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Antioxidant and insect growth regulatory activities of stilbenes and extracts from *Yucca periculosa*☆

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

The methanol extract from the bark of *Yucca periculosa* F. Baker afforded 4,4′-dihydroxstilbene, resveratrol and 3,3′,5,5′-tetrahydroxy-4-methoxystilbene and had growth regulatory activity against the Fall Army worm (*Spodoptera frugiperda* J.E. Smith, Lepidoptera:Noctuidae) an insect pest of corn. The most active compound was 3,3′,5,5′-tetrahydroxy-4-methoxystilbene which had significant effects at 3 μg/g in diets. In addition to the inhibitory activity on bleaching of crocin induced by alkoxyl radicals, these compounds also demonstrated scavenging properties toward 2,2-diphenyl-1-picrylhydrazyl in TLC autographic and spectrophotometric assays. Our results indicate that these compounds could be involved in interference of sclerotization and moulting. These compounds appear to have selective effects on the pre-emergence metabolism of the insect. The results were fully comparable to known natural insect growth inhibitors such as gedunin and *Cedrela* extracts and have had a possible role as natural insecticidal agents.

Keywords: Stilbenes; Insect-growth-regulation; Insect pest; IPM; *Spodoptera frugiperda*; Fall armyworm; *Yucca periculosa*; Agavaceae

1. Introduction

Yucca species (Agavaceae) are widely distributed in arid lands, but only a few are medicinally or agronomically important. *Yucca periculosa* F. Baker, known commonly as ''palmitos'' or ''izote'' is a tree, endemic to Mexico, that grows in the semi-arid regions of this Tehuacan–Cuicatlan Valley, Puebla–Oaxaca States. The area is especially rich in ''Izotal'', which is an endemic vegetation dominated by *Y. periculosa*, that also includes pine forest, oak-pine forest, chaparral, tropical deciduous forest and several associations of column cacti. The commercial cultivation of *Y. periculosa* is limited due to its exploitation by native people as a wood source, as well as for handicrafts (leaves), and food (flowers). In the wild, these plants survive under different environmental stress conditions (Casas, et al., 2001), with a lifespan of up to 100 years or so. The only insect (*Digonogastra spp*.) found on this plant is a specialist of the fruits of *Yucca spp*., a solitary ectoparasitoid of larvae (Althoff, 2001).

In the present study, stilbenes were isolated from bark of *Y. periculosa*. These compounds are known antioxidants, whose occurrence in plants has been reported (Christensen et al., 1988; Cuendet et al., 2000; Pacher et al., 2002; Su et al., 2002) and mainly in grapes and wines (Burns et al., 2002). These substances have been shown to have some important biological activities (Williams et al., 1996), as antifungal (Schultz et al., 1992) and as antimicrobial inhibitors (Chan, 2002; Docherty et al., 2001). In addition, they possess COX-1 and COX-2 inhibitory effects (Su et al., 2002), as well as affecting

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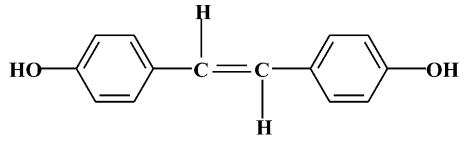
lipid peroxidation (Stivala et al., 2001), LDL oxidation (Frankel et al., 1993), arachidonate metabolism (Kimura et al., 1985), root growth (Arichi et al., 1982), antioxidant and vasodilation capacities (Burns et al., 2000); they can also function as phytoalexins (Siemann and Creasey, 1992) and tyrosinase inhibitors (Shimizu et al., 2000).



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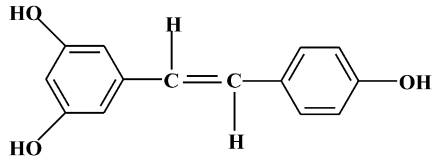

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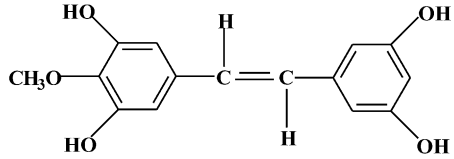

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The stilbenes play important roles in plants, especially in heartwood protection as part of both constitutive and inducible defense mechanisms, and in dormancy and growth inhibition. Certain stilbenoids, besides being toxic to insects and other organisms, have mammalian antifeedant and nematicidal properties (Croteau et al., 2000; Gorham et al., 1995; Schroder, 1999)

Some investigations on sites and mechanism of insecticidal or IGR action report that different phenolic compounds are enzyme and metabolism inhibitors (Calderón et al., 2001; Kubo et al., 2000; Kubo, 2000; Kubo and Kinst-Hori, 1999; Hammond and Kubo, 1999; Panzuto et al., 2002; Shimizu et al., 2000). In addition, these broactin compounds are ubiquitous in angiosperms and have antifeedant effects on phytophagous insects as well (Feeny, 1968; Feeny, 1976; Rhoades and Cates, 1976; Swain, 1979). It is also known that phenols can bind to proteins, acting as nutritional protein precipitating agents, thus reducing their digestibility (Feeny, 1976; Rhoades, 1979).

We have previously demonstrated that diverse secondary metabolites have different sites of action and different molecular targets when they interact with enzymes involved in metamorphosis (Calderón et al., 2001; Céspedes et al., 2000; 2001).

Our field observations indicate that this species has strong resistance to insect attack. The aim of this work was thus to correlate phytochemical composition with the inhibition of growth and development of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), as a model insect pest.

The present paper reports the effects of the MeOH bark extract, as well as its isolates: stilbene **1** (4, 4′-dihydroxy stilbene), stilbene **2** (resveratrol), and stilbene **3** (3,5,3′,5′-tetrahydroxy-4-methoxy-stilbene), on the Fall armyworm (*Spodoptera frugiperda* (J.E. Smith), Lepidoptera: Noctuidae). Our data indicate that it is possible to correlate some antioxidant activities (i.e. crocin, DPPH) with insect growth and development; these parameters also are being accepted as indirect measures of other different physiological processes (Camps, 1988; Macías et al., 2000; Galindo et al., 1999). Aspects such as insecticidal and growth regulatory activities, rate of development, time of pupation, adult emergence and deformity were evaluated and compared with those of gedunin and *Cedrela* MeOH extract, a known growth inhibitor of *S. frugiperda* (Calderón et al., 2001; Céspedes et al., 2000).

2. Results and discussion

In our screening program for biological activities of Mexican plants from arid regions, the extracts of *Y. periculosa* displayed insecticidal activity in a preliminary trial. Based on this information we carried out several studies of bark of *Y. periculosa* as follows:

The MeOH extract of bark of *Y. periculosa* (Me-Yuc) gave, together with the previously reported 4,4′-dihydroxystilbene **1** and resveratrol **2**, an additional methoxy stilbene **3**, that was identified as 3,3′,5,5′-tetrahydroxy-4-methoxy-stilbene (Fig. 1). ^{1}H and ^{13}C NMR resonances are in accordance with previously reported data (Christensen et al., 1988; Cuendet et al., 2000; Su et al., 2002; Oleszek et al., 2001; Schultz et al., 1992; Shimizu et al., 2000).

2.1. Insecticidal activity against larvae

The insecticidal effects of **1**–**3**, gedunin, Me-Yuc and MeOH-Cedrela (Me-Ced) extracts against larvae of first instars of *Spodoptera frugiperda* are summarised in Table 1. Compounds **2** and **3**, as well as the Me-Yuc and Me-Ced extracts, at 10 ppm concentration, produced significant larval mortalities (> 60%), whereas **3** produced higher mortality (95%) at the same concentration (Tables 1 and 2). On the other hand, **3** and the MeOH extract, had the highest insecticidal activities producing 100% of larval mortality at >25.0 ppm. It is

noteworthy that, when larvae were fed with a diet containing 25 ppm of **3** all larvae died (Table 3). The 50% lethal concentration (LC_{50}) of larvae at 7 days for these compounds and extracts are outlined in Table 2. It is important to point out that **3** and the Me-Yuc extract were more active than gedunin and the Me-Ced extract used as positive controls, with LC_{50} value of 5.4 ppm and 7.18 ppm, respectively.

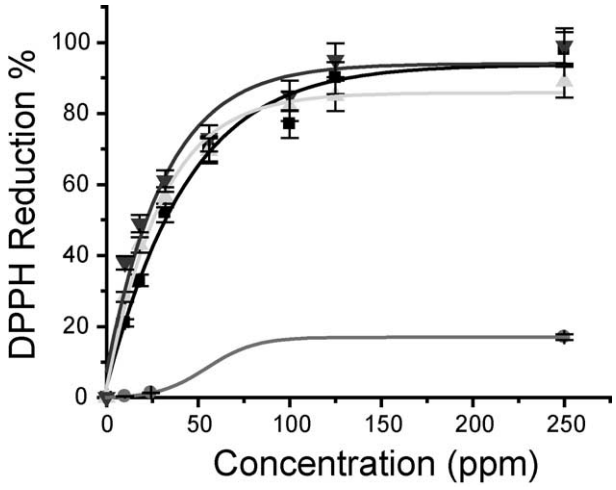

Fig. 1. Scavenging activity of Me-Yuc (■), 1 (•), 2 (▲), 3 (▼) on radical reduction of DPPH. Measurements at 517 nm, determination after 30 min.

2.2. Insect growth inhibitory activity

The compounds **1**, **2**, **3**, gedunin, and the Me-Yuc and Me-Ced extracts inhibited each larval stage, i.e. growth when incorporated into diets at 25 ppm (up 80% of length). On the other hand **3**, Me-Yuc and gedunin produced total inhibition (100% of weight gain) above 25 and 50 ppm, respectively (see Tables 4 and 3). However, **1** clearly showed lower larval inhibition than **2**, **3**, gedunin, Me-Yuc and Me-Ced extracts at high concentration (25 ppm) (Table 3). At 21 days, this growth reduction was clearly significant between 7 and 10 ppm ($P < 0.05$). However, only **3** and Me-Yuc showed the highest larval growth inhibition at the same concentrations (Table 3).

The percentage of larvae that reached pupation decreased in all tested compounds (**1**–**3**, gedunin and Me-Yuc and Me-Ced extracts) in comparison to control. Thus, **2**, **3**, gedunin, Me-Yuc and Me-Ced extracts showed significant delay of pupation by 37.5, 45.8, 41.7, 37.5 and 33.3% at 25, 10, 25, 25 and 25 ppm, respectively. The most important effect was observed with **3**

Table 1
Growth inhibitory effects of Me-Yuc, Me-Ced extracts, compounds **1**, **2**, **3** and gedunin on Fall Armyworm growth bioassay[a]

Treatment	µg/ml (ppm)	Mean weight gained (mg)[b]	% of weight[c]	Mean length (cm)[d]	% of length[c]	Mortality%
Control		101.9±7.1a	100	1.20±0.045	100	5.6
1	1.0	91.7±2.7a	90	1.10±0.051	89	8
	3.0	86.6±2.1b	85	1.03±0.049	86	15
	7.0	72.3±1.5b	71	0.85±0.047	71	21
	10.0	63.1±1.1b	62	0.78±0.051	65	32
2	1.0	90.7±3.9a	89	1.09±0.049	91	11
	3.0	76.4±3.1b	75	0.99±0.052	83	21
	7.0	63.2±2.8b	62	0.80±0.039	67	58
	10.0	41.7±1.7b	41	0.54±0.028	45	60
3	1.0	89.6±2.7a	88	1.03±0.051	86	14
	3.0	71.3±2.1b	70	0.97±0.040	81	22
	7.0	57.1±1.1b	56	0.61±0.033	51	67
	10.0	31.6±0.9c	31	0.38±0.015	32	70
	25.0	15.3±0.7c	15	0.13±0.009	11	90
Me-Yuc	10.0	67.2±1.9a	66	0.73±0.051	61	63
	15.0	49.9±1.8b	49	0.56±0.044	47	71
	25.0	28.5±1.1c	28	0.26±0.036	22	81
	50.0	11.2±0.9c	11	0.11±0.005	9	98
Gedunin	10.0	7.2±0.4c	7.1	0.39±0.019	32.5	33
	25.0	3.40±0.2c	3.3	0.24±0.012	20.0	38
	50.0	1.90±0.1c	1.9	0.20±0.010	16.7	70.8
Me-Ced	2.0	60.8±3.8a	59.7	0.93±0.046	77.5	45.5
	10.0	24.3±1.5b	23.8	0.65±0.032	54.2	51.8
	25.0	8.9±0.6c	8.7	0.46±0.023	38.3	78.9
	50.0	6.5±0.4c	6.4	0.40±0.020	33.3	98.6

[a] after 7 days of incubation, mean of three replicates.
[b] Means followed by the same letter within a column are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). Means are±standard error. 95% Confidence limits.
[c] percentage with respect to control.
[d] Mean length total increase from *eclosion*.

Table 2
Insect growth regulatory activities of the compounds **1**–**3** and MeOH extract from *Y. periculosa* and MeOH-Ced and gedunin against *S. frugiperda* larvae in a no-choice bioassay[a]

Treatment	7 days			21 days			
	GWI_{50}[b]	GLI_{50}[c]	LC_{50}[d]	GI_{50}[b]	EI_{50}[g]	pI_{50}[e]	PI_{50}[f]
1	26.54	32.74	27.6	9.24	N. D.	0.96	38.26
2	8.75	11.6	6.4	5.94	12.29	0.77	7.19
3	6.97	7.24	5.4	3.45	4.83	0.54	9.02
Me-Yuc	14.99	13.22	7.18	5.13	5.79	0.71	18.82
Gedunin	2.87	5.53	30.08	1.90	3.95	0.27	14.01
Me-Ced	3.56	10.87	8.22	14.17	1.43	1.15	3.84

[a] The parameters in ppm values.
[b] The GWI_{50} and GI_{50} correspond to the growth inhibition in weight at 7 and 21 days, respectively, and was calculated as the dose corresponding to midpoint between complete inhibition (100% of control) and no effect by PROBIT analysis ($P<0.05$).
[c] GLI_{50} correspond to the growth inhibition in length at 7 days, and was calculated as the dose corresponding to midpoint between complete inhibition (100% of control) and no effect by PROBIT analysis ($P<0.05$).
[d] LC_{50} is the lethal concentration producing 50% mortality in "no choice" test calculated by PROBIT analysis ($P<0.05$).
[e] pI_{50} correspond to log GI_{50}.
[f] PI_{50} correspond to concentration producing 50% of pupation, and was calculated as the dose corresponding to midpoint between complete inhibition (100% of control) and no effect by PROBIT analysis ($P<0.05$).
[g] EI_{50} correspond to concentration producing 50% of emergence and was calculated as the dose corresponding to midpoint between complete inhibition (100% of control) and no effect by PROBIT analysis ($P<0.05$).

Table 3
Activity of Me-Yuc, Me-Ced extracts, compounds **1**, **2**, **3** and gedunin on pupation and emergences parameters of Fall Armyworm (after 21 days of incubation)[a]

Treatment	Conc (ppm)	Mean time pupation (days)	Pupation SP [%][c,d]	Mean emergence (days)	Emergence (%)[e]
Control		22.0	91.7	30	100
1	1.0	22.0	87.5a	30	89
	3.0	22.0	87.5a	30	89
	7.0	22.5[b]	62.5	31	80
	10.0	22.5[b]	58.3	30	80
2	1.0	22.0	79.2a	30	87
	3.0	22.0	79.2a	31	84
	7.0	22.5[b]	41.7b	31	82
	10.0	22.5[b]	41.7b	31	74
3	1.0	22.0	91.2	31	75
	3.0	22.5[b]	79.2	32	58
	7.0	23.5[b]	70.8	32	41
	10.0	25.0[b]	45.8	35	27
	25.0	0	0	–	–
Me-Yuc	10.0	23.5	83.2	30	20
	15.0	24.0[b]	62.5	35	20
	25.0	25.0[b]	37.5	35	18
	50.0	0	0	–	–
Gedunin	10.0	24.0[b]	54.2	32	15
	25.0	24.5[b]	41.7	33	13
	50.0	25.0[b]	25.0	–	–
Me-Ced.	2.0	23.5	54.2	31	15
	10.0	24.5[b]	41.7	32	13
	25.0	25.0[b]	33.3	36	8
	50.0	25.0[b]	25.0	–	–

[a] Mean of three experiments.
[b] Means within a column are significantly different from control in a Kruskal–Wallis chi-squared approximation test at $P<0.005$.
[c] Means followed by the same letter within a column after±standard error values are not significantly different in a Student–Newman–Keuls (SNK) test at $P<0.05$ (treatments are compared by concentration to control), 95% Confidence limits.
[d] SP: Survival Pupation = Number of survival pupae × 100 / Total larvae for pupation.
[e] Emergence (%) = Number of adults emerged × 100 / Total number of pupae.

Table 4
Fall armyworm bioassay results from Me-Yuc, Me-Ced extracts, compounds **1**, **2**, **3** and gedunin on growth[a]

Treatment	Concentration (ppm)	Mean weight gained (mg)[b]	%[c]	Mean length gained (mm)	%[c]
Control		481.5±19.92a	100	43.8 1.68	100
1	1.0	454.1±20.51a	94.3	35.1 1.01	80.1
	3.0	379.1±13.96a	78.7	23.1 0.98	52.7
	7.0	297.3±11.80a	61.7	19.0 0.71	43.4
	10.0	214.9±6.90b	44.6	17.3 0.60	39.5
2	1.0	410.1±20.51a	85.2	38.9 1.43	88.8
	3.0	329.1±13.96a	68.3	36.1 1.31	82.4
	7.0	236.6±8.83a	49.1	27.8 1.22	63.5
	10.0	110.2±5.51b	22.9	15.71 0.99	35.9
3	1.0	333.8±11.30a	69.3	29.5 1.58	67.4
	3.0	316.5±15.82a	65.7	22.1 1.11	50.1
	7.0	123.2±6.16a, b	25.6	11.8 0.81	26.9
	10.0	7.41±0.370c	1.54	10.1 0.41	23.1
	25.0	4.01±0.201c	0.83	4.9 0.28	11.2
Me-Yuc	10.0	111.0±5.55a, b	23.05	15.4 0.66	35.2
	15.0	45.0±2.25b	9.35	8.1 0.41	18.5
	25.0	23.0±1.15b	4.77	3.0 0.19	6.8
	50.0	0.0	0	0	0
Gedunin	10.0	9.86±0.55c	2.05	5.1 0.22	11.6
	25.0	6.50±0.19c	1.35	3.7 0.18	8.4
	50.0	3.81±0.11c	0.79	2.9 0.11	6.6
Me-Ced	2.0	421.1±22.50a	87.45	31.1 1.20	71.0
	10.0	289.1±14.90a	60.04	22.9 1.11	52.3
	25.0	166.6±7.83a	34.60	15.9 0.98	36.3
	50.0	101.2±4.51b	21.01	12.1 0.67	27.6

[a] Values taken at 21 days larval growth, mean of three replicates.
[b] Means followed by the same letter within a column after±standard error values are not significantly different in a Student-Newman-Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control), 95% Confidence limits.
[c] Percentage with respect to control.

and Me-Yuc extract at 25 and 50 ppm, which reduced survival pupation to 0.0% in both cases, respectively. Significant delays in time to pupation (25 days) were observed at 10, 25, 50 and 25 ppm for **3**, Me-Yuc, gedunin and Me-Ced extract, respectively. Furthermore, gedunin and the Me-Ced extract, significantly reduced pupae weights at 25 ppm, respectively (data not shown), while the Me-Yuc extract showed the greatest effect on pupae weights at 10 ppm (data not shown), in accordance with previously reported data (Calderón et al., 2001; Céspedes et al., 2000).

Percentage of emergence, as compared to the pupal stage, showed further reductions with compounds **2**, **3**, Me-Yuc, gedunin, and Me-Ced extract at 25, 10, 10, 25, and 25 ppm with 0.0, 27.0, 18.0, 13.0 and 8.0% of emergence, respectively (Table 3). However, **2**, **3**, Me-Yuc, gedunin and Me-Ced extract drastically reduced the percentage of adult emergence to 0% at 25, 25, 50, 50 and 50 ppm, respectively. These facts could be correlated with EI_{50} and pEI_{50} values, i.e. parameters that showed a strong growth inhibition of compounds **2**, **3**, Me-Yuc, gedunin and Me-Ced with pEI_{50} of 0.77, 0.54, 0.71, 0.27 and 1.15 values, respectively, underlining the potency of **3**, Me-Yuc and gedunin, respectively (Table 2).

2.3. Growth inhibition (GI) and regulatory growth index (RGI)

In many of the treatments, the mean adult weight was significantly delayed in the average time to reach the adult stage relative to the control larvae. The GI and RGI values clearly showed (Table 5) that 3 and the Me-Yuc extract, with RGI values of 0.25 and 0.45 at 10 and 15 ppm, had the strongest effect. Gedunin also showed a pronounced effect with RGI values of 0.51 and 0.10 at 25 and 50 ppm, respectively. These parameters, together with the LD_{50} values (Table 6), corroborated the highest effect with the methoxy-stilbene **3**, since it caused the greatest inhibition of growth (98.46 and 69.0%) in weight at 7 and 21 days, at 10 ppm, respectively (Tables 1 and 4). On the other hand, this compound showed an inhibitory effect in length at 10 ppm (68.0 and 76.9%), at 7 and 21

Table 5
GI and RGI of S. *frugiperda* as a function of increased concentrations of **1**–**3** and MeOH extract from *Y. periculosa*, gedunin and MeOH extract from *Cedrela ciliolata*[a]

Compounds	Concentration (ppm)	GI[b]	RGI[c]
Control		0.99±0.045[a]	
1	1.0	0.99±0.050b	1.00
	3.0	0.99±0.050b	1.00
	7.0	0.84±0.085b	0.85
	10.0	0.83±0.055b	0.84
	25.0	0.75±0.031b	0.75
2	1.0	0.99±0.050b	1.00
	3.0	0.99±0.050b	1.00
	7.0	0.94±0.040b	0.95
	10.0	0.92±0.046b	0.93
	25.0	0.89±0.044b	0.90
3	1.0	0.75±0.031b	0.75
	3.0	0.59±0.040b	0.60
	7.0	0.39±0.065b	0.40
	10.0	0.25±0.035c	0.25
	25.0	0.15±0.038c	0.20
Me-Yuc	10.0	0.68±0.050b	0.68
	15.0	0.45±0.039b	0.45
	25.0	0.0	
Me-Ced	2.0	0.99±0.050b	1.00
	10.0	0.69±0.055b	0.70
	25.0	0.59±0.040b	0.60
	50.0	0.39±0.065b	0.40
Gedunin	10.0	0.77±0.060b	0.77
	25.0	0.51±0.040b	0.51
	50.0	0.10±0.010c	0.10

[a] Mean of three replicates.
[b] Means followed by the same letter within a column after±standard error values are not significantly different in a Student–Newman–Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control), 95% Confidence limits.
[c] $RGI_{treatment} = GI_{treated} / GI_{control}$.

Table 6
Acute toxicity of compounds **1**–**3**, MeOH extracts against larval of last stage of S. *frugiperda*.[a]

Compounds	Concentration (ppm)	% Survival[b]	LD_{50}[c]
Control	0.0	100.0	
1	1.0	98.1±4.75b	38.0
	3.0	95.0±3.90b	
	7.0	78.9±3.51b	
	10.0	66.3±3.11c	
	25.0	59.3±2.96c	
2	1.0	99.5±4.90b	24.1
	3.0	90.2±4.55b	
	7.0	81.4±4.25b	
	10.0	77.1±3.85b	
	25.0	48.2±2.8	
3	1.0	85.5±3.91b	10.1
	3.0	80.0±3.10b	
	7.0	74.3±2.82b	
	10.0	45.1±2.4	
	25.0	27.9±1.9	
Me-Yuc	10.0	38.4±5.1	8.0
	15.0	14.1±3.0	
	25.0	5.0±1.8	
Me-Ced	2.0	93.9±4.69b	n. d.
	10.0	78.9±3.95b	
	25.0	70.2±3.51c	
	50.0	69.1±3.45	
Gedunin	10.0	54.7±2.73b	10.78
	25.0	14.1±0.71c	
	50.0	0	

[a] After 24 h, survival of adults was recorded (percent relative to controls).
[b] Mean of three replicates. Means followed by the same letter within a column after±standard error values are not significantly different in a Student–Newman–Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control), 95% Confidence limits.
[c] The LD_{50} is the lethal dose producing 50% survival.

days, respectively (Tables 1 and 4). In addition, this compound, at 25 and 50 ppm, was noteworthy insecticidal with 100% mortality, respectively (data not shown).

It is important to note that similar insect growth regulatory activity on *S. litura* (Common cutworm) was studied by Morimoto et al. (2000). These authors reported that the flavonoids, 5-hydroxy-3, 6, 7, 8, 4′-pentamethoxyflavone; 5-hydroxy-3, 6, 7, 8-tetramethoxyflavone; 5, 6-dihydroxy-3, 7-dimethoxyflavone and 4, 4′, 6′-trihydroxy-2′-methoxychalcone are insect antifeedant flavonoids against the common cutworm (*Spodoptera litura*), these compounds being detected in small amounts in *Gnaphalium affine* (Asteraceae) with ED_{50} values between 1.1×10^{-7} and 2.5×10^{-8} mol/cm^2. These values are, however, not comparable with our bioassay. Nevertheless, it is possible to infer that the substitution pattern of flavones induce an increase in the activity of those flavones (phenolic compounds). There are no reports of insecticidal stilbenes, but only those having tyrosinase inhibitory (Shimizu et al., 2000), and antifeedant activities of such phenolic compounds (Kubo et al., 2000; Kubo, 2000; Kubo and Kinst-Hori, 1999). The presence of hydroxyl, methoxyl and furan moieties, in similar forms to cathecol, seems to be necessary for insecticidal activity as in limonoids containing this chemical group (Céspedes et al., 2000; 2001; Calderón et al., 2001; Nakatani et al., 1994) and in other phenolic compounds as alkanols and tannic acid, respectively (Hammond and Kubo, 1999; Panzuto et al., 2002).

2.4. Acute toxicity on larvae of last stage of S. frugiperda and antioxidant activity

Stilbenes **1**–**3** showed moderate acute toxicity with a range 45–77% of survival at 10 ppm, respectively (Table 6). However, **3**, gedunin and Me-Yuc extract showed a potent acute toxicity of 27.9, 14.1, and 5.0% of survival on larvae at the last stage of *S. frugiperda* at 25 ppm, respectively. The LD_{50} values of **2**, **3**, Me-Yuc

and gedunin were 24.1, 10.1, 8.0, and 10.8 ppm, respectively. In order to establish correlation between insect growth regulatory (IGR) and acute toxicity activities with the antioxidant properties of these phenolic compounds, crocin and DPPH radical scavenging test of these stilbenes were carried out.

In addition, in these stilbenes the presence or absence of a methoxyl group increases or decreases, respectively the strength of these compounds upon inhibition of DPPH (Fig. 1, Table 7). We suggest that insect growth inhibitory activity of Me-Yuc extract could be caused not by one strong inhibitor, but by a synergistic effect of the stilbene composition. Inhibition of DPPH activity by stilbenes has been reported on related bioassays (Rimando et al., 2002; Burns et al., 2002). Therefore, the plant stilbenes may be considered as efficient IGR and radical scavengers (Kim et al., 2002; Rimando et al., 2002; Stilava et al., 2001). It is worth mentioning that the strong antioxidant caffeic acid, a phenolic acid with two hydroxyl groups, was used as a reference compound together with α-tocopherol, where is commonly used as an antioxidant in food (Fig. 2).

The antioxidant activity of these compounds was also evaluated spectrophotometrically on the bleaching of the H_2O-soluble crocin (Bors et al., 1984). Alkoxyl radicals were generated from *t*-BuOOH by UV photolysis of aqueous solutions containing 10 μM crocin and 1 mM *t*-BuOOH. *t*-BuOH (0.5 M) was added to scavenge the hydroxy radicals produced. Gallic acid was also added as reference compound. Compounds **1**, **2**, **3**, and Me-Yuc extract were all active, with activities comparable to gallic acid (Fig. 3).

Resveratrol **2** and the methoxylated stilbene **3** had more potent insecticidal inhibitory activity. It is obvious that the nature of the substituent at C-3 plays an important role for the insecticidal activity of this compound. The most active compound (**3**) contained a small and relatively lipophilic group at C-3, whereas compound **2** with two hydrophilic hydroxyl groups exhibited minor activity. These results confirm previous findings on the quantitative structure activity relationship of stilbene derivatives, namely that the inhibitory tyrosinase activity of the respective natural product depends on the polarity of ring A and on the size of the substituent (Shimizu et al., 2000). The stilbene **3**, with the presence of a methoxyl group in the A ring, seems to be the cause of growth inhibitory activity with a 1.54% of weight gained at 10 ppm and 23.1% of length gained at 10 ppm, respectively (Table 4).

These facts show that acute toxicity and growth inhibition observed may be due to the inhibition of tyrosinase, and this target was demonstrated also for other

Table 7
Effect of natural compounds **1**–**3**, and extracts on DPPH reduction[a], I_{50}[b] values[e]

Compound	I_{50}
Tocopherol	11.86
Caffeic acid	2.13
1	n.d.[c]
2	25.27
3	22.22
MeOH-Yuc	33.53
MeOH-Ced	n.t.[d]
Gedunin	n.t.

[a] Means of three experiments.
[b] Concentration that produce 50% of scavenging radicals from DPPH.
[c] Not determined.
[d] Not tested.
[e] Values in ppm.

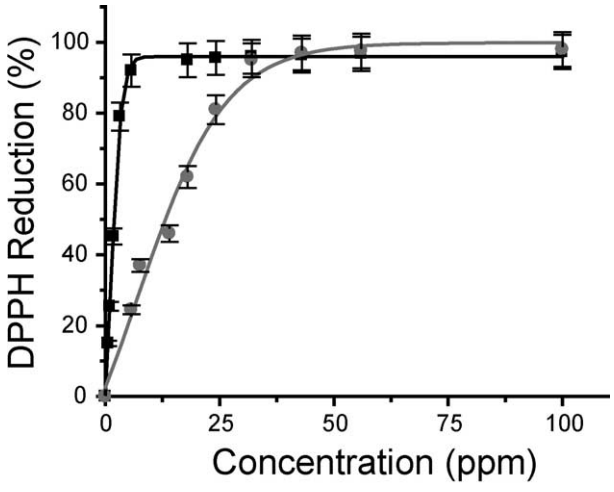

Fig. 2. Scavenging activity of pattern compounds caffeic acid (■), tocopherol (●) on radical reduction of DPPH. Measurements at 517 nm, determination after 30 min.

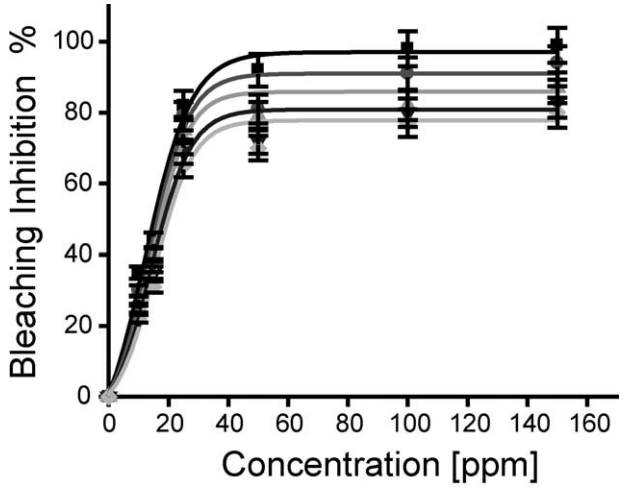

Fig. 3. Inhibitory activity of compounds **2**, **3**, Me-Yuc, Gallic acid, and caffeic acid on the bleaching of crocin measurement at 440 nm of fluorimetric emission, determination after 20 min: Caffeic acid (■), Me-Yuc extract (●), 3 (▲), gallic acid (▼), 2 (◆). Values of compounds and extract in ppm.

stilbenes from natural sources (Kim et al., 2002; Shimizu et al., 2000; Gilly et al., 2001). In summary, the insecticidal activity of the Me-Yuc extract from bark parts of *Y. periculosa* may be due to synergistic effects shown by the phenolic components of the mixture in the test system used in this investigation. These facts are indicative of the potency of the methanol extract from *Y. periculosa*.

Thus, the effect of the compounds **2**, **3** and MeOH extract on reducing insect growth, increasing development time and mortality of *S. frugiperda* is similar to that of gedunin and more potent than the MeOH extract from *Cedrela salvadorensis* (Calderón et al., 2001; Céspedes et al., 2000). The mode of action of these compounds is being investigated and may be due to a combination of antifeedant action as midgut phenoloxidase inhibition and moulting sclerotization toxicity, as found for other phenolics (Kubo et al., 2000; Kubo, 2000; Kubo and Kinst-Hori, 1999) and extracts (Feng et al., 1995). In addition, the presence of an orcinol group seems to be important for these activities as showed for the most potent compounds **2** and **3** in this study. Furthermore, a great percentage of larvae that reached pupation decreased with the application of **2** and **3** in comparison to control, which might be due to the inhibition by tyrosinase as well. The sites and mode of action of this extract and its compounds is being investigated.

The activity of this desert plant, their metabolites and MeOH extracts is comparable to the insect growth regulator gedunin, which suggests potential for further development of these materials.

3. Experimental

3.1. Plant material

Bark from *Y. periculosa* was collected from the ''Helia Bravo Hollis'' Ecological Reserve in Zapotitlan de Las Salinas, Tehuacan–Cuicatlan Valley, Puebla–Oaxaca States, Mexico, November 2001. Voucher specimens can be found at the ethnobotanical collection of the herbarium IZTA-UNAM (key: IZTA 27516), Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonoma de Mexico, Mexico D.F., Mexico.

3.2. Chemicals and solvents

All reagents used were either analytical or chromatographic grade. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), α-tocopherol, caffeic acid, gallic acid, ethylenediaminetetraacetic acid (EDTA), bovine serum albumin, Percoll, quercetin, saffron, sorbitol, tricine and trizma-hydrochloride were purchased from Sigma-Aldrich Química, S.A. de C.V., Toluca, Mexico. MeOH, CH_2Cl_2, $CHCl_3$, NaCl, KCl, NaOH, KOH, *tert*-butanol, *tert*-butyl hydroperoxide, $CuSO_4$, NH_4Cl, $MgCl_2$, acetic anhydride, silica gel GF_{254} analytical chromatoplates, silica gel grade 60, (70-230, 60A°) for cc, *n*-hexane, and ethyl acetate were purchased from Merck-Mexico, S.A., Mexico.

3.3. Apparatus

^{1}H NMR ^{13}C NMR spectra were recorded and 300 MHz, 75 MHz respectively, on Varian VXR-300S spectrometer, will chemical shifts (ppm) being referenced to $(CH_3)_4Si$. $CDCl_3$, MeOD and acetone-d_6 from Aldrich Chemical Co. were used as solvents, with coupling constants being quoted in Hz. IR spectra were obtained in KBr on a Perkin Elmer 283-B and a FT-IR Nicolet Magna 750 spectrophotometers, whereas UV spectra were determined on a Shimadzu UV-160. The Spectronic model Genesys 5 spectrophotometer was also used for measuring biological activities. Optical rotations were measured on a JASCO DIP-360 spectropolarimeter, whereas melting points were obtained on a Fisher-Johns hot-plate apparatus and remain uncorrected. Fluorimetric measurements were determined with TURNER Barnstead-Thermolyne, model Quantech S5 Fluorometer, with 420, 440, 470, 550, and 650 Turner filters.

3.4. General experimental procedures

The plant material (leaves, bark and flowers) were dried and milled. These samples were extracted with hexane, dichloromethane, and methanol for bioassays evaluation. The most active extract of each of the samples was the MeOH bark extract, which was tested for insecticidal activity and then submitted to cc using SiO_2 (G 60, Merck) as solid phase. Elution, carried out with $CHCl_3$:methanol mixtures, afforded the active fractions, which were analyzed by TLC and antioxidant bioautographic assay (Céspedes et al., 2002) using different solvent systems (*n*-hexane:ethyl acetate and DCM:MeOH mixtures). Repeated TLC of these fractions led to the isolation of the secondary metabolites, which were purified by prep-TLC. Identical compounds were collected and identified by TLC with authentic samples. Stilbene **1** (70 mg), stilbene **2** (100 mg) and stilbene **3** (250 mg) were obtained as pure natural products, were was analyzed and characterized by their R_f, IR, UV, ^{1}H NMR and ^{13}C NMR spectroscopy data.

Identification of stilbenes **1**–**3** was made by both spectroscopic analyses and direct comparison with authentic samples (Christensen et al., 1988; Cuendet et al., 2000; Pacher et al., 2002; Su et al., 2002), only **1**, **2**, and **3** being purified in sufficient amount for use bioassays. Analytical TLC was performed on Silica gel 60 F_{254} E. Merck plates with compounds a interest visualized by spraying with a 10% solution of H_2SO_4, followed by heating at 110 °C.

3.5. Bioassays with fall armyworm

Larvae used for the experiments were obtained from the culture at the Centro de Investigación en Biotecnología at the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México, maintained under previously described conditions, (Céspedes et al., 2000). An artificial diet containing sterile water (800 ml), agar (10 g), soy meal (50 g), corn meal (96 g), yeast extract (40 g), wheat germ (4 g), sorbic acid (2 g), choline chloride (2 g), ascorbic acid (4 g), *p*-hydroxybenzoic acid methyl ester (2.5 g), Wesson salt mixture (7 ml), Vanderzant vitamin mixture for insects (15 ml), formaldehyde (2.5 ml), streptomycin (0.1 unit), aureomycin (5 g), and milled ear (20 g) of corn grain (for 1 kg of diet) were used for the bioassays, thus being prepared using the procedure described earlier (Mihm, 1987). 24-Well polystyrene multidishes were filled with the liquid diet, and then left for 20 min at room temp under sterile conditions. The 3.4 ml wells measure 17 mm in depth×15 mm in diameter with a 1.9 cm^2 culture area. All test compounds were dissolved in 95% of ethanol and layered on top of each well with the artificial diet using up to six concentrations and a control (1 ml 95% ethanol) allowing evaporation of solvent. In addition was used 1.0 and 3.5 ppm of the hexane and MeOH extracts, since these extracts showed the highest inhibitory activity in the preliminary bioassay (data not shown). For each concentration used and for the control, a single *S. frugiperda* neonate first instar larva was placed on the diet mixture in each well for 7 days, thus each one experiment contains 72 larvae in total (each plate of 24 well with three replicates). After 7 days, surviving larvae were measured and weighed and then transferred to separate vials containing fresh stock diet. Larval weight gains and mortality were recorded after 21 days of incubation, since pupation average is 23±1 days. Other lifecycle measurements were recorded, such as time to pupation, mortality of larvae and adult emergence and deformities. All treatments were carried out in a controlled environment chamber with an 18L:6D photoperiod, at 25 °C day and 19 °C night temp regime, and a relative humidity of 80%±5%. There were three replicate for each assay. Control assays (24-wells) contained the same numbers of larvae, volume of diet, and ethanol as the test solutions (Céspedes et al., 2000).

3.6. Acute toxicity on Spodoptera frugiperda

Acute toxicity was determined by topical application to larvae of last stage of *S. frugiperda*. The larvae of *S. frugiperda* were iced to stop their movement and treated on their abdomens with each of the test compounds, at concentrations of 1, 3, 7, 10, 25 and 50 ppm. Additional concentrations (15 and 2 ppm) were used for Me-Yuc extract and Me-Ced extract, respectively (Table 6). The solvent used was 10.5 μl of acetone, with controls only using the solvent. After 24 h survival levels were recorded. Five larvae were used for each concentration, respectively. LD_{50} is the lethal dose producing 50% survival (Calderón et al., 2001).

3.7. Relative growth index and growth index

The relative growth index (RGI) and growth index (GI) were calculated according to Zhang et al., 1993.

3.8. Reduction of 2,2-diphenyl-1-picrylhydrazyl (=2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; DPPH) Radical

TLC autographic assay: after developing and drying, TLC plates were sprayed with a 0.2% DPPH solution in MeOH. The plates were examined 30 min after spraying. Active compounds appear as yellow spots against a purple background. In similar form, TLC plates were sprayed with 0.05% β-carotene solution in $CHCl_3$. The plates were examined under UV_{254} light until the background becomes discolored (bleached). Active compounds appeared as pale yellow spots against a white background. Spectrophotometric assay (Bors et al., 1992; Cuendet et al., 1997): A soln (50 ml) containing the compound to be tested were added to 5 ml of a 0.004% MeOH soln. of DPPH, with quercetin used as an internal standard reference. Absorbance at 517 nm was measured after 30 min, and the percent of activity was calculated.

3.9. Bleaching of crocin

Crocin was isolated from commercial saffron (SIGMA) by extraction with MeOH followed by HPLC (RP-18, MeOH/H_2O 1:1), and identified by comparison of its ^1H and ^{13}C NMR data literature values. The test was carried out according to Bors et al. (1992). Aq. solutions containing 10 μM crocin, 1 mM *t*-BuOOH, 0.5 M *t*-BuOH and various dilutions of the compounds to be tested were prepared. The solutions were placed under UV_{254} light. Following the decrease of absorbance monitored bleaching of crocin and fluorescence emission at 440 and 470 nm with time each 5 min (Céspedes et al., 2002).

3.10. Statistical analysis

Data shown in figures and tables are the mean results obtained with means of three replicates and independent seeds, crocin and DPPH preparations and are presented as mean±standard errors of the mean (SEM). Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM Procedures (SAS, 1982). The results are given in the text as probability values, with $P < 0.05$ adopted as

the criterion of significance, differences between treatment means were established with a Student–Newman–Keuls (SNK) test. The GI_{50}, RI_{50} and I_{50} values for each activity were calculated by PROBIT analysis on the basis of the percentage of inhibition obtained at each concentration of the samples. I_{50} is the concentration producing 50% inhibition. Complete statistical analysis was performed by means of the MicroCal Origin 5.0 statistical and graphs PC program.

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References

Althoff, D., 2001. http://www.cas.vanderbilt.edu/bio239/dmaweb/ymp.html.

Arichi, H., Kimura, Y., Okuda, H., Baba, M., Kozawa, M., Arichi, S., 1982. Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb. Et Zucc. on lipid metabolism. Chem. Pharm. Bull. 30, 1766–1770.

Bors, W., Saran, M., Eltsner, E.F., 1992. Modern Methods Plant Analysis. New Series, Acad. Press, N.Y.

Burns, J., Yokota, T., Ashihara, H., Lean, M.E.J., Crozier, A., 2002. Plant foods and herbal sources of resveratrol. J. Agric. Food Chem. 50, 3337–3340.

Casas, A., Valiente-Banuet, A., Viveros, J.L., Caballero, J., Cortés, L., Dávila, P., Lira, R., Rodríguez, I., 2001. Plant Resources of the Tehuacan–Cuicatlán Valley, México. Economic Botany 55 (1), 129–166.

Calderón, J.S., Céspedes, C.L., Rosas, R., Gómez-Garibay, F., Salazar, J.R., Lina, L., Aranda, E., Kubo, I., 2001. Acetylcholinesterase and insect growth inhibitory activities of *Gutierrezia microcephala* on fall armyworm *Spodoptera frugiperda* J. E. Smith. Z. Naturforschung 56c, 382–394.

Camps, F.M., 1988. Relaciones planta-insecto. Insecticidas de origen vegetal. In: Bellés, X. (Ed.), Insecticidas Bioracionales. CSIC, Madrid, pp. 69–86.

Céspedes, C.L., Calderón, J.S., Lina, L., Aranda, E., 2000. Growth inhibitory effects on fall armyworm *Spodoptera frugiperda* of some LIMONOIDS ISOLAted from *Cedrela* spp (Meliaceae). J. Agric. Food Chem. 48, 1903–1908.

Céspedes, C.L., Alarcón, J., Aranda, E., Becerra, J., Silva, M., 2001. Insect growth regulador and insecticidal activity of β-dihydroagarofurans from *Maytenus* spp. (Celastraceae). Z. Naturforsch 56c, 603–613.

Céspedes, C.L., Uchoa, A., Salazar, J.R., Perich, F., Pardo, F., 2002. Plant growth inhibitory activity of p-hydroxyacetophenones and tremetones from Chilean endemic Baccharis species and some analogous: a comparative study. J. Agric. Food Chem. 49, 4243–4251.

Chan, M.M., 2002. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem. Pharmacol. 63, 99–104.

Christensen, L.P., Lam, J., Sigsgaard, T., 1988. A novel stilbene from the wood of *Chlorophora excelsa*. Phytochemistry 27, 3014–3016.

Croteau, R., Kutchan, T.M., Lewis, N.G., 2000. Natural Products (secondary metabolites). In: Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp. 1250–1318.

Cuendet, M., Hostettmann, K., Potterat, O., Dyatmiko, W., 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helv. Chim. Acta 80, 1144–1152.

Cuendet, M., Potterat, O., Salvi, A., Testa, B., Hostettmann, K., 2000. A stilbene and dihydrochalcones with radical scavenging activities from *Loiseleuria procumbens*. Phytochemistry 54, 871–874.

Docherty, J.J., Fu, M.M., Tsai, M., 2001. Resveratrol selectively inhibits *Neisseria gonorrhoeae* and *Neisseria meningitides*. J. Antimicrob. Chemotherapy 47, 239–246.

Feeny, P.P., 1968. Effect of oak leaf tannins on larval growth of the winter moth Operophtera brumata. J. Insect Physiol 14, 805–817.

Feeny, P.P., 1976. Plant apparency and chemical defense. In: Wallace, J.W., Mansell, R.L. (Eds.), Biochemical Interactions between Plants and Insects. Plenum Press, New York, pp. 1–40.

Feng, R.Y., Chen, W.K., Isman, M.B., 1995. Synergism of malathion and inhibition of midgut esterase activities by an extract from *Melia toosendan* (Meliaceae). Pestic. Biochem. Physiol 53, 34–41.

Frankel, E.N., Waterhouse, A.L., Kinsella, J.E., 1993. Inhibition of human LDL oxidation by resveratrol. Lancet 341, 1004–1103.

Galindo, J.C., Hernandez, A., Dayan, F.E., Tellez, M.R., Macias, F.A., Paul, R.N., Duke, S.O., 1999. Dehydrozaluzanin C, a natural sesquiterpenolide, causes rapid plasma membrane leakage. Phytochemistry 52, 805–813.

Gilly, R., Mara, D., Oded, S., Zohar, K., 2001. Resveratrol and a novel tyrosinase in carignan grape juice. J. Agric. Food Chem. 49, 1479–1485.

Gorham, J., Tori, M., Asakawa, Y., 1995. The biochemistry of the stilbenoids. In: Harborne, J.B. (Ed.), Biochemistry of Natural Products Series. Chapman and Hall, London.

Hammond, D., Kubo, I., 1999. Structure-activity relationship of alkanols as mosquito larvicides with novel findings regarding their mode of action. Bioorganic and Medicinal Chemistry 7, 271–278.

Kim, Y.-M., Yun, J., Lee, Ch.-K., Lee, H., Min, K.R., 2002. Oxyresveratrol and hydroxystilbene compounds inhibitory effect on tyrosinase and mechanism of action. J. Biol. Chem. 277, 16340–16344.

Kimura, Y., Okuda, H., Arichi, S., 1985. Effects of stilbenes on arachidonate metabolism in leukocytes. Biochim. Biophys. Acta 834, 275–278.

Kubo, I., Kinst-Hori, I., Chauduri, S.K., Kubo, Y., Sánchez, Y., Ogura, T., 2000. Flavonols from *Heterotheca inuloides*: tyrosinase inhibitory activity and structural criteria. Bioorg. Med. Chem. 8, 1749–1755.

Kubo, I., Kinst-Hori, I., 1999. Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. J. Agric. Food. Chem. 47, 4121–4125.

Kubo, I., 2000. Tyrosinase inhibitors from plants. Rev. Latinoamer. Quim 28, 7–20.

Macias, F.A., Galindo, J.C., Molinillo, J.M., Castellano, D., 2000. Dehydrozaluzanin C: a potent plant growth regulator with potential use as a natural herbicide template. Phytochemistry 54, 165–171.

Mihm, J.A., 1987. Mass rearing stem borers, fall armyworms and corn earworms at CIMMYT. In: Toward Insect Resistant Maize for the Third World. Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. CIMMYT-Mexico, Mexico, pp: 5–21.

Morimoto, M., Kumeda, S., Komai, K., 2000. Insect antifeedant fla-

vonoids from Gnaphalium affine D. Don. J. Agric Food Chem. 48, 1888–1891.

Nakatani, M., Huang, R.C., Okamura, H., Naoki, H., Iwagawa, T., 1994. Limonoid antifeedants from Chinese *Melia azedarach*. Phytochemistry 36, 39–41.

Oleszek, W., Sitek, M., Stochmal, A., Piacente, S., Pizza, C., Cheeke, P., 2001. Resveratrol and other phenolics from the bark of *Yucca schidigera* Roezl. J. Agric. Food Chem. 49, 747–752.

Pacher, T., Seger, C., Engelmeier, D., Vajrodaya, S., Hofer, O., Greger, H., 2002. Antifungal stilbenoids from *Stemona collinsae*. J. Nat. Prod 65, 820–827.

Panzuto, M., Mauffette, Y., Albert, P.J., 2002. Developmental, gustatory, and behavioral responses of leafroller larvae, *Choristoneura rosaceana*, to tannic acid and glucose. J. Chem. Ecol 28, 145–160.

Rhoades, D.F., 1979. Evolution of plant chemical defense against herbivores. In: Rosenthal, G.A., Janzen, D.H. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, New York, pp. 3–54.

Rhoades, D.F., Cates, R.G., 1976. Toward a general theory of plant antiherbivore chemistry. Recent Adv. Phytochemistry 10, 168–213.

Rimando, A., Cuendet, M., Desmarchelier, C., Metha, R.G., Pezzuto, J.M., Duke, S.O., 2002. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. J. Agric. Food Chem. 50, 3453–3457.

Williams, R.L., Elliot, M., Perry, R., Greaves, B., 1996. The strogenic activity of the polyphenolic resveratrol, benefits of moderate consumption of red wine. K. Polyphenols Communications 96, Bordeaux France 1996 P210, 489–490.

Schroder, J., 1999. The chalcone/stilbene synthase-type family of condensing enzymes. In: Barton, D.H.R., Nakanishi, K., Meth-Con, O. (Eds.), Comprehensive Natural Products Chemistry, Polyketides and other Secondary Metabolites including Fatty Acids and their Derivatives. Elsiever, Amsterdam, pp. 749–772.

Schultz, T.P., Boldin, W.D., Fischer, T.H., Nicholas, D.D., McMurtrey, K.D., Pobanz, K., 1992. Structure-fungicidal properties of some 3- and 4-hydroxylated stilbenes and bibenzyl analogues. Phytochemistry 31, 3801–3806.

Sieman, E.H., Creasy, L.L., 1992. Concentration of the phytoalexin resveratrol in wine. Am. J. Enol. Vitic 43, 49–52.

Shimizu, K., Kondo, R., Sakai, K., 2000. Inhibition of tyrosinase by Flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigations. Planta Medica 66, 11–15.

Stivala, L.A., Savio, M., Carafoli, F., Perucca, P., Bianchi, L., Maga, G., Forti, L., Pagnoni, U.M., Albini, A., Prosperi, E., Vannini, V., 2001. Specific structural determinants are responsible for the antioxidant activity and the cell effects of resveratrol. J. Biol. Chem. 276 (25), 22586–22594.

Su, B.-N., Cuendet, M., Hawthorne, M.E., Kardono, L.B.S., Riswan, S., Fong, H.H.S., Metha, R.G., Pezzuto, J.M., Kinghorn, A.D., 2002. Constituents of the bark and twigs of Artocarpus with cyclooxygenase inhibitory activity. J. Nat. Prod 65, 163–169.

Swain, T., 1979. Tannins and lignins. In: Rosenthal, G.A., Janzen, D.H. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, New York, pp. 657–682.

SAS Institute, 1982. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Zhang, M., Chaudhuri, S.K., Kubo, I., 1993. Quantification of insect growth and its use in screening of naturally occurring insect control agents. J. Chem. Ecol 19, 1109–1118.