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SEPARATION AND CHARACTERIZATION OF *METOPIUM BROWNEI* URUSHIOL COMPONENTS

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Key Word Index—*Metopium brownei*; Anacardiaceae; urushiol; 3-pentadecylcatechol; 3-(10′*Z*-pentadecenyl)catechol; 3-(10-′*Z*, 13′*E*-pentadecadienyl)catechol; *Fusarium oxysporum*; *Helminthosporium longirostratum*; antifungal activity; brine shrimp test.

Abstract—The composition of *Metopium brownei* 3-*n*-alk(en)ylcatechols (urushiol) was established by GC-mass spectrometry analysis of their corresponding TMSi ether derivatives. Bioactivity-directed fractionation of the acetone extract from the fresh stem bark of *M. brownei* rendered a mixture of three 3-*n*-penta-dec(en)ylcatechols with antifungal properties and toxicity to brine shrimp. HPLC separation of the methylated mixture allowed the isolation of 3-(10′*Z*,13′*E*-pentadecadienyl)catechol, a new natural product, 3-penta-decylcatechol and 3-(10′*Z*-pentadecenyl)catechol as their methyl derivatives. The structures of the isolated compounds were elucidated by spectral means.

INTRODUCTION

Metopium brownei (Jacq.) Urban (Anacardiaceae) is a medicinal tree (8–25 m high) which grows exclusively in the Gulf of México, from Veracruz to Quintana Roo. The plant is locally known by the names of 'chechém negro', 'box chechém', 'kabal chechém' and 'madera negra venenosa' [1, 2]. *M. brownei*, like poison ivy and many other plants of the Anacardiaceae family, causes irritation, inflammation, and blistering of the skin of sensitive individuals. Several medicinal properties such as antiviral, cathartic, diaphoretic, antirheumatic and sedative are attributed to this species [1, 2]. The beautiful lustre, colour and close graining of its wood makes it one of the most attractive timber tree species of the region for cabinet work [1]. Previous phytochemical work with the wood of this species led to the detection of eight flavonoids by paper chromatography [3, 4]. In our continuing search for bioactive metabolites from Mexican medicinal plants [5–7], the isolation and characterization of the major allergenic and fungitoxic compounds from the bark of *M. brownei* are described.

* Taken in part from the M.Sc. thesis of José F. Rivero-Cruz.

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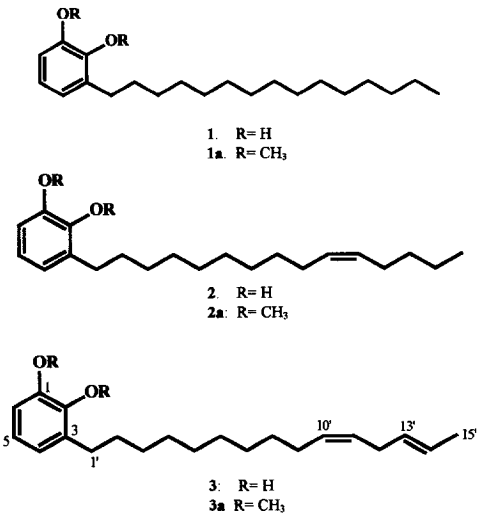
RESULTS AND DISCUSSION

Composition of urushiol from the stem bark of M. brownei

The primary technique for the detection of 3-*n*-alk(en)ylcatechols (urushiol) present in the stem bark of *M. brownei* was GC-mass spectrometry. The components were analyzed as their TMSi derivatives, which were obtained by treatment of the crude acetone extract with Sigma Sil-A [8]. The suitability of GC-mass spectrometry for the analysis of TMSi derivatives of 3-*n*-alk(en)yl catechols has been previously demonstrated [8–10, *inter alia*]. The urushiol composition of a sample obtained from the stem bark, as well as the relative concentration and R_t of the components are given in Table 1. According to the results summarized in Table 1 the sample consisted largely of 3-*n*-pentadec(en)ylcatechols (99.11%), while the homologous *n*-C_{17}-substituted catechols were present in trace levels (0.89%). With respect to the relative proportions of each alk(en)ylcatechol, the mono- and diolefinic congeners of 3-*n*-pentadecylcatechol were predominant. A similar composition has been described for the urushiols from poison ivy and poison wood [8, 11, 12]. All of the catechol TMSi ethers exhibited typical mass frag-

Table 1. Composition of urushiols from the fresh stem bark of *Metopium brownei*

Type of alk(en)ylcatechols	R_t (min)	% of each alk(en)ylcatechol in the mixture
bis-TMSi-*n*-C_{15}-catechols		
Saturated	24.23	21.16
Monoene	24.54	38.52
Diene	24.69	39.43
bis-TMSi-*n*-C_{17}-catechols		
Saturated	26.22	0.30
Monoene	26.31	0.59



mentation patterns (see Experimental) previously described for similar compounds [8–10].

Bioactivity guided isolation of 3-*pentadecylcatechol*; (**1**), 3-(10′Z-*pentadecenyl*)*catechol* (**2**); *and* 3(10′Z, 13′E-*pentadecadienyl*)*catechol* (**3**) *as their methyl derivatives from the stem bark of* M. brownei.

The acetone extract from the stem bark was evaluated for its potential antifungal property against *Fusarium oxysporum* and *Helminthosporium longirostratum* [5–7]. The extract inhibited the radial growth of *H. longirostratum* by 30% at the concentration of 200 μg ml^{-1}. *Fusarium oxysporum* was less sensitive to the extract since at the same concentration the radial growth was only inhibited by 5% (Fig. 1). The extract also displayed significant toxicity to brine shrimp larvae (BST) [13]; the LC_{50} was 123.93 μg ml^{-1}. The active extract was fractionated, using the BST and the bioautographic antifungal test [14] at each step for activity directed fractionation, to yield only one active fraction (F1-SB-2). F1-SB-2 was toxic to *Artemia salina* (BST LC_{50} = 89.13 μg ml^{-1}) and also exhibited antifungal activity against *H. longirostratum* and *F. oxysporum* (Fig. 1) at the concentration range between 150 and 700 μg ml^{-1}; the inhibition of the radial growth of the phytopathogenic fungi was comparable to that induced by Captan, a commercial fungicide employed as a positive control. According to the GC-mass spectrometry analysis, active fraction F1-SB-2 contained the three *n*-C_{15} alk-(en)yl catechols detected in the crude extract. HPLC separation of the methylated catechol active mixture allowed the isolation of compounds **1**–**3** as their methyl derivatives and their structures were determined by spectroscopic means. Compound **3** is a new natural product, while compounds **1** and **2** have been previously isolated from *Rhus vernicifera* and *Gluta renghas* [15–17].

The MS spectrum of methyl derivative **3a** exhibited an intense molecular ion at *m/z* 344, as well as prominent peaks at *m/z* 151, 136, 121 and 91, in harmony with the general fragmentation pattern described for 3-*n*-alk(en)yl catechols [8–10, 15–17]. The NMR spectra of **3a** (Experimental) displayed typical signals for a 1,2,3-trisubstituted benzene ring [ABC system, δ_H 6.9 (*dd*, *J* = 7.9, 7.9 Hz, H-5), 6.8 (*dd*, *J* = 7.9, 1.8 Hz, H-6), and 6.53 (*dd*, *J* = 7.9, 1.8 Hz, H-4); δ_C 149 (C-1), 148.14 (C-2), 136.0 (C-3), 123.69 (C-4), 122.4 (C-5) and 110.9 (C-6)] and for two methoxyl groups [δ_H 3.76 (*s*) and 3.35 (*s*); δ_C 60.2 and 55.32]. In addition, the spectra showed the presence of a 3-*n*-C_{15} alkenyl chain possessing two disubstituted double bonds [δ_H 5.5–5.45 (*m*, H-10′, H-11′, H-13′ and H-14′); δ_C 130.7 (C-13′ and C-14′), 130.2 (C-10′ and C-11′)], and one terminal allylic methyl group [δ_H 1.59 (*d*, *J* = 4.0 Hz, H-15′); δ_C 17.9 (C-15′)], which in turn was consistent with the disposition of one of the unsaturations between C-14′ and C-13′. The methylene signal at δ_H 2.73 (*t*, *J* = 8.0 Hz, H-1′), which showed a cross peak with the aromatic proton at C-4 in the NOESY spectrum, was assigned to the benzilic protons. On the other hand, the methylene observed at the lowest field was attributable to that of C-12′ [δ_H 2.80 (*m*, H-12′); δ_C 22.7 (C-12′)], and was consonant with the second unsaturation being located between C-11′ and C-10′. The second allylic methylene was observed at δ_H 2.05 (*m*, H-9′) and δ_C 27.49 (C-9′). Finally, the signals for the remaining methylenes were observed as an apparent broad singlet in the region δ_H 1.23–1.39 (H-3′–H-8′) in the ^{1}H NMR, and at δ_C 30.0 (C-3′–C-8′) in the ^{13}C NMR spectra. The stereochemistry of the double bond $\Delta^{10'-11'}$ was determined to be *Z* from the diagnostic shifts of the two allylic methylene carbons (δ 27.4 and 22.7), as these would have appeared at *ca* δ_C 33.0 if the bond were *E* [18, 19]. In the case of the $\Delta^{13'-14'}$ unsaturation, the *E* configuration was assumed on the basis of the carbon chemical shift observed for the terminal methyl group [δ_C 17.4] [18, 19]. It is important to note that the double bond positioning at the alkenyl side chain of compound **3** is unusual.

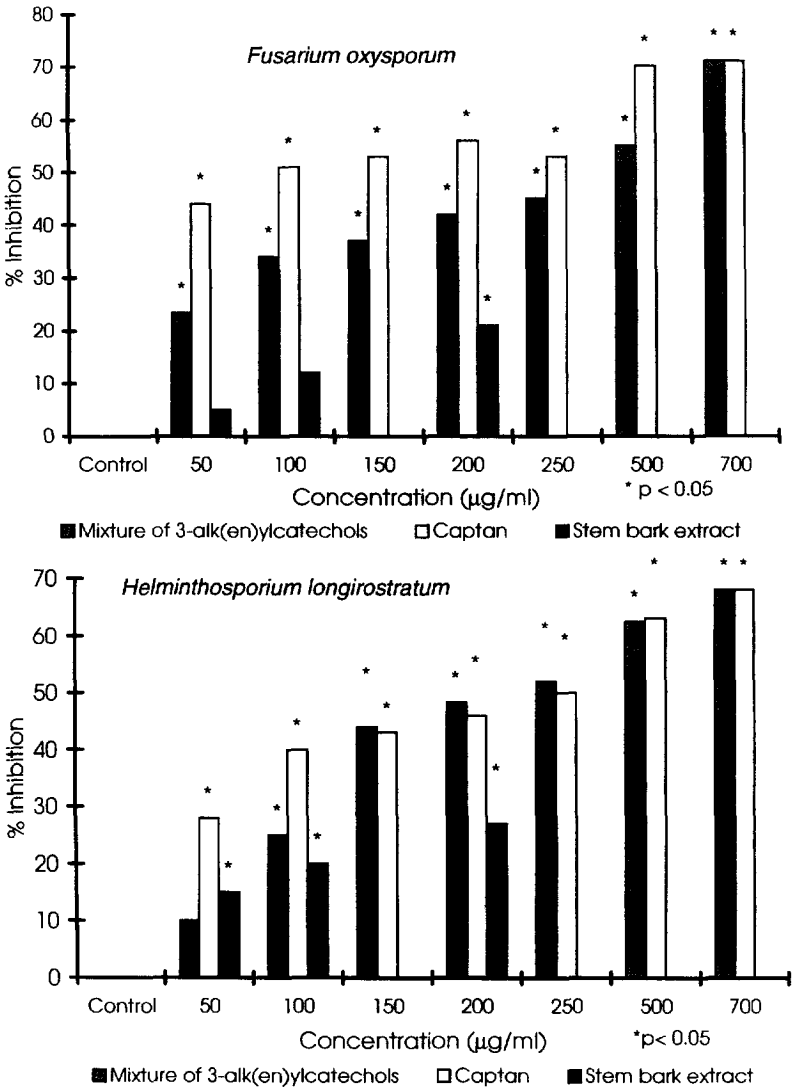


Fig. 1. Antifungal activity of the acetone extract and urushiol from *Metopium brownei* against *Helminthosporium longistratum* and *Fusarium oxysporum*.

The NMR spectra (Experimental) of derivatives **1a** and **2a** (not previously described) showed features in common with those of compound **3a**. Comparative analysis indicated that the 10′*Z*, 13′*E*-pentadecadienyl grouping in **3a** was replaced by pentadecyl and 10′*Z*-pentadecenyl moieties at C-3 in **1a** and **2a**, respectively. Complete characterization of compound **2a** required localization of the unsaturation which was accomplished by epoxidation of the double bond using *m*-chloroperbenzoic acid, followed by GC-mass spectrometry analysis of the resulting epoxide. Cleavage between carbons bearing the epoxide function yielded fragments at *m/z* 305, 263, 99 and 57, indicating that the double bond of the parent compound **2a** occurred between C-10′ and C-11′. As in the case of compound **3a**, the stereochemistry of the double bond was determined to be *Z* because of the carbon chemical shift [δ_C 27.6] displayed by the allylic methylene carbons [18, 19]. The mass spectra of compounds **1a** and **2a** were in agreement with those previously described [10].

CONCLUSIONS

The results from the present investigation indicated that the stem bark of *M. brownei* contains alk(en)ylcatechol-type allergens, having mainly 15 carbons unsaturated alkyl side chains, as do most of the allergenic Anacardiaceae species that have been studied [8, 11, 12, *inter alia*]. Therefore, use of this plant as a medicinal agent represents a considerable hazard in

Southeastern México, where the plant is particularly widespread. It is important to point out that the brine shrimp test turned out to be a simple and efficient method to detect the presence of alk(en)ylcatechols.

The urushiol from *M. brownei* exhibited remarkable antifungal activity against two phytopathogenic fungi; however, the allergenic properties of these compounds likely preclude their development into environmentally safe fungicides. Nevertheless, the antifungal activity elicited by the urushiol could be of importance in the defence mechanism of the plant, preventing attack by some fungi and bacteria.

EXPERIMENTAL

General. Mps uncorr. IR KBr pellets (solids) or NaCl discs (oils films). ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-3005 instrument operating at 300 and 75 MHz, respectively, in $CDCl_3$ or C_6D_6 with TMS as an int. standard. GC-MS analyses were conducted on a Hewlett–Packard Model 5890 gas chromatograph interfaced with a Jeol JMS AX50HA mass spectrometer. The GC column was PAS-1701 tested silicone (25 m × 0.32 mm i.d.). The linear temperature programming was from 150–260°, at the rate of 10° min^{-1}; and the carrier gas was He (0.6 psi, 2 ml min^{-1}). The total duration of analysis was 40 min per injection. EI mass spectra were obtained using ionization voltage of 70 eV. Semi-prep. HPLC was performed on a μBondapack RP-18 column (10 μm, 3.9 i.d. × 300 mm, Waters) at a flow rate of 2 ml min^{-1}. Analytical and prep. TLC were performed on precoated silica gel 60 F_{254} plates (Merck). Argentation TLC was carried out with silica gel plates impregnated with a 10% soln of $AgNO_3$. TLC spots were visualized by spraying with a 10% soln of $Ce(SO_4)_2$ in 2N H_2SO_4, followed by heating at 110° or with a 1% soln $FeCl_3$ in EtOH. For open CC, silica gel 60 (70–230 mesh, Merck) was used; for flash CC, silica gel (40 μm, Baker) was employed.

Plant material. The stem bark of *M. brownei* was collected in Municipio de Tulum, Quintana Roo, México, in November 1995. A voucher specimen (A.L.95-16) is deposited in the National Herbarium (MEXU), Instituto de Biologia (UNAM).

Extraction of the fresh stem bark of M. brownei. Fresh stem bark (574 g wet wt) was extracted × 3 at room temp for 24 hr with Me_2CO. The mixt. was filtered and the Me_2CO evapd *in vacuo* (BST LC_{50} = 123.93 μg ml^{-1}).

GC-MS analysis of urushiol from the stem bark of M. brownei. An aliquot of the acetone extract (1 mg) was treated with 1 ml of Sigma Sil-A (trimethylchlorosilane–hexadimethyl silane–pyridine 1:3:9) for 30 min at room temp under N_2 atmosphere and then directly subjected to GC-MS analysis. *bis*-TMSi-3-pentadecylcatechol. R_t = 24.23 min; EIMS *m/z* (rel. int.): 464 [M^+ (48)], 449 (6), 269 (4), 268 (8), 179 (10), 79 (100). *bis*-TMSi-3-(10′*Z*-pentadecenyl)catechol. R_t = 24.54 min; EIMS *m/z* (rel. int.): 462 [M^+ (100)], 447 (25), 268 (17), 267 (20), 179 (18), 147 (5), 73 (95), 52 (57). *bis*-TMSi-3-(10′ *Z*, 13 *E*-pentadecadienyl)-catechol R_t = 24.69 min; EIMS *m/z* (rel. int.): 460 [M^+ (100)], 445 (10), 267 (12), 268 (4), 179 (8), 147 (12), 79 (100), 73 (50), 52 (58). *bis*-TMSi-3-heptadecylcatechol. R_t = 26.22 min, EIMS *m/z* (rel. int.): 490 [M^+ (42)], 355 (10), 268 (9), 267 (7), 179 (8), 73 (64). *bis*-TMSi-3-heptadecenylcatechol. R_t = 26.31 min, EIMS (70 eV) *m/z* (rel. int.): 488 [M^+ (100)], 473 (10), 268 (11), 267 (12), 179 (12), 73 (82).

Isolation of 3-*pentadecylcatechol* (**1**), 3-(10′*Z*-*pentadecenyl*)*catechol* (2) *and* 3-(10′*Z*, 13′*E*-*pentadecadienyl*)*catechol* (3) *as their methyl derivatives.* The Me_2CO extract (6 g) was partitioned between $CHCl_3$ (FoSB-1) and water (FoSB-2). After elimination of the solvent *in vacuo*, FoSB-1 yielded 4.12 g of a brown residue (BST LC_{50} = 110.19 μg ml^{-1}) while FoSB-2 yielded 2.25 g (BST LC_{50} > 1000 μg ml^{-1}). According to the bioautographic bioassay, the antifungal activity was concd in the organic phase FoSB-1, and was localized at R_f 0.45 in the $CHCl_3$–MeOH (95:5) system. FoSB-1 (4 g) was further fractionated by flash CC on silica gel (32 g) using a solvent gradient of 40% $CHCl_3$–hexane to 50% MeOH–$CHCl_3$. Frs were combined based on TLC profiles to give eight pooled frs (F1SB-1 to F1SB-8). Biological testing showed that F1-SB-2 (677 mg) was toxic to *A. salina* (BST LC_{50} = 89.2 μg ml^{-1}) and significantly inhibited the radial growth of the phytopathogenic fungi. This fr. also showed a positive $FeCl_3$ test. A portion of F1SB-2 (250 mg) in Et_2O (5 ml) was allowed to react with an excess of an Et_2O soln. of CH_2N_2 during 1 week at 4° to yield, after evapn of the solvent, 268 g of methylated F1SB-11. The methylated fraction was column chromatographed on silica gel (23 g) impregnated with $AgNO_3$ (15%) using hexane gradually enriched with EtOAc as the eluant. Elution with hexane–EtOAc (95:5) yielded a mixt. of **1a**–**3a** (162.5 mg), which were sepd by RP-HPLC (see General) with CH_3CN–H_2O–CH_3COOH (80:20:2) to give **3a** (65 mg, R_t = 8.99 min), **3a** (43.89 mg, R_t = 15.38 min) and **1a** (15.1 mg, R_t = 25 min). A second portion of F1SB-2 (5 mg) was treated with 0.5 ml of Sigma Sil-A for 30 min at room temp, under N_2 atmosphere and directly subjected to GC-MS analysis.

O-*Dimethyl*-3-*pentadecylcatechol* (**1a**). Oil ^1H NMR (benzene-d_6): δ 6.94 (*dd*, *J* = 7.5 Hz, H-5), 6.68 (*m*, H-4 and H-6), 3.85 (*s*, –OCH_3), 3.81 (*s*, –OCH_3), 2.60 (*t*, *J* = 8.0, H-1′), 1.57–1.31 (*br s*, H-2′–H-14′), 0.89 (*t*, *J* = 5.1 Hz, H-15′); ^{13}C NMR (benzene-d_6): δ 153.8 (C-1), 148.1 (C-2), 136.9 (C-3), 123.7 (C-4), 122.4 (C-5), 110.9 (C-6), 60.22 (–OCH_3), 55.34 (–OCH_3), 30.50 (C-1′) 29.58 (C-2′–C14′), 14.2 (C-15′).

O-*Dimethyl*-3-(10′*Z*-*pentadecenyl*)*catechol* (**2a**). Oil. IR ν_{max} cm^{-1}: 2929, 1600, 1468, 1371, 1161, 1014, 770 and 730; ^1H NMR (benzene-d_6): δ 6.90 (*dd*, *J* = 7.9 Hz, H-5), 6.80 (*dd*, *J* = 7.9, 1.8 Hz, H-6), 6.53 (*dd*, *J* = 7.9, 1.8 Hz, H-4), 5.58 (*t*, *J* = 5.6 Hz, H-10′ and H-11′), 3.76 (*s*, –OCH_3), 3.35 (*s*, –OCH_3), 2.7 (*t*,

$J = 7.9$, H-1′), 2.02 (*m*, H-9′ and H-12′), 1.60 (*m*, H-2′), 1.27–1.40 (*m*, H-3′–H-8′, H-13′ and H-14′), 0.88 (*t*, $J = 5.1$ Hz, H-15′); ^{13}C NMR (benzene-d_6): δ 153.8 (C-1), 148.1 (C-2), 136.9 (C-3), 130.0 (C-10′, C-11′), 123.7 (C-4), 122.4 (C-5), 110.9 (C-6), 60.22 (–OCH_3), 55.34 (–OCH_3), 30.50 (C-1′), 30.0 (C-3′–C-8′, C-13′ and C-14′), 27.6 (C-9′ and C-10′), 14.2 (C-15′); EIMS *m/z* (rel. int.): 346 [M^+ (100)], 191 (5), 136 (50), 121 (15), 91 (20), 55 (13), 41 (10).

O-*Dimethyl*-3-(10′Z, 13′E-*pentadecadienyl*)*catechol* (**3a**). Oil. IR ν_{max} cm^{-1}: 2727, 2856, 1600, 1450, 1172, 1014, 770 and 730; ^{1}H NMR (benzene-d_6): δ 6.9 (*dd*, $J = 7.9$ Hz, H-5), 6.8 (*dd*, $J = 7.9$, 1.8 Hz, H-6), 6.53 (*dd*, $J = 7.9$, 1.8 Hz, H-4), 5.5–5.45 (*m*, H-10′, H-11′, H-13′ and H-14′), 3.76 (*s*, –OCH_3), 3.35 (*s*, –OCH_3), 2.80 (*m*, H-12′), 2.73 (*t*, $J = 8$ Hz, H-1′), 2.05 (*m*, H-9′), 1.68 (H-2′), 1.59 (*d*, $J = 4.0$ Hz, H-15′), 1.23–1.39 (*m*, H-3′–H-8′); ^{13}C NMR (benzene-d_6): δ 149 (C-1), 148.14 (C-2), 136.0 (C-3), 130.7 (C-13′ and C-14′), 130.2 (C-10′ and C-11′), 123.69 (C-4), 122.4 (C-5), 110.9 (C-6), 60.2 (–OCH_3), 55.32 (–OCH_3), 30.8 (C-1′), 30.2 (C-2′), 30.0 (C-3′–C-8′, 27.49 (C-9′), 22.7 (C-12′), 17.9 (C-15′); EIMS *m/z* (rel. int.): 344 [M^+ (55)]. 276 (9), 262 (11), 248 (6), 191 (23), 164 (25), 151 (100), 136 (60), 121 (18), 95 (17), 91 (28), 81 (17), 55 (12).

Epoxidation of compound **2a**. **2a** (5 mg) was dissolved in CH_2Cl_2 (5 ml) and allowed to react with *m*-chloroperbenzoic acid (5 mg) at room temp for 24 hr. The reaction mixt. was processed using standard techniques to afford an oily surface (3 mg). EIMS *m/z* (rel. int.): 362 [M^+ (30)], 305 (10), 276 (25), 262 (11), 207 (1), 191 (15), 177 (35), 151 (100), 137 (70), 136 (60), 121 (18), 91 (25), 81 (15), 77 (11), 57 (11), 43 (12).

Brine shrimp lethality test (*BST*) *bioassay*. The extracts, frs, isolated compounds and derivatives were evaluated for lethality to brine shrimp larvae as described [13].

Bioassay with phytopathogenic fungi. The target species were *Fusarium oxysporum* and *Helminthosporium longirostratum*. The bioassays were carried out using the procedures previously described [14, 20]. The measurements were made after 72 hr of incubation. The mean value of three applications were analyzed by ANOVA ($P < 0.05$). The extract was evaluated at 50, 100 and 200 μg ml^{-1}. Finally, the catechol mixt. F1SB-2, was evaluated at 50, 100, 200, 300, 500 and 700 μg ml^{-1}. Captan, a commercial fungicide, was used as a positive control.

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