Juvenomimetic and Insecticidal Activities of Senecio salignus (Asteraceae) and Salvia microphylla (Lamiaceae) on Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Research

Juvenomimetic and insecticidal activities of *Senecio salignus* (Asteraceae) and *Salvia microphylla* (Lamiaceae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

Plant extracts can be used as an alternative to synthetic insecticides for the control of insect pests. Based on this knowledge, juvenomimetic and insecticidal activities of *n*-hexane extracts of the aerial parts of *Senecio salignus* DC. (Asteraceae) and *Salvia microphylla* Kunth (Lamiaceae) collected in Mexico were evaluated against 1st instar larvae of *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae). *Senecio salignus* extract showed insecticidal activity at 500 ppm, resulting in larval mortality of 52.5% and pupal mortality of 62.5%. *Salvia microphylla* extract at the same concentration caused larval mortality of 65.0% and pupal mortality of 82.5%. The LC50 was 440 ppm for *S. salignus* extract and 456 ppm for *S. microphylla* extract based on the total larval period. The juvenomimetic activity of *S. salignus* extract at 500 ppm increased the duration of the larval period to 17.3 d and of the pupal period to 1.4 d. It also reduced pupal weight by 34.7% with respect to the control (241 mg). For *S. microphylla* extract at 500 ppm, the duration of the larval and pupal periods were increased by 2.0 and 12.1 d, respectively, and the pupal weight was reduced by 14.1% with respect to the control (243 mg). The major compounds of *S. salignus* extract were γ-sitosterol, palmitic acid, lupeol, and β-amyrin, and those of *S. microphylla* extract were oleic acid, γ-sitosterol, (*Z,Z,Z*)-9,12,15-octadecatrien-1-ol, and palmitic acid. These results indicate that both extracts have potential to be used to control *S. frugiperda* due to their juvenomimetic and insecticidal activities.

Key Words: chilca; mirto; fall armyworm; γ-sitosterol; lupeol; β-amyrin

Resumen

Los extractos de plantas pueden ser usados como alternativa al empleo de insecticidas químico sintéticos para el control de insectos plaga. Basándose en este conocimiento, se evaluaron las actividades insecticida y juvenomimética del extracto *n*-hexano de las partes aéreas de *Senecio salignus* DC. (Asteraceae) y de *Salvia microphylla* Kunth (Lamiaceae) colectadas en Mexico, contra larvas del primer estadio de *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae). El extracto de *S. salignus* mostró actividad insecticida a 500 ppm, ocasionando 52.5% de mortalidad larval y 62.5% de mortalidad pupal. El extracto de *S. microphylla* causó una mortalidad larval de 65.0% a 500 ppm y una mortalidad pupal de 82.5% con la misma concentración. La concentración letal media (LC50) fue de 440 ppm para el extracto de *S. salignus* y de 456 ppm para el extracto de *S. microphylla* para todo el periodo larval. La actividad juvenomimética de *S. salignus* a 500 ppm resultó en el incremento de la fase larval de 17.3 dias y para el periodo pupal fue de 1.4 días, también redujo el peso pupal 34.7% respecto al control (241 mg). Para el extracto de *S. microphylla*, a 500 ppm causó el incremento de las fases larval y pupal 2.0 y 12.1 días respectivamente y reducción del peso pupal de 14.1% respecto al control (243 mg). Los principales compuestos del extracto de *S. salignus* fueron el γ-sitosterol, ácido palmítico, lupeol y β-amirina, y para el extracto de *S. microphylla* fueron el ácido oleico, γ-sitosterol, (*Z,Z,Z*)-9,12,15-octadecatrien-1-ol, y el ácido palmítico. Estos resultados indican que ambos extractos pueden ser empleados en el control de *S. frugiperda* por presentar actividades juvenomimética e insecticida.

Palabras Clave: chilca; mirto; gusano cogollero; γ-sitosterol; lupeol; β-amirina

The fall armyworm, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), is one of the most destructive insect pests of maize, *Zea mays* L. (Poaceae), in the tropical and subtropical regions of the western hemisphere (Andrews 1988; Santos et al. 2003). Currently, the principal control method for this species is through the use of synthetic insecticides (Tagliari et al. 2010). However, integrated pest man-

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agement programs for noctuid insects have been well demonstrated. As a result, there has been increased interest in research related to the identification of botanical extracts that demonstrate insecticidal activity, so as to reduce the use of synthetic insecticides (Pavela & Chermenskaya 2004).

Many plants have insecticidal or juvenomimetic activities against insects. The genus *Senecio* (Asteraceae) comprises about 1,500 species with 165 species found in Mexico. These species are known to produce many insecticidal compounds such as alkaloids, sesquiterpenes, chalcones, and flavonoids (Romo de Vivar et al. 2007). This genus has also been associated with anti-inflammatory, vasodilator, antiemetic, and antimicrobial activities (Rodríguez & López 2001; Rosa et al. 2004). Studies of insecticidal activity have been conducted with *Senecio umbrosus* Waldst. & Kit and *Senecio otites* Kunze ex DC., and extracts have been shown to affect larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Domínguez et al. 2008; Pavela 2011).

The genus *Salvia* is the most diverse genus of the family Lamiaceae, with over 1,000 species around the world distributed in tropical and subtropical zones. In Mexico, there are at least 300 reported species (Fernández 2006). Many species of this genus produce various bioactive compounds such as sesquiterpenes, diterpenes, triterpenes, sterols, and polyphenols (Yi-Bing et al. 2012). Antioxidant, antimicrobial, analgesic, anticancer, antipyretic, and anti-inflammatory activities have also been reported (Kamatou et al. 2008; Akin et al. 2010). In addition, insecticidal activity on *S. frugiperda* and *S. littoralis* has been reported (Pavela 2004; Zavala-Sánchez et al. 2013).

The aim of this study was to determine the insecticidal and juvenomimetic activities of the *n*-hexane extracts of the aerial parts of "chilca," *Senecio salignus* DC. (Asteraceae), and "mirto," *Salvia microphylla* Kunth (Lamiaceae), on *S. frugiperda*.

Materials and Methods

INSECT REARING

Fall armyworm larvae were reared in the Laboratory of Natural Insecticide Compounds at the Universidad Autónoma de Querétaro, in Querétaro, Mexico. The larvae were reared at 25 ± 2 °C and 70% relative humidity with a 12:12 h L:D photoperiod. For preparation of the fall armyworm diet, the following mixture was made: 800 mL distilled water, 30 g ground beans, 90 g ground corn (dried beans and corn were ground with a Thomas-Wiley Model 4 mill with a particle size of 1 mm), 20 g brewer's yeast, 10 g vitamins (vitamin mix, Lepidoptera # 722, Bio-Serv), 10 g agar, 1.7 g ascorbic acid (dissolved in 17 mL ethanol), 2.5 mL formaldehyde, 1.7 g methyl *p*-hydroxybenzoate, and 0.6 g neomycin sulfate (Bergvinson & Kumar 1997).

PLANT MATERIAL AND EXTRACTION

Aerial parts (leaves, stems, and flowers) of *S. salignus* and *S. microphylla* were collected in Tenancingo County, Mexico, at 18.9514° to 19.0403° north latitude and 98.5958° to 99.6436° west longitude, and 2,060 m asl. in Sep 2013. Taxonomic authentication was performed by Abigail Aguilar Contreras, and vouchers were deposited at Herbarium of Instituto Mexicano del Seguro Social (IMSS). The voucher specimen for S*. salignus* was IMSS M 15,546 and for *S. microphylla* was IMSS M 15,821.

After thorough cleaning of aerial material from each plant, it was shade dried at room temperature for a minimum of 20 d. The dried plant was then made into powder with a Thomas-Wiley Model 4 mill with a particle size of 1 mm. Dried and powdered aerial material from *S. salignus* or *S. microphylla* (250 g) were extracted with 2 L *n*-hexane under reflux for 4 h. The extract was filtered and the solvent removed under reduced pressure by using a rotatory evaporator. The yield weight of *S. salignus* extract was 2.31% and of *S. microphylla* 2.09%.

PHYTOCHEMICAL TEST

The extracts were tested in triplicate for various phytochemical classes by using the following methods: 1) alkaloids: Meyer, Wagner, and Dragendorff reagents; 2) cardiotonics: Raymond and Baljet reagents; 3) flavonoids: H_2SO_4 concentrate; 4) saponins: H_2O at boiling temperature; 5) sterols and triterpenes: Salkowsky reagent, Liebermann/Burchard reagent; 6) tannins: ferric chloride; and 7) terpenes: Noller reagent (Tiwari et al. 2011; Wadood et al. 2013).

IDENTIFICATION OF THE PRINCIPAL COMPOUNDS FROM *N*-HEXANE EXTRACTS OF *S. SALIGNUS* AND *S. MICROPHYLLA*

Twenty µL *n*-hexane extracts of aerial portions of *S. salignus* and *S. microphylla* were diluted with 1 mL acetone. The extracts were analyzed on an Agilent Technologies (Santa Clara, California) 6890N GC equipped with an HP-5MS column (30 m in length; 25 mm internal diameter; 0.25 µm film thickness) equipped with an Agilent MS 5973 detector, at 250 °C. The carrier gas was helium, with a flow rate of 1 mL/min; the split ratio was 2:1. The column temperature was initially 50 °C (for 3 min) and was gradually increased to 240 °C, at 3 °C/min; this temperature was maintained for 2 min. The injector temperature was 250 °C, and 1 µL *n*-hexane extract was injected and analyzed in duplicate. The spectra were collected at 71 eV ionization voltages, and the analyzed mass range was 15 to 600 m/z. The identification of the components was confirmed by comparison of the retention indices with those of authentic compounds and with the Wiley09/NIST11 library.

BIOASSAY

Bioassays were conducted using for each concentration 40 replicates (larvae) divided in 5 experimental units with 8 larvae each, selected randomly. Preliminary screening of each extract was carried out at 5 concentrations ranging from 0.5 to 5,000 ppm, and a control, by following the previously described method (Santiago-Santiago et al. 2009), altogether using 240 larvae for each plant. The extracts were mixed with the larval diet ingredients during preparation. For the final bioassays, 6 concentrations of extracts were tested (0, 50, 500, 1,000,

Table 1. Phytochemical test of the *n*-hexane extracts of *Senecio salignus* and *Salvia microphylla*.

	ALK			CAR		FLA	SAP	STE	TAN	TER	TRI
Extract	Mayer	Wagner	Dragendorff	Raymond	Beljet	H_2SO_4	H_2O	Salkowski	$FeCl_3$	Noller	Liebermann-Burchad
S. microphylla	–	–	–	+	+	–	+	+	+	+	+
S. salignus	–	–	–	–	–	+	–	+	+	+	+

ALK = alkaloids; CAR = cardiotonics; FLA = flavonoids; SAP = saponins; STE = sterols; TAN = tannins; TER = terpenes; TRI = triterpenes.

Table 2. Insecticidal and juvenomimetic activities of aerial parts *n*-hexane extract of *Senecio salignus* against *Spodoptera frugiperda*.

	Mortality (%)		Duration (d)		
Concentration (ppm)	Larva	Pupa	Larva	Pupa	Pupal weight (mg)
5,000	100 ± 0*	100 ± 0*	—	—	—
2,000	95.0 ± 3.5*	97.5 ± 2.5*	54.5 ± 0.5*	20.0 ± 0*	87.5 ± 14.5*
1,000	90.0 ± 4.8*	95.0 ± 3.5*	52.0 ± 1.8*	17.0 ± 1.0*	110.0 ± 5.8*
500	52.5 ± 8.0*	62.5 ± 7.8*	40.2 ± 1.8*	12.5 ± 0.3*	157.3 ± 6.3*
50	12.8 ± 5.3	20.0 ± 6.4	23.2 ± 0.2	11.1 ± 0.2	226.5 ± 3.1
Control	10.0 ± 4.8	15.0 ± 5.7	22.9 ± 0.2	11.1 ± 0.2	241.0 ± 4.1
LC50	0.440×10^3 (0.24442–0.60503) ppm				

Results are the mean of at least 40 determinations ± standard error. * Significantly different from control ($P < 0.001$).

2,000, and 5,000 ppm) according to the method used by Rodríguez-Hernández & Vendramim (1996) as modified by Romero-Origel et al. (2012), and 240 larvae were used for each plant. The larvae were maintained at 27 ± 2 °C, 70 ± 5% relative humidity, and a 14:10 h L:D photoperiod. The pupae were weighed 24 h after pupation and then moved to another container for development to the adult stage. The insecticide parameters evaluated were larval and pupal mortality, and the juvenomimetic parameters were the length of the larval and pupal period and the pupal weight at 24 h after formation. The median lethal concentration (LC50) to the larval population of *S. frugiperda* was calculated for each extract by using data for total larval period mortality.

STATISTICAL ANALYSIS

A statistical analysis was conducted, and data were tested for normality and homoscedasticity before analysis. In some cases, Kruskal–Wallis non-parametric analysis of variance (ANOVA) was used when data violated these assumptions and could not be corrected using a transformation. ANOVA, followed by Tukey's test, was performed, and the LC50 was calculated by probit analysis, using the SYSTAT statistical analysis program (SYSTAT 1998).

Results

PHYTOCHEMICAL TEST

The extract of *S. salignus* tested positive for tannins, flavonoids, terpenes, triterpenes, and sterols (Table 1). The *S. microphylla* extract tested positive for cardiotonics, saponins, tannins, terpenes, triterpenes, and sterols (Table 1).

INSECTICIDAL ACTIVITY OF *S. SALIGNUS* AND *S. MICROPHYLLA* EXTRACTS

Exposure to the *n*-hexane extract of the aerial parts of *S. salignus* (Table 2) induced 100% larval mortality at 5,000 ppm, and 95, 90, and 52.5% at 2,000, 1,000, and 500 ppm, respectively. Mortality was 10% in the control treatment (LC50 = 440 ppm). Pupal mortality was 100, 97.5, 95, and 62.5% at 5,000, 2,000, 1,000, and 500 ppm, respectively, and the control mortality was 15%. The *S. microphylla* extract (Table 3) resulted in a larval mortality of 100% at 5,000 ppm and 97.5, 87.5, and 65% at 2,000, 1,000, and 500 ppm, respectively. The control showed 7.5% mortality (LC50 = 456 ppm). Pupal mortality was 100, 100, 95, and 82.5% at 5,000, 2,000, 1,000, and 500 ppm, respectively, and 15% in the control.

JUVENOMIMETIC ACTIVITY OF *S. SALIGNUS* AND *S. MICROPHYLLA* EXTRACTS

The juvenomimetic activity of the *S. salignus n*-hexane extract (Table 2) extended the larval period by 31.6, 29.1, and 17.3 d at 2,000, 1,000, and 500 ppm, respectively, and increased the pupal period by 8.9, 5.9, and 1.4 d at 2,000, 1,000, and 500 ppm, respectively, compared with the controls (22.9 and 11.1 d). It also reduced the pupal weight by 63.7, 54.4, and 34.7% at 2,000, 1,000, and 500 ppm when compared with the control weight (241 mg).

The *S. microphylla* extract (Table 3) prolonged the larval period by 12.5, 10.9, and 2.0 d at 2,000, 1,000, and 500 ppm, respectively, and increased the pupal period by 16.5 and 12.1 d at 1,000 and 500 ppm, respectively, compared with controls (22.5 and 10.5 d). It also reduced the pupal weight by 74.9, 39.2, and 14.1% at 2,000, 1,000, and 500 ppm, respectively, when compared with the control (243 mg).

Table 3. Insecticidal and juvenomimetic activities of aerial plant tissue *n*-hexane extract of *Salvia microphylla* against *Spodoptera frugiperda*.

	Mortality (%)		Duration (d)		
Concentration (ppm)	Larva	Pupa	Larva	Pupa	Pupal weight (mg)
5,000	100.0 ± 0*	100.0 ± 0*	—	—	—
2,000	97.5 ± 2.5*	100.0 ± 0*	35.0 ± 0*	—	61.0 ± 0*
1,000	87.5 ± 5.3*	95.0 ± 3.5*	33.4 ± 3.2*	27.0 ± 1.0*	148.0 ± 22.0*
500	65.0 ± 7.6*	82.5 ± 6.1*	24.5 ± 0.4*	22.6 ± 0.6*	209.1 ± 3.8*
50	15.0 ± 5.7	20.0 ± 6.4	23.1 ± 0.2	11.1 ± 0.2	232.1 ± 2.0
Control	7.5 ± 4.2	15.0 ± 5.7	22.5 ± 0.2	10.5 ± 0.2	243.4 ± 3.9
LC50	$0.456.2 \times 10^3$ (0.32647–0.58455) ppm				

Results are the mean of at least 40 determinations ± standard error. * Significantly different from control ($P < 0.001$).

IDENTIFICATION OF PRINCIPAL COMPOUNDS

We found 48 compounds in *n*-hexane aerial extracts of *S. salignus* identified by GC-MS analysis, representing 99.95% of the extracted material (Table 4); the retention times ranged between 3.88 and 67.20 min. The major components and their respective retention times were: palmitic acid (12.23%) 44.66 min, γ-sitosterol (16.10%) 54.05 min, β-amyrin (5.18%) 61.51 min, and lupeol (6.44%) 61.72 min. The GC-MS analysis of *n*-hexane aerial parts extracts of *S. microphylla* showed 59 compounds, which accounted for 99.96% of the extracted material (Table 5); the retention times ranged between 3.72 and 63.39 min. The major components and retention times were: palmitic acid (7.12%) 44.8 min, (*Z*,*Z*,*Z*)-9,12,15-octadecatrien-1-ol (11.11%) 49.76 min, oleic acid (14.76%) 49.98 min, and γ-sitosterol (12.77%) 54.74 min.

Discussion

To our knowledge, this is the first report to demonstrate the insecticidal and juvenomimetic activities of the *n*-hexane extracts of aerial

Table 4. Composition of the n-hexane aerial plant tissue extract of *Senecio salignus*.

No.	Retention time (min)	Peak area (%)[a]	Compound name
1	3.88	0.73	p-Xylene
2	4.08	1.03	Acid 2-methyl-butanoic
3	30.43	0.86	(−)-Spathulenol
4	30.55	1.75	Caryophyllene oxide
5	31.77	0.21	1-Benzoxepin-3-ol, 2,3,4,5-tetrahydro-
6	32.44	0.51	Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
7	32.59	1.26	Hexadecane
8	33.89	0.80	Longifolenaldehyde
9	36.76	0.23	Alloaromadendrene oxide-(1)
10	38.06	0.75	Tetradecanoic acid
11	39.41	0.58	Benzene, 1,4-diethyl-2,3,5,6-tetramethyl-
12	39.64	2.12	Octadecane
13	40.55	1.21	6,10,14-Trimethylpentadecane-2-one
14	41.56	0.30	(1S,5R,10S)-1,5,8,8-Tetramethylbicyclo |8.1.0| undecano-2,6-dione
15	44.31	1.73	Dicyclooctanopyridazine
16	44.66	12.23	Palmitic acid
17	46.06	1.29	Eicosane
18	48.03	1.08	1-Octadecene
19	49.38	3.88	Linoleic acid
20	49.64	1.66	Trans-oleic acid
21	49.73	0.52	Ledol
22	50.49	1.85	Stearic acid
23	51.77	0.31	1-Docosene
24	51.94	2.10	Docosane
25	52.56	0.26	2-Oxabicyclo[2.2.1]heptane-1-carboylic acid-4,7,7-trimethyl-3-oxo-(1,2-dimethyl-1-ethynyl) propylester
26	52.66	0.21	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-1,1ar-(1aα,4β,4aβ,7α,7aβ,7bα)-1-
27	53.70	0.47	β-Sitosterol
28	53.82	3.24	Stigmasterol, 22,23-dihydro-
29	54.05	16.10	γ-Sitosterol
30	56.30	0.82	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-
31	56.56	1.14	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one
32	57.35	1.17	Heptacosane
33	57.50	0.40	Cholestan-3-one, 4,4-dimethyl-,(5β)-
34	57.66	4.32	Triphenylphosphine oxide
35	59.17	1.05	Z-14-Nonacosane
36	59.80	0.31	Bis(2-ethylhexyl) phthalate
37	59.91	1.73	Triacontane
38	60.31	1.22	4-Androsten-6β-ol-3,17-dione
39	60.35	0.42	2-benzoylguaiazulene
40	60.82	4.85	Baurenol
41	61.16	2.94	1-Hexacosene
42	61.51	5.18	β-Amyrin
43	61.72	6.44	Lupeol
44	62.36	1.08	Heptacosane, 1-chloro-
45	62.59	1.14	Z-11(13-Methyl)tetradecen-1-ol acetate
46	64.74	4.30	Heptacosane
47	66.53	0.19	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpenthyl)-
48	67.20	1.98	Nonacosane

[a]Values reported as a percentage of the total area.

Table 5. Composition of the n-hexane aerial plant tissue extract of *Salvia microphylla*.

No.	Retention time (min)	Peak area (%)a	Compound name
1	3.72	0.14	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo(5,4,0,0,8,8) undecane
2	3.90	0.49	p-Xylene
3	4.37	0.21	m-Xylene
4	4.73	0.16	Ethanol,2-butoxy-
5	5.18	0.10	Cumene
6	5.53	0.45	α-Thujene
7	6.79	0.63	β-Phellandrene
8	8.29	0.23	2-Carene
9	9.83	0.21	Crithmene
10	12.51	0.16	L-Camphor
11	13.88	0.61	Borneol
12	14.98	0.28	(+)-α-Terpineol
13	22.82	0.17	(+)-Cyclosativene
14	23.02	0.89	Ylangene
15	23.46	0.24	β-Baurbonene
16	23.65	0.16	β-Cedrene
17	24.61	0.17	Isoledene
18	24.76	1.24	Caryophyllene
19	25.57	0.16	[+]-Aromadendrene
20	26.01	0.45	Cadinene
21	26.47	1.52	α-Copaene
22	27.22	0.68	Isoledene
23	27.32	0.56	α-Curcumene
24	27.61	0.45	Naphthalene,hexahydro-1,6-dime
25	27.74	1.40	Aristolene
26	28.04	0.28	α-Muurolene
27	28.45	0.77	Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene
28	28.57	1.12	Naphthalene,1,2,3,4-tetrahydro
29	28.88	1.15	-Cadiene
30	29.02	0.21	β-Sesquiphellandrene
31	29.29	2.61	Quinoline,5,8-dimethyl-
32	29.62	0.47	Germacrene
33	29.74	2.76	Copaene
34	29.99	0.56	Naphthalene,1,2-dihydro-1,1,6-trimethyl-
35	30.65	3.86	Caryophyllene oxide
36	31.53	1.22	Guaiol
37	31.64	2.50	1-Naphthalenol,decahydro-4a-methyl-8-methylene-2-[methylethyl]-[1-,1R(1α,2β,4aβ,4aα]-
38	32.02	0.61	Eremophilene
39	32.66	1.63	10,10-Dimethyl-2,6-dimethylenebicyclo-|7.2.0| undecan-5β-ol
40	33.02	0.23	-Gurjunene
41	33.14	1.12	β-Endesmol
42	33.27	0.73	1,4-Methano-1H-indene,octahydro-1,7a-dimethyl-4-(1-methylenyl)-11s-(1α,3aβ.,4α,7aβ)-
43	33.36	0.80	δ-Selinene
44	34.00	1.33	Cadalene
45	43.92	1.57	Hexadecenoic acid, Z-11-
46	44.80	7.12	Palmitic acid
47	48.15	0.44	Methyllinoleate
48	49.02	2.08	Phytol
49	49.76	11.11	9,12,15-Octadecatrien-1-ol,(Z,Z,Z)-
50	49.98	14.76	Oleic acid
51	52.52	3.20	Selenolo[3,4-b][1]benzoselenophen-3-(1H)-one
52	55.74	12.77	γ-Sitosterol
53	57.11	0.35	Cyclopropanecarbonitrile,1-(p-bromophenyl)-2-1b-(dimethylamino) phenyl
54	58.03	3.08	Triphenylphosphine oxide
55	59.17	0.93	Benzene, 1-(4-phenyl-1,3-butadinylil)-3-|2-(trimethylsilyl) ethynyl-
56	59.93	3.83	Bis(2-ethylhexyl) phthalate
57	60.02	1.10	4-[3-Pyridyl]-3-thiosemicarbazone piperonal
58	60.25	0.54	Androst-2-en-17-one,4,4-dimeth
59	63.39	1.36	α-Monoolein

[a]Values reported as a percentage of the total area.

parts of *S. salignus* and *S. microphylla* against *S. frugiperda* larvae. These extracts demonstrated strong insecticidal activity and showed an LC50 of 440 ppm and 456 ppm, respectively. In similar studies, 0.5% of root powder of *S. salignus* caused 100% mortality in *Zabrotes subfasciatus* Boheman (Coleoptera: Bruchidae) in stored beans (López-Pérez et al. 2007; López et al. 2010). Moreover, the extracts of *Lepidaploa lilacina* Mart. ex DC. (Asteraceae), *Ageratum fastigiatum* Gardner (Asteraceae), and *Lychnophora ramosissima* Gardner (Asteraceae) caused 72.0, 65.9, and 61.0% egg mortality, respectively (Rodríguez & López 2001). Rodríguez & López (2001) also showed that after 2 d, *Lychnophora* sp. (Asteraceae) and *Vernonia holosericea* Mart. (Asteraceae) extracts caused 8.7 and 87% larval mortality, respectively, in *Z. subfasciatus*. The extracts of *Lychnophora ericoides* Mart. (Asteraceae) and *Trichogonia villosa* Sch. Bip. ex Baker (Asteraceae) caused 97.7% egg mortality in *S. frugiperda* after 1 d (Tavarez et al. 2009).

Additionally, the chloroform extract of aerial parts of *S. microphylla* showed insecticide activity against S. *frugiperda* (LC50 = 919 ppm) (Zavala-Sánchez et al. 2013). On the other hand, Ramírez-Moreno et al. (2001) reported that aqueous extracts of aerial parts at 5% concentration of *Salvia karwinskii* Benth (Lamiaceae) and *Salvia polystachya* Epling (Lamiaceae) had low insecticidal activity (13% with both species) against *Leptophobia aripa elodia* Boisduval (Lepidoptera: Pieridae). Rashid et al. (2009) showed 80% mortality in adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) with dichloromethane extract of the aerial parts of *Salvia cabulica* Benth (Lamiaceae).

The juvenomimetic activities of *S. salignus* and *S. microphylla n*-hexane extracts against *S. frugiperda* larvae began at 500 ppm, wherein each extract increased the length of the larval and pupal periods and decreased the pupal weight. Ramírez-Moreno et al. (2001) tested the repellent activity of aqueous extract using the powder of the entire *S. salignus* plant on *L. aripa elodia*. However, this plant extract had no effect on this insect. Domínguez et al. (2008) showed the antifeedant activity of the ethanolic extract of aerial parts of *S. otites* against *S. littoralis* and reported a feeding inhibition of 43% at 100 µg/cm^2 in *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Rhopalosiphum padi* L. (Hemiptera: Aphididae). The same study also showed that only 30% of *M. persicae* and 13% of *R. padi* settled to feed at a concentration of 50 µg/cm^2. Moreover, the chloroform extract of the aerial parts of *S. microphylla* showed juvenomimetic activity against *S. frugiperda* beginning at 500 ppm, which increased the pupal duration to 2 d and reduced the pupal weight by 13.3% with respect to the control (Zavala-Sánchez et al. 2013). Ramírez-Moreno et al. (2001) observed 7% repellency with 5% aqueous extracts of *S. karwinskii* and *S. polystachya* against *L. aripa elodia* larvae.

The GC-MS analysis showed that the principal components of *S. salignus n*-hexane extract were: palmitic acid, γ-sitosterol, β-amyrin, and lupeol, in addition to caryophyllene oxide. Sánchez-Muñoz et al. (2012) reported *n*-hexane extracts of aerial parts of *S. salignus* from the Mexican state of Guerrero to contain caryophyllene oxide, whereas Pérez-González et al. (2013) reported nonacosane (10.11%), (*Z*,*Z*)-9.12-octadecadienoic acid (7.5%), squalene (5.17%), and (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (5%) as principal components of the chloroform extract of aerial parts of *S. salignus*.

The principal components of *S. microphylla* extract were: palmitic acid, (*Z*,*Z*,*Z*)-9,12,15-octadecatrien-1-ol, oleic acid, and γ-sitosterol, in addition to caryophyllene and caryophyllene oxide. Lima et al. (2012) found (*E*)-caryophyllene (15.35%), α-eudesmol (14.06%), β-eudesmol (8.74%), and γ-eudesmol (7.64%) as principal components of essential oil from aerial parts of *S. microphylla*.

This study showed potential for the use of *S. salignus* and *S. microphylla n*-hexane extracts against *S. frugiperda* larvae. Both plants contain bioactive compounds such as flavonoids, essential oils, diterpenes, and triterpenes, which can act as an antifeedants (Tomás-Barberán & Wollenweber 1990). Also, palmitic acid and oleic acid showed insecticidal and juvenomimetic acivities against *S. frugiperda* larvae with larval viability values of 33.3 and 48.5%, respectively, when exposed to 1,600 ppm of palmitic and oleic acid, with respective LC50 values of 989 and 1,353 ppm, respectively (Pérez-Gutiérrez et al. 2011). These acids were present as principal components of *n*-hexane extract of *S. microphylla*, and palmitic acid was extracted from *S. salignus*. The γ-sitosterol was reported as an active principle of acetone extract of stem bark of *Vitex schliebenii* Moldenke (Lamiaceae) against 3rd and 4th instar larvae of *Anopheles gambiae* Giles (Diptera: Culicidae) (Nyamoita et al. 2013), and this phytosterol also was a principal component of *n*-hexane extracts of *S. salignus* and *S. microphylla*. The β-amyrin and lupeol isolated from *Inula japonica* (Asteraceae) were determined to have acaricidal activity against *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) by Duan et al. (2011). In this study, these compounds were also present in *S. salignus* extract. Therefore, it is possible that the presence of palmitic acid, γ-sitosterol, β-amyrin, and lupeol in *S. salignus n*-hexane extract and that of palmitic acid, oleic acid, and γ-sitosterol in *S. microphylla n*-hexane extract are responsible for insecticidal and juvenomimetic activities in the present study. These extracts could provide a botanical source of insecticides for alternative pest management of *S. frugiperda*.

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