

Research Article

Evaluation of the Toxicity of *Virola sebifera* Crude Extracts, Fractions and Isolated Compounds on the Nest of Leaf-Cutting Ants

Keylla Utherdyany Bicalho,¹ Ana Paula Terezan,¹ Denise Cristina Martins,¹ Taís Garcia Freitas,² João Batista Fernandes,¹ Maria Fátima das Graças Fernandes da Silva,¹ Paulo Cezar Vieira,¹ Fernando Carlos Pagnocca,² and Odair Côrrea Bueno²

¹Departamento de Química, Universidade Federal de São Carlos, CP 13565-905 São Carlos, SP, Brazil

²Centro de Estudos de Insetos Sociais, Universidade Estadual Paulista, Campus Rio Claro, CP 199, 13506-900 Rio Claro, SP, Brazil

Correspondence should be addressed to João Batista Fernandes, djbf@power.ufscar.br

Received 30 April 2011; Accepted 5 June 2011

Academic Editor: Jacques H. C. Delabie

Copyright © 2012 Keylla Utherdyany Bicalho et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The phytochemical study of *Virola sebifera* leaves led to the isolation of three lignans: (+)-sesamin, (–)-hinokinin, and (–)-kusunokinin and three flavonoids: quercetin-3-*O*- α -L-rhamnoside, quercetin-3-*O*- β -D-glucoside, and quercetin-3-methoxy-7-*O*- β -D-glucoside by using techniques as high-speed counter-current chromatography and high-performance liquid chromatography. The crude extracts, fractions, and isolated compounds were evaluated for their insecticidal and fungicidal potential against *Atta sexdens rubropilosa* and its symbiotic fungus *Leucoagaricus gongylophorus*. The bioassay results showed a high insecticidal activity for the methanol crude extract of the leaves of *V. sebifera* and its *n*-hexane, dichloromethane and ethyl acetate fractions. The fungicidal bioassay revealed high toxicity of the lignans against *L. gongylophorus*.

1. Introduction

Virola sebifera is one of the most widely spread Myristicaceae species through Brazil [1, 2]. Although it has been mainly known by its hallucinogenic effects [3, 4], this species is also employed, in folk medicine, as antiulcer and in treatment of rheumatism [5, 6]. Previous phytochemical investigations revealed a high diversity of secondary metabolites, where were found 12 lignans [5, 7–10], 23 neolignans [2, 6, 8, 11, 12], one dimeric neolignan [8], 5 polyketides [6, 13, 14], and two alkaloids [3].

Leaf-cutting ants (Hymenoptera), dominant herbivores in the tropics, are considered a serious pest for agriculture, especially when they attack cultivated plants [15]. Control of this pest is still challenging and mostly made by using synthetic insecticides that have often temporary effects and affect nontarget species [16].

They cut high amounts of vegetal matter to feed its symbiotic fungus, *Leucoagaricus gongylophorus* that produce

enzymes which are necessary to metabolize polysaccharides to mono and disaccharides, which supplies the major part of the energy needs of adult workers [17]. This relationship between the leaf-cutting ants and its symbiotic fungus is essential to their survival.

Due to the difficulty in controlling this pest, an intense search for alternative methods have been made and may involve both the search for insecticides compounds, or chemical fungicides that inhibit the symbiotic fungus growth. In the present work was evaluated the toxicity of crude extract, fractions, and isolated compounds against *Atta sexdens rubropilosa* and its symbiotic fungus *L. gongylophorus*.

2. Experimental

2.1. General Procedures. The high-speed counter-current chromatography (HSCCC) system employed in the present work was a P. C. Inc. (Potomac, MD, USA) instrument equipped with a quadruple multilayer coil of 1.68 mm

TABLE 1: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing crude extracts (2000 $\mu\text{g}\cdot\text{mL}^{-1}$) from *V. sebifera*.

Crude extracts	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	2	8	14	22	36	36	42	52	62	72	17 ^a
Dichloromethane leaves (I)	0	10	18	40	64	92	98	100	100	100	7.5 ^b
Methanol leaves (II)	0	2	4	20	38	60	70	70	76	78	10 ^a
Ethanol branches (III)	2	10	14	32	42	48	58	64	64	66	11 ^a

^a Control or no significant and ^b significant difference according to long-rank test ($P < 0.05$).

TABLE 2: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing the fractions (1000 $\mu\text{g}\cdot\text{mL}^{-1}$) of the methanol crude extract of leaves of *V. sebifera*.

Fractions	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	0	0	2	6	8	14	18	30	42	62	23 ^a
<i>n</i> -Hexane (IV)	0	8	18	36	54	72	82	88	92	94	8 ^b
Dichloromethane (V)	2	12	20	60	82	92	94	94	94	94	5 ^b
Ethyl acetate (VI)	0	0	4	14	36	40	60	64	82	92	12 ^b
Hydroalcoholic (VII)	2	3	10	14	26	32	46	50	62	76	17 ^b

^a Control or no significant and ^b significant difference according to long-rank test ($P < 0.05$).

I.D. polytetrafluoroethylene (PTFE) tubing and had a total capacity of 443 mL. The β value varied from 0.50 at the internal terminal to 0.85 at the external terminal, and the revolution radius was 10 cm ($\beta = r/R$, where r is the distance from the coil to the holder and R the revolution radius or the distance between the holder axis and the central shaft). The loop volume of the sample injection was 5 mL, and the revolution speed of the apparatus was regulated with a speed controller in the range between 0 and 1000 rpm. The flow rate was controlled with an FMI-50 QD SSY, BS/BS (Fluid Metering, New York, USA) constant flow pump. The fractions were obtained by an automated fraction collector DC-1200 (Eyela, Sunnyvale, CA, USA).

High-performance liquid chromatography (HPLC) analyses were performed using Shimadzu pump LC-6AV and a SPD 6AV UV detector set at 254 nm. HPLC grade solvents were obtained from Tedia, and H₂O was purified in a Milli-Q system.

LC-UV-electrospray ionization (ESI) MS/MS data were obtained using an Alliance 2695 liquid chromatography equipped with a Waters (Milliford, MA, USA) PDA2996 photodiode array detection (DAD) system; mass spectral data were acquired in negative ion mode on a triple quadrupole Micromass Quattro LC spectrometer (Manchester, U.K.), equipped with a Z-Spray API ion source and a megaflo electro spray probe.

The ¹H NMR, ¹³C NMR, and 2D correlations spectra were obtained using Bruker DRX-400 spectrometer, with CDCl₃ and DMSO-*d*₆.

2.2. *Plant Material.* *V. sebifera* was collected at the cerrado reserve of Canchim Farm, São Carlos, São Paulo state, Brazil and identified by Dra. Maria Helena Antunes de Oliveira e Souza from the Botanic Department of Federal University of São Carlos, where can be found the voucher specimens.

2.3. *Extraction and Isolation of Compounds.* The extracts were prepared from leaves and branch. The leaves air-dried powered (362 g) of *V. sebifera* were subsequently extracted with dichloromethane (I) and methanol (II), and the branches air-dried powered (407 g) were extracted with ethanol (III). The crude methanol extract (II) was submitted to a liquid-liquid partition with *n*-hexane (IV), dichloromethane (V), ethyl acetate (VI), remaining, the hydroalcoholic phase (VII). The dichloromethane fraction was purified through high-speed counter-current chromatography (HSCCC) obtaining three lignans (1–3), and the ethyl acetate fraction was purified through exclusion chromatography using Sephadex LH-20 as stationary phase and high-performance liquid chromatography (HPLC) obtaining three flavonoids (4–6). The compounds were identified using NMR and MS techniques.

2.4. *Fungicidal Bioassay.* The experiments with the symbiotic fungus *L. gongylophorus* were conducted in Bioassays Natural Products Laboratory, Federal University of São Carlos (UFSCar). The fungus *L. gongylophorus* (Singer) Möller (syn *Rozites gongylophorus*) was isolated from an *A. sexdens rubropilosa* laboratory nest and maintained in laboratory in a culture medium composed of malt extract (20 g·L⁻¹), bacto-peptone (5 g·L⁻¹), yeast extract (2 g·L⁻¹), and agar (20 g·L⁻¹) [19]. The samples submitted for assay with the symbiotic fungus were incorporated into the culture medium and followed by the addition of distilled water. Then, in each tube were added 10 mL of culture medium with sample or only culture medium. All the material was autoclaved under the conditions 120°C and 1.0 atm. for 20 minutes. After the sterilization of the material, culture media were poured in Petri plates (80 × 15 mm) inside the laminar flow

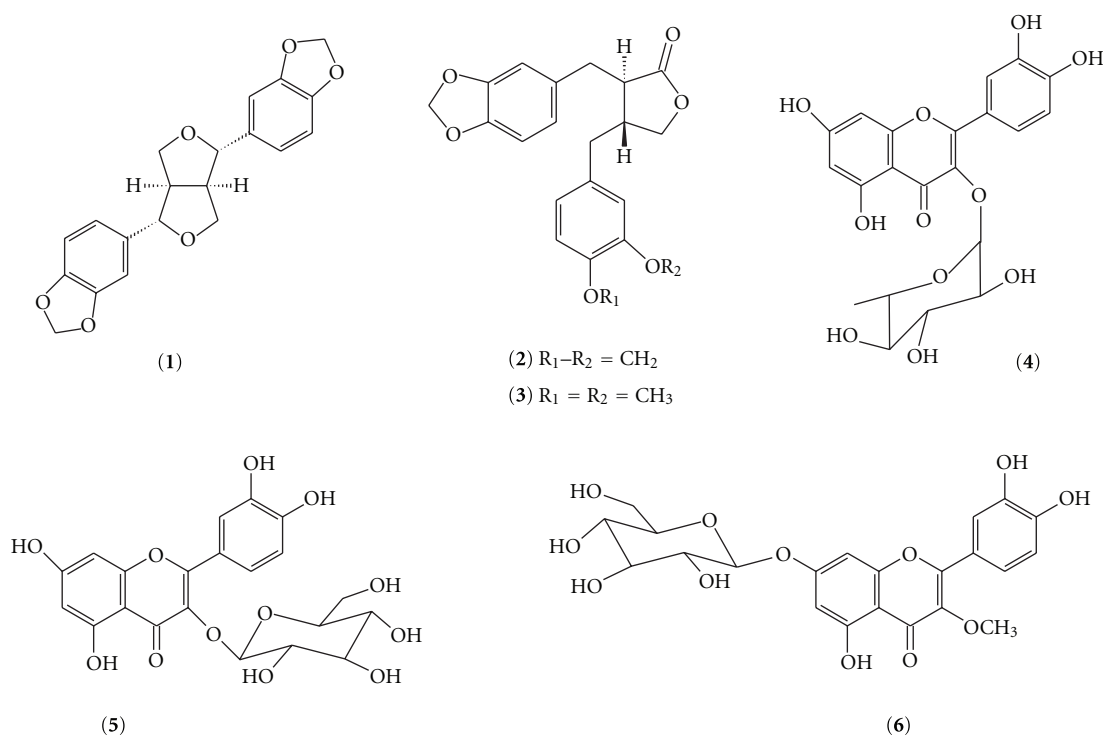


FIGURE 1: Chemical structures of the isolated compounds from *V. sebifera*.

cabinet, previously sterilized for 30 minutes by ultraviolet light. After solidification of the culture medium, each Petri plate was inoculated with a disc of agar (8 mm) at the center position, previously colonized by the symbiotic fungus *L. gongylophorus*. After the incubation period of 30 days at 25°C, calculations were performed in the areas of mycelial growth of the symbiotic fungus in each sample.

2.5. Leaf-Cutting Ant Insecticide Bioassay. The *A. sexdens rubropilosa* workers used in the assays were randomly removed from laboratory nests. They had a body mass of 20–25 mg. Before the assays, the nests were supplied daily with leaves of *Eucalyptus* sp., oat seed and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp., or rose petals. Fifty ants were removed from the nests and put into five Petri dishes (ten ants each) for each treatment. During the assay, the ants were maintained on an artificial diet prepared with glucose (50.0 g·L⁻¹), bacto peptone (10.0 g·L⁻¹), yeast extract (1.0 g·L⁻¹), and agar (15.0 g·L⁻¹) in distilled water (100 mL) [20]. The diets (0.4–0.5 g per dish) with the addition of compounds (experiment) or without it (control) were offered daily in a small plastic cap. The control was prepared with the diet and the solvent. To ensure that undetectable remaining amounts of the solvent did not affect the ants, a comparison was made with another set of dishes in which water was used instead of solvent. As expected, the same survival rates were obtained with both systems (data not shown). The compounds were poured into the hot diet immediately after it was autoclaved. The final concentration of the extracts added to the diet was 2000 µg·mL⁻¹, of the fractions were 1000 µg·mL⁻¹, and of the compounds

were 200 or 400 µg·mL⁻¹. During the assays, the material was maintained in an incubator at the temperature 25 (±1)°C and relative humidity ranging between 70 and 80%. The maximum length of observation was 25 days, and the number of dead ants was registered daily.

Percentage survival was plotted as a function of time in a survival curve which was then used to calculate the median survival time (S50, the time at which 50% of the ants in each experiment remained alive). The S50 was calculated and survival curves were compared using the nonparametric log-rank test at the 95% significant level [21].

3. Results and Discussion

The compounds isolated from the dichloromethane fraction of the methanol crude extract of the leaves of *V. sebifera* were the lignans (+)-sesamin (1), (-)-hinokinin (2), and (-)-kusunokinin (3) (Figure 1). These compounds were identified by the comparison of NMR spectral data with those described in the literature [7, 22–24]. The compounds isolated from the ethyl acetate were the flavonoids quercetin-3-*O*-α-*L*-rhamnoside (4, synonyms: quecetrin or quecitrin), quercetin-3-*O*-β-*D*-glucoside (5), and quercetin-3-methoxy-7-*O*-β-*D*-glucoside (6) (Figure 1). These flavonoids were also identified by the comparison of NMR spectral data with those described in the literature [25–27].

The *V. Sebifera* crude extracts (I, II, and III) were evaluated by their insecticidal potential, and only the methanol crude extract of the leaves (II) presented insecticidal activity

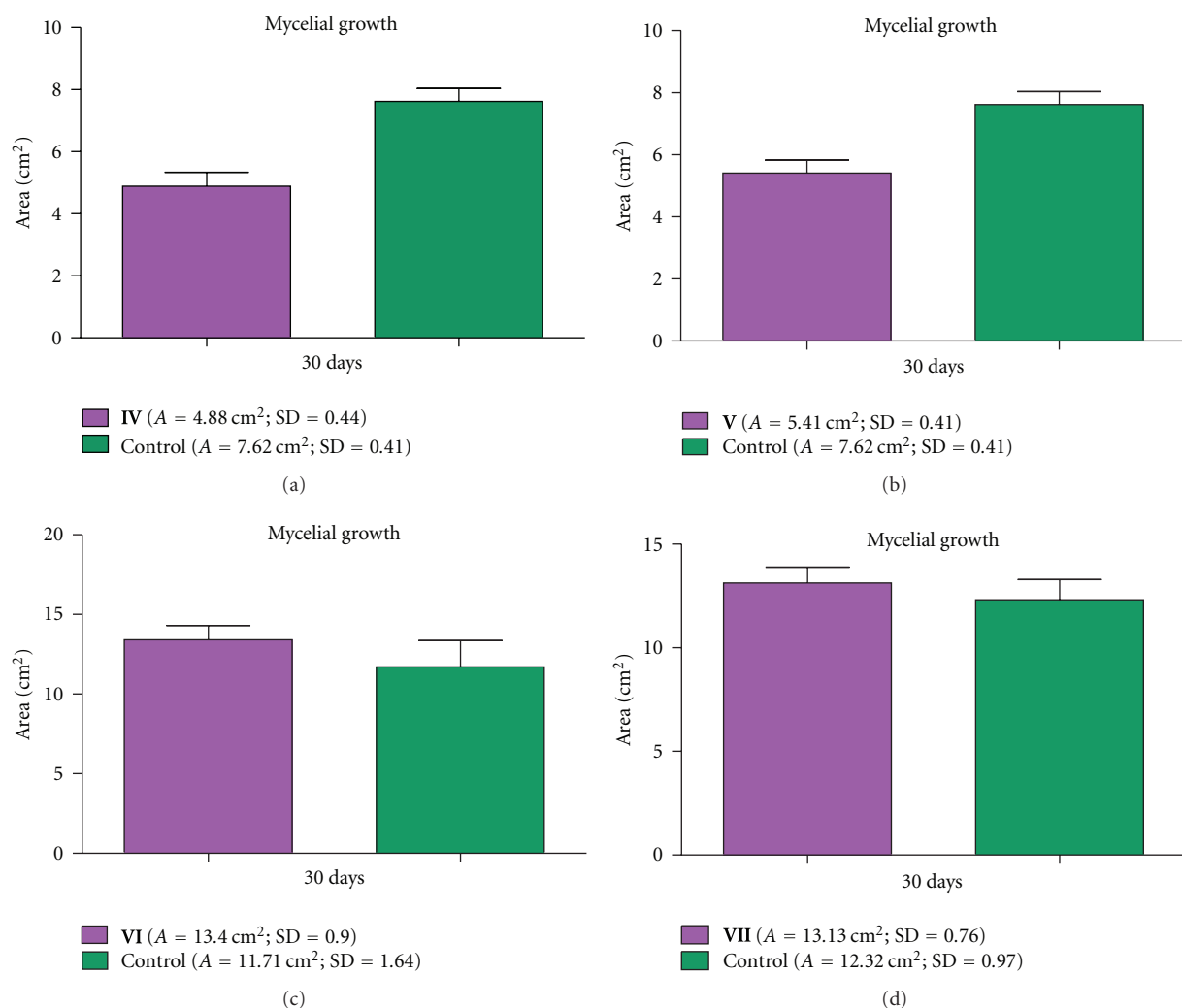


FIGURE 2: Effect of the fractions IV ($600 \mu\text{g}\cdot\text{mL}^{-1}$), V ($600 \mu\text{g}\cdot\text{mL}^{-1}$), VI ($600 \mu\text{g}\cdot\text{mL}^{-1}$), and VII ($600 \mu\text{g}\cdot\text{mL}^{-1}$) on the mycelial growth of the symbiotic fungus *L. gongylophorus*.

on *A. sexdens rubropilosa* (Table 1), and it was chosen for the beginning of the fractionation studies. In this treatment, the mortality of ants reached 100% at the 17th day while the control with the pure diet did not exceed 72% of mortality at the end of the 25 days of experiment.

The *n*-hexane (IV), dichloromethane (V), ethyl acetate (VI), and hydroalcoholic (VII) fractions from partition of methanol extract II were tested in ants (*A. sexdens rubropilosa*) ingestion bioassay (Table 2). All the fractions presented statistic difference in comparison with control; however, *n*-hexane, dichloromethane, and ethyl acetate fractions showed high cumulative mortality, 94%, 94%, and 92% of dead ants on the 25th day of experiment, respectively. The *n*-hexane and dichloromethane fractions showed the highest insecticidal activity in a short time, presenting 82% and 94% of mortality in only 12 days of experiment.

In the *in vivo* fungicidal bioassay of these fractions, the *n*-hexane and dichloromethane fractions showed 36% and 29% of inhibitory activity on symbiotic fungus; respectively, while

the ethyl acetate and hydroalcoholic fractions promoted the mycelial growth of the fungus in 14% and 7%, respectively (Figure 2).

All the six compounds 1–6 were evaluated as their insecticidal potential against *A. sexdens rubropilosa* workers by an ingestion bioassay (Table 3). Although the compounds 2, 3, 5, and 6 presented statistic difference in comparison with control, only the lignan 3 resulted in higher cumulative mortality, 90%, than the control with pure diet. These results indicate that none of the others substances were toxic to the ants workers, and only the lignan (–)-kusunokinin (3) was considered biologically active as an insecticide.

In contrast with the weak activity presented by most of the compounds tested, the three lignans showed a high fungicidal potential against the symbiotic fungus *L. gongylophorus*. The lignans (+)-sesamin (1), (–)-hinokinin (2), and (–)-kusunokinin (3) inhibited the mycelial growth in 74%, 72%, and 100%, respectively (Figures 3 and 4). In previous studies [18] were already evaluated the fungicidal

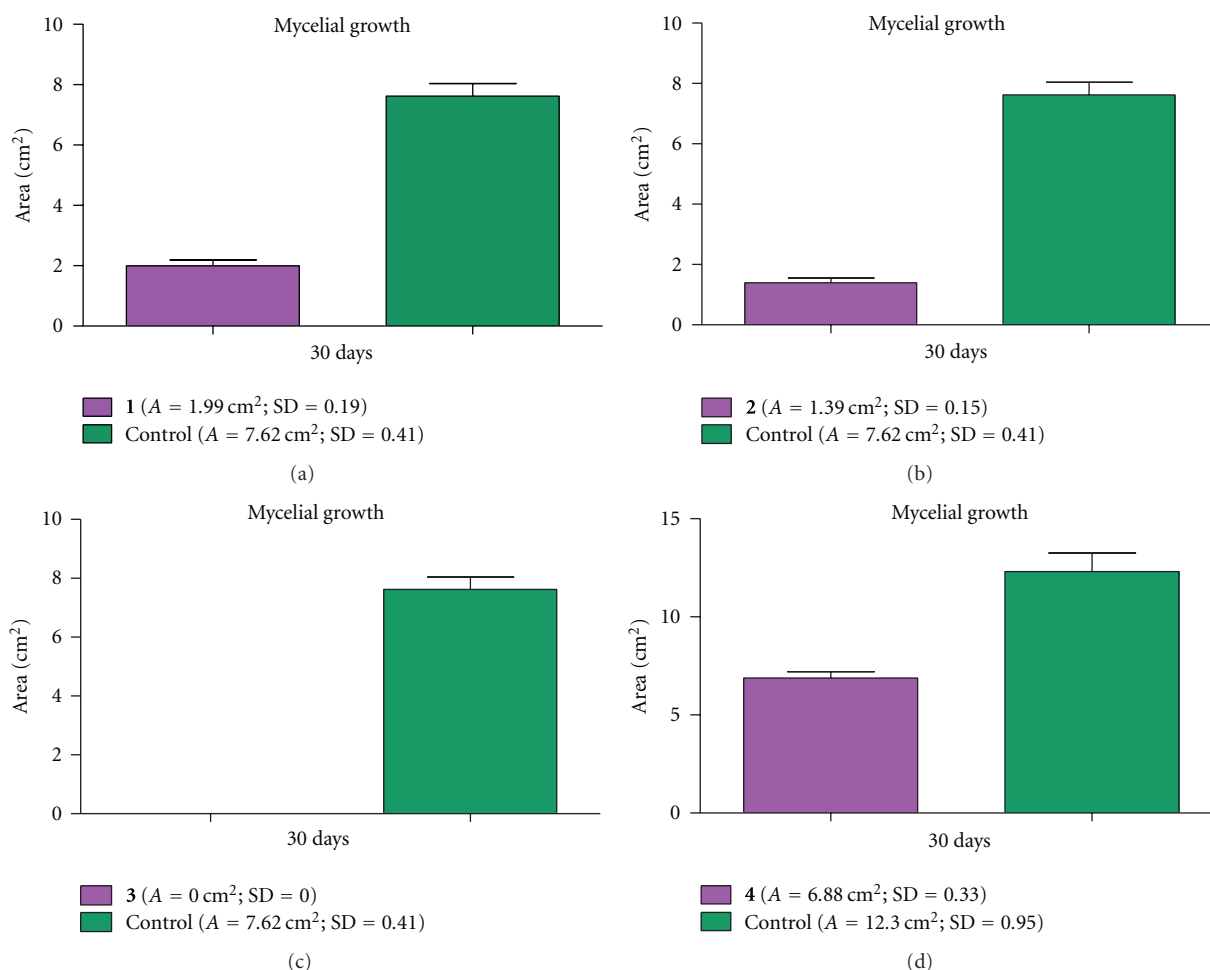


FIGURE 3: Effect of the compounds (+)-sesamin (1) ($100 \mu\text{g}\cdot\text{mL}^{-1}$), (–)-hinokinin (2) ($100 \mu\text{g}\cdot\text{mL}^{-1}$), (–)-kusunokinin (3) ($120 \mu\text{g}\cdot\text{mL}^{-1}$), and quercetin-3-*O*- α -L-rhamnoside (4) ($100 \mu\text{g}\cdot\text{mL}^{-1}$) [18] on the mycelial growth of the symbiotic fungus *L. gongylophorus*.

TABLE 3: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing the compounds 1–4 ($400 \mu\text{g}\cdot\text{mL}^{-1}$) and 5, 6 ($200 \mu\text{g}\cdot\text{mL}^{-1}$).

Fractions	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	0	0	0	6	10	10	24	44	78	84	18.5 ^a
(+)-sesamin (1)	0	2	2	4	4	6	16	44	56	62	19 ^a
(–)-hinokinin (2)	0	2	4	4	6	10	18	22	42	64	23 ^b
(–)-kusunokinin (3)	2	6	6	18	22	24	34	70	82	90	16 ^b
Quercetin-3- <i>O</i> - α -L-rhamnoside (4)	0	2	4	10	12	20	34	42	58	74	19 ^a
Quercetin-3- <i>O</i> - β -D-glucoside (5)	0	0	2	2	4	14	26	36	40	60	25 ^b
Quercetin-3-methoxy-7- <i>O</i> - β -D-glucoside (6)	0	2	2	6	8	14	24	30	48	54	23 ^b

^aControl or no significant and ^bsignificant difference according to long-rank test ($P < 0.05$).

potentials for furofuran and dibenzylbutyrolactone lignans such as (+)-sesamin and (–)-kusunokinin, respectively. For (+)-sesamin was confirmed its high toxicity against the fungus *L. gongylophorus*, but the results were quite different for (–)-kusunokinin. The actual bioassay uses a different methodology than that used initially, and the fact that the lignan (–)-hinokinin, another dibenzylbutyrolactone lignan,

also showed toxicity against the fungus validates the results of this bioassay.

The flavonoids quercetin-3-*O*- β -D-glucoside (5) and quercetin-3-methoxy-7-*O*- β -D-glucoside (6) were not tested in this bioassay, and the flavonoid quercetin-3-*O*- α -L-rhamnoside (4) showed weak inhibition of fungal growth, only 44% [28].

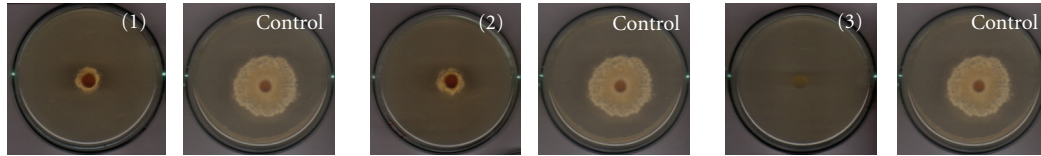


FIGURE 4: Images of the symbiotic fungus in the presence of the lignans (+)-sesamin (1), (-)-hinokinin (2), and (-)-kusunokinin (3) after 30 days of inoculations and the respective controls.

4. Conclusions

The results observed in the ingestion bioassay with ants workers for the methanol crude extract of the leaves of *V. sebifera* and the *n*-hexane, dichloromethane, and ethyl acetate fractions suggest the use of this plant to control nests of *A. sexdens rubropilosa*. Besides, the high fungicidal potentials of the lignans reveal a rich source for new fungicides.

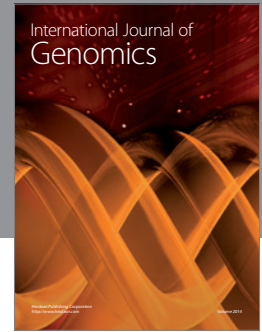
Acknowledgments

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Nacional de Ciência e Tecnologia de Controle Biorracional de Insetos Pragas, and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support and scholarships (CNPQ).

References

- [1] W. A. Rodrigues, "Revisão taxonômica das espécies de *Virola* Aublet (Myristicaceae) do Brasil," *Acta Amazonica*, vol. 10, pp. 1–123, 1980.
- [2] C. T. Rizzini and W. B. Mors, *Botânica econômica Brasileira*, Epu, Edusp, São Paulo, Brazil, 1976.
- [3] K. Kawanishi, Y. Uhara, and Y. Hashimoto, "Alkaloids from the hallucinogenic plant *Virola sebifera*," *Phytochemistry*, vol. 24, no. 6, pp. 1373–1375, 1985.
- [4] C. Denny, M. E. Zacharias, K. L. Kohn, M. A. Foglio, and J. E. Carvalho, "Atividade antiproliferativa dos extratos e da fração orgânica obtidos das folhas de *Virola sebifera* Aubl. (Myristicaceae)," *Revista Brasileira de Farmacognosia*, vol. 17, pp. 598–603, 2007.
- [5] J. C. V. Martínez, M. I. J. Aldana, and L. E. S. Cuca, "Dibenzylbutane lignans from *Virola sebifera* leaves," *Phytochemistry*, vol. 50, no. 5, pp. 883–886, 1999.
- [6] L. M. X. Lopes, M. Yoshida, and O. R. Gottlieb, "1,11-diarylundecan-1-one and 4-aryltetralone neolignans from *Virola sebifera*," *Phytochemistry*, vol. 21, no. 3, pp. 751–755, 1982.
- [7] L. M. X. Lopes, M. Yoshida, and O. R. Gottlieb, "Dibenzylbutyrolactone lignans from *Virola sebifera*," *Phytochemistry*, vol. 22, no. 6, pp. 1516–1518, 1983.
- [8] L. M. X. Lopes, M. Yoshida, and O. R. Gottlieb, "Further lignoids from *Virola sebifera*," *Phytochemistry*, vol. 23, no. 11, pp. 2647–2652, 1984.
- [9] J. C. V. Martínez, L. E. S. Cuca, and P. M. Martínez, "Lignano furofurânicos em corteza de *Virola sebifera* (Aublet)," *Revista Colombiana de Química*, vol. 14, pp. 117–125, 1985.
- [10] R. V. R. Rotz, L. E. S. Cuca, and J. C. V. Martínez, "Linanos em hojas de *Virola sebifera*," *Revista Colombiana de Química*, vol. 16, pp. 51–55, 1987.
- [11] L. M. X. Lopes, M. Yoshida, and O. R. Gottlieb, "Aryltetralone and arylindanone neolignans from *Virola sebifera*," *Phytochemistry*, vol. 23, no. 9, pp. 2021–2024, 1984.
- [12] K. R. Rezende and M. J. Kato, "Dibenzylbutane and aryltetralone lignans from seeds of *Virola sebifera*," *Phytochemistry*, vol. 61, no. 4, pp. 427–432, 2002.
- [13] M. J. Kato, L. M. X. Lopes, H. F. Paulino Fo, M. Yoshida, and O. R. Gottlieb, "Acylresorcinols from *Virola sebifera* and *Virola elongata*," *Phytochemistry*, vol. 24, no. 3, pp. 533–536, 1985.
- [14] C. Denny, M. E. Zacharias, A. L. T. G. Ruiz et al., "Antiproliferative properties of polyketides isolated from *Virola sebifera* leaves," *Phytotherapy Research*, vol. 22, no. 1, pp. 127–130, 2008.
- [15] C. G. S. Marinho, T. M. C. Della Lucia, and M. C. Picanço, "Fatores que dificultam o controle das formigas cortadeiras," *Bahia Agrícola*, vol. 7, pp. 18–21, 2006.
- [16] J. B. Fernandes, V. David, P. H. Facchini, M. F. G. F. Silva, E. Rodrigues Filho, and P. C. Vieira, "Extrações de óleos de sementes de citros e suas atividades sobre a formiga cortadeira *Atta sexdens* e seu fungo simbiote," *Química Nova*, vol. 25, pp. 1091–1095, 2002.
- [17] A. Silva, M. Bacci Jr., C. Gomes de Siqueira, O. Correa Bueno, F. C. Pagnocca, and M. J. Aparecida Hebling, "Survival of *Atta sexdens* workers on different food sources," *Journal of Insect Physiology*, vol. 49, no. 4, pp. 307–313, 2003.
- [18] F. C. Pagnocca, S. B. Ribeiro, V. L. V. Torkomian et al., "Toxicity of lignans to symbiotic fungus of leaf-cutting ants," *Journal of Chemical Ecology*, vol. 22, no. 7, pp. 1325–1330, 1996.
- [19] C. H. Miyashira, D. G. Tanigushi, A. M. Gugliotta, and D. Y. A. C. Santos, "Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa* Forel in two culture media," *Brazilian Journal of Microbiology*, vol. 41, no. 2, pp. 506–511, 2010.
- [20] O. C. Bueno, M. S. C. Morini, F. C. Pagnocca, M. J. A. Hebling, and O. A. Silva, "Sobrevivência de operárias de *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) isoladas do formigueiro e alimentadas com dietas artificiais," *Anais da Sociedade Entomológica do Brasil*, vol. 26, pp. 107–113, 1997.
- [21] R. Elandt-Johnson and N. L. Johnson, *Survival Models and Data Analysis*, John Wiley & Sons, New York, NY, USA, 1980.
- [22] X. Wang, Y. Lin, Y. Geng, F. Li, and D. Wang, "Preparative separation and purification of sesamin and sesamol from sesame seeds by high-speed counter-current chromatography," *Cereal Chemistry*, vol. 86, no. 1, pp. 23–25, 2009.
- [23] D. Enders and M. Milovanovic, "Asymmetric synthesis of (+)-hinokinin, (+)-dehydrocubebin and cubebin dimethyl ether, a new lignin from *Phyllanthus niruri*," *Zeitschrift für Naturforschung*, vol. 62B, pp. 117–120, 2007.

- [24] G. B. Messiano, L. Vieira, M. B. Machado, L. M. X. Lopes, S. A. De Bortoli, and J. Zukerman-Schpector, "Evaluation of insecticidal activity of diterpenes and lignans from *Aristolochia malmeana* against *Anticarsia gemmatalis*," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 8, pp. 2655–2659, 2008.
- [25] P. M. L. Santos, J. Schripsema, and R. M. Kuster, "Flavonoídes O-glicosilados de *Croton campestris* St. Hill. (Euphorbiaceae)," *Revista Brasileira de Farmacognosia*, vol. 15, pp. 321–325, 2005.
- [26] B. Bennini, A. J. Chulia, M. Kaouadji, and F. Thomasson, "Flavonoid glycosides from *Erica cinerea*," *Phytochemistry*, vol. 31, no. 7, pp. 2483–2486, 1992.
- [27] L. Krenn, A. Miron, E. Pemp, U. Petr, and B. Kopp, "Flavonoids from *Achillea nobilis* L.," *Zeitschrift für Naturforschung C*, vol. 58, no. 1-2, pp. 11–16, 2003.
- [28] L. Nebo, A. P. Terezan, S. P. Cardoso et al., "Activity of extracts and fractions from *Anacardium humile* in the nest of leaf-cutting ants," in *Proceedings of the 1st American Meeting of Chemical Ecology*, p. 141, Colonia del Sacramento, Uruguai, 2010.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

