Antifungal Amides from *Piper scutifolium* and *Piper hoffmanseggianum*

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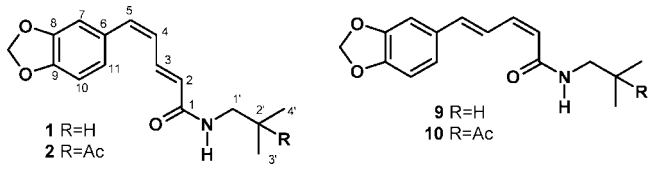
Chromatographic fractionation of a dichloromethane extract from the leaves of *Piper scutifolium* yielded two new isobutyl amides, scutifoliamide A (**1**) and scutifoliamide B (**2**), together with the known compounds piperolactam C (**3**), piperovatine (**4**), piperlonguminine (**5**), corcovadine (**6**), isopiperlonguminine (**7**), and isocorcovadine (**8**). From the dichloromethane extract from the leaves of *P. hoffmannseggianum* two new isobutyl amides, hoffmannseggiamide A (**9**) and hoffmannseggiamide B (**10**), were obtained together with the known compounds isopiperlonguminine (**7**) and isocorcovadine (**8**), sitosterol, and stigmasterol. The structures of the new compounds were established on the basis of spectroscopic data analysis. The inhibitory activity of compounds **1**−**10** against the growth of the fungi *Cladosporium sphaerospermum* and *C. cladosporioides* was determined by bioautography.

Chemical studies carried out on Piperaceae species have revealed the occurrence of amides,[1–3] chromenes,[4] pyrones,[5] prenylated hydroquinones,[6] and benzoic acids,[4] of which some show fungitoxic potential.[3–6] In the course of a search for antifungal derivatives from *Piper* species, crude extracts from the leaves of *Piper scutifolium* Jack. and *P. hoffmannseggianum* Roem. & Schult. showed potential against the fungi *Cladosporium cladosporioides* and *C. sphaerospermum* and were submitted to fractionation procedures aimed at the isolation of antifungal compounds. Chromatographic procedures yielded scutifoliamide A (**1**), scutifoliamide B (**2**), piperolactam C (**3**), piperovatine (**4**), piperlonguminine (**5**), corcovadine (**6**), isopiperlonguminine (**7**), and isocorcovadine (**8**) from *P. scutifolium* and compounds **7**, **8**, hoffmannseggiamide A (**9**), hoffmannseggiamide B (**10**), sitosterol, and stigmasterol from *P. hoffmaseggianum*. The structures of the new compounds were determined on the basis of their spectroscopic data, and these new compounds were found to contain the rare 2,5-*trans,cis* (**1** and **2**) and 2,5-*cis,trans* (**9** and **10**) configurations for the conjugated double bonds.



The CH_2Cl_2 extract from leaves of *P. scutifolium* was submitted to chromatographic separation over silica gel to afford scutifoliamide A (**1**), scutifoliamide B (**2**), piperolactam C (**3**), piperovatine (**4**), piperlonguminine (**5**), corcovadine (**6**), isopiperlonguminine (**7**), and isocorcovadine (**8**).

The ^{1}H NMR spectrum of **1** displayed signals of aromatic hydrogens at δ 6.70 (m, 2H) and 7.10 (m, 1H), conjugated double bond protons at δ 6.00 (d, J = 14.5 Hz, 1H), 6.23 (t, J = 11.4 Hz, 1H), 6.62 (d, J = 11.4 Hz, 1H), and 7.72 (dd, J = 14.5 and 11.4 Hz), and one singlet at δ 6.02 (s, 2H) assigned to a methylenedioxy group. The presence of signals at δ 3.17 (t, J = 6.6 Hz, 2H), 1.80 (m, 1H), and 0.93 (d, J = 6.6 Hz, 6H) associated with the data described above was indicative of an isobutylamide derivative.[7] The ^{13}C NMR and DEPT 135° spectra showed signals for one carbonyl at δ 166.2, one methylenedioxy group at δ 101.2, 10 sp^2 carbons at δ 147.9−108.4, and three aliphatic carbons at δ 47.0 (CH_2), 28.6 (CH), and 20.1 (CH_3). Comparison of the NMR data with those reported for piperlonguminine[8] was indicative of a similar structure but with a different stereochemistry due to the differences in the chemical shifts of the C-2/C-5 signals. Analysis of the coupling constant of the signal at δ 6.00 (J = 14.5 Hz) in the ^{1}H NMR spectrum, assigned to H-2, indicated a *trans* configuration at δ.[2] Similarly, the doublet attributed to H-5 showed J = 11.4 Hz, which is indicative of a *cis* configuration at the C-4/C-5 double bond.[9,10] Therefore, the structure of **1** was determined as 5-(8,9-methylenedioxy)-2-*trans*-4-*cis*-pentadienyl-*N*-(2′-methylpropyl)amide, and this compound has been named scutifoliamide A.

The ^{1}H NMR spectrum of **2** showed a similar profile to that obtained for compound **1** as a result of the signals of the conjugated double bond hydrogens at δ 5.90 (d, J = 14.7 Hz, H-2), 6.25 (t, J = 11.4, H-4), 6.65 (t, J = 11.4 Hz, H-5), and 7.74 (dd, J = 14.7 and 11.4 Hz, H-3), the aromatic hydrogens at δ 6.74−6.98 (m), and the methylenedioxy group at δ 5.97 (s, 2H). However, this spectrum showed a doublet at δ 3.58 (J = 6.4 Hz, 2H), assigned to H-1′, an intense singlet at δ 1.45 (6H), attributed to H-3′/H-4′, and an additional signal at δ 2.03 (s, 3H), suggesting the presence of an acetoxyl group in the structure of **2**, as observed previously for corcovadine.[8] The ^{13}C NMR and DEPT 135° spectra showed sp^2 carbon peaks at δ 147.9−108.4 and resonances for a methylenedioxy group at δ 101.2 and two carbonyl groups at δ 166.1 (amide) and 170.9 (ester). These data were associated with signals of three aliphatic carbons at δ 22.4 (CH_3), 24.1 (CH_3), and 82.3 (C, a carbinolic carbon), which confirmed the presence of an acetoxyl group linked at C-2′. Comparison of the NMR data of **1**, **2**, and corcovadine[8] allowed the characterization of **2** (scutifoliamide B) as 5-(8,9-methylenedioxy)-2-*trans*-4-*cis*-pentadienyl-*N*-(2′-acetoxy-2′-methylpropyl)amide.

The CH_2Cl_2 extract from leaves of *P. hoffmannseggianum* was subjected to chromatographic separation over silica gel and Sephadex LH-20 to afford isopiperlonguminine (**7**), isocorcovadine (**8**), sitosterol, stigmasterol, and two fractions composed of mixtures of **7** + **10** (fraction A) and **8** + **9** (fraction B). Although these

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fractions appeared to be homogeneous by TLC, analysis of their ^{1}H NMR spectroscopic data indicated that they contained a mixture of closely related derivatives, not readily separable by reversed-phase HPLC procedures. Since compounds **7** and **8** were isolated in pure form, the individual resonance signals of **9** and **10** could be discerned.

The molecular formula $C_{15}H_{19}O_3N$ assigned to compound **9** was determined by analysis of ^{13}C NMR spectroscopic data associated with HRESIMS. The IR spectrum showed a strong absorption band at 1627 cm^{-1}, which was indicative of a carbonyl group of a conjugated amide. Similar to compounds **1** and **2**, the ^{1}H NMR spectrum showed a 1,3,4-trisubstituted aromatic ring due to the signals at δ 7.02 (d, J = 1.8 Hz), 6.70 (d, J = 7.8 Hz), and 6.89 (dd, J = 7.8 and 1.8 Hz) and a methylenedioxy group at δ 5.98 (s, 3H). The presence of an isobutyl moiety was determined by signals at δ 3.10 (d, J = 6.6 Hz, 2H), 1.80 (m, 1H), and 0.89 (d, J = 6.9 Hz, 6H) assigned to H-1′, H-2′, and H-3′/4′, respectively. This spectrum also showed signals referring to hydrogens of double bonds at δ 5.61 (d, J = 11.4 Hz, 1H), 6.47 (t, J = 11.4 Hz, 1H), 6.66 (d, J = 15.8 Hz, 1H), and 8.08 (dd, J = 15.8 and 11.4 Hz, 1H), which were indicative of an $\alpha,\beta,\gamma,\delta$-unsaturated system. Comparison of the ^{1}H NMR data of **9** with those reported for 1-[1-oxo-5-(3,4-methylenedioxyphenyl)-2-*cis*-4-*trans*-pentadienyl]pyrrolidine isolated from *Piper nigrum*[11] suggested a *trans,cis* geometry for double bonds Δ^2 and Δ^4, since the chemical shift for H-4 (δ 8.08) is characteristic of C-3/C-4 double bonds, which was confirmed by values of the coupling constants for H-5 (15.8 Hz) and H-2 (11.4 Hz), respectively. Comparison of ^{13}C NMR spectroscopic data described for a pyrrolidine model,[11] to **5** and **7**, allowed the determination of **9** as 5-(8,9-methylenedioxy)-2-*cis*-4-*trans*-pentadienyl-*N*-isobutylamide, which has been named hoffmannseggiamide A.

The ^{1}H NMR data of compound **10** showed similarities to those observed for compound **9**, especially in the low-field region of the spectrum. The signals at δ 5.61 (d, J = 11.2 Hz, H-2), 6.54 (t, J = 11.2 Hz, H-3), 8.03 (dd, J = 15.8 and 11.2 Hz, H-4), and 6.67 (d, J = 15.8 Hz, H-5) were also assigned to the $\alpha,\beta,\gamma,\delta$-unsaturated system containing *trans,cis* geometry as proposed for compound **9**. However, a difference between **9** and **10** was based on the observation of one singlet at δ 1.39 (s, 6H) in **10**, assigned to methyl groups (H-3′ and H-4′) connected to a tertiary carbinolic carbon. Comparison between ^{13}C NMR data of **10** and **9** indicated two additional peaks at δ 21.3 (CH_3) and 169.8 (C=O), which were assigned to an acetoxyl group at C-2′. Finally, comparison of NMR spectroscopic data to the 2′-acetoxy-2′-methylpropyl moiety of compounds **10** and **6**, along with the HREIMS, which showed a molecular ion peak at m/z 354.1311 [M = Na]$^+$, corresponding to a molecular formula of $C_{18}H_{21}O_5N$, corroborated the proposed structure. Thus, compound **10** (hoffmannseggiamide B) was determined as 5-(8,9-methylenedioxy)-2-*cis*-4-*trans*-pentadienyl-*N*-(2′-acetoxy-2′-methylpropyl)amide.

The known compounds **3**−**8**, **11**, and **12** were identified by comparison of their spectroscopic data with reported values.[8,12−14]

All compounds were assayed by means of a bioautographic method against *Cladosporium sphaerospermum* and *C. cladosporioides* (Table 3) and exhibited considerable activity with a detection limit of 1.0 μg for the known compounds **4**−**6**, similar to the controls nystatin (1.0 μg) and miconazole (1.0 μg). Compound **7** showed an antifungal activity 4 times higher than the reference compounds. The new amides **1** and **2** as well as the mixtures composed of **7** + **10** and **8** + **9** indicated moderate activity, with the threshold activity limit determined as 5.0 μg for these amides.

The geometry of double bonds appears to be involved in antifungal activity, since compound **7** (0.25 μg), with a *cis,cis* configuration, is 20 times more active than the *trans,cis* isomer **1** (5.0 μg). In the case of the acetate derivatives, the detection limit for **8** (2.5 μg) was half of that determined for **2** (5.0 μg). When compound **7** (*cis,cis*) was mixed with **10** (*cis,trans*), the minimum amount required to inhibit the fungal growth was determined as 5.0 μg, 20 times higher than pure **7**. A similar response was observed with the mixture **8** + **9** (4:1, determined by ^{1}H NMR spectroscopy), in which the threshold limit (5.0 μg) was higher than pure **8** (2.5 μg).

P. scutifolium and *P. hoffmannseggianum* accumulate isomeric isobutyl amides containing 2,4-*trans,cis* and 2,4-*cis,trans* configurations, respectively. Although such stereochemical features for unsaturated amides are quite rare in nature, they have previously been observed in other *Piper* species such as *P. arboreum,*[10] *P. tuberculatum,*[10] *P. brachystachyum,*[15] *P. longum,*[16] *P. nigrum,*[11,17] and *P. officinarum.*[18]

Experimental Section

General Experimental Procedures. UV spectra were recorded in a UV/visible Shimadzu UV-1601PC spectrophotometer using $CHCl_3$ as solvent. IR spectra were obtained in a Perkin-Elmer model 1750 spectrometer. ^{1}H NMR spectra were recorded at 200 and 300 MHz and ^{13}C NMR at 50 and 75 MHz in Bruker DPX-300 and DRX-500 spectrometers, respectively. $CDCl_3$ (Aldrich) was used as solvent and TMS (Aldrich) as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (J) in Hz. HREIMS were obtained on a Bruker Daltonics MicroTOF mass spectrometer. LREIMS were measured in a HP 5990/5988A mass spectrometer, and GCLREIMS were measured in a Shimadzu GC-17A chromatograph interfaced with a MS-QP-5050A mass spectrometer. Temperature programming was performed from 150 to 300 °C at 15 °C/min, then isothermal at 300 °C for 5 min. The injector and detector temperatures were 150 and 320 °C, respectively, and helium was used as the carrier gas. Analytical HPLC was performed using a Dionex C_{18} (3 μm, 150 × 5 mm) column with a UVD-DAD 340U as detector. Silica gel (Merck, 230–400 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatographic separation, while silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) and preparative TLC (1.0 mm).

Plant Material. *Piper hoffmannseggianum* Roem. & Schult. and *P. scutifolium* Jack. leaves were collected in Ubatuba, SP, Brazil, in December 2000 and September 2002, respectively, and were identified by Dr. Elsie Franklin Guimarães (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro). Voucher specimens (Kato-127 and Kato-281) have been deposited at Herbarium of Instituto de Botânica−SEMA, São Paulo, SP, Brazil, and at the Herbarium of the Jardim Botânico do Rio de Janeiro, RJ, Brazil, respectively.

Extraction and Isolation. Dried and powdered leaves of *P. scutifolium* (10 g) were extracted with CH_2Cl_2 (three times at room temperature). Part of the crude extract (3 g) was subjected to flash silica gel column chromatography eluted with *n*-hexane containing increasing amounts of EtOAc (up to 90%), to give 17 fractions. Fractions 2 (12 mg) and 4 (40 mg) were individually purified using Sephadex LH-20 columns eluted with MeOH to afford pure **3** (3 mg) and **4** (20 mg), respectively. Fraction 6 (150 mg) was purified by silica gel column chromatography eluted with *n*-hexane containing increasing amounts of EtOAc (up to 100%), yielding 16 fractions (I−XVI). Compounds **6** (5 mg), **1** (1 mg), and **8** (7 mg) were obtained from fraction V (40 mg) after purification by silica gel preparative TLC (hexane−CH_2Cl_2−EtOAc, 1:1:1), while compounds **5** (8 mg), **7** (10 mg), and **2** (0.8 mg) were obtained from fraction VII (45 mg) after purification by silica gel preparative TLC (*n*-hexane−CH_2Cl_2−EtOAc, 1:1:1).

Dried and powdered leaves of *P. hoffmannseggianum* (7 g) were extracted with CH_2Cl_2 (three times at room temperature). After concentration in vacuo 770 mg of crude extract was obtained. A part of this extract (700 mg) was applied to a Sephadex LH-20 column and eluted with mixtures of *n*-hexane in CH_2Cl_2 (1:4) followed by CH_2Cl_2−Me_2CO (3:2 and 1:4) and finally with Me_2CO to give 100 fractions (10 mL each). After TLC analysis, these fractions were combined into nine groups (I–IX). Group III (111 mg) was submitted to silica gel column chromatography and eluted with *n*-hexane containing increasing amounts of EtOAc to give 13 fractions. Fraction 5 was composed of a mixture of **11** and **12** (5 mg). Fraction 9 (40 mg) was purified by silica gel preparative TLC eluted with CH_2Cl_2−MeOH (98:2) to give four bands (A–D). Band A (8 mg) was composed of pure **8**, and band C (15 mg) was composed of a mixture of **7** (64%) and **10** (36%). Group V (30 mg) was subjected to Sephadex LH-20

Table 1. ^{1}H and ^{13}C NMR (200 and 50 MHz, δ ppm, $CDCl_3$) Spectroscopic Data for **1** and **2**

	1		**2**	
position	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1		166.2 (C)		166.1 (C)
2	6.00 (d, 14.5)	123.5 (CH)	5.90 (d, 14.7)	123.6 (CH)
3	7.72 (dd, 14.5 and 11.4)	136.7 (CH)	7.74 (dd, 14.7 and 11.4)	137.1 (CH)
4	6.23 (t, 11.4)	126.4 (CH)	6.25 (t, 11.4)	126.4 (CH)
5	6.62 (d, 11.4)	136.1 (CH)	6.65 (d, 11.4)	136.3 (CH)
6		130.6 (C)		130.6(C)
7	6.70−7.10 (m)	108.4 (CH)	6.74−6.98 (m)	108.4 (CH)
8		147.9 (C)		147.9 (C)
9		147.8 (C)		147.9 (C)
10	6.70−7.10 (m)	109.2 (CH)	6.74−6.98 (m)	109.3 (CH)
11	6.70−7.10 (m)	125.8 (CH)	6.74−6.98 (m)	125.6 (CH)
1′	3.17 (t, 6.6)	47.0 (CH_2)	3.58 (d, 6.4)	48.2 (CH_2)
2′	1.80 (m)	28.6 (CH)		82.3 (C)
3′	0.93 (d, 6.6)	20.1 (CH_3)	1.45 (s)	24.1 (CH_3)
4′	0.93 (d, 6.6)	20.1 (CH_3)	1.45 (s)	24.1 (CH_3)
C(O)				170.9 (C)
CH_3			2.03 (s)	22.4 (CH_3)
OCH_2O	6.02 (s)	101.2 (CH_2)	5.97 (s)	101.2 (CH_2)

Table 2. ^{1}H and ^{13}C (300 and 75 MHz, δ ppm, $CDCl_3$) Spectroscopic Data for **9** and **10**

	9		**10**	
position	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1		165.1 (C)		165.5 (C)
2	5.61 (d, 11.4)	118.3 (CH)	5.61 (d, 1.2)	118.3 (CH)
3	6.47 (t, 11.4)	138.9 (CH)	6.54 (t, 11.2)	138.6 (CH)
4	8.08 (dd, 15.8, 11.4)	123.7 (CH)	8.03 (dd, 15.8, 11.2)	123.8 (CH)
5	6.66 (d, 15.8)	140.5 (CH)	6.67 (d, 15.8)	140.7 (CH)
6		131.2 (C)		130.1 (C)
7	7.02 (d, 1.8)	107.2 (CH)	7.07 (d, 1.7)	105.2 (CH)
8		147.1 (C)		148.2 (C)
9		147.0 (C)		148.1 (C)
10	6.70 (d, 7.8)	107.5 (CH)	6.72 (d, 8.1)	108.5 (CH)
11	6.89 (dd, 7.8, 1.8)	122.1 (CH)	6.90 (dd, 8.1, 1.7)	122.7 (CH)
1′	3.10 (t, 6.6)	45.9 (CH_2)	3.51 (d, 6.0)	46.7 (CH_2)
2′	1.80 (m)	28.6 (CH)		81.3 (C)
3′	0.89 (d, 6.9)	19.2 (CH_3)	1.39 (s)	23.1 (CH_3)
4′	0.89 (d, 6.9)	19.2 (CH_3)	1.39 (s)	23.1 (CH_3)
C(O)				169.8 (C)
CH_3			1.94 (s)	21.3 (CH_3)
OCH_2O	5.89 (s)	100.2 (CH_2)	5.88 (s)	100.2 (CH_2)

column chromatography and eluted with $CHCl_3$−MeOH (1:1) to afford 16 fractions (10 mL each). After TLC analysis, these fractions were combined into four groups (I–IV). Group II (3 mg) was composed of pure **7**, while group IV (4 mg) by a mixture of compounds **8** (79%) and **9** (21%).

Scutifoliamide A (1): white, amorphous solid; UV λ_{max} ($CHCl_3$) (log ε) 337 (3.0), 308 (3.0), 293 (3.0), 241 (3.2) nm; IR ν_{max} (KBr) 3304, 1649, 1254 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; LREIMS *m/z* 273 (27) [M$^+$], 216 (14), 201 (54), 173 (53), 143 (33), 135 (10), 122 (6), 115 (100), 96 (28), 89 (19), 83 (5), 63 (20), 57 (21), 41 (33); HRESIMS *m/z* 274.1439 [M + H]$^+$ (calcd for $C_{16}H_{20}O_3N$ 274.1444).

Scutifoliamide B (2): white, amorphous solid; UV λ_{max} ($CHCl_3$) (log ε) 342 (3.6), 310 (3.5), 296 (3.5), 241 (3.7) nm; IR ν_{max} (KBr) 3327, 1732, 1657, 1255 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; LREIMS *m/z* 331 (1) [M$^+$], 271 (8), 216 (9), 230 (5), 201 (70), 172 (23), 150 (29), 143 (19), 115 (73), 89 (14), 59 (29), 43 (100); HRESIMS *m/z* 332.1492 [M + H]$^+$ (calcd for $C_{18}H_{22}O_5N$ 332.1498).

Hoffmannseggiamide A (9): white, amorphous solid (mixture with **8**); IR ν_{max} (KBr) 3433, 1627, 1260 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 2; GCLREIMS *m/z* 273 (37) [M$^+$], 228 (26), 216 (21), 201 (90), 173 (84), 143 (39), 122 (9), 115 (100), 96 (35), 85 (56), 83 (70), 63 (23), 57 (35), 41 (44); HRESIMS *m/z* 296.1261 [M + Na]$^+$ (calcd for $C_{16}H_{20}NO_3Na$ 296.1264).

Hoffmannseggiamide B (10): white, amorphous solid (mixture with **7**); IR ν_{max} (KBr) 2873, 1641, 1622, 1265 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 2; GCLREIMS *m/z* 331 (1) [M$^+$], 271 (6), 216 (10), 230 (8), 201 (69), 172 (25), 150 (27), 143 (15), 115 (72), 89 (13), 59 (29), 43 (100); HRESIMS *m/z* 354.1311 [M + Na]$^+$ (calcd for $C_{16}H_{20}NO_3Na$ 354.1318).

Table 3. Antifungal Activity of Compounds **1**–**10** against *C. cladosporioides* and *C. sphaerospermum*

	antifungal activity (μg)[a]	
compound(s)	*C. cladosporioides*	*C. sphaerospermum*
1	5.0	5.0
2	5.0	5.0
3	5.0	50.0
4	1.0	1.0
5	1.0	1.0
6	1.0	1.0
7	0.25	0.25
8	2.5	1.0
7 + **10**	5.0	5.0
8 + **9**	5.0	5.0
nystatin	1.0	1.0
miconazole	1.0	1.0

[a] Minimum amount required for the inhibition of fungal growth on a thin-layer chromatographic plate (TLC).

Antifungal Assay. The microorganisms used in the antifungal assay, *Cladosporium cladosporioides* (Fresen) de Vries SPC 140 and *C. sphaerospermum* (Perzig) SPC 491, have been maintained at the Instituto de Botânica, São Paulo, SP, Brazil. Ten microliters of the solutions were prepared, in different concentrations, corresponding to 20, 10, 5, and 1 μg for pure compounds and 100 μg for the crude extracts or fractions. The samples were applied to TLC plates, with these being eluted with $CHCl_3$−MeOH (99:1) or *n*-hexane−EtOAc (4:1) followed by complete removal of the solvent at room temperature.

The chromatograms were then sprayed with a spore suspension of fungi in glucose and salt solution and incubated for 72 h in the darkness in a moistened chamber at 25 °C. Clear inhibition zones appeared against a dark background, indicating the minimal amount of compound required (Table 3). Nystatin and miconazole were used as positive controls, whereas ampicillin and chloramphenicol were used as negative controls.[10,19,20]

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References and Notes

(1) Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597–673.
(2) Greger, H. *Planta Med.* **1984**, *50*, 366–375.
(3) Danelutte, A. P.; Lago, J. H. G.; Young, M. C. M.; Kato, M. J. *Phytochemistry* **2003**, *64*, 555–559.
(4) Lago, J. H. G.; Ramos, C. S.; Casanova, D. C. C.; Morandim, A. A.; Bergamo, D. C. B.; Cavalheiro, A. J.; Bolzani, V. S.; Furlan, M.; Guimarães, E. F.; Young, M. C. M.; Kato, M. J. *J. Nat. Prod.* **2004**, *49*, 1783–1788.
(5) Alécio, A. C.; Bolzani, V. S.; Young, M. C. M.; Kato, M. J.; Furlan, M. *J. Nat. Prod.* **1998**, *61*, 637–639.
(6) Lago, J. H. G.; Tanizaki, T. M.; Young, M. C. M.; Guimarães, E. F.; Kato, M. J. *J. Braz. Chem. Soc.* **2005**, *16*, 153–156.
(7) Navickiene, H. M. D.; Alécio, A. C.; Kato, M. J.; Bolzani, V. S.; Young, M. C. M.; Cavalheiro, A. J.; Furlan, M. *Phytochemistry* **2000**, *6*, 621–626.
(8) Costa, S. S.; Mors, W. B. *Phytochemistry* **1981**, *20*, 1305–1307.
(9) Cleyn, R. de; Verzele, M. *Bull. Soc. Chim. Belg.* **1975**, *84*, 435–438.
(10) Silva, R. V.; Navickiene, H. M. D.; Kato, M. J.; Bolzani, V. S.; Méda, C. I.; Young, M. C. M.; Furlan, M. *Phytochemistry* **2002**, *59*, 521–527.
(11) Wei, K.; Li, W.; Koike, K.; Pei, Y.; Chen, Y.; Nikaido, T. *J. Nat. Prod.* **2004**, *67*, 1005–1009.
(12) Antunes, P. A.; Chierice, G. O.; Constantino, C. J. L.; Aroca, R. F. *Vib. Spectrosc.* **2001**, *27*, 175–181.
(13) Facundo, A. V.; Morais, S. M.; Braz Filho, R. *Quím. Nova* **2003**, *27*, 79–83.
(14) Ahmad, V. U.; Aliya, R.; Perveern, S.; Shameel, M. *Phytochemistry* **1992**, *31*, 1429–1431.
(15) Koul, S. K.; Taneja, S. C.; Agarwal, V. K.; Dhar, K. L. *Phytochemistry* **1988**, *27*, 3523–3527.
(16) Tabuneng, W.; Bando, H.; Amiya, T. *Chem. Pharm. Bull.* **1983**, *31*, 3562–3565.
(17) Siddiqui, B. S.; Begum, S.; Gulzar, T.; Noor, F.; Noor, F. *Phytochemistry* **1997**, *45*, 1619–1621.
(18) Gupta, O. P.; Gupta, S. C.; Dhar, K. L.; Atal, C. K. *Phytochemistry* **1977**, *16*, 1436–1437.
(19) Homans, A. L.; Fuchs, A. *J. Chromatogr.* **1970**, *51*, 327–329.
(20) Rahalison, L.; Hamburger, M.; Monod, M.; Frenk, E.; Hostettmann, K. *Planta Med.* **1994**, *60*, 41–44.

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