steam distillation is also aromatic of good smell. The difficulty in isolation of pure fractions containing only a single minor component has largely restricted the use of IR, NMR in the identification.

The advent of fused quartz, bonded-phase capillary column has led to very reproducible retention times [**Jennings (1987)**]. Given reproducible retention times, the unequivocal identification of essential oil components is possible by retention time/mass spectroscopy/computer searching algorithms [**Adams (1995)**].

GC/MS analysis of the steam-volatile fraction, Ca1, and the methylene chloride extract fractions, Ca21-Ca24, revealed the identification of thirty seven compounds, which could be classified into eight monoterpenoids **1-8**, twenty three sesquiterpenoids **9-31** and six fatty acids **32-37**. Spectral identification of the separated compounds was based on good agreement with the expected fragmentation patterns as well as with authentic spectra [**Adams (1995)**] and with those of the NIST library.

Molluscicidal activity of certain plant extracts against *M. cartusiana* land snails as leaf dipping and residue film applications had been studied under laboratory conditions.

Data presented in Table 7 showed that *Cyperus articulatus* L. extract exhibited high molluscicidal activities against *M. cartusiana* land snails in both dipping and residue film applications at 76.6 and 70 % mortality after 48 hrs, while *Cyperus alopecuroids* Rottb. showed 23 % and 20 % mortality. *Cynanchum acutum* L., *Malva parviflora* L. and *Bombax malybricum* L. extracts faild to exhibit molluscicidal activity.

Results in Table 8 indicated that the molluscicidal activity of the crude extract of *C. articulatus* as well as its fraction containing sesquiterpene lactones, fraction Ca32e, exhibited a high toxic action against *M. cartusiana* with mortality of 76.6%, 100 % and LC_{50} of 0.470%, 0.056 %. respectively.

EXPERIMENTAL

Instrumentation

GC/MS instrument was a Varian GC interfaced to Finnigan SSQ 7000 Mass Selective Detector (MSD) 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 polydimethylsiloxane (0.5 μm film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C / min. to 250°C and isothermally for 10 min., at 250°C. Injector temperature was 200°C and the volume injected was 0.5 μl. Transition–line and ion source temperature were 250°C and 150°C respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

The Plant material

Cyperus articulatus L., family Cyperaceae, was collected at a canal bank on the Mediterranean coastal strip near New Damietta at September 2004. It was identified by Prof. Dr. Mashaly, I., Botany Department, Faculty of Science, Mansoura University.

Extraction

The underground plant parts (rhizomes), 930 g, were dried in shadow, cutted into small pieces and macerated in methanol and left overnight at room temperature, then filtered off. The marc was washed several times by methanol then the filterate was evaporated under reduced pressure to afford the crude extract, 43.6 g, as a brown residue.

Fractionation

The crude extract (43.675 g) was dissolved in small amounts of methanol and diluted with water, then it was exhaustively extracted by petroleum ether and the extract was separated into steam-volatile (Ca1, 0.645 g) and steam-nonvolatile (Ca2, 0.321 g) fractions. The MeOH-extract was then extracted by CH_2Cl_2 to give the third fraction (Ca3, 5.153 g), followed by $CHCl_3$ extraction to give the forth fraction (Ca4, 0.964 g). Finally, the aqueous layer was evaporated to dryness and extracted by MeOH to give the fifth fraction (Ca5, 19.358 g).

Separation of Compounds

Fraction Ca3 was separated on silica gel CC into five subfractions as indicated by Table 1.

The subfraction Ca32a was reseparated by preparative TLC (silica gel, pet.ether/ethyl acetate 3:1) into three bands Ca32a1 (30 mg, R_f 0.97), Ca32a2 (82 mg, R_f 0.92) and Ca32a3 (34 mg, R_f 0.68). Fraction Ca1 gave by GC/MS 16 compounds as indicated by Table2. Fraction Ca32a1 afforded by GC/MS 12 compounds as indicated by Table 3. Fraction Ca32a2 afforded by GC/MS 12 compounds as indicated by Table 4. Fraction Ca32a3 afforded by GC/MS 14 compounds as indicated by Table 5. Fraction Ca32e afforded by GC/MS 7 compounds as indicated by Table 6.

Tested snails

Adult snails of *M. cartusiana* were collected from Egyption clover fields at El-Mansoura district, Dakahlia Governorate. The collected snails were transferred in cloth bags to laboratory. Healthy individuals were kept in glass boxes containing moistened soil and fed on fresh lettuce leaves for two weeks for acclimatization [**El-Okda (1981) and Mortada *et al.*,** (2005)].

Residue film application

The tested surface exposure method was used to evaluate the toxicity effect of plant extracts. Serial concentrations of crude extracts (2ml) were deposited on bottom and cover of Petri-dishes (9.5 cm in diameter). After evaporation, snails were placed in each dish, covered and kept at room temperature for 1, 2 and 3 days. Three replicates were used per each concentration. Mortality percentages were counted.

Leaf dipping technique

The crude extract of *C. articulatus* as well as pet. ether, CH_2Cl_2, $CHCl_3$, MeOH extracts were tested at serial concentrations of 0.125, 0.25, 0.50 and 1.0 %. Similar pieces of green lettuce leaves were dipped in a glass jar containing 100 ml of the tested extract for 5 second, then left until solution dropping stopped before being offered to the snails.

Ten adult individuals were exposed to each treatment leaf in disposable plastic box (24 × 16 ×10 cm). Three replicates of ten snails for each treatment were carried out, in addition to an untreated check. Mortality percentages were recorded after 1, 3, 5 and 7 days post treatment and corrected for natural mortality according to Abbott's formula [**Abbott (1925)**] then subjected to Probit analysis by Finney's method [**Finney (1952)**].

Table (1): CC Separation data of fraction Ca3.

Fraction	Eluent	Weight
Ca31	CH_2Cl_2	0.259 g
Ca32a-Ca32e	CH_2Cl_2/acetone 9:1	0.544, 0.180, 0.121, 0.147, 0.491 g
Ca33	CH_2Cl_2/acetone 8:2	0.297 g
Ca34	CH_2Cl_2/acetone 7:3	1.655 g

Table (2): GC/MS peak identification data of fraction Ca1.

Identification	R_t, min	%
Trans-pinocarveol, **1**	8.00	0.29
Cis-verbenol, **3**	8.13	0.29
Myrtenal, **6**	8.95	0.23
α-Cubebene, **13**	11.31	0.21
α-Copaene, **11**	11.56	0.18
β-Cubebene, **14**	11.78	8.77
Cyperene, **22**	12.23	10.23
γ-Cadinene, **9**	13.15	4.09
τ-Cadinol, **10**	13.71	5.85
Spathulenol. **25**	13.85	7.31
Caryophyllene oxide, **29**	14.20	4.08
1-Valeren-3-one, **21**	14.91	3.51
Humulene epoxide II, **28**	14.99	4.39
Cyperane, **23**	15.45	6.50
Methyl palmitate, **36**	18.32	2.05
Palmitic acid, **34**	18.80	1.75

Table (3): GC/MS peak identification data of fraction Ca32a1.

Identification	**R_t, min**	**%**
α-Copaene, **11**	11.56	7.50
Cyperene, **22**	12.23	10.00
γ-Cadinene, **9**	13.15	2.50
τ-Cadinol, **10**	13.71	5.00
Spathulenol, **25**	13.85	12.50
Caryophyllene oxide, **29**	14.20	4.17
1-Valeren-3-one, **21**	14.91	4.58
Humulene epoxide II, **28**	14.99	13.33
α-Copaen-8-ol, **12**	15.08	15.00
Cyperane, **23**	15.45	3.33
Methyl palmitate, **36**	18.32	1.17
Palmitic acid, **34**	18.80	0.83

Table (4): GC/MS peak identification data of fraction Ca32a2.

Identification	**R_t, min**	**%**
α-Copaene, **11**	11.56	6.25
Cyperene, **22**	12.23	8.75
γ-Cadinene, **9**	13.15	2.50
τ-Cadinol, **10**	13.71	5.00
Spathulenol, **25**	13.85	6.00
Caryophyllene oxide, **29**	14.20	3.00
Aromadendrene oxide, **26**	14.52	12.50
Allo-Aromadendrene oxide, **27**	14.79	2.25
Humulene epoxide II, **28**	14.86	4.50
Dehydrocyperotundone, **20**	14.99	5.35
Ledol, **24**	15.44	5.50
4(15),11-Eudesmadien-3-one, **17**	15.95	10.22

Table (5): GC/MS peak identification data of fraction Ca32a3.

Identification	R_t, min	%
Trans-pinocarveol, **1**	8.00	2.44
Cis-verbenol, **3**	8.13	2.32
Pinocarvone, **2**	8.38	1.46
Myrtenol, **5**	8.97	1.95
Verbenone, **4**	9.18	2.24
Trans-carveol, 7	9.35	0.98
Trans-limonene diepoxide, **8**	10.68	1.10
Cyperol, **15**	11.48	1.82
Isocyperol, **16**	11.65	1.71
τ-Cadinol, **10**	13.71	2.20
Aromadendrene oxide, **26**	14.52	7.32
4(15),11-Eudesmadien-3-one, **17**	15.95	17.07
α-Cyperone, **18**	16.00	14.63
Corymbolone, **19**	18.23	17.56

Table (6): GC/MS peak identification data of fraction Ca32e.

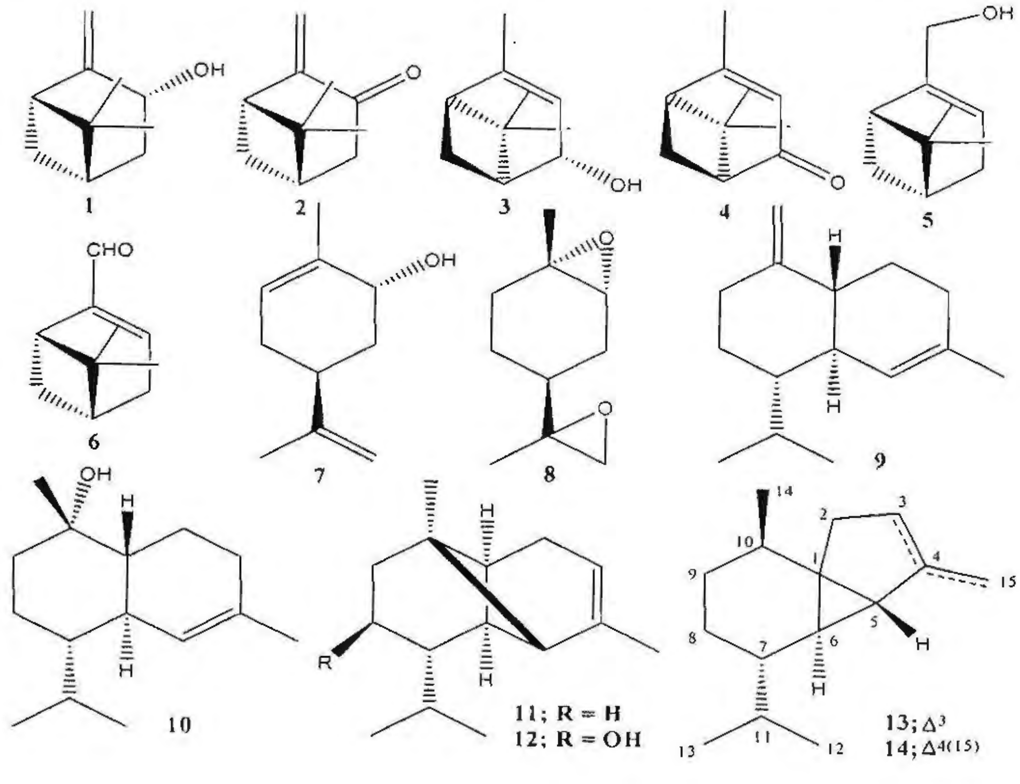
Identification	R_t, min	%
Dodecanoic acid, **32**	14.48	4.90
Tetradecanoic acid, **33**	16.85	9.80
4-Germacren-12,6-olide, **31**	16.94	14.71
Dihydrocostunolide, **30**	18.05	8.33
Palmitic acid, **34**	18.80	15.69
Oleic acid, **37**	20.53	15.20
Stearic acid, **36**	20.73	6.86

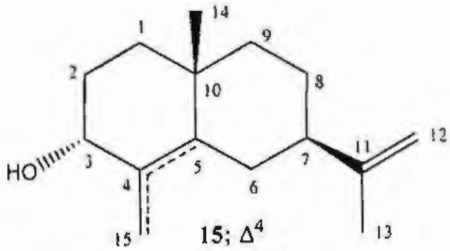
Table (7): Preliminary screening of crude plant extracts against *M. cartusiana* snails using two methods of application.

Plant extracts	% mortality	
	Dipping application	Residue film application
Cyperus articulatus L.	76.6	70.0
Cyperus alopecuroids Rottb.	23.0	20.0
Cynanchum acutum L.	0.0	0.0
Malva parviflora L.	0.0	0.0
Bombax malybricum L.	0.0	0.0

Table (8): Molluscicidal activity of sesquiterpene lactones fraction Ca32e and the crude extract of *Cyperus articulatus* L. against *M. cartusiana* land snail.

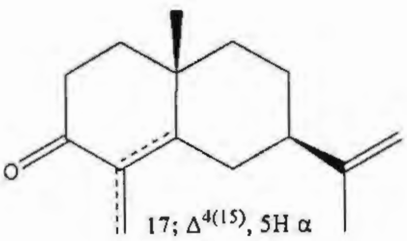
Extracts	Conc. %	Mortality %	LC_{50} %	Fiducial limits at 95 %		Slope & ± variance
				Lower	Upper	
Crude extract	0.25 0.50 1.0	23.3 56.6 76.6	0.470	0.343	0.627	5.79 ± 0.23
Sesquiterpene lactones fraction, Ca32e	0.125 0.25 0.50	80.0 86.6 100	0.056	0.004	0.112	7.68 ± 0.69





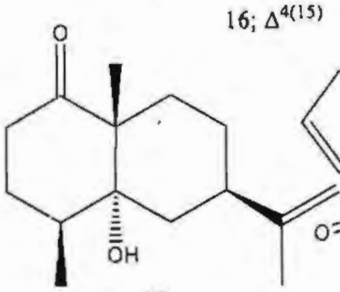
15; Δ^4

16; $\Delta^{4(15)}$



17; $\Delta^{4(15)}$, 5H α

18; Δ^4



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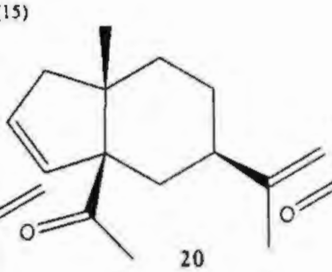

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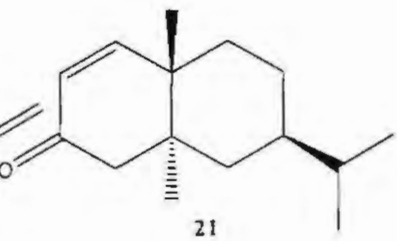

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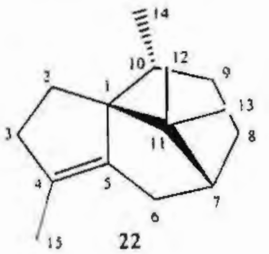

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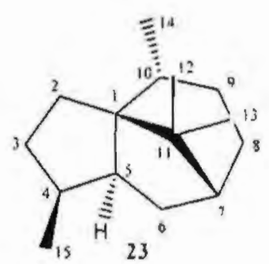

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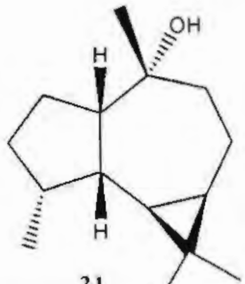

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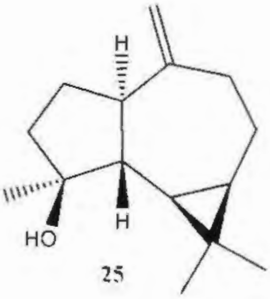
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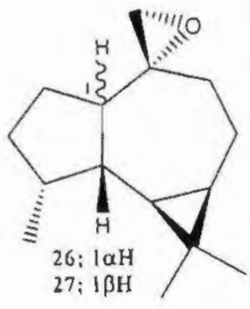
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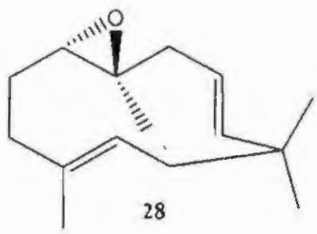
27; 1βH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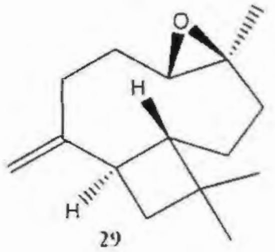
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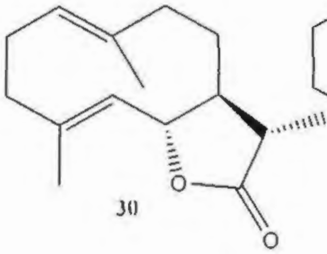
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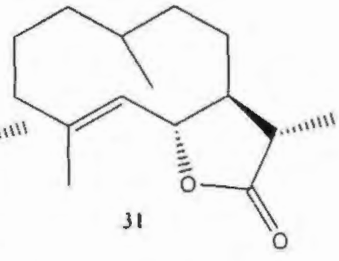
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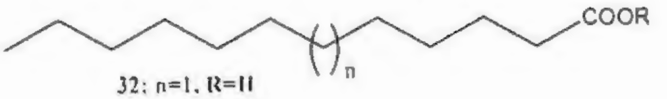
32: n=1, R=H
33: n=3, R=H
34: n=5, R=H
35: n=5, R=CH_3
36: n=7, R=H


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تربينات نصف ثلاثية قاتلة للرخويات من نبات سيبرس أرتكيولاتس ضد القوقع الأرضي موناكا كارتزيانا

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[١] قسم الكيمياء – كلية العلوم – جامعة المنصورة

[٢] معهد بحوث وقاية النباتات – المركز القومي للبحوث الزراعية

في مسح لخمسة خلاصات نباتية بحثا عن الفعالية ضد الرخويات على القوقع الأرضي موناكا كارتزيانا، فقد أعطت خلاصة نبات سيبرس أرتكيولاتس الخام فعالية عالية (نسب موت 3.3%, 56.6%, 76.6% للتركيزات 1.0%, 0.5%, 0.25% علي الترتيب). وكانت قيمة LC_{50} مساوية 0.47%. أما خلاصة نبات سيبرس ألوبكرويدس فأعطت فعالية ضعيفة، بينما خلاصات سينانكم أكيوتم ومالفا بارفي فلورا وبومبكس ماليبريكم فكانت غير فعالة.

تم تجزيىء خلاصة نبات سيبرس أرتكيولاتس الفعالة إلى أربع جزئيات بالاستخلاص المتتالي بمذيبات إيثر بترولي، داي كلوروميثان، كلوروفورم وميثانول. كما تم فصل خلاصة الايثر البترولي بالتقطير البخاري إلى جزء متطاير و آخر غير متطاير. وبعد ذلك تم إختبار الجزئيات المختلفة ضد القوقع ولكن لم تتضح فعالية أي منهم.

أدى الفصل الكروماتوجرافي المتبوع بتحليل GC/MS للجزئيات المختلفة إلى تعريف سبعة وثلاثين مركبا، تشتمل على ثمانية تربينات أحادية وواحد وعشرين من تربينويدات نصف ثلاثية واثنين من تربينات نصف ثلاثية لاكتونية وستة أحماض دهنية.

إعاده فحص الجزئية المحتوية على التربينات النصف ثلاثية اللاكتونية ضد القواقع أكد فعاليتها العالية. فقد كانت نسب موت القواقع 100%, 86.6%, 80.0% للتركيزات 0.50%, 0.25%, 0.125% على الترتيب. وكانت قيمة LC_{50} مساوية 0.056%. وقد أعزيت فاعلية التربينات نصف الثلاثية اللاكتونية لعدم وجود ارتباط بين الأحماض الدهنية (المرافقة للاكتونات) وبين الفاعلية ضد القواقع. وتم تعريف هذين اللاكتونين ١(١٠)،٤-جيرماكراداين-١٢،٦-أولايد و ٤-جيرماكرين-١٢،٦-أولايد.