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Molluscicidal and insecticidal properties of sesquiterpene lactones and extracts of *Magnolia grandiflora* L.

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ABSTRACT

Leaves and the stem bark of *Magnolia grandiflora* L. were extracted with dichloromethane. Repeated column chromatography followed by crystallization of two extracts led to isolate the two germacronolide sesquiterpene lactones costunolide and parthenolide. Epoxidation of parthenolide with *m*-chloroperbenzoic acid yielded 1,10-epoxyparthenolide. The structure of the compounds was identified by extensive spectroscopic analysis and compared with those found in the literature. Extracts and sesquiterpenes were evaluated for their molluscicidal and mosquitocidal activities on the fresh water snail, *Biomphalaria alexandrina* (Ehrenberg) and terrestrial snails, *Theba pisana* (Muller) and *Eobania vermiculata* (Muller), and mosquito larvae of *Culex pipiens* L. Parthenolide revealed the strongest molluscicidal activity among the tested extracts and compounds against the fresh water snail with LC_{50} value of 14.65 μg ml^{-1}. This compound also showed the most potent molluscicidal activity against terrestrial snails. It was more toxic than a reference pesticide methomyl to *T. pisana* after 24 hours of treatment. Extracts and sesquiterpenes were less toxic to *C. pipiens* larvae than the fresh water snail. Costunolide exhibited the highest mosquitocidal activity among the extracts and the isolated compounds with LC_{50} value of 58.48 μg ml^{-1}. This is the first report on the molluscicidal and insecticidal activities of extracts and sesquiterpene lactones of *M. grandiflora*.

Keywords: *Magnolia grandiflora*; Sesquiterpene lactones; *Biomphalaria alexandrina*; *Theba pisana*; *Eobania vermiculata*; *Culex pipiens*; Molluscicidal activity; Mosquitocidal activity.

INTRODUCTION

Tropical diseases are a major threat with approximately more than half of the world's population run the risk of becoming infected by one of these diseases. Every year at least 500 million people suffer from one of these infections that include malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis, and leishmaniasis (WHO 1995 and 1996). The diseases not only cause high levels of morbidity and mortality, but they also inflict great economic losses and social disruption on countries with high disease endemicity, such as the less-developed countries of Africa, Asia and Latin America (Joseph *et al.*, 2004).

Sesquiterpene lactones constitute a large group of the secondary metabolites, which are important constituents of essential oils. A large number of sesquiterpene lactones are found in members of the Compositae (Asteraceae) where they are characteristic constituents of the family. However, they have been reported from several plant families, such as Acanthaceae, Amaranthaceae, Apiaceae, Magnoliaceae and others. Sesquiterpene lactones possess a wide range of biological activities, including plant growth regulating, insect antifeedant, antibacterial, antifungal, cytotoxic and antitumougenic properties. Therefore, they play an important role on protection of plants against pathogens, herbivorous insects and mammals and function as allelopathic agents (Picman, 1986; Baruah *et al.*, 1994; Goren *et al.*, 1996 and Mansilla and Palenzuela, 1999).

Magnolia grandiflora L. (Magnoliaceae) is a large evergreen ornamental tree widely distributed throughout many countries around the world. The bark, wood and other parts of the plant have been featured in American Indian medicine and listed in the United States Pharmacopoeia and pharmacognosy texts as bitter tonics, antimalarials and diaphoretics. In Chinese traditional medicine, the plant is also used for treatment of cold,

headache and stomachache (Rao and Davis, 1982 and Wu *et al.*, 1988). This species has been the object of exhaustive phytochemical research and has yielded a variety of natural products, including alkaloids (Nakano, 1954), terpenoids (sesquiterpenes and sesquiterpene lactones (El-Feraly and Chan, 1978; El-Feraly *et al.*, 1979; El-Feraly, 1984 and Luo *et al.*, 2001), lignan glycosides (Rao and Wu, 1978), and biphenyls (El-Feraly and Li, 1978).

The fresh water snail, *Biomphalaria alexandrina* (Ehrenberg), is the main vector of human bilharziasis in northern Egypt. Land snails, *Theba pisana* (Muller) and *Eobania vermiculata* (Muller), cause great damage to ornamental plants, vegetables and citrus trees (Miller *et al.*, 1988). *Culex pipiens* L. is one of *Culex* mosquitoes responsible of transmission of lymphatic filariasis. There is no data available about the toxicity of extracts and sesquiterpene lactones of *Magnolia grandiflora* L. to *B. alexandrina*, *T. pisana*, *E. vermiculata* and *C. pipiens*. Therefore, the present study describes the bioactivity of *M. grandiflora* extracts and their isolated sesquiterpene constituents against the three selected pests.

MATERIALS AND METHODS

1. General experimental procedures

Ultraviolet (UV) spectra were recorded in MeOH on a Shimadzu UV-160A spectrophotometer. Mass spectra (HR-FAB-MS) were measured on JMS-102A mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a JEOL FX-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Optical rotation was measured at 22 °C using JASCO P-1030 spectropolarimeter. Infrared (IR) spectra (KBr) were measured on a JASCO FT/IR 5300. Melting points were determined using a Mitamura melting point apparatus and were uncorrected. Silica gel 70 - 230 mesh (Merck) was used on open column chromatography. Solvents were purchased from Wako (Japan) and were HPLC grade.

2. Plant materials

Leaves and stem bark of *Magnolia grandiflora* L. were collected in April 2004 from Alexandria Public Garden, Alexandria, Egypt. The plant material was identified by Dr. Ahmed Moharib of Alexandria University. Voucher specimens are deposited in Faculty of Agriculture, Alexandria University.

3. Test organisms

Biomphalaria alexandrina (Ehrenberg), one of the snail vectors of schistosomiasis, was collected from fresh water ponds in Kafr Eldawar, Behera Governorate, Egypt. The snail was kept in dechlorinated water supplied with fresh lettuce leaves at laboratory conditions for one week before bioassay tests. *Theba pisana* (Muller) and *Eobania vermiculata* (Muller) terrestrial snails were collected from Faculty of Agriculture Garden, Alexandria, Egypt. The snails were acclimatized to laboratory conditions for one week and were fed on fresh lettuce leaves. *Culex pipiens* L. was reared in our laboratory according to Al-Sharook *et al.* (1991).

4. Extraction and isolation of sesquiterpene lactones from *Magnolia grandiflora*

The air-dried stem bark (1.9 Kg) of *M. grandiflora* was extracted with dichloromethane (10 L). The extraction was conducted at room temperature for one week and was concentrated under reduced pressure to yield 32 g of the extract. The extract was suspended in 10 % water/methanol (500 ml) and then extracted with hexane (300 ml × 3). The aqueous methanolic extract (10 g) was subjected to silica gel column (500 g) and eluted with the mixture of 1, 2 and 10 % acetone/dichloromethane (v / v). Thirty five fractions of 200 ml were collected and pooled into two main fractions, fr. 1 (Fr. nos 1~5, 2.6 g) and fr. 2 (Fr. nos 14~24, 3.8 g), on the basis of the similar TLC profiles. The first fraction was recrystallized from hexane to

offer 1169.0 mg of costunolide. The second fraction was recrystallized from ether to give 2.7 g of parthenolide.

The air-dried leaves (1.5 kg) were extracted with dichloromethane (10 L) at room temperature for one week to afford 43 g of oily extract. The extract was suspended in 10 % water/methanol (600 ml) and extracted with hexane (400 ml × 3). The aqueous methanolic fraction (22 g) was chormatographed on silica gel (1 kg) using 20, 35, 50, 25 and 100 % ethyl acetate / hexane (v / v) to give 63 fractions (each fraction 200 ml). Fractions 9~12 were further purified by silica gel column eluted with 5 % acetone/dichloromethane (v / v) and recrystallized from hexane to give 754.0 mg of pure costunolide. Fractions 19~22 were eluted on silica gel column eluted with 2 and 5 % methanol/chloroform (v / v) to yield parthenolide, which was recrystallized from ether to give 4.9 g of pure crystals.

Costunolide 1. Colourless needles from hexane, mp 105-106 °C; $[\alpha]_D$ 127° (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} nm: 220 (ε 9000); IR (KBr) ν_{max} cm^{-1}: 2926, 1766, 1660, 1439, 1138, 966, 814 and 706; ^1H NMR ($CDCl_3$): δ 1.43 (3H, s, Me-14), 1.70 (3H, s, Me-15), 4.58 (1H, t, J = 9.9 Hz, H-6), 4.75 (1H, d, J = 9.9 Hz, H-5), 4.86 (1H, dd, J = 10.3 and 3.3 Hz, H-1), 5.55 (1H, d, J = 2.9 Hz, H-13), 6.22 (1H, d, J = 3.7 Hz, H-13); FAB-MS *m/z* 233 $[M + H]^+$.

Parthenolide 2. Colourless prisms from ether, mp 115-116°C; $[\alpha]_D$ -94.8° (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} nm: 217 (ε 4000); IR (KBr) ν_{max} cm^{-1}: 2932, 1765, 1444, 1290, 1249, 1141, 985 and 715; ^1H NMR ($CDCl_3$): δ 1.30 (3H, s, Me-15), 1.71 (3H, s, Me-14), 2.80 (1H, d, J = 8.8 Hz, H-5), 3.87 (1H, t, J = 8.8 Hz, H-6), 5.22 (1H, dd, J = 9.9 and 2.5 Hz, H-1), 5.64 (1H, d, J = 3.3 Hz, H-13), 6.29 (1H, d, J = 3.7 Hz, H-13); FAB-MS *m/z* 249 $[M + H]^+$.

5. Epoxidation of parthenolide 2.

Epoxidation of parthenolide 2 was carried out according to the method described by El-Feraly and Chan (1978) with slight modifications. Parthenolide (500 mg) was dissolved in 30 ml of chloroform, and then 575 mg of *m*-chloroperbenzoic acid (75 %) was added. The mixture was allowed to stand at 5 °C for 3 hr. Dichloromethane (100 ml) was added to the reaction mixture and then washed with 5 % Na_2SO_3 (50 ml), 5 % $NaHCO_3$ (50 ml) and then water (50 ml). Dichloromethane/chloroform phase was dried over anhydrous sodium sulphate and concentrated to give 609 mg of the white residue, which was chromatographed on silica gel using 40 % ethyl acetate / hexane (v / v) to yield 408 mg of pure 1,10-epoxyparthenolide 3.

1,10-epoxyparthenolide 3. Colourless prisms from ethanol, mp 166-168°C; $[\alpha]_D$ -40.8° (c 0.50, ethanol); UV (MeOH) λ_{max} nm: 220 (ε 3500); IR (KBr) ν_{max} cm^{-1}: 2941, 1763, 1392, 1296, 1251, 1147, 949, 765; ^1H NMR ($CDCl_3$): δ 1.29 (3H, s, Me-15), 1.33 (3H, s, Me-14), 2.42 (1H, dd, J = 14.1 and 7.1 Hz, H-7), 2.81 (1H, dd, J = 10.6 and 2.4 Hz, H-1), 2.86 (1H, d, J = 9.4 Hz, H-5), 3.88 (1H, t, J = 9.9 Hz, H-6), 5.57 (1H, d, J = 3.3 Hz, H-13), 5.64, 6.27 (1H, d, J = 3.9 Hz, H-13); FAB *m/z* 265 $[M + H]^+$.

6. Bioassay against *Biomphalaria alexandrina*

Molluscicidal activity of *M. grandiflora* extracts and isolated sesquitepenes was carried out against *B. alexandrina* according to World Health Organization procedure (Anonymous, 1965). Adult snails with similar shell size (8-10 mm diameter) were used. Extracts and isolated sesquiterpenes were dissolved in acetone and were added to 50 ml dechlorinated tap water taken in glass beakers (100 ml capacity) to obtain a various desired concentrations up to 100 μg ml^{-1}. Five snails were introduced into each glass beaker. Two controls, one with 50 ml dechlorinated tap water alone and other with 50 ml dechlorinated tap water

containing the maximum volume of acetone in the test medium (0.5 ml) were maintained. Each concentration was tested in triplicate. Niclosamide was used as standard molluscicide for aquatic snails. The mortality percentages were recorded after 24 hours. No response to a needle probe was taken as evidence of death (Singh *et al.*, 1998). The LC_{50} values expressed as $\mu g\ ml^{-1}$ were calculated from log-concentration mortality regression lines (Finney, 1971).

7. Bioassay against terrestrial snails

The efficiency of extracts and isolated sesquiterpenes was evaluated on adult snails (Hussein *et al.*, 1994 and Radwan and El-Zemity 2001) of *T. pisana* (17mm shell diameter) and *E. vermiculata* (26 mm shell diameter). Extracts and isolated sesquiterpenes were prepared in acetone and tested at doses of 0.1, 0.25, 0.5 mg / snail and 0.25, 0.5, 1 mg / snail against *T. pisana* and *E. vermiculata*, respectively. These doses contained in 5 µl of acetone solution in the case of *T. pisana* and 25 µl in the case of *E. vermiculata* were gently applied on the surface of the snail body inside the shell using a micropipette. Three replicates (five snails in each one) of each concentration were used. Control snails were treated with the same volumes of acetone. The snails were fed on fresh lettuce leaves during the course of experiment. Methomyl (98 %, Wako, Japan) was used at same concentrations as reference pesticide for comparison. The mortality percentages were recorded after 24 and 48 hours of treatment.

8. Bioassay against *Culex pipiens*

The fourth instar larvae of *C. pipiens* were chosen to evaluate the insecticidal activity of extracts and isolated sesquiterpenes. Appropriate volumes of stock solutions of tested extracts and compounds in acetone were added to dechlorinated water (50 ml) taken in glass beakers (100 ml capacity) to obtain a range of concentrations up to 500 $\mu g\ ml^{-1}$. Ten larvae were used per glass beaker, with each concentration tested in triplicate

controls, one with 50 ml of water and the other with 50 ml of water containing the maximum volume of acetone in the tested medium, were carried out. Malathion (95 %, American Cyanamid Co, USA) was used as reference insecticide. The mortality percentages were recorded after 24 hr of treatment and LC_{50} values were calculated according to Finney (1971).

RESULTS AND DISCUSSION

1. Isolation and structure determination of sesquiterpene lactones from *Magnolia grandiflora*

Repeated chromatographic separation on open silica gel columns followed by recrystallization of *M. grandiflora* leaves and stem bark extracts led to isolate the two sesquiterpene lactones, costunolide 1 and parthenolide 2. Epoxidation of parthenolide 2 with *m*-chloroperbenzoic acid gave 1,10-epoxyparthenolide 3. The chemical structure (Fig.1) of the isolated sesquiterpene lactones; costunolide 1 and parthenolide 2 and parthenolide derivative, 1,10-epoxyparthenolide 3 was established by UV, IR, ^{1}H and ^{13}C NMR analysis and mass spectral, as well as comparison of NMR data with those reported in the literature (El-Feraly and Chan, 1978).

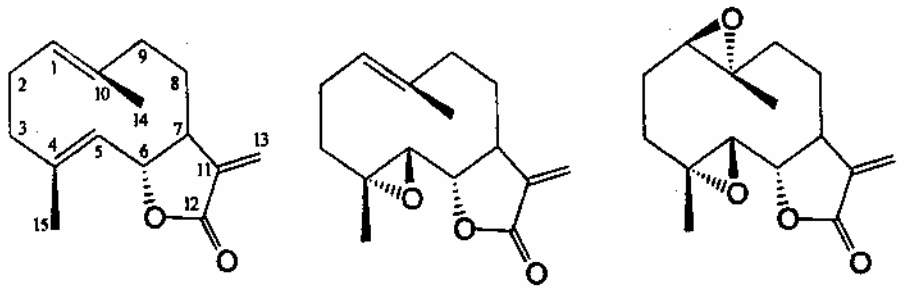

Fig. 1. Chemical structure of sesquiterpene lactones (1-3).

2. Molluscicidal activity of *Magnolia grandiflora* extracts and isolated sesquitepenes against fresh water snail *Biomphalaria alexandrina*

Results of molluscicidal activity of dichloromethane extracts and sesquiterpene lactones on *B. alexandrina* are shown in Table (1). Leaves extract displayed stronger molluscicidal activity than stem bark extract with LC_{50} of 48.32 μg ml^{-1}. These extracts showed higher toxicity than water extracts of different parts of *Calendula officinalis* and *Ammi majus* tested against the same snail (Rawi *et al.*, 1996). Sesquiterpenes (1-3) were more

Table (1): Comparative toxicity of dichloromethane extracts and sesquiterpene lactones of *Magnolia grandiflora* against *Biomphalaria alexandrina* adult snail

Extract/Compound	LC_{50} (μg ml^{-1})	95% Confidence limits		Slope
		Upper	Lower	
Leaves extract	48.32	52.96	44.27	8.17
Stem bark extract	57.82	65.45	52.48	7.01
Costunolide 1	17.83	19.08	16.56	3.24
Parthenolide 2	14.65	15.19	14.12	7.00
1,10-epoxyparthenolide 3	28.61	30.32	26.94	4.16
Niclosamide	0.28	0.32	0.24	6.14

toxic to the snail than the crude extracts. Parthenolide 2 was the most toxic compound to the snail with LC_{50} of 14.65 μg ml^{-1} followed by costunolide 1 and 1,10-epoxyparthenolide 3 with LC_{50} values of 17.83 and 28.61 μg ml^{-1}, respectively. The molluscicidal activities of isolated sesquiterpenes were weaker than a reference moluscicide, niclosamide. Parthenolide 2 was also shown to be the strongest antibacterial activity among these compounds against six food borne bacteria (Abdelgaleil and Hashinaga, unpublished data). The molluscicidal activities of these sesquiterpenes were higher than

those of monoterpenes (El-Zemity *et al.*, 2001), segetane diterpenes and jatrophene diterpenes (Abdelgaleil *et al.*, 2002), and comparable with those of paraliane diterpenes, and weaker than those of sesquiterpenes ambrosin and damsin (Marston and Hostettmann, 1985) evaluated on the same snail. Parthenolide 2 with an 4,5-epoxy group displayed the highest molluscicidal activity among these compounds indicating that the presence of this epoxy group was necessary for enhancement of the activity. In contrast, introduction of the second 1,10-epoxy group strongly decreased the molluscicidal activity of compound 3 comparing with the compounds 1 and 2. It is obvious that the molluscicidal activity of these sesquiterpenes lactones may vary because of the presence of specific functional groups in the molecule. The same finding was established in different naturally occurring compounds (Hostettmann *et al.*, 1982; Dimetry *et al.*, 1992 and Nakatani, 1999).

3. Molluscicidal activity of *Magnolia grandiflora* extracts and isolated sesquitepenes against terrestrial snails *Theba pisana* and *Eobania vermiculata*

Table (2) presents mortality percentages of dichloromethane extracts and sesquiterpene lactones of *M. grandiflora* for the two snails *T. pisana* and *E. vermiculata*. The results showed that parthenolide 2 exhibited higher mortality percentages than costunolide 1 and extracts on both snails at all of the tested concentrations after 24 and 48 hours. In the mean time, it showed higher mortality than a reference pesticide, methomyl against *T. pisana* after 24 hours at the tested concentrations. Extracts and parthenolide 2 were more toxic to *T. pisana* than *E. vermiculata* after 24 and 48 hours at concentrations of 0.25 and 0.5 mg / snail while costunolide 1 was more toxic to *E. vermiculata* than *T. pisana* at the same concentrations. Molluscicidal activity of parthenolide 2 against *T. pisana* was stronger than

Table (2): Mortality percentages of *Theba pisana* and *Eobania vermiculata* after 24 and 48 hours of treatment with dichloromethane extracts and sesquiterpene lactones of *Magnolia grandiflora*

Extract/Compound	Conc. (mg /snail)	*T. pisana*		*E. vermiculata*	
		Mortality (%)			
		24 hr	48 hr	24 hr	48 hr
Leaves extract	0.1	0	0	-[a]	-
	0.25	6.7	33.3	0	6.7
	0.5	20.0	33.3	6.7	26.7
	1	-	-	6.7	40.0
Stem bark extract	0.1	0	13.3	-	-
	0.25	0	20.0	0	13.3
	0.5	33.3	40.0	6.7	33.3
	1	-	-	6.7	33.3
Costunolide 1	0.1	0	13.3	-	-
	0.25	0	13.3	13.3	33.3
	0.5	6.7	20.0	13.3	40.0
	1	-	-	26.7	46.7
Parthenolide 2	0.1	40.0	46.7	-	-
	0.25	53.3	66.7	0	60.0
	0.5	73.3	86.7	6.7	60.0
	1	-	-	53.3	80.0
Methomyl	0.1	20.0	73.3	-	-
	0.25	40.0	93.3	53.3	100.0
	0.5	53.3	100.0	60.0	100.0
	1	-	-	86.7	100.0
Control	0	0	0	0	6.67

[a]- = not tested.

that of monoterpenoids (α-terpineol, pulegone and anisole) but close to thymol and eugenol (El-Zemity, 2001).

4. Insecticidal activity of extracts and isolated sesquiterpenes against *Culex pipiens*

Larvicidal activity of extracts and sesquiterpenes was examined by dipping method against *C. pipiens*. Costunolide 1 exhibited the highest insecticidal activity with LC_{50} value of 58.48 μg ml^{-1} followed by parthenolide 2 with LC_{50} value of 119.59 μg ml^{-1}. Leaves extract was more toxic than the stem bark extract and 1,10-epoxyparthenolide 3 (Table 3). The insecticidal activities of the tested extracts were stronger than those of acetone extracts of *Citrullus colocynthis* and *Datura stramonium* seeds but were weaker than those of acetone extracts of *Azadirachta indica* fruits and *Piper nigrum* seeds tested against the same insect (Abdelgaleil, 1995).

Table (3): Comparative toxicity of dichloromethane extracts and sesquiterpene lactones against *Culex pipiens* fourth instar larvae

Extract/Compound	LC_{50} (μg ml^{-1})	95% Confidence limits		Slope
		Upper	Lower	
Leaves extract	286.38	298.55	274.29	6.66
Stem bark extract	349.67	382.48	315.02	6.49
Costunolide 1	58.48	60.96	56.06	5.93
Parthenolide 2	119.59	126.43	113.25	6.01
1,10-epoxyparthenolide 3	>300			
Malathion	0.26	0.31	0.21	2.01

The biological activity of sesquiterpene lactones is attributed to the presence in the molecule of an exocyclic methylene conjugated to the γ-lactone as well as to other electrophilic functional groups that can react with sulfydryl proteins (Rodriguez *et al.*, 1976). This study demonstrates that extracts and sesquiterpene lactones of *M. grandiflora* have molluscicidal and insecticidal activities for the first time. Extracts and isolated sesquiterpenes were more toxic to the fresh water snail *B. alexandrina* than larvae of *C. pipiens*. These findings suggest that the extracts of this plant can be utilized for the fresh water snail (*B. alexandrina*) control and the isolated sesquiterpenes may serve as template for synthetic elaboration leading to useful molluscicides.

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الخصائص الابادية لمركبات السيسكيوتربين لاكتون ومستخلصات نبات الماجنوليا ضد القواقع والبعوض

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أوراق وقلف ساق نبات الماجنوليا استخلصت بمذيب الميثيلين كلوريد. الفصل الكروماتوجرافى المتكرر متبوع بعملية البلورة أدى الى عزل مركبين من مجموعة السيسكيوتربين لاكتون هما Porthenolide، Costunolide. عملية الاكسده لمركب Parthenolide باستخدام حمض الميتاكلوربربنزويك أعطت 1,10-epoxparthenolide . تم التعرف على تركيب هذه المركبات باستخدام طرق التحليل الطيفى المختلفة وكذلك بمقارنة البيانات المتحصل عليها بتلك الموجودة فى المراجع العلمية. المستخلصات والمركبات المعروفة تم تقييمها ضد قوقع المياه العذبة البيموفلاريا اليكسندرينا والقواقع الارضية الاوبانيا فرمكيولاتا (قوقع الحدائق البنى) والتيبا بيسانا (قوقع الحدائق الابيض) وكذلك على بعوضة الكيولكس بيبنس. مركب parthenolide أظهر أعلى فاعلية ضد قوقع المياه العذبة حيث كانت قيمة LC_{50} هى ١٤,٧٥ ميكروجرام / مل. هذا لمركب أيضا أظهر أعلى فاعلية ضد القواقع الارضية المختبرة حيث أنه كان أكثر فاعلية من الميثوميل على قوقع التيبا بيسانا بعد ٢٤ ساعة من المعاملة. المستخلصات والمركبات المعزولة كانت أقل سمية على بعوضة الكيولكس من قوقع المياه العذبه. مركب costunolide أظهر أعلى فاعلية ضد البعوضة المختبرة حيث كانت قيمة LC_{50} له هى ٥٨,٤٨ ميكروجرام/ مل. هذه أول دراسة على النشاط الابادى لمركبات السيسكيوتربين لاكتون ومستخلصات نبات الماجنوليا ضد القواقع والبعوض.