J. Pest Cont. & Environ. Sci. 15 (1): 101 -112 (2007).

Evaluation of molluscicidal properties of extracts and active principles from two Egyptian plants against the terrestrial snail, *Eobania vermiculata*

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ABSTRACT

Extracts and compounds derived from *Artemisia judaica* (L.) and *Simmondsia chinensis* (Link) Schneider were evaluated for their molluscicidal activities against the terrestrial snail, *Eobania vermiculata* (Muller). Of eight non-polar and polar tested extracts, petroleum ether extract of *A. judaica* was the most potent with LD_{50} value of 0.18 mg/snail, while diethyl ether extract of *S. chinensis* was the least effective one with LD_{50} value of 4.81 mg/snail. Bioassay-guided fractionation of *A. judaica* essential oil and *S. chinensis* chloroform extract on silica gel columns provided a monoterpene piperitone and a phenylpropanoid *trans*-ethyl cinnamate, and two glucosides simmondsin and simmondsin 2`ferulate. The structure of these compounds was determined by using spectroscopic methods of UV, IR, ^{1}H NMR, ^{13}C NMR and MS. When tested for their molluscicidal effects against *E. vermiculata*, the isolated compounds showed a pronounced activity, particularly, piperitone and *trans*-ethyl cinnamate. *trans*-Ethyl cinnamate showed the highest activity with LD_{50} value of 0.055 mg/snail. To the best of our knowledge, this is the first study on molluscicidal potential of the extracts and the active principles from *A. judaica* and *S. chinensis*.

Keywords: *Artemisia judaica*; *Simmondsia chinensis*; Plant extracts; piperitone; *trans*-ethyl cinnamate; simmondsin; simmondsin 2`ferulate; Molluscicidal activity; *Eobania vermiculata*

INTRODUCTION

The increasing dependence on chemicals for pest control poses a serious threat to human health and environment. Further, the heavily use of pesticides lead to the development of resistance among pests. Plants-derived secondary metabolites have a great potential as alternatives to the currently used pesticides. Tens of thousands of secondary products of plants have been identified and there are estimates that hundreds of thousands of these compounds exist (Duke, 1990). There is growing evidence that most of these compounds are involved in the interaction of plants with other species, primarily the defence of the plant from plant pests. Thus, these secondary compounds represent a large reservoir of chemical structures with biological activity. The current research in pest control agents is focused on finding new classes of compounds with novel modes of action. Natural plant products that are biodegradable, exhibit structural and biological diversity and complexity, and rarely contain halogenated atoms constitute such class of chemicals (Duke, 1990 and Dayan *et al*., 1999).

Artemisia judaica (L.) is a perennial fragrant shrub which grows widely in the deserts and Sinai Peninsula of Egypt (Tackholm, 1974). Many *Artemisia* species have a characteristic scent and taste, caused by monoterpenes and sesquiterpenes, which in many cases, are the reason for their application in folk medicine. Mixture of the dry leaves of *A. judaica*, *A. monosperma* and *A. hera alba* is a very common anthelmintic drug in the most of North African and Middle Eastern countries under Arabic name of Shih. These *Artemisia* species have been used worldwide in tonic, stomachic and stimulant beverage and as antiseptic oils or tinctures for the relief of rheumatic pains (El-Massry *et al.*, 2002). It has been reported that the volatile components of *A. judaica* possess a repellent effect on snakes (Boulos, 1983).

Simmondsia chinensis (Link) Schneider is a semi-arid evergreen shrub. It grows wild in the desert south-western United States and north-western Mexico. However, the plant is cultivated in some of the Middle Eastern and Latin American countries (Borlaug *et al.*, 1985 and Bellirou *et al*., 2005). Jojoba oil has a good market in cosmetics and lubricants (Cokelaere *et al*., 1992).

Recently, it has been reported that the jojoba seeds possess anti-inflammatory activity (Habashy *et al*., 2005). The defatted jojoba meal contains 20 to 32 % of protein which consists mainly from albumins (79 %) and globulins (21 %) (Shrestha *et al*., 2002). This meal also contains approximately 15 % of a group of glucosides, known as simmondsins (Elliger *et al.*, 1973 and Van Boven *et al.*, 2000). Eight glucoside compounds (simmondsin and seven simmondsin derivatives) have been isolated and identified form the jojoba seeds (Bellirou *et al.*, 2005). Among these the methylated compounds simmondsin and simmondsin 2`-ferulate exhibited food-intake inhibition to rodents and chickens.

The terrestrial snail, *Eobania vermiculata* Muller (Mollusca: Gastropoda: Stylommatophora: Helicidae), is a destructive pest widely distributed in Egypt. It attacks the different parts of plants (leaves, roots, buds, flowers) of many field crops, vegetables, fruits and ornamentals, causing a great damage in quality and production (El-Okda, 1980).

As part of our search to find out new naturally occurring pest control agents from plants growing in Egypt, the molluscicidal activity of the extracts of *A. judaica* and *S. chinensis* was evaluated against the land snail, *E. vermiculata*. In addition, the isolation and identification of the two active principles piperitone and *trans*-ethyl cinnamate from *A. judaica* and the two glucosides, simmondsin and simmondsin 2`ferulate from *S. chinensis* and their molluscicidal effects were also studied.

MATERIALS AND METHODS

Plant materials: Aerial parts of *Artemisia judaica* (L.) (Shih plant) and seeds of *Simmondsia chinensis* (Link) Schneider (jojoba plant) were collected from Sinai Peninsula and Al-Bostan Region**,** respectively, in September, 2004. The plant materials were identified by Prof. Dr. Fath Allah Zaitoon, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, where voucher No. 06/6 and 06/7 are deposited.

Instruments: ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on a JEOL JNM ECD 500 Spectrometer in $CDCl_3$. IR spectra were performed with Perkin Elmer 1430 Ratio Recording Inferared Spectrometer in KBr disks. UV spectra were measured with HEλIOSα UV-VL Spectrophotometer V4.60 in methanol.

Used animal: The brown garden snail, *Eobania vermiculata* (Muller), was collected from gardens in Abohomos region, Behera Governorate, Egypt. The snail was acclimatized to laboratory conditions (26 ± 1°C) for two week and was fed on fresh lettuce leaves.

Preparation of plant extracts: Dried and powdered aerial parts of *A. judaica* (0.5 Kg) and seeds of *S. chinensis* (1Kg) were exhaustively extracted with petroleum ether (60-80° C), diethyl ether, chloroform and ethanol, respectively, using Soxhlet apparatus for four hours. Evaporation of the solvents under reduced pressure gave 51.7 and 240.0 g of petroleum ether extracts, 34.5 and 98.9 g of diethyl ether extracts, 40.0 and 45.4 g of chloroform extracts, and 44.0 and 141.3 g of ethanol extracts for *A. judaica* and *S. chinensis*, respectively.

Extraction and isolation of active principles from *A. judaica*: Dried and powdered aerial parts of *A. judaica* were hydrodistilled in a Clevenger-type apparatus. The resulting essential oil (18 g) was chromatographed on silica gel column using hexane, 2.5% acetone/hexane, 10 % acetone/hexane and acetone solvent systems. The resulting fractions were concentrated under reduced pressure and examined by TLC to offer two main fractions. The first fraction was further purified on silica gel column eluted with 1% acetone/hexane to give 2.05 g of *trans*-ethyl cinnamate (**2**) (Figure 1). The second fraction was subjected to silica gel column eluted with chloroform to offer 2.52 g of piperitone (**1**).

Piperitone (1): δ ^{1}H NMR 0.50 and 0.60 (each 3H, t, J = 6.9 Hz, Me-8 and Me-9), 0.74 (1H, m, H-4a), 0.94 (1H, m H-5), 1.35 (1H, m, H-4b), 1.46 (m, H-6b), 1.59 (3H, d, J = 11.5 Hz, H-10), 1.66 (1H, m, H-7), 1.99 (1H, m, H-6a) and 5.46 (1H, d, J = 11.5 Hz, H-3).

***trans*-Ethyl cinnamate (2)**: δ ^{1}H NMR 1.06 (3H, t, J = 6.9 Hz, $CH_2\underline{CH_3}$), 3.98 (2H, q, J = 6.9 Hz, $\underline{CH_2}CH_3$), 6.18 (1H, d, J = 17.6 Hz, H-2), 7.08 (3H, m, H-3`, 4`and 5`), 7.23 (2H, m H-2`and H-6`) and 7.44 (d, J = 19.4 Hz, H-3).

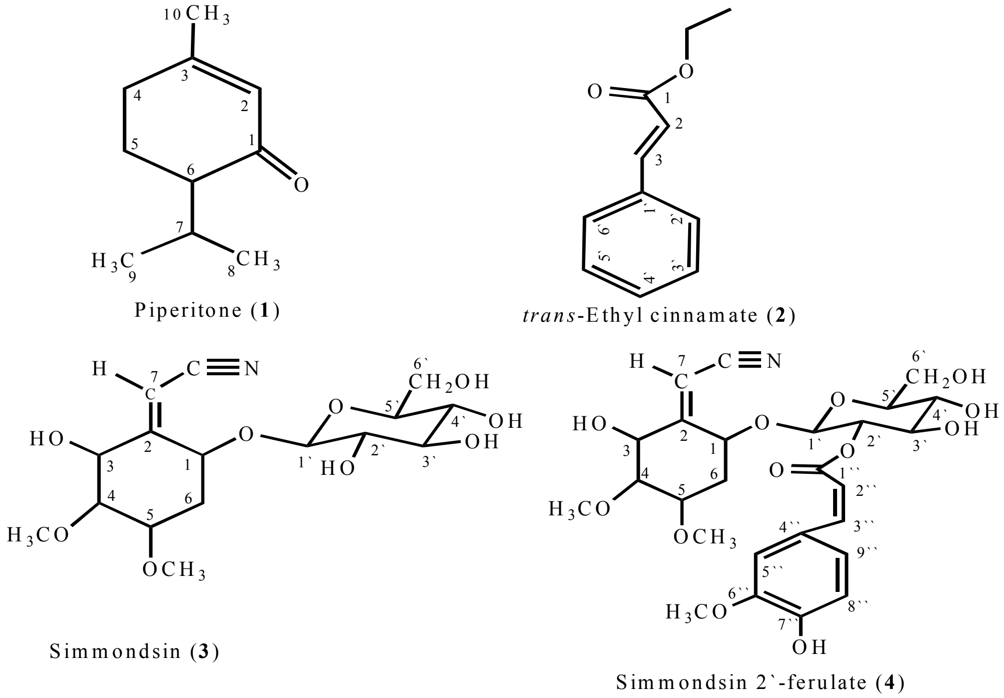
Extraction and isolation of active principles from *S. chinensis*: The chloroform extract of *S. chinensis* (45.4 g) was subjected to silica gel column chromatography eluted by chloroform, 10% methanol/chloroform, 20% methanol/chloroform and methanol solvent systems. Thirty fractions of 200 ml were collected and pooled to two main fractions based on their TLC profiles. Fraction 1 (3.4 g) was further purified by silica gel column chromatography using 10% chloroform/methanol solvent system to give 2.1 g of simmondsin 2`-ferulate (**4**), (Figure 1). Similar purification of the second fraction (7.3 g) followed by recrystallization from acetone/chloroform (1:4) gave 5.4 g pure prisms of simmondsin (**3**).

Simmondsin (3): δ ^{1}H NMR 1.68 (1H, dt, J = 14.5 and 3.9Hz, H-6a), 2.48 (1H, dt, J = 15.3 and 3.9 Hz, H-6b) 3.13 (1H, dd, 9.2 and 3.1 Hz H-2`), 3.22 (1H, m H-4), 3.29 (1H, t, J = 8.4 Hz, H-5`), 3.32 (1H, t, J = 9.2, H-4`), 3.36 (1H, t, J = 8.6 Hz, H-3`), 3.43 (3H, s, 5-OCH_3), 3.45 (3H, s, 4-OCH_3), 3.64 (1H, dd, J = 12.3 and 5.4 Hz, H-6`a), 3.81 (1H, brd, J = 12.2 Hz, H-6`b), 3.90 (1H, q, J = 3.7 Hz, H-5), 4.37 (1H, d, J = 8.4 Hz, H1`), 4.71 (1H, brd, J = 8.4, H-3), 4.86 (1H, t, J = 3.9, H-1) and 5.69 (1H, s, H-7).

**Simmondsin 2`-ferulate (4):** δ $^1$H NMR 1.47 (1H, d, J = 12.3 Hz, H-6a), 2.39 (1H, d, J = 14.5 Hz, H-6b) 3.00 (1H, dd, 10.0 and 3.3 Hz, H-4), 3.23 (3H, s, 5-$OCH_3$), 3.35 (1H, m H-5`), 3.37 (3H, s, 4-OCH_3), 3.45 (1H, dd, J = 19.1 and 9.9, H-4`), 3.60 (1H, m, H-3`), 3.72 (2H, m, H-6`), 3.84 (3H, s, O-$CH_3$), 3.84 (1H, H-1`), 4.60 (1H, d, J = 7.7 Hz, H-3), 4.73 (1H, dd, J = 21.4 and 11.5 Hz, H-2`), 4.86 (1H, t, J = 9.2 Hz, H-1), 5.71 (1H, s, H-7), 6.34 (1H, d, J = 15.3, H-2``), 6.80 (1H, d, J = 5.1, H-8``), 7.04 (1H, d, J = 8.4 Hz, H-9``), 7.63 (1H, d, J = 15.3 Hz, H-3``), 7.76 (1H, s, H-5``).

Molluscicidal bioassay: The molluscicidal activity of the extracts and the isolated compounds was evaluated against the adult snail of *E. vermiculata* (26 mm shell diam.) using the method described by Hussein *et al.* (1994) and

Radwan and El-Zemity (2001). Stock solutions in acetone of the test compounds were prepared. A concentration series of each compound was prepared by dilution with distilled water containing 0.05% of Triton-X 100. The tested doses (0.03, 0.15, 0.35, 0.7, 1.5 and 3.0 mg/snail) contained in 30µl of these dilutions were gently applied on the surface of the snail body inside the shell using a micropipette. Three replicates (ten snails in each) of each concentration and control were used. Control snails were treated with acetone/water/Triton-X 100 solution. The snails were fed on fresh lettuce leaves during the course of experiment. Methomyl (90%, Kafr El-Ziat Co., Egypt) was used as a reference pesticide for land snails. The mortality percentages were recorded after 48 hours of treatment. The lethal dose causing 50% mortality (LD_{50}) expressed as mg/snail were calculated from log-concentration mortality regression lines (Finney, 1971).



Piperitone (**1**)

trans-Ethyl cinnamate (**2**)

Simmondsin (**3**)

Simmondsin 2`-ferulate (**4**)

Figure 1. Chemical structure of isolated compounds from *Artemisia judaica* (**1** and **2**) and *Simmondsia chinensis* (**3** and **4**).

RESULTS AND DISSCUSION

Molluscicidal effect of *A. judaica* and *S. chinensis* extracts: The molluscicidal potential of *A. judaica* and *S. chinensis* extracts against *E. vermiculata* expressed as LD_{50} values is summarized in Table 1. The eight extracts were effective against the tested snail. Petroleum ether extract of *A. judaica* was the most potent among all of the tested extracts with LD_{50} value of 0.18 mg/snail followed by chloroform extract of *S. chinensis* (LD_{50} = 0.39 mg/snail). Diethyl ether and petroleum ether extracts of *S. chinensis* showed the least mollusicidal activity.

Table1. Molluscicidal activity of *Artemisia judaica* and *Simmondsia chinensis* extracts against *Eobania vermiculata* after 48 h.

Extract	LD_{50} (mg/snail)	Confidence limits		Slope
		Lower	Upper	
Artemisia judaica				
Petroleum ether	0.18	0.14	0.23	1.15
Diethyl ether	0.93	0.59	1.58	0.63
Chloroform	1.89	1.11	3.96	0.60
Ethanol	2.10	1.41	3.55	0.85
Simmondsia chinensis				
Petroleum ether	3.06	1.69	7.06	0.62
Diethyl ether	4.81	2.42	13.84	0.61
Chloroform	0.39	0.25	0.59	0.64
Ethanol	0.96	0.58	1.76	0.56
Methomyl	0.0071	0.0052	0.0097	1.27

Despite the extensive studies on the molluscicidal activities of plant oils and extracts against fresh water snails and other land snails, there have been only limited studies on the activity of plant extracts against *E. vermiculata* (Kassem *et al*., 1993; Ghamry, 1997; Saad, 1998; Abdelgaleil, 2005 and Abdelgaleil and Badawy 2006). The non-polar extracts (petroleum ether and diethyl ether) of *A. judaica* were more effective than the polar extracts (chloroform and ethanol) against the tested snail. In contrary, the polar extracts of *S. chinensis* were more effective than the non-polar extracts. A possible explanation of this observation is that the non-polar extracts of *A. judaica* contain mainly volatile oil which possesses pesticidal activity due to the presence of biologically active monoterpenes. In the case of *S. chinensis*, the polar extracts contain a higher concentration of toxic glucosides than the non-polar extracts.

Isolation and identification of active principles: A monoterpenoide piperitone (**1**) and a phenylpropanoid *trans*-ethyl cinnamate (**2**) were isolated from *A. judaica* essential oil by repeated silica gel column chromatography. In addition, the chloroform extract of *S. chinensis* was fractionated on silica gel column chromatography and the most active fractions were further purified on a silica gel column followed by recrystallization to give the two glucosides; simmondsin (**3**) and simmondisn 2`-ferulate (**4**). The chemical structures of the isolated compounds (**1-4**) were elucidated by using spectroscopic methods, including UV, IR, ^{1}H NMR ,^{13}C NMR and MS. Extensive studies of spectroscopic data of the isolated compounds allowed us to confirm the four isolated compounds as piperitone (**1**), *trans*-ethyl cinnamate (**2**), simmondsin (**3**) and simmondsin 2`-ferulate (**4**). The two isolated compounds **1** and **2** represented the major constituents of the oil of *A. judaica* as stated by El-Massry *et al.*, 2002. On the other hand, the two glucosides **3** and **4** isolated from *S. chinensis* have been isolated before from jojoba seed (Van Boven *et al.*, 1994 and Van Boven *et al.*, 1995).

Molluscicidal effect of the isolated active principles: The results of molluscicidal activity of the four isolated compounds expressed as LD_{50} against *E. vermiculata* are shown in Table 2. *trans*-Ethyl cinnamate (**2**) revealed the highest activity with LD_{50} value of 0.055 mg/snail followed by piperitone (**1**) while simmondsin 2`ferulate (**4**) exhibited the lowest activity with LD_{50} value of 0.18 mg/snail. In general, compounds **1** and **2** were more toxic to the snail than glucosides **3** and **4**. These compounds were more potent than sesquiterpenes isolated from *Magnolia grandiflora* (Abdelgaleil, 2005) and essential oils of *Mentha microphylla* and *Lantana camara* (Abdelgaleil and Badawy, 2006).

In summary, the results of the present study indicate that the petroleum ether extract of *A. judaica* and chloroform extract of *S. chinensis* possess a reasonable mollusicicidal potential against *E. vermiculata* snail. Moreover, the isolated compounds piperitone (**1**) and *trans*-ethyl cinnamate (**2**) have a remarkable molluscicidal activity. This may considered as a starting point for using such compounds in control of terrestrial snails.

Table 2. Molluscicidal activity of the isolated compounds against *Eobania vermiculata* after 48 h.

Compound	LD_{50} (mg/snail)	Confidence limits		Slope
		Lower	Upper	
Piperitone (**1**)	0.067	0.047	0.092	1.13
trans-Ethyl cinnamate (**2**)	0.055	0.038	0.077	1.16
Simmondsin (**3**)	0.11	0.08	0.15	0.93
Simmondsin 2`ferulate (**4**)	0.18	0.13	0.25	0.89
Methomyl	0.0071	0.0052	0.0097	1.27

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الملخص العربى

تقييم الخصائص الابادية لمستخلصات والمركبات الفعاله لنباتين مصريين على قوقع الحدائق البنى *Eobania vermiculata*

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تم تقييم النشاط الابادى لمستخلصات والمركبات المعزولة من نباتى الشيح والجوجوبا ضد قوقع الحدائق البنى *Eobania vermiculata* . وقد أوضحت النتائج أن مستخلص البتروليم ايثر لنبات الشيح كان اعلى المستخلصات نشاطاً حيث كانت قيمة LD_{50} تساوى 0.18 مجم / قوقع بينما كان مستخلص الداى ايثيل ايثر لنبات الجوجوبا هو أقل المستخلصات الثمانية المختبرة فاعلية حيث كانت قيمة LD_{50} تساوى 4.81 مجم/ قوقع . وأعطى الفصل الكروماتوجرافى متبوع بالتقييم الحيوي لزيت نبات الشيح والمستخلص الكلوروفورمى لنبات الجوجوبا أربع مركبات وهى piperitone و *trans*-ethyl cinnamate وsimmondsin وsimmondsin 2`ferulate. التركيب الكيماوى لهذه المركبات تم تقديره باستخدام طرق التحليل الطيفى المختلفة وهى UV و IR و ^{1}H NMR و ^{13}C NMR و MS . و أن المركبات المعزولة قد أظهرت فاعليه جيدة ضد القوقع المختبر خاصة الـ piperitone و الـ *trans*-ethyl cinnamate . حيث أن مركب *trans*-ethyl cinnamate قد أظهر أعلى نشاط حيث كانت قيمة الـ LC_{50} له تساوى 0.055 مجم/ قوقع و هذه الدراسة تعتبر الاولى فى تقييم مستخلصات والمركبات الفعالة من نباتى الشيح و الجوجوبا على قوقع الحدائق البنى .