

Allelochemicals from *Stauranthus perforatus*, a Rutaceous tree of the Yucatan Peninsula, Mexico

Ana Luisa Anaya ^{a,*}, Martha Macías-Rubalcava ^a, Rocío Cruz-Ortega ^a,
Clara García-Santana ^a, Perla N. Sánchez-Monterrubio ^b,
Blanca E. Hernández-Bautista ^a, Rachel Mata ^b

^a Instituto de Ecología, UNAM. Circuito Exterior, Ciudad Universitaria, 04510, México, D.F.

^b Facultad de Química, UNAM. Ciudad Universitaria, 04510, México, D.F.

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Abstract

Aqueous leachates and a CHCl₃–MeOH (1:1) extract of roots of *Stauranthus perforatus* showed a significant phytotoxic effect on *Amaranthus hypochondriacus* and *Echinochloa crus-galli*. Bioassay-directed fractionation of the active organic extract led to the isolation and characterization of ten secondary metabolites, which included two pyranocoumarins [xanthyletin (**1**) and 3-(1',1'-dimethylallyl)-xanthyletin (**2**)], four furanocoumarins [chalepinsin (**3**), ammirin (**4**), chalepin (**5**) and 2'-isopropyl-psoralene (**6**)], two lignans [asarinin (**7**) and fargesin (**8**)], one sesquiterpene [4,5-epoxy-β-caryophyllene (**9**)], and one alkamide [pellitorine (**10**)]. From these compounds, 2'-isopropyl-psoralene (**6**) or anhydromarmesin, is reported for the first time as a natural product, whereas compounds **4–10** are now reported as being present in *S. perforatus*. Metabolites **1**, **3–5** and **10** caused significant inhibition of radicle growth of *A. hypochondriacus* and *E. crus-galli*. Furthermore, in a greenhouse experiment the decomposition of the leaves and roots in the soil had a significant inhibitory effect on the growth of weeds. The allelopathic action of the decomposition of roots was evident up to the sixth week of the experiment. The effect of leaves was comparable to that of DPCA (dimethyl tetrachloroterephthalate), a commercial herbicide. Finally different concentrations of *Stauranthus* root powder were combined with maize kernels and used to feed corn weevil. The treatments resulted in high mortality of this insect.

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Keywords: *Stauranthus perforatus*; Rutaceae; *Amaranthus hypochondriacus*; Amaranthaceae; *Echinochloa crus-galli*; Poaceae; Allelochemicals; Corn weevil; Xanthyletin; 3-(1',1'-Dimethylallyl)-xanthyletin; Chalepinsin; Ammirin; Chalepin; 2'-Isopropyl-psoralene; Asarinin; Fargesin; 4,5-Epoxy-β-caryophyllene; Pellitorine; Cytotoxicity

1. Introduction

Stauranthus perforatus Liebm. (Rutaceae), as well as other species of the Rutaceae family (*Esenbeckia yaxhoob*, *Zanthoxylum fagara*, and *Pilocarpus* spp.), is known as 'tankasché' in the Mexican states of Quintana Roo and Yucatán. This tree, up to 30 m high, grows

only in the coast of the Gulf of Mexico and the Yucatan Peninsula from sea level to 700 m altitude. It is found in the medium or high canopy of semi-evergreen tropical forests (Martínez, 1994). The leaves and roots of this species are used by local people to treat gastrointestinal diseases, headaches, epilepsy and common colds, as well as as a diuretic agent (Téllez-Valdez et al., 1989).

Setzer et al. (2000) reported that the crude dichloromethane extract of *S. perforatus* bark showed *in vitro* cytotoxic activity against Hep-G2 (human hepatocellular carcinoma) and MDA-MB-231 (human mammary

* Corresponding author. Tel.: +52 55 5622 9032; fax: +52 55 5622 9043.

E-mail address: alanaya@miranda.ecologia.unam.mx (A.L. Anaya).

adenocarcinoma) cells. The cytotoxic compounds proved to be the quinoline alkaloids skimmianine and veprisine, and the furocoumarin heraclenin. They also isolated isopimpinellin, and xanthotoxin. Skimmianine, a ubiquitous alkaloid in the Rutaceae family, showed antileishmanial, mutagenic and anti-platelet aggregation activities, and behaves as a 5-HT antagonist. More recently Setzer et al. (2003) reinvestigated this species by using HPLC–MS and HPLC–NMR to obtain six furanocoumarins and nine quinoline alkaloids.

Continuing our study of the flora of the Ecological Reserve El Eden, Quintana Roo, Mexico (Anaya, 1999; Anaya et al., 1992, 1995; Caamal-Maldonado et al., 2001) we investigated the allelochemical potential of *S. perforatus* and isolated the bioactive compounds.

2. Results and discussion

2.1. Effects of aqueous leachates and organic extracts on plant root growth

Aqueous leachates of leaves and roots of *S. perforatus* inhibited the root growth of *Amaranthus hypochondriacus* and *Echinochloa crus-galli* (Fig. 1). The former species was more sensitive to the treatments than the latter. The extract of roots produced a higher phytotoxic

effect, particularly on *A. hypochondriacus*. The leaf extract was significantly inhibitory only on the root growth of *E. crus-galli* at the highest concentration. The phytotoxicity of the leaves significantly decreased with the organic extraction. These results were the guidelines for selecting the roots for a bioactivity fractionation investigation.

2.2. Isolation and characterization of compounds from *S. perforatus* roots

Extensive chromatography of the crude active extract furnished 10 known compounds (Fig. 2), which included two pyranocoumarins [xanthyletin (1) and 3-(1',1'-dimethylallyl)-xanthyletin (2)], four furanocoumarins [chalepentin (3), ammirin (4), chalepin (5) and 2'-isopropyl-psoralene (6)], two lignans, [asarinin (7) and fargesin (8)], one sesquiterpene [4,5-epoxy- β -caryophyllene (9)] and one amide [pellitorine (10)]. The structures of these compounds were determined by comparison of the spectral and physical data with those reported in the literature. Compounds 4–10 are reported for the first time in *S. perforatus* and 2'-isopropyl-psoralene (6) or anhydromarmesin, is reported for the first time as a natural product. However, it was obtained previously by dehydration of marmesin (Chatterjee et al., 1978; Elgamal et al., 1979).

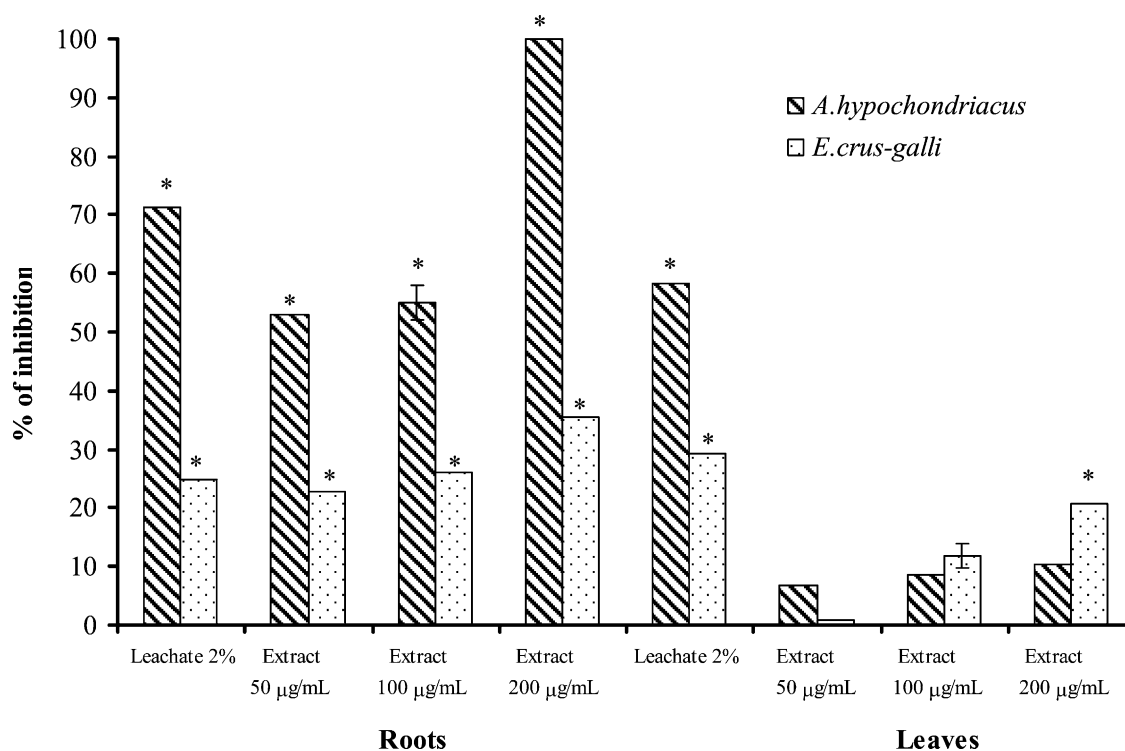


Fig. 1. Effect of aqueous leachates and CHCl_3 –MeOH extracts of the roots and leaves of *S. perforatus* on the root growth of *A. hypochondriacus* and *E. crus-galli*. The vertical bar represents maximum standard deviation, $n = 4$. * $P < 0.05$.

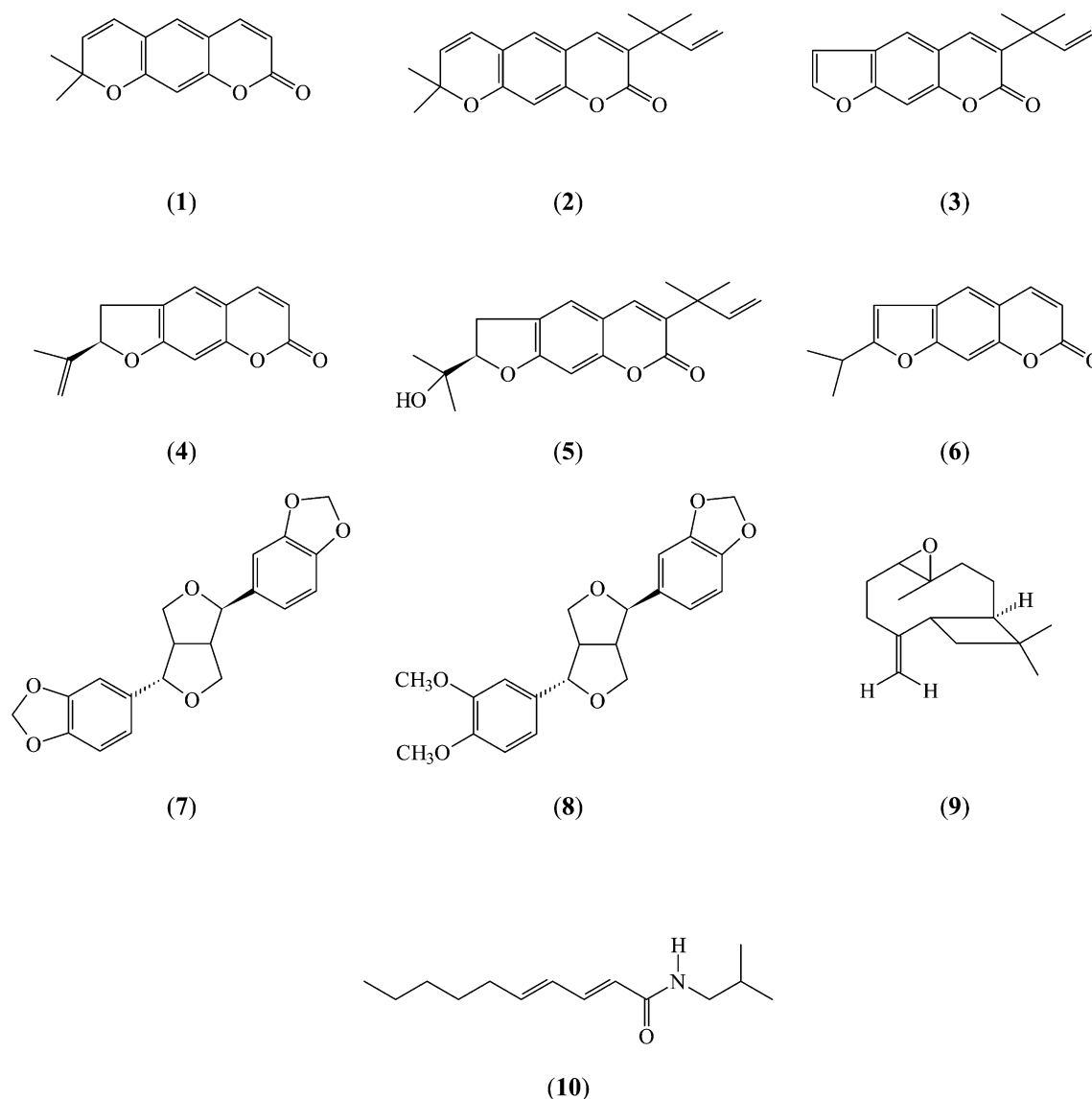


Fig. 2. Structures of compounds from *S. perforatus* roots: xanthyletin (**1**), 3-(1',1'-dimethylallyl)-xanthyletin (**2**), chalepensisin (**3**), ammirin (**4**), chalepin (**5**) and 2'-isopropyl-psoralene (**6**), asarinin (**7**), fargesin (**8**), 4,5-epoxy- β -caryophyllene (**9**), and pellitorine (**10**).

2.3. Phytogrowth-inhibitory activity of chromatographic fractions and compounds

Phytogrowth-inhibitory activity of the CHCl_3 –MeOH (1:1) extract and primary fractions I–X of the roots of *S. perforatus* are shown in Table 1. The bioactivity of natural products **1**–**5**, **7**, **8** and **10** (100 $\mu\text{g/ml}$) on *A. hypochondriacus* and *E. crus-galli* are summarized in Table 2. The pyranocoumarin xanthyletin (**1**) was the most active compound against *A. hypochondriacus* (100% inhibition). This coumarin reduced the root growth and germination of this plant in a concentration-dependent manner. The IC_{50} were 69.5 and 59.8 $\mu\text{g/ml}$ for the root growth and germination inhibition, respectively. The amide pellitorine (**10**), and the

furanocoumarins chalepensisin (**3**) and chalepin (**5**) also produced a strong inhibitory effect on *A. hypochondriacus*. On the other hand, *E. crus-galli* was less sensitive to these compounds.

The common pyranocoumarin xanthyletin **1** has been isolated from other plant families and Rutaceae species. In a previous study several related bioactive compounds from *E. yaxhoob* were identified (Mata et al., 1998). The bioactivity and some modes of action on photosynthesis of three important coumarins of *S. perforatus*: xanthyletin (**1**), 3-(1',1'-dimethylallyl)-xanthyletin (**2**), and chalepensisin (**3**) were studied. These coumarins inhibited ATP synthesis from water to methylviologen in spinach thylakoids in a concentration-dependent manner (Macías et al., 1999). Macías et al. (1993) reported the phytotoxic

Table 1

Phytogrowth-inhibitory activity of the CHCl₃–MeOH (1:1) extract and primary fractions from *S. perforatus* roots on the root growth of *A. hypochondriacus* and *E. crus-galli*

Treatments (100 µg/ml)	Control root growth (%)	
	<i>A. hypochondriacus</i>	<i>E. crus-galli</i>
Extract	44.9 ± 0.6*	73.9 ± 1.3*
Fractions		
I	63.0 ± 0.7*	103 ± 1.9
II	80.6 ± 0.5*	110 ± 1.0
III	68.1 ± 0.8*	84.8 ± 2.7
IV	31.4 ± 0.3*	98.4 ± 2.9
V	42.4 ± 0.7*	69.1 ± 1.7*
VI	32.4 ± 0.2*	60.3 ± 2.3*
VII	30.5 ± 0.3*	62.1 ± 1.6*
VIII	21.9 ± 0.8*	70.2 ± 3.2*
IX	37.1 ± 0.6*	93.3 ± 3.0
X	96.5 ± 0.7	91.5 ± 2.0
2,4-D	0.0 ± 0.0*	0.0 ± 0.0*

* $P < 0.05$.

Table 2

Phytogrowth-inhibitory activity of compounds from *S. perforatus* roots on the root growth of *A. hypochondriacus* and *E. crus-galli*

Compounds ^a (100 µg/ml)	Control root growth (%)	
	<i>A. hypochondriacus</i>	<i>E. crus-galli</i>
1	0.0 ± 0.0*	69.7 ± 2.1*
2	74.9 ± 1.5	83.7 ± 1.9
3	22.0 ± 0.6*	108 ± 2.0
4	61.1 ± 1.7*	61.6 ± 1.9*
5	45.1 ± 1.3*	88.5 ± 1.8
7	93.2 ± 1.9	89.5 ± 0.6
8	92.1 ± 1.8	83.7 ± 2.2
10	20.3 ± 0.7*	55.6 ± 2.2*
2,4-D	0.0 ± 0.0*	0.0 ± 0.0*

^a Xanthyletin (1), 3-(1',1'-dimethylallyl)-xanthyletin (2), chalepentin (3), ammirin (4), chalepin (5), asarinin (7), fargesin (8), pellitorine (10).

* $P < 0.05$.

activity of xanthyletin (1) and nine other coumarins, isolated from the leaves of *Pilocarpus goudotianus*, on *Lactuca sativa*. Pavao et al. (2002) observed that

glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) from *Trypanosoma cruzi* complexed with chalepin (3), a coumarin from *Pilocarpus spicatus*. This complex was characterized by X-ray crystallography. The final refined model of the complex shows extensive conformational changes when compared with the native structure. There are other studies on the different bioactivities and ecological roles of coumarins (Berenbaum, 1995; Berenbaum et al., 1991; Mafezoli et al., 2000; El-Shafae and Ibrahim, 2003; Smith et al., 2004; Hale et al., 2004; Ahua et al., 2004).

2.4. Cytotoxicity activity

The level of phytotoxic activity of 1, 3, 4 and 10 prompted us to assess their cytotoxicity in order to determine potential toxic effects on human and animals. Thus the effect of these compounds against six human solid tumor cell lines in a 7-day MTT test using adriamycin as a positive control was evaluated. The results, summarized in Table 3, indicated that only pellitorine (10) was cytotoxic, particularly against the PC-3 (human prostate adenocarcinoma) cell line. On the other hand, chalepentin (3) showed a marginal cytotoxicity on A-549 (human lung carcinoma) and MCF-7 (human breast carcinoma) cell lines. These results allow us to assess that phytotoxicity is a strong bioactivity showed by xanthyletin (1), chalepentin (3), and ammirin (4), and to propose them as promising molecules for future development of natural herbicides.

2.5. Bioassay with *Sitophilus zeamais* (corn weevil)

Fig. 3 shows that powdered roots of *S. perforatus* mixed into maize kernels at 1% and 2% caused a mortality of 'corn weevil' of 91% and 95.5%, respectively, whereas complete mortality occurred at 3% and 4%. The insecticidal effect was probably due to the mixture of coumarins, pellitorine, and lignans, among other

Table 3

Cytotoxicity data for the selected compounds from *S. perforatus*

Compounds	Cytotoxicity					
	Tumor cell line ED ₅₀ (µg/ml)					
	A-549 ^a	MCF-7 ^b	HT-29 ^c	A-498 ^d	PC-3 ^e	PACA-2 ^f
1	79.8	18.4	47.4	64.8	45.2	5.5
3	7.7	5.7	3.5	9.5	17.8	5.2
4	27.5	46.9	34.8	34.1	37.9	37.0
10	16.3	9.3	3.5	3.9	4.8	2.2
Adriamycin	3.22 × 10 ⁻²	2.04 × 10 ⁻²	4.21 × 10 ⁻²	3.48 × 10 ⁻³	2.41 × 10 ⁻¹	1.20 × 10 ⁻²

^a Human lung carcinoma.

^b Human breast carcinoma.

^c Human colon adenocarcinoma.

^d Human kidney carcinoma.

^e Human prostate adenocarcinoma.

^f Human pancreatic carcinoma.

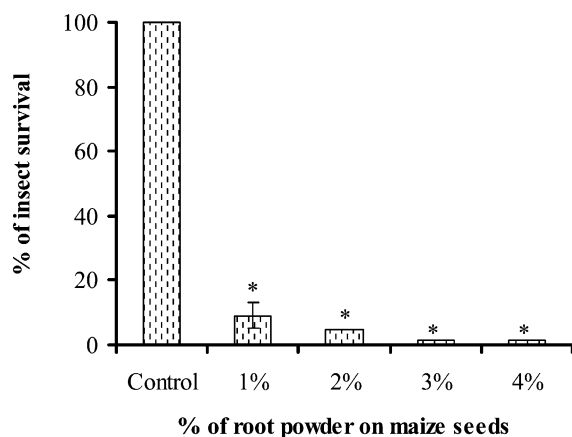


Fig. 3. Effect of *S. perforatus* root powder (% w/w) sprinkled on maize kernels, on the survival (%) of *Sitophylus zeamais*. The vertical bar represents maximum standard deviation, $n = 4$. * $P < 0.05$.

toxic compounds present in the roots of this plant, with known insecticidal activity (Saadali et al., 2001; He et al., 2002; Navickiene et al., 2003; Rios et al., 2002; Rowell and Blinn, 2003; Smith et al., 2004).

2.6. Greenhouse experiment

With the aim to find some natural techniques to control weeds using allelopathic plants as green manures, the effect of the decomposition of the leaves and roots of *S. perforatus* was tested on the emergence of weeds in the soil of pots in a greenhouse experiment (Fig. 4). Pots with *Stauranthus* leaves showed the highest allelopathic activity on the growth of weeds. Some differences between the effect of the leaves and the commercial herbicide were observed from the third to the fifth week, however, in both cases the highest decrease in weed population was observed between weeks 6 and 7. Thus, the leaves of *S. perforatus* might have a potential as plant mulch for weed control in some crops. Decomposition of roots behaves in a different way since its allelopathic effect was detected up to the 6 week and persists during week 7. It is important to point out that the release of allelopathic compounds during decomposition of roots was slower perhaps because their content in recalcitrant compounds as cellulose, hemicellulose, and lignin (Paul and Clark, 1989). Oliva et al. (2002) investigated the effects of rue (*Ruta graveolens*) leaves on soil characteristics and on germination and seedling growth of four crop species (radish, zucchini, cauliflower and tomato). Results indicate that seedling development for all the species was suppressed in incorporated and amended soil, compared to the rue-free soil, indicating a very strong allelopathic effect of *R. graveolens* on these crops.

The different bioassays performed show that some of the isolated compounds and mixtures of them in extracts and chromatographic fractions possess a strong and/or

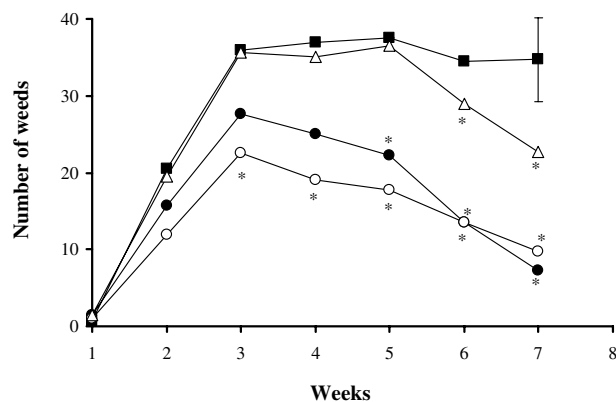


Fig. 4. Effects of the leaves and roots of *S. perforatus* incorporated as green manures (2%) into the soil of pots on the number of emerging weed seedlings. Leaves (○), roots (△), positive control [DPCA; 11.2 kg/Ha] (●), and control (■). The vertical bar represents maximum standard deviation, $n = 4$. * $P < 0.05$.

specific bioactivity on different test organisms and cells. Water is a good solvent to extract phytotoxic allelochemicals from entire leaves and roots of *S. perforatus*, but the bioactivity of leaves significantly decreased when an organic solvent was used to extract allelochemicals from them. However, in the greenhouse experiment, leaves were the most allelopathic treatment on the emergence of weeds during their decomposition in the soil.

As in other Rutaceae species, *S. perforatus* possesses a rich diversity of secondary metabolites with different biological properties that could be involved in many metabolic and ecological processes. Substances that transmit information between organisms are a fundamental part of the regulatory chemicals of nature (Eisner and Meinwald, 1995). The chemical richness of *S. perforatus* provides multiple defense mechanisms that this tree has developed against herbivores and competitors.

3. Experimental

3.1. Plant material

Roots and leaves of *S. perforatus* were collected in Quintana Roo, México, in July 1995. Voucher specimens (ALA95-8) were deposited at the Herbarium of the University of Yucatan, Merida.

3.2. Aqueous leachates

Two grams of leaves and roots of *S. perforatus* were soaked for 3 h in 100 ml of distilled water. The leachates were filtered through Whatman paper (No. 4) and then through a sterile Millipore membrane (0.45 µm). The osmotic potential was measured with a freezing-point

osmometer (Osmette A. Precision System, Inc.). The values ranged from 15 to 17 mosm/l.

3.3. General experimental procedures

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks on a Perkin–Elmer 599-B spectrophotometer. UV spectra were obtained on a Shimadzu 160 UV spectrometer in MeOH solution. Optical rotations were taken on a JASCO DIP 360 polarimeter. NMR spectra were recorded on a Bruker DMX500, in CDCl₃, either at 500 (¹H) or 125 (¹³C) MHz, using tetramethylsilane (TMS) as an internal standard. EI mass spectra were performed using a Hewlett–Packard 5890 mass spectrometer. Open column chromatography: silica gel 60 (70–230 mesh, Merck). Analytical and preparative TLC were performed on pre-coated silica gel 60 F254 plates (Merck).

3.4. Extraction and isolation of allelochemicals of the roots

The dried roots of *S. perforatus* (2.6 kg) were exhaustively extracted (3 times) with CHCl₃–MeOH (1:1) [32 l] for 4 days at room temperature. Evaporation to dryness in vacuum of the combined CHCl₃–MeOH extracts afforded a dark green residue (232.8 g). The crude extract was subjected to silica gel cc (1 kg) with a gradient of *n*-hexane–EtOAc–MeOH, with 115 fractions (1000 ml each) obtained. Ten fractions, I–X, were pooled according to thin layer chromatography (TLC) analysis. Root growth bioassay showed nine active pools (I–IX). Primary fraction I (62.1 g, eluted with 100% *n*-hexane) was purified by silica gel cc with solvent mixtures of increasing polarity (*n*-hexane, *n*-hexane–EtOAc to pure EtOAc) to yield eight secondary fractions (I-A to II-H). From the secondary fraction I-C (eluted with 97% *n*-hexane and 3% EtOAc), 4,5-epoxy- β -caryophyllene (**9**) (149.1 mg) spontaneously crystallized (Mata et al., 1997). Primary fraction II (2.4 g, eluted with 95% *n*-hexane and 5% EtOAc) was further subjected to Si gel cc using the same elution system as for fraction I to yield eleven secondary fractions (II-A to II-K). Fraction II-D (eluted with 100% *n*-hexane), was resolved by successive preparative TLC (85% *n*-hexane and 15% EtOAc) to yield the pyranocoumarin 3-(1',1'-dimethylallyl)-xanthyletin (**2**) (63.2 mg) (Hernández et al., 1988; Kamaraj and Sembulingam, 2003). From primary fraction III (3.9 g, eluted with 85% *n*-hexane and 15% EtOAc), the furanocoumarin chalepensis (**3**) (2.0 g) spontaneously crystallized (Kumar et al., 1995). From primary fraction V (6.4 g eluted with 65% *n*-hexane and 35% EtOAc), the pyranocoumarin xanthyletin (**1**) (2.0 g) spontaneously crystallized (Rudiño-Piñera et al., 1995) and the lignan asarinin (**7**) (96.5 mg) (Brown et al., 2001). The mother

liquors from this phytotoxic fraction were subjected again to Si gel cc eluting with a gradient system (*n*-hexane–CHCl₃–EtOAc–MeOH). Thirteen secondary fractions were obtained (V-A to V-M). From fraction V-G (eluted with 100% EtOAc), crystallized a mixture of the furanocoumarin 2-isopropyl-psoralene (**6**) (Zubía et al., 1992; Masuda et al., 1998) and β -sitosterol. The mixture was resolved by preparative TLC (99% benzene and 1% EtOAc, 50% *n*-hexane and 50% CHCl₃) to yield **6** (14.4 mg). Primary fraction VI (2.6 g, eluted with 50% *n*-hexane and 50% EtOAc) was further resolved by Si gel cc eluted with a gradient system (benzene–EtOAc–MeOH), to yield seven secondary fractions (VI-A to VI-G). From secondary fraction VI-D (eluted with 100% EtOAc) the furanocoumarin ammirin (**4**) (25.2 mg) spontaneously crystallized (Zubía et al., 1992). Further purification of mother liquors by preparative TLC (90% benzene and 10% EtOAc) provided the amide pellitorine (**10**) (123.5 mg) (Saadali et al., 2001). Active fraction VII (1.8 g, eluted with 25% *n*-hexane and 75% EtOAc) was further purified by Si gel cc, using the same elution system as for fraction VI, to yield eleven fractions (VII-A to VII-K). Secondary fraction VII-E (eluted with 100% EtOAc) was purified by successive preparative TLC (85% *n*-hexane and 15% EtOAc) afforded the lignan fargesin (**8**) (13.3 mg) (Gibbons et al., 1997). Primary fraction VIII (14.7 g, eluted with 100% EtOAc) was applied to a Si gel column eluted with a gradient system (*n*-hexane–EtOAc). Nine secondary fractions were obtained (VIII-A to VIII-I). Finally, fraction VIII-G (eluted with 100% EtOAc) was resolved by successive preparative TLC (65% benzene and 35% EtOAc) to provide the furanocoumarin chalepin (**5**) (2.8 g) (Kumar et al., 1995).

3.5. Bioassays with seeds

Phytotoxicity of aqueous leachates, organic extracts, and pure compounds were tested on seeds of *A. hypochondriacus* L. (Amaranthaceae) and *E. crus-galli* L. (Beauv.), (Poaceae). *A. hypochondriacus* seeds were purchased from a local market in Tulyehualco, Mexico, D.F. Seeds of *E. crus-galli* were from Valley Seed Service, Fresno, California, USA.

Aqueous leachates (4%) were mixed (1:1 v/v) with agar (2%) to obtain a 2% test solution. Pure agar (2%) was used as negative control, and agar (4%) with 200 μ g/ml of 2,4-dichlorophenylacetic acid (2,4-D) (1:1 v/v) with a final concentration of 100 μ g/ml, was used as positive control. Germination and seedling root growth bioassays for the aqueous leachates were carried out according to procedures published previously (Anaya et al., 2003). Bioassays were set up in 6-cm Petri dishes. Ten seeds of each test plant were sown directly on the agar of each Petri dish following a completely random design with four replicates per treatment. Petri

dishes were kept in darkness at 27 °C. Root lengths were measured 24 h after treatment for *A. hypochondriacus*; and 48 h for *E. crus-galli*. Data were analyzed by ANOVA and Tukey's tests (Mead et al., 2002). Bioassays with organic extracts and pure compounds were set up using the same conditions as for aqueous leachates, but with filter paper as a substrate. The filter paper (Whatman 42) was impregnated with 1.5 ml of the solutions (100 µg/ml) or 2,4-D (100 µg/ml) as a positive control. After total evaporation of the solvents, filter paper was moistened with distilled water (1.5 ml). Filter paper with only distilled water was used as negative control. Test seeds and time of harvesting for these bioassays were the same.

3.6. Cytotoxicity assay

Cytotoxicity determinations of pure compounds against A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), and PACA-2 (human pancreatic carcinoma) human solid tumor cell lines were performed in a seven-day 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) assay at the Purdue Cell Culture Laboratory using standard protocols, with adriamycin as a positive control (Anderson et al., 1991; Rieser et al., 1992; He et al., 1996).

3.7. Bioassay with *Sitophilus zeamais* Motsch. (corn weevil)

The insects were provided by the Toxicology Laboratory of Colegio de Posgraduados, (Texcoco, México), and they were brought to the laboratory in a jar with a pierced cap and 500 g of the susceptible land race "cacahuacintle" maize. Insects were separated from the maize by screening the seeds through a sieve No. 16; they were placed in jars filled to 3/4 of their capacity with "cacahuacintle" maize and incubated for 8 days at room temperature (25 °C). After this time, the maize was screened again to eliminate all adult insects. Jars were kept under observation until the first adult insects appeared. After 8 days, the bioassays were started with 1 week old insects.

The experiments were performed in a complete randomized block design, with 4 repetitions per treatment, using small jars with 100 g of "cacahuacintle" maize kernels. Twenty four insects, 12 males and 12 females, were added to each jar. The treatments used were: (A) kernels of maize (100 g); (B)–(E) kernels of maize (100 g) sprinkled with 1, 2, 3, and 4 g of root powder of *S. perforatus*. The percent of insect survival was evaluated after 15 days of incubation at room temperature (≈25 °C). Results were analyzed by ANOVA.

3.8. Greenhouse experiment using leaves and roots of *S. perforatus* as green manures

Because of the phytotoxicity showed by *S. perforatus*, a greenhouse experiment was conducted to observe the effect of the decomposition of leaves and roots of *Stauranthus* on the growth of weeds. Soil for pots was collected in a crop field in San Pablo Oztotepec, Mexico. Physical and chemical characteristics of this soil show that it is a clay-loam soil with a pH slightly acid (6.3), with 2.4% of organic matter, extremely poor in potassium (1.12 meq/100 g), poor in available calcium (0.8 meq/100 g) and magnesium (0.20 meq/100 g), rich in phosphorus (500 ppm) and nitrogen (3010 ppm). The soil was sieved (1.41 mm) and hand homogenized. The experiment was performed in a complete randomized block design with four repetitions. The treatments used were: (1) 1 kg of soil (negative control); (2) 1 kg of soil + 2% of leaves; (3) 1 kg of soil + 2% of roots; (4) 1 kg of soil + DPCA (dimethyl tetrachloroterephthalate) 11.2 kg/Ha as formulated product (positive control). Pots were watered when necessary. The number of weeds in the soil of pots was recorded every week. The experiment was ended after seven weeks. Data were analyzed by ANOVA ($P < 0.05$).

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