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Insecticidal and antifeedant activites of two glucosides isolated from the seeds of *Simmondsia chinensis* (link) Schneider against Cotton leafworm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Insecticidal and antifeedant activities of four extracts of the seeds of jojoba plant, *Simmondsia chinensis* (link) Schneider, were evaluated against the third instar larvae of *Spodoptera littoralis* (Boisd) using topical application and non-choice leaf disk assays, respectively. Chloroform extract showed the strongest insecticidal and antifeedant effects among the tested extracts. Bioassay-driven fractionations of chloroform extract over silica gel columns followed by recrystallizations afforded two glucosides. The structure of the isolated glucosides was established by physico-chemical properties and spectroscopic analyses. The isolated glucosides, simmondsin and simmondsin 2`-ferulate, were tested for their insecticidal and antifeedant activities. In topical application assay, simmondsin and simmondsin 2`-ferulate showed strong insecticidal activity with LD_{50} values of 1.49 and 2.58 µg / larva, respectively. When tested for antifeedant activity, both compounds showed pronounced activity in a concentration-dependent manner. This is the first study on the insecticidal and antifeedant activities of *S. chinensis* seed extracts as well as the isolated glucosides.

Keywords: *Simmondsia chinensis*, insecticidal activity, antifeedant activity, glucosides, *Spodoptera littoralis*.

INTRODUCTION

The jojoba plant, *Simmondsia chinensis* (link) Schneider, is an arid perennial evergreen shrub indigenous to Arizona, California and North-western Mexico (Hogan, 1978). It is also grown in some Middle East and Latin America countries (Borlaug *et al*., 1985 and Bellirou *et al.*, 2005). The

jojoba seeds contains about 50 - 60 % of unique wax ester oil which is composed mainly of straight chain monoesters in the range of C_{40}-C_{44} (Elliger *et al*., 1973). Jojoba oil has a good market in cosmetics and lubricants (Cokelaere *et al*., 1992). Recently, it has been reported that the jojoba oils possess anti-inflammatory activity (Habashy *et al*., 2005).

A meal of the jojoba seeds is very rich with protein (20 – 32 %) which consisted mainly from albumins (79 %) and globulins (21 %) (Shrestha *et al*., 2002). This meal also contains approximately 15% of group of glucosides, known as simmondsins (Elliger *et al*., 1973 and Van Boven *et al*., 2000). Eight glucosides compounds (simmondsin and seven simmondsin derivatives) have been isolated and identified so far 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.

This plant is unknown in the literature as a plant of any pesticidal activity. Therefore, The present work was designed to:1) preparation of jojoba crude extracts and test their insecticidal and antifeedant activities against cotton leafworm, *Spodoptera littoralis*; 2) isolation, purification and identification of active glucosides, simmondsin and simmondsin 2`-ferulate, and evaluate their insecticidal and antifeedant activities against the same pest.

MATERIALS AND METHODS

1. Plant material: Seeds of *Simmondsia chinensis* (link) Schneider (jojoba plant) were collected from Al-Bostan Region, Behera Governorate in September, 2004. The plant material was identified with guidance of Flora of Egypt Book (Tackholm, 1974) and confirmed by Prof. Dr. Fath Allah Zaiton of Plant Pathology Department, Faculty of Agriculture (El-Shatby), Alexandria University.

2. Test insect: A susceptible cotton leafworm, *Spodoptera littoralis* (Boisd), strain was obtained from Bioassay Laboratory, Pesticide Chemistry Department, Faculty of Agriculture, Alexandria University. The colony was reared under laboratory conditions on caster bean, *Ricinus communis* L., leaves at 26 ° C ± 2 and 70 ± 5 RH (Eldefrawi *et al.*, 1964).

3. 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 CD_3OD. 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. Spectroscopic analyses were conducted at Faculty of Science Central Laboratory, Alexandria University. Mass spectrometry measurements were performed with JEOL JMS-AX500 at National Research Center, Giza.

4. Extraction and isolation of active glycosides from the seed of *Simmondsia chinensis*: Dried and powdered seeds of *Simmondsia chinensis* (1Kg) were exhaustively extracted with petroleum ether (60 – 80 °C), diethyl ether, chloroform and ethanol (3 L of each solvent), respectively, using Soxhlet apparatus for four hours (Van Boven *et al.*, 1995). Evaporation of the solvents under reduced pressure gave 240.0 gm of petroleum ether extract, 98.9 gm of diethyl ether, 45.4 gm of chloroform extract and 141.3 gm of ethanol extract. The chloroform extract was subjected to silica gel (1kg) column chromatography eluted by chloroform/methanol solvent system; starting with chloroform then 10 % methanol / chloroform then 20 % methanol/chloroform and finally with methanol. Thirty fractions of 200 ml have been collected and pooled to two main fractions based on their TLC profiles. Fraction 1 (3.4 gm) was further purified on silica gel column chromatography using 10 % chloroform/methanol solvent system to give 2.1 gm of simmondsin 2`-ferulate (2). Similar purification of the second fraction (7.3 gm) followed by recrystallization from acetone/chloroform (1:4) gave 5.4 gm pure prisms of simmondsin (1)

5. Topical application bioassay: The third instar larvae of *Spodoptera littoralis* (Boisd.) were used to assess the larvicidal activity of jojoba plant seed extracts and the isolated glycosides, simmondsin (1) and simmondsin 2`-ferulate (2). A series of concentrations of the extracts, the isolated compounds and a reference insecticide, chlorpyrifos were prepared in acetone. One microliter of test solution was applied on the dorsum of larvae by microapplicator. Three replicates of 10 larvae in each one were maintained for each dose and control treatment. The treated larvae were transferred to glass cups and supplied with fresh castor oil bean leaves. The percentages of mortality were recorded after 24h of treatment and corrected if necessary by Abbott`s formula (Abbott, 1925). The lethal dose causing 50

% mortality (LD_{50}) expressed as µg / larva were calculated from log-concentration mortality regression lines (Finney, 1971).

6. Antifeedant bioassay: The antifeeding activity of johoba plant and the isolated glycosides was tested against the third-instar larvae of cotton leafworm, *S. littoralis*, by using a non-choice leaf disk method as described by Kubo and Nakanishi (1977). Stock solutions in acetone of test extracts and compounds were prepared. A series of concentrations of each extract and compound were prepared by dilution with distilled water then a wetting agent (Triton-X 100) was added at constant concentration of 0.05%. Leaf disks (15 mm diam) of castor oil bean plant were immersed in test solutions for 10 seconds and left for dry. Five treated disks were transferred into glass cup with 10 third instar larvae. Three replicates of each concentration and control were arranged. Control disks were immersed in a solution containing water, acetone and Triton-X 100. The larvae were left to feed for 24 h at constant condition of 26 °C ± 2 and 70 ± 5 RH. The eaten areas in control and treated disks were measured by Dethier`s method (Dethier 1947). The percentage of feeding inhibition was determined after 24 h by the formula of Abivardi and Benz (1984): antifeeding index (AFI) = 100 ($C – T/C$), where C is the eaten area of leaf discs in the control and T is the eaten area of leaf discs in the treatment.

RESULTS AND DISCUSSION

1. Insecticidal activity of jojoba seeds extracts: The insecticidal activity of four extracts (petroleum ether, ether, chloroform and ethanol extracts) of the jojoba seeds was tested against the third instar larvae of *S. littoralis* by topical application method. Values of LD_{50} expressed as µg / larva, confidence limits and regression line slopes of the four tested extracts are shown in Table 1. The tested extracts revealed pronounced insecticidal activity against the tested insect. Chloroform extract was the most active one with LD_{50} value of 4 µg / larva followed by petroleum ether extract with LD_{50} value of 12.96 µg / larva, while ethanol and ether extracts were less active since their LD_{50} values of 23.42 and 28.16 µg / larva, respectively. The insecticidal activity of chloroform and petroleum ether extracts was higher than that of petroleum ether extracts of *Piper nigrum* and *Datura stramonium* (El-Doksh *et al*., 1984) and anise oil (Abbassy *et al*., 1998a) but weaker than that of acrtone / ethanol extract of *Pancratium maritimum* (Abbassy *et al*., 1998b) tested against the same insect using the same assay.

Table (1): Insecticidal activity of *Simmondisa chinensis* extracts against the third instar larvae of *Spodoptera littoralis* using topical application assay

Extract	LD_{50} (µg/larva)	Confidence limits		Slope
		Lower	Upper	
Petroleum ether	12.96	8.70	19.41	0.68
Ether	28.16	16.37	58.33	0.50
Chloroform	4.00	2.62	5.66	0.84
Ethanol	23.42	16.12	34.51	0.75

2. Antifeedant activity of jojoba seeds extracts: The antifeedant potential of Jojoba seeds extracts expressed as antifeedant percentage is presented in Table 2. All of tested extracts revealed antifeedant activity in a concentration-dependant manner. Chloroform extract exhibited the strongest antifeedant potency among the tested extracts at all of tested concentrations with antifeedant percentages of 51.95, 60.16 and 72.11 at concentrations of 0.1, 0.5 and 1 %, respectively. The tested extracts showed higher antifeedant activity than the extracts of nine plants evaluated by the same method against the fourth instar of *S. littoralis* (Abdelgaleil, 1995). A large number of plant extracts was reported to possess antifeedant activity against insects (Jilani and Saxena, 1990; Swidan, 1994 and Ben Jannet *et al.*, 2001). We could not compare the antifeedant potency of tested extracts with those reported in the literature due to the differences of assay method, insect, insect stage, concentration and results presentation.

Table (2): Antifeedant activity of *Simmondisa chinensis* extracts against the third instar larvae of *Spodoptera littoralis*

Concentration (%)	Antifeedant index (AFI %)			
	Petroleum ether extract	Ether extract	Chloroform extract	Ethanol extract
0.1	9.24	16.38	51.95	22.61
0.5	25.01	26.86	60.16	25.30
1	42.45	37.54	72.11	47.71

3. Isolation and structure elucidation of glucosides from the chloroformic extract of *S. chinensis*: The chloroformic extract of *S. chinensis* seeds displayed the highest insecticidal and antifeedant activity

against the third instar larvae of *S. littoralis*. Therefore, this extract has been selected for isolating the active compounds responsible for this biological activity. The chloroformic extract was subjected to silica gel column chromatography using chloroform/methanol solvent system as eluents. The most active fractions were further purified by repeated column chromatography followed by recrystallization to give two glucosides, simmondsin (1) and simmondisn 2`-ferulate (2) (Fig. 1). For both isolated compounds, TLC analysis showed only one violet spot after the plates were sprayed with 1-naphthol reagent along with intense blue fluorescence under short UV- wavelength radiation (254 nm).

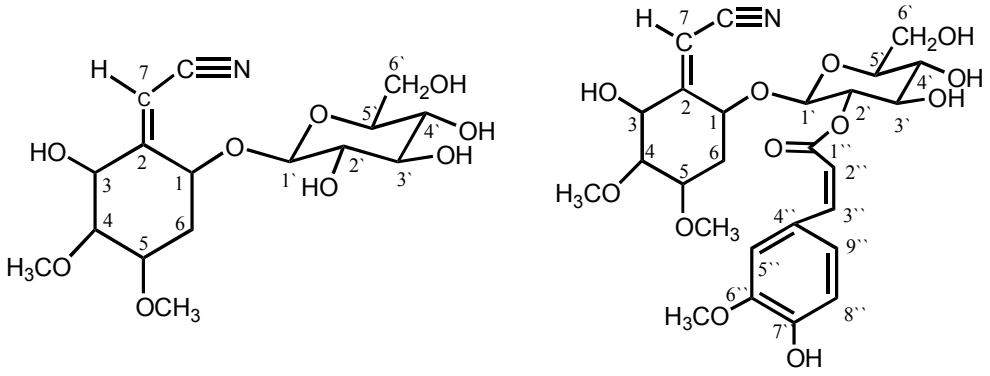

Figure 1. Chemical structure of glycosides isolated from the seeds of *Simmondisa chinensis*.

The first isolated compound, simmondsin (1), which is a major glucoside constituent of the seeds of *S. chinensis*, showed strong UV absorbance at 220 nm (ε = 11000) which can be attributed for double and triple bonds in conjugated system. The IR signals at 3600-3200, 2930-2900, 2215 and 1640-1600 cm^{-1} are matched with the presence of OH, CH, C≡N, C=C moieties in the molecule. Spectra of ^{1}H NMR and ^{13} C NMR displayed this compound contained 2 methyls, 2 methylene, 10 methenes and two carbons not bearing hydrogen. Also the two spectra showed two methoxy groups (^{1}H NMR δ 3.43 (5-OCH_3) and δ 3.45 (4-OCH_3); ^{13} C NMR δ 56.9 (5-OCH_3) and 57.3 (4-OCH_3), one olefinic proton at ^{1}H NMR δ 5.69 and nine protons on carbons attached directly to oxygen. A downfield proton and carbon signals at δ 4.37 (H1`) and δ 102.8 (C1`) indicated that this carbon is attached with two oxygen atoms. Mass spectra of this compound showed

two characteristic peaks at *m/z* [M-glucose] and *m/z* 163 (glucose), base peak. Detailed spectroscopic data are shown in Table 3.

Table (3): Physical and spectroscopic data of the isolated glycosides

	Simmondsin (**1**)	Simmondsin 2`ferulate (**2**)
Crystal shape	Colorless prisms	Colorless gum
UV λ_{max} nm (ε)	220 (11000)	220 (24000) and 326 (19500)
IR ν_{max} cm^{-1}	3600-3200, 2930, 2900, 2215, 1660, 1640, 1450, 1430, 1110, 1090, 1040, 990.	3600-3200, 2930, 1725, 1710, 1630, 1600, 1515, 1270, 1180, 1120, 1080, 1040, 760
δ ^{1}H NMR	δ 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), 5.69 (1H, s, H-7).	δ 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``)
MS *m/z*	*m/z* 212 $[M\text{-glucose}]^+$, *m/z* 163 [glucose, base peak), $C_{16}H_{25}NO_9$.	*m/z* 214 [M-glucose 2`-ferulate]$^+$, *m/z* 64 [base peak), $C_{26}H_{33}NO_{12}$.

The second isolated glucoside (simmondsin 2`-ferulate, 2) showed two UV maximum absorbances at 220 nm (ε = 24000) and 326 nm (ε = 19500). The strong UV absorbance and the absorbance at longer wavelength (326) are constant with the presence of aromatic ring substituted at glucose moiety. The IR spectrum of this compound revealed signals for OH groups (3600-3200 cm^{-1}), C-H (2930 cm^{-1}), C=O (1725 and 1710 cm^{-1}) and benzene ring (1630, 1600 and 760 cm^{-1}). The NMR data revealed the presence of 3 methyls, 2 methelene, 15 methenes and six carbons not attached with protons. The NMR spectra of this compound was similar to those of the first compound simmondsin with some additional proton and carbon signals of 2`ferulate moiety substituted on glucose ring. Thus, the chemical structures of simmondsin and simmondsin 2`-ferulate were

elucidated by using the previously physico-chemical properties and spectroscopic methods, including UV, IR, ^{1}H NMR ,^{13} C NMR and MS, as well as comparison of NMR data with those reported in the literature (Van Boven *et al*., 1994 and Van Boven *et al*., 1995).

**4. Insecticidal effects of isolated glucosides; simmondsin and simmondsin 2`-ferulate:** The insecticidal activity of isolated compounds, simmondsin and simmondsin 2`-ferulate was evaluated by topical application assay on the third instar larvae of *S. littoralis*. Table 4 presents the LD_{50} values (µg / larva) of the two compounds and a reference insecticide, chlorpyrifos. Both compounds revealed a pronounced insecticidal activity but were less active than chlorpyrifos. Simmondsin (LD_{50} = 1.49 µg / larva) showed higher insecticidal activity than simmondsin 2`-ferulate (LD_{50} = 2.58 µg / larva). Comparing the toxicities of these compounds with those of monoterpenes (geijerene and pregeijerene) tested against the larvae of *S. litura* (F.) by the same assay (Kiran *et. al.*, 2006). Results showed that the isolated compounds were more potent than those of monoterpenes.

Table (4): Insecticidal activity of isolated glucosides, (simmondsin and simmondsin 2`ferulate, against the third instar larvae of *Spodoptera littoralis* using topical application assay

Compound	LD_{50} (µg/larva)	Confidence limits		Slope
		Lower	Upper	
Simmondsin (1)	1.49	0.92	2.31	0.63
Simmondsin 2`ferulate (2)	2.58	1.71	3.84	0.69
Chlorpyrifos	0.12	0.10	0.14	1.55

**5. Antifeedant efficacy of isolated glucosides; simmondsin and simmondsin 2`-ferulate:** The isolated compounds, simmondsin and simmondsin 2`-ferulate, were tested for their antifeedant activity using non-choice leaf disk assay against the third instar larvae of *S. littoralis*. Antifeedant percentages of the two compounds are shown in Table 5. The antifeedant activity for both compounds was concentration-dependent. The isolated compounds exhibited strong antifeedant activity even at low concentration of 10 µg / ml with antifeedant percentages of 38.05 and 32.30 for simmondsin and simmondsin 2`-ferulate (2), respectively. The highest antifeedant activity was observed at 1000 µg/ml where simmondsin (1) and

simmondsin 2`-ferulate caused antifeedant percentages of 92.12 and 90.0, respectively. The isolated compounds exhibited stronger antifeedant activity than limonoids isolated from *Khaya senegalensis* (Abdelgaleil and Nakatani, 2003), *Khaya ivorensis*, *Chukrasia tabularis* and *Swietenia mahogani* (Abdelgaleil and Al-Aswad, 2005). However, they showed comparable antifeedant activity to neo-clerodane diterpenoids isolated from *Salvia* spp. In addition, some of these of neo-clerodane diterpenoids were more active than the isolated compounds (Simmonds *et. al*., 1996).

Table (5): Antifeedant activity of isolated glucosides, (simmondsin and simmondsin 2`ferulate, against the third instar larvae of *Spodoptera littoralis* using non-choice leaf disk assay

Concentration	Antifeedant index (AFI %)	
(µg/ml)	Simmondsin (1)	Simmondsin 2`ferulate (2)
10	38.05	32.30
50	46.22	44.20
100	63.40	60.90
500	78.90	76.88
1000	92.12	90.00

Comparing the insecticidal and antifeedant activities of the isolated glucosides, simmondsin and simmondsin 2`-ferulate, with chloroform extract of *S. chinensis* seeds revealed that the isolated compounds were more active than the crude extract. This result indicating that the bioactivity of this extract may be attributed to the isolated glucosides. This finding is supported by those of Cockelaere *et al.*, 1992 and Flo *et al.*, 1997 who found that simmondsin and simmondsin 2`-ferulate exhibit biological activity as food-intake inhibitors.

In summary, this study demonstrated the chloroform extract of jojoba, *S. chinensis*, seeds and its isolated glucosides, simmondsin and simmondsin 2`-ferulate, possessed remarkable insecticidal and antifeedant activities against the cotton leafworm. The feasibility of use of these extract and compounds under field conditions, however, is still questionable. Therefore, further studies for toxicology, formulation and evaluation of these compounds under field conditions are necessary.

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