Artigo

CHEMICAL CONSTITUENTS FROM THE STEM BARK OF *Annona pickelii* (Annonaceae)

Emmanoel Vilaça Costa[a,b,*], Marília Fernanda Chaves Sampaio[c], Marcos José Salvador[d], Angelita Nepel[e] and Andersson Barison[e]

[a]Departamento de Química, Universidade Federal de Sergipe, Avenida Vereador Olímpio Grande, S/N, Campus Prof. Alberto Carvalho, 49500-000 Itabaiana – SE, Brasil

[b]Departamento de Química, Universidade Federal do Amazonas, Avenida General Rodrigo Otavio Jordão Ramos, 6200, Campus Sen. Arthur Virgílio Filho, 69077-000 Manaus – AM, Brasil

[c]Departamento de Química, Universidade Federal de Sergipe, Av. Marechal Rondon, S/N, Cidade Universitária Prof. José Aloísio de Campos, 49100-000 São Cristóvão – SE, Brasil

[d]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, 13083-970 Campinas – SP, Brasil

[e]Departamento de Química, Universidade Federal do Paraná, Centro Politécnico, CP 19081, 81531-990 Curitiba – PR, Brasil

Recebido em 23/10/2014; aceito em 05/03/2015; publicado na web em 28/04/2015

The phytochemical investigation of the stem bark of *Annona pickelii* yielded four steroids (β-sitostenone, β-sitosterol, stigmasterol and campesterol), three lignans (eudesmin, magnolin and yangambin), twelve alkaloids (liriodenine, lysicamine, atherospermidine, anonaine, analobine, asimilobine, discretamine, stepholidine, coclaurine, orientaline, juziphine and stepharine), and a benzenoid (2-methoxybenzoic acid). These compounds support their recent reclassification from *Rollinia* to *Annona*, and that it is a typical species of the family Annonaceae. Significant antifungal and antioxidant activities were found for the methanol crude extract as well as for the lignans eudesmin, magnolin, yangambin and the alkaloid discretamine. In addition, several items of NMR data for the alkaloids were reviewed and unequivocally described in this work.

Keywords: Annonaceae; *Annona pickelii*; steroids; lignans; alkaloids; NMR.

INTRODUCTION

Annona L. belongs to the family Annonaceae and comprises approximately 175 species of trees and shrubs, found predominantly in lowland tropical regions.[1] Economically, this genus is the most important of the family Annonaceae due to its edible fruits and medicinal properties.[2] Previous chemical and pharmacological investigations on some species of this genus revealed the presence of important bioactive compounds, exhibiting several pharmacological activities, including cytotoxicity against tumor cell lines,[3-5] antimicrobial,[6-8] antioxidant,[6-8] antiparasitic properties, mainly against *Leishmania* sp. and *Trypanosoma cruzi*,[5-11] and analgesic and anti-inflammatory activities.[12] These activities generally are attributed to alkaloids, acetogenins, and terpenes.

In this context, *Annona pickelii* (Diels) H. Rainer [synonym: *Rollinia pickelii* Diels] is a small tree endemic to Brazil, popularly known as 'araticum-do-mato', 'araticum-da-mata', 'jaquirinha-do--mato', and 'jussara' found in Paraíba, Pernambuco and recently in Sergipe, Brazil,[13] where this species is endemic and commonly found in Atlantic forest remainders. Previous phytochemical and pharmacological investigations on this species described the isolation of terpenes, lignans and alkaloids.[6-11,13,14]

In our continuous search for bioactive natural products from Sergipe annonaceous plants, four steroids (**1-4**), three lignans (**5-7**), twelve alkaloids (**8-19**) and one benzenoid (**20**) (Figure 1) were obtained according to a bio-guided systematic investigation from the stem bark of *A. pickelii*. Their structures were established on basis of spectrometric data, including 1D (^{1}H and ^{13}C) and 2D (HSQC and HMBC) NMR experiments, as well as 1D NOE and MS analysis. Moreover, many of these compounds were isolated a long time ago, and their ^{1}H and ^{13}C NMR chemical shifts were performed only on basis of general chemical shifts arguments. The NMR chemical shift assignments based on two-dimensional NMR experiments have not been performed. Therefore, their previous ^{13}C assignments contain some ambiguities. In the same way, their previous ^{1}H NMR chemical shifts as well as the scalar coupling constants values have not been specifically assigned and/or obtained. In this work, the complete and unequivocal assignments of ^{1}H and ^{13}C NMR chemical shifts are described for several alkaloids. Additionally some biological activities were demonstrated for the pure compounds. This is the first report on the phytochemical investigation and biological activities of the stem bark of this plant.

EXPERIMENTAL

General procedures

GC-MS analyses were performed on a Shimadzu QP5050A GC-MS system equipped with an AOC-20i auto-injector, and an Rtx®-5Sil MS fused capillary chromatography column (30 m x 0.25 mm x 0.25 µm film thickness) coated with 5%-diphenyl-95%-dimethylpolysiloxane. MS analysis were taken at 70 eV with a scan interval of 0.5 s and fragments from 40-500 Da. LR-ESI-MS were obtained in the positive ion mode on an ultra-high performance Waters Acquity UHPLC-TQD LC-MS system, equipped with an ESI source. 1D and 2D NMR data were acquired at 303 K in $CDCl_3$ or $CDCl_3$ plus some drops of CD_3OD on a Bruker AVANCE III 400 NMR spectrometers, operating at 9.4 Tesla, observing ^{1}H and ^{13}C at 400.13 and 100.61 MHz, respectively. The spectrometer was equipped with a 5-mm multinuclear direct detection probe with *z*-gradient. One-bond and long-range ^{1}H-^{13}C correlation from HSQC and HMBC NMR experiments were optimized for an average coupling constant $^1J_{H,C}$ and $^{LR}J_{H,C}$ of 140 and 8 Hz, respectively. All ^{1}H and ^{13}C NMR chemical

*e-mail: emmanoelvc@gmail.com

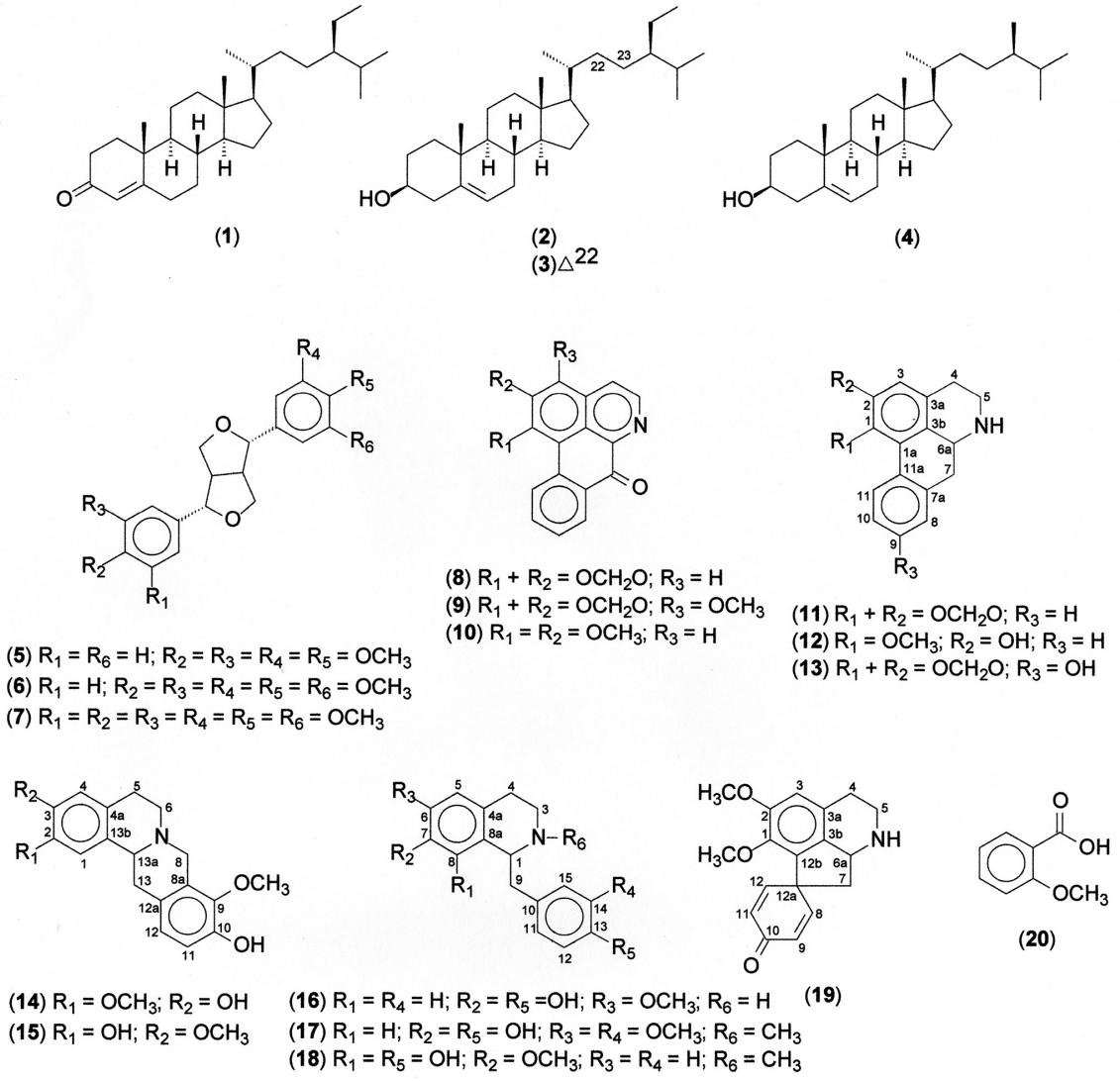

Figure 1. *Chemical constituents from the stem bark of Annona pickelii*

shifts (δ) are given in ppm related to the TMS signal at 0.00 ppm as internal reference, and the coupling constants (*J*) in Hz. Silica gel 60 (70-230 mesh) was used for column chromatography, while silica gel 60 F_{254} was used for analytical (0.25 mm), and preparative (1.00 mm) TLC. Compounds were visualized by exposure under $UV_{254/365}$ light and spraying of *p*-anisaldehyde reagent followed by heating on a hot plate, and Dragendorff's reagent.

Botanical material

The stem bark of *Annona pickelii* were collected at 'Mata do Crasto', in the city of Santa Luzia do Itanhy [coordinates: 11° 23' 01" S, 37° 25' 13" W], Sergipe, Brazil, in March 2010. The identity of the plant was confirmed by Dr. A. P. N. Prata, Department of Biology, Sergipe Federal University (UFS), Brazil, and a voucher specimen (#15442) has been deposited in the Herbarium of Sergipe Federal University (ASE/UFS).

Extraction and isolation

Dried at room temperature and powdered stem bark of *A. pickelii* (790 g) was successively extracted with *n*-hexane (2.5 L, five times) followed by MeOH (2.5 L, five times), yielding hexane (5.53 g) and MeOH (129.32 g) extracts, after solvent removal under reduce pressure. An aliquot of the hexane extract (5.0 g) was initially subjected to silica gel column chromatography (CC) eluted with increasing concentrations of CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*), followed by EtOAc in CH_2Cl_2 (0 to 100, *v/v*), and MeOH in EtOAc (0 to 70, *v/v*), affording 177 fractions (25 mL each), that were pooled in 23 groups (**G1 to G23**), according to TLC analysis. **Group G17** (446.0 mg) eluted with CH_2Cl_2-EtOAc (80:20, *v/v*), was submitted to a new silica gel CC eluted with increasing concentrations of CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*) followed by EtOAc in CH_2Cl_2 (0 to 100, *v/v*), giving 201 fractions (each 20 mL) that were pooled in 31 groups (G17.1 to G17.31), according to TLC analysis. Groups G17.6 to G17.8 were also pooled (213.0 mg) and submitted to preparative TLC eluted with *n*-hexane-EtOAc (80:20, *v/v*, two times), affording **1** (5.3 mg) and a mixture of **2**, **3** and **4** (48.9 mg). **Group G18** (346.0 mg) eluted with CH_2Cl_2-EtOAc (70:30, 60:40 and 50:50, *v/v*) was submitted to a new silica gel CC eluted with the same eluent system as described for initial CC, yielding 78 fractions (25 mL each), that were subsequently pooled into 14 groups (G18.1 to G18.14). Group G18.10 (76.1 mg) was submitted to preparative TLC eluted with *n*-hexane-EtOAc (95:05, *v/v*, four times), giving **5** (4.3 mg), **6** (27.4 mg), and **7** (20.1 mg).

TLC investigations indicated a high concentration of alkaloids in the MeOH extract. Therefore, an aliquot of this extract (120.0 g)

was initially subjected to an acid-base extraction to give alkaloid (0.68 g) and neutral (3.04 g) fractions.[15] An aliquot of alkaloid fraction (0.60 g) was submitted to silica gel CC previously treated with a 10% $NaHCO_3$ solution,[15] and eluted with increasing concentrations of CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*), followed by EtOAc in CH_2Cl_2 (0 to 100, *v/v*), and MeOH in EtOAc (0 to 50, *v/v*), giving 130 fractions (15 mL each), that were pooled in 12 groups (**G1** to **G12**), according to TLC analysis. **Group G4** (195.0 mg) eluted with CH_2Cl_2 (100%) and CH_2Cl_2-EtOAc (95:05 and 90:10, *v/v*) was submitted to a new silica gel CC eluted with increasing concentrations of CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*) followed by MeOH in CH_2Cl_2 (0 to 50, *v/v*), affording 27 fractions (25 mL each) that were subsequently pooled into 5 groups (G4.1 to G4.5). Group G4.2 (29.9 mg) was submitted to preparative TLC eluted with CH_2Cl_2-MeOH (95:05, *v/v*, two times) yielding a mixture of **8** and **20** (3.1 mg) and **11** (18.3 mg). **Group G6** (27.3 mg) eluted with EtOAc (100%) was also submitted to preparative TLC eluted with CH_2Cl_2-MeOH (95:05, *v/v*, two times) giving **14** (4.8 mg) and **15** (9.5 mg), respectively. **Group G7** (128.8 mg) eluted with EtOAc-MeOH (95:05, *v/v*) was submitted to a new silica gel CC eluted with increasing concentrations CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*) followed by MeOH in CH_2Cl_2 (0 to 100, *v/v*) resulting in 38 fractions (25 mL each), that were subsequently pooled into 7 groups (G7.1 to G7.7). Group G7.2 (58.8 mg) was submitted to preparative TLC eluted with CH_2Cl_2-MeOH (90:10, *v/v*, three times) yielding a mixture of **12** and **16** (9.9 mg), and a mixture of **17** and **18** (6.1 mg). **Group G8** (50.2 mg) eluted with EtOAc-MeOH (95:05 and 90:10, *v/v*) was subjected to a new silica gel CC eluted with increasing concentrations of CH_2Cl_2 in *n*-hexane (0 to 100, *v/v*) followed by MeOH in CH_2Cl_2 (0 to 100, *v/v*) resulting in 30 fractions (20 mL each) that were pooled into 7 groups (G8.1 to G8.7). Groups G8.3 and G8.4 were also pooled (21.0 mg) and submitted to preparative TLC eluted with CH_2Cl_2-MeOH (90:10, *v/v*, two times) yielding a mixture of **8**, **9** and **10** (7.4 mg), **8** (2.7 mg), **12** (5.1 mg), and **19** (1.7 mg), respectively.

β-sitostenone (1) and Mixture of β-sitosterol (2), stigmasterol (3) and campesterol (4): white needles; identified by comparison with literature data (co-TLC, mp, ^{1}H NMR and ^{13}C NMR);[16,17] EI-MS *m/z* 412, 414, 412, and 400 $[M]^{+\cdot}$, respectively.

Eudesmin (5), **Magnolin (6)** and **Yangambin (7)**: Yellow amorphous powder ($CHCl_3$); identified by comparison with literature data (^{1}H NMR and ^{13}C NMR);[18,19] EI-MS *m/z* 386, 416 and 446 $[M]^{+\cdot}$, respectively.

Liriodenine (8), Atherospermidine (9) and lysicamine (10): Orange crystals (CH_2Cl_2:MeOH 3:1); identified by comparison with literature data (^{1}H NMR and ^{13}C NMR).[9-11]

Anonaine (11): Brown amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Table 1; LR-ESI-MS $[M+H]^+$ *m/z* 266.5.

Asimilobine (12): Brown amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Table 1. LR-ESI-MS $[M+H]^+$ *m/z* 268.4.

Analobine (13) and coclaurine (16): Brown amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively.

Discretamine (14): Yellow amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Table 2. LR-ESI-MS $[M+H]^+$ *m/z* 328.6.

Stepholidine (15): Yellow amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Table 2; LR-ESI-MS $[M+H]^+$ *m/z* 328.6.

Mixture of orientaline (17) and juziphine (18): Brown amorphous powder (MeOH); ^{1}H and ^{13}C NMR data, see Table 3.

Stepharine (19): Brown amorphous powder ($CHCl_3$:MeOH 3:1); ^{1}H and ^{13}C NMR data, see Table 4. EI-MS $[M]^{+\cdot}$ *m/z* 297.

Mixture of 2-Methoxybenzoic acid (20) and liriodenine (8): Mixture of yellow and white needles (CH_2Cl_2:MeOH 3:1); identified by comparison with literature data (^{1}H and ^{13}C NMR).[9-11,20-22]

Antifungal and antibacterial activities (in vitro)

Crude extracts, fractions and isolated compounds were evaluated for antifungal and antibacterial activities using the broth microdilution method (96-well microtiter plates), as previously described by Salvador *et al*.[23] in the concentration of 12 to 5000 μg mL^{-1}. The minimal inhibitory concentration (MIC) was the lowest concentrations that completely inhibit a tested strain. In these assays, chloramphenicol and ketoconazole were used as positive controls, while a DMSO solution in distilled water (5:95, *v/v*) was the negative control. Each assay was performed in duplicate for each microorganism evaluated and repeated three times. The strains of microorganisms utilized are shown in Table 5.

Antioxidant assay by ORAC-FL kinetic assay (in vitro)

The antioxidant capacities of the extracts, fractions and pure compounds were assessed through the Oxygen Radical Absorbance Capacity (ORAC) assay (Table 5). This technique measures the scavenging activity against the peroxyl radical [Azobis (2-amidinopropane) dihydrochloride (AAPH), using fluorescein as the fluorescent probe. The ORAC assays were carried out on a Biotek Synergy 2 multidetection microplate reader system. The incubator temperature was set at 37 °C. The procedure was carried out according to the method established by Ou *et al*.[24] with modifications Salvador *et al*.[25] The data are expressed as μmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TE) per gram of extracts and fractions on a dry basis (μmol of TE g^{-1}) and as the relative Trolox equivalent for pure compounds. In these evaluations, quercetin, isoquercitrin, and caffeic acid were used as positive controls and the assays were performed in triplicate.

RESULTS AND DISCUSSION

Once a time the crude extracts of the stem bark of *A. pickelii* were found to have antimicrobial and antioxidant activities (Table 5), they were subjected to successive chromatographic separations as described in the Experimental leading to the isolation and identification of 20 chemical constituents, being four steroids (**1-4**), three lignans (**5-7**), six aporphinoid alkaloids (**8-13**), two tetrahydroprotoberberine alkaloids (**14-15**), three benzyltetrahydroisoquinoline alkaloids (**16-18**), one proaporphine alkaloid (**19**), and one benzenoid (**20**) (Figure 1). Compounds **2**, **5-8**, **10** and **13** were recently described in the leaves of this species,[13] while the other compounds are described here for the first time in this species.

All compounds were identified by comparing their spectrometric data with those reported in the literature, as well as, extensive analysis of NMR data as β-sitostenone (**1**),[16,17] β-sitosterol (**2**),[16,17] stigmasterol (**3**),[16,17] campesterol (**4**),[16,17] eudesmin (**5**),[13,18] magnolin (**6**),[13,18] yangambin (**7**),[13,19] liriodenine (**8**),[9-11,13] atherospermidine (**9**),[9-11] lysicamine (**10**),[26-30] and 2-methoxybenzoic acid (**20**).[24] Although, as the alkaloids **11-19** have been described a long time ago, their ^{1}H and ^{13}C NMR data are incomplete and showed ambiguities according

to literature data. In this work, the complete and unequivocal ^{1}H and ^{13}C NMR data were revised according to 1D and 2D NMR experiments (Tables 1-4).

Compound **11** displayed two spin system in the ^{1}H NMR spectrum which is characteristic of aporphine alkaloids, one consisting of the signals at δ 2.67 and δ 3.01 (1H each, *m*, H-4) and δ 3.02 and δ 3.41 (1H each, *m*, H-5), and the other comprising the signals at δ 3.99 (1H, *m*, H-6a) and δ 2.83 and δ 2.96 (1H each, *m*, H-7). The singlet at δ 6.57 (1H, H-3) indicated a 1,2,3,4,5-pentasubstituted benzene ring, while the other aromatic signals revealed any substitution on the D ring (Table 1). The location of the methylenedioxy at C-1 and C-2 were established on the basis of the long-range ^{1}H-^{13}C correlation map from HMBC NMR experiment. In this, the hydrogens at δ 6.57 (H-3) as well as the methylenedioxy hydrogens showed correlation with the carbons at δ 142.5 (C-1) and δ 146.8 (C-2). The overall analysis of 1D and 2D NMR data enable the complete and unequivocal ^{1}H and ^{13}C NMR chemical shifts assignments (Table 1).

Compound **12** presented ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra very similar to those of **11**. However, in this case, the methylenedioxy signals were replaced by signals suggesting the presence of a methoxyl and hydroxyl groups at C-1 and C-2. The location of the methoxyl group at C-1 was established on the basis of the long-range ^{1}H-^{13}C correlation map from HMBC NMR experiment. In this, the hydrogen at δ 6.68 (H-3) as well the methoxyl hydrogens at δ 3.60 showed strong correlation with the carbon at δ 143.5 (C-1) (Table 1).

Compound **14** showed several signals in the aliphatic region of ^{1}H NMR spectrum that along with ^{1}H-^{1}H correlation map from COSY NMR experiment revealed three spin systems, which are typical for tetrahydroprotoberberine alkaloids (Table 2). The signals at δ 3.82 and δ 3.88 (3H each, *s*) indicated the presence of two methoxyl groups in the structure. Furthermore, the ^{1}H NMR spectrum exhibited two signals at δ 6.71 (1H, H-1) and δ 6.64 (1H, H-4), indicating a 1,2,4,5-tetrasubstituted benzene ring and two doublets at δ 6.81 (1H, *J* 8.2 Hz, H-12) and δ 6.77 (1H, *J* 8.2 Hz, H-11) suggesting a 1,2,3,4-tetrasubstituted benzene ring. The complete assignments of the ^{1}H and ^{13}C NMR chemical shifts were established on basis of one-bond and long-range ^{1}H-^{13}C NMR correlation experiments and 1D experiments (Table 2). In this, the hydrogen at δ 6.64 (H-4) and the methoxyl hydrogens at δ 3.88 showed long-range ^{1}H-^{13}C correlation with the carbon at δ 145.8 (C-2), while the hydrogen at δ 6.77 (H-11) and the methoxyl hydrogens at δ 3.82 showed long-range ^{1}H-^{13}C correlation with the carbon at δ 143.6 (C-9). The hydroxyl groups at C-3 and C-10 were established on the basis of the long-range ^{1}H-^{13}C correlations of hydrogen at δ 6.71 (H-1) with the carbon at δ 144.4 (C-3) and the hydrogen at δ 6.81 (H-12) with the carbon at δ 147.2 (C-10), that showed any correlation with the methoxyl groups (Table 2). Moreover, the selective irradiation of the resonance frequency of the methoxyl hydrogens at δ 3.88 caused a NOE enhancement in the signal at δ 6.71 (H-1), while the selective irradiation of the resonance frequency of the methoxyl hydrogens at δ 3.82 caused a NOE intensification in the signal at δ 4.20 (H-8 pseudoequatorial) (Table 2).

Compound **15** showed ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra very similar to those of **14**. However, in this case, the hydrogen at δ 6.77 (H-1) as well as the methoxyl hydrogens at δ 3.86 showed ^{1}H-^{13}C long-range correlation with the same carbon at δ 146.3 (C-3), while the hydrogen at δ 6.61 (H-4) showed ^{1}H-^{13}C long-range correlation with the carbon at δ 144.6 (C-2), that showed any correlation with methoxyl groups. Thus, the methoxyl group at C-1 in **14** was replaced by a hydroxyl group in **15**. In addition, the selective irradiation of the resonance frequency of the methoxyl hydrogens at δ 3.86 showed a NOE intensification in the signal of H-4 at δ 6.61 and any enhancement in the signal of H-1 at δ 6.77. The overall NMR data are described in (Table 2).

Compounds **13** and **16** were obtained in a mixture once its NMR data indicated the presence of two set of signals with different ratios. The first set of signals in the ^{1}H NMR spectrum displayed two spin

Table 1. NMR data (400 and 100 MHz for ^{1}H and ^{13}C, respectively) for the aporphine alkaloids **11-13**

Position	Anonaine (**11**)		Asimilobine (**12**)		Analobine (**13**)	
	δ_C mult.[a,c]	δ_H mult. (*J* in Hz)[a]	δ_C mult.[b,c]	δ_H mult. (*J* in Hz)[b]	δ_C mult.[b,c]	δ_H mult. (*J* in Hz)[b]
1	142.5, C		143.5, C		143.0, C	
1a	116.1, C		125.9, C		115.7, C	
2	146.8, C		148.9, C		148.3, C	
3	107.9, CH	6.57 *s*	115.1, CH	6.68 *s*	107.6, CH	6.50 *s*
3a	126.4, C		129.2, C		127.2, C	
3b	128.1, C		127.4, C		127.3, C	
$4_{pseudoeq}$	29.0, CH_2	3.01 *m*	28.3, CH_2	3.01 *m*	29.4, CH_2	2.67 *m*
$4_{pseudoax}$		2.67 *m*		2.70 *m*		2.30 *m*
$5_{pseudoeq}$	43.1, CH_2	3.41 *m*	42.8, CH_2	3.35 *m*	43.9, CH_2	3.33 *m*
$5_{pseudoax}$		3.02 *m*		3.02 *m*		2.96 *m*
6a	53.3, CH	3.99 *dd* (14.2; 4.9)	53.4, CH	3.81 *dd* (13.8; 4.6)	54.5, CH	3.89 *m*
$7_{pseudoeq}$	36.8, CH_2	2.96 *dd* (14.2; 4.9)	36.9, CH_2	2.86 *dd* (13.8; 4.6)	37.4, CH_2	2.84 *m*
$7_{pseudoax}$		2.83 *dd* (14.2; 14.2)		2.76 *brd* (13.8)		2.74 *m*
7a	134.9, C		135.7, C		137.6, C	
8	128.1, CH	7.25 *m*	127.3, CH	7.31 *ddd* (6.7, 2.0, 0.6)	115.8, CH	6.70 *d* (2.7)
9	127.5, CH	7.22 *m*	127.6, CH	7.22 *m*	158.0, C	
10	127.00 CH	7.31 *m*	128.0, CH	7.22 *m*	114.9, CH	6.71 *dd* (8.3, 2.7)
11	127.03, CH	8.07 *brd* (7.7)	127.6, CH	8.32 *dd* (8.1, 0.6)	129.5, CH	7.91 *d* (8.3)
11a	131.1, C		132.9, C		123.9, C	
1-OCH_2O-2	100.6, CH_2	5.94 *d* (1.4)			101.7, CH_2	5.90 *d* (1.2)
		6.09 *d* (1.4)				6.07 *d* (1.2)
H_3CO-1			60.2, CH_3	3.60 *s*		

The experiments were acquired at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$[a] or $CDCl_3$ with some drops of CD_3OD[b]. [c] Multiplicities determined by DEPT 135, HSQC e HMBC experiments.

Table 2. NMR data (400 and 100 MHz for ^{1}H and ^{13}C, respectively) for tetrahydroprotoberberine alkaloids **14** and **15**

Position	Discretamine (**14**)			Stepholidine (**15**)		
	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]	NOE	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]	NOE
1	108.3, CH	6.71 *s*	H_3CO-2; H-$13_{pseudoeq}$	112.0, CH	6.77 *s*	
2	145.8, C			144.6, C		
3	144.4, C			146.3, C		
4	114.7, CH	6.64 *s*	H-$5_{pseudoeq}$	111.4, CH	6.61 *s*	H_3CO-3; H-$5_{pseudoeq}$
4a	127.0, C			125.5, C		
$5_{pseudoeq}$ $5_{pseudoax}$	28.4, CH_2	3.08 *ddd* (17.0; 12.0; 5.0) 2.67 *m*		28.7, CH_2	3.11 *m* 2.69 *m*	
$6_{pseudoeq}$ $6_{pseudoax}$	51.6, CH_2	3.19 *ddd* (10.9; 5.0; 1.6) 2.63 *m* (11.9; 10.8; 2.9)		52.0, CH_2	3.21 *m* 2.67 *m*	
$8_{pseudoeq}$ $8_{pseudoax}$	53.9, CH_2	4.20 *d* (15.5) 3.56 *d* (15.5)	H-$8_{pseudoax}$	54.1, CH_2	4.20 *d* (15.6) 3.55 *d* (15.6)	H-$8_{pseudoax}$
8a	127.8, C			127.9, C		
9	143.6, C			143.8, C		
10	147.2, C			147.5, C		
11	115.1, CH	6.77 *d* (8.2)		115.4, CH	6.76 *d* (8.3)	
12	124.7, CH	6.81 *d* (8.2)		124.7, CH	6.80 *d* (8.3)	
12a	126.5, C			126.4, C		
$13_{pseudoeq}$ $13_{pseudoax}$	35.9, CH_2	3.28 *dd* (16.0; 3.8) 2.82 *dd* (16.0; 11.4)		35.8, CH_2	3.28 *dd* (16.0; 3.9) 2.78 *dd* (16.0; 11.4)	
13a	59.6, CH	3.59 *dd* (11.4; 3.8)		59.6, CH	3.57 *dd* (11.4; 3.9)	
13b	128.7, C			129.9, C		
H_3CO-2	56.2, CH_3	3.88 *s*	H-1			
H_3CO-3				56.0, CH_3	3.86 *s*	H-4
H_3CO-9	60.3, CH_3	3.82 *s*	H-$8_{pseudoeq}$	60.2, CH_3	3.83 *s*	H-$8_{pseudoeq}$

[a]The experiments were acquired at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$ + drops of CD_3OD. [b]Multiplicities determined by DEPT 135, HSQC e HMBC experiments.

system characteristic of aporphine alkaloids at δ 2.30 and δ 2.67 (H-4), δ 2.96 and δ 3.33 (H-5), δ 3.89 (H-6a) and δ 2.84 and δ 2.74 (H-7) (Table 1). The singlet at δ 6.50 (1H, H-3) indicated a 1,2,3,4,5-pentasubstituted benzene ring and the spins system at δ 6.70 (1H, *d*, *J* 2.7 Hz, H-8), 6.71 (1H, *dd*, *J* 8.3 and 2.7 Hz, H-10) and 7.91 (1H, *d*, *J* 8.3 Hz, H-11), indicating a 1,2,4-trisubstituted benzene ring. The location of the hydroxyl and the methylenedioxy at C-9 and C-1 and C-2 were established on the basis of the long-range ^{1}H-^{13}C correlation map from HMBC NMR experiment. In this, the hydrogens at δ 6.50 (H-3) as well as the methylenedioxy hydrogens showed correlation with the carbons at δ 143.0 (C-1) and δ 148.3 (C-2). On the other hand, the hydrogen at 7.91 (H-11) showed ^{1}H-^{13}C long-range correlation with the carbon at 158.0 (C-9), that showed no correlation with hydrogens from methoxyl group. The overall analysis of 1D and 2D NMR experiments enable the complete and unequivocal ^{1}H and ^{13}C NMR chemical shifts assignments (Table 1).

The ^{1}H NMR spectrum showed also a second NMR dataset consisting of two spin system characteristic of a benzyltetrahidroisoquinoline alkaloid at δ 4.13 (H-1), δ 2.93 and δ 3.22 (H-3), δ 2.78 (H-4) and δ 2.84 and δ 3.17 (H-9) (Table 3). The signals at δ 6.65 (1H, *s*, H-5) and δ 6.69 (1H, *s*, H-8) indicated a 1,2,4,5-tetrasubstituted benzene ring, while the signals at δ 7.07 (2H, *d*, *J* 8.5 Hz, H-11 and H-15) and δ 6.77 (2H, *d*, *J* 8.5 Hz, H-12 and H-14) indicted presence of a *p*-substituted benzene ring in the structure. The location of the two hydroxyl groups at C-7 and C-13 and the methoxyl group at C-6, were established on the basis of the long-range ^{1}H-^{13}C correlation map from HMBC NMR experiment. In this, the hydrogens at δ 6.69 (H-8) as well as the methoxyl hydrogens showed correlation with the same carbon at δ 148.0 (C-6). The hydroxyl groups at C-7 and C-13, were established through the ^{1}H-^{13}C long-range correlations of hydrogens at δ 6.65 (H-5) and δ 7.07 (H-11 and H-15) with the carbons at δ 145.7 (C-7) and δ 157.2 (C-13), respectively, that showed no correlation with hydrogens from methoxyl groups. The overall analysis of 1D and 2D NMR experiments enable the complete and unequivocal ^{1}H and ^{13}C NMR chemical shifts assignments (Table 3).

Compounds **17** and **18** presented NMR data indicating the presence of two set of signals from two benzyltetrahydroisoquinoline alkaloids. The first set of signals in the ^{1}H NMR spectrum displayed two spin system at δ 3.82 (H-1) and δ 2.77 and δ 3.07 (H-9) and δ 2.84 and δ 3.22 (H-3) and δ 2.73 and δ 2.88 (H-4) (Table 3). The signal at 2.54 (3H, *s*) revealed the presence of an *N*-CH_3 group. The singlet at δ 6.64 (1H, *s*, H-5) and δ 6.14 (1H, *s*, H-8) indicated a 1,2,4,5-tetrasubstituted benzene ring and the spins system at δ 6.54 (1H, *dd*, *J* 8.2 and 2.1 Hz, H-11), δ 6.80 (1H, *d*, *J* 8.2 Hz, H-12) and δ 6.63 (1H, *d*, *J* 2.1 Hz, H-15), indicating a 1,2,4-trisubstituted benzene ring. The overall analysis of 1D and 2D NMR experiments enable to locate the methoxyl and hydroxyl groups in the structure as well as to perform the complete and unequivocal ^{1}H and ^{13}C NMR chemical shifts assignments (Table 3).

The second NMR dataset in the ^{1}H NMR spectrum showed two very similar spin system (Table 3). However, for this compound only one signal for a methoxyl group was observed at δ 3.86 (3H, s), while the signal at δ 2.43 (3H, s) revealed the presence of an *N*-CH_3 group, as well. The main difference was the two doublets at δ 6.61 (H-5) and δ 6.83 (H-6) (1H each, *J* 8.4 Hz) that indicated a 1,2,3,4-tetrasubstituted benzene ring and the two doublets at δ 7.11

Table 3. NMR data (400 and 100 MHz for ^{1}H and ^{13}C, respectively) for the benzyltetrahydroisoquinoline alkaloids **16-18**

Position	Coclaurine (**16**)		Orientaline (**17**)		Juziphine (**18**)	
	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]
1	57.8, CH	4.13 *dd* (9.4; 4.4)	65.8, CH	3.82 *m*	61.9, CH	4.39 *dd* (8.9; 2.8)
$3_{pseudoeq}$	41.2, CH_2	3.22 *m*	47.4, CH_2	3.22 *m*	46.0, CH_2	3.31 *m*
$3_{pseudoax}$		2.93 *m*		2.84 *m*		2.82 *m*
$4_{pseudoeq}$	28.8, CH_2	2.78 *m*	25.5, CH_2	2.88 *m*	24.2, CH_2	2.91 *m*
$4_{pseudoax}$		2.78 *m*		2.73 *m*		2.63 *m*
4a	126.1, C		124.6, C		126.5, C	
5	112.7, CH	6.65 *s*	112.4, CH	6.64 *s*	120.1, CH	6.61 *d* (8.4)
6	148.0, C		148.0, C		111.4, CH	6.83 *d* (8.4)
7	145.7, C		145.2, C		146.6, C	
8	114.0, CH	6.69 *s*	115.5, CH	6.14 *s*	144.2, C	
8a	129.6, C		129.3, C		124.0, C	
9	41.7, CH_2	3.17 *dd* (14.2, 4.4)	40.9, CH_2	3.07 *m*	38.8, CH_2	3.08 *m*
		2.84 *dd* (14.2, 9.4)		2.77 *m*		2.93 *m*
10	128.8, C		132.9, C		132.9, C	
11	131.3, CH	7.07 *d* (8.5)	121.8, CH	6.54 *dd* (8.2; 2.1)	131.2, CH	7.11 *d* (8.6)
12	116.6, CH	6.77 *d* (8.5)	112.7, CH	6.80 *d* (8.2)	116.0, CH	6.70 *d* (8.6)
13	157.2, C		147.3, C		156.6, C	
14	116.6, CH	6.77 *d* (8.5)	147.5, C		116.0, CH	6.70 *d* (8.6)
15	131.3, CH	7.07 *d* (8.5)	117.4, CH	6.63 *d* (2.1)	131.2, CH	7.11 *d* (8.6)
H_3CO-6	56.4, CH_3	3.83 s	56.3, CH_3	3.81 *s*		
H_3CO-7					56.6, CH_3	3.86 *s*
H_3CO-14			56.4, CH_3	3.83 *s*		
H_3C-*N*			42.3, CH_3	2.54 *s*	42.7, CH_3	2.43 *s*

[a]The experiments were acquired at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$ + drops of CD_3OD. [b]Multiplicities determined by DEPT 135, HSQC e HMBC experiments.

(H-11 and H-15) and δ 6.70 (H-12 and H-14) (2H each, *J* 8.4 Hz) indicted the presence of a *p*-substituted benzene ring in the structure. The overall analysis of 1D and 2D NMR experiments enable to locate the methoxyl and hydroxyl groups in the structure as well as to perform the complete and unequivocal ^{1}H and ^{13}C NMR chemical shifts assignments (Table 3).

Compound **19** showed a ^{1}H NMR spectrum and HSQC correlation map consistent with six typical signals for a proaporphine alkaloid at δ 2.21 (H-7 pseudoaxial), δ 2.41 (H-7 pseudoequatorial), δ 2.79 (H-4 pseudoaxial/pseudoequatorial), δ 3.14 (H-5 pseudoaxial), δ 3.44 (H-5 pseudoequatorial), and δ 4.30 (H-6a). Furthermore, the ^{1}H NMR spectrum exhibited one singlet at δ 6.66 indicated a 1,2,3,4,5-penta-substituted benzene ring, two double doublets at δ 6.30 and δ 6.42 (each 1H, *dd*, *J* 10.0 and 1.9 Hz), and two other double doublets were observed at δ 6.93 and δ 7.06 (each 1H, *dd*, *J* 10.0 and 2.9 Hz). The HSQC and HMBC correlations maps showed 14 signals, being one at δ 186.7 from a carbonyl group, ten between δ 154.3 and δ 112.7, two methoxyl at δ 56.6 and δ 61.4, three methylenes at δ 48.2, δ 44.8 and δ 26.1, one methine at δ 57.9, and a quaternary carbon at δ 51.4 (Table 4). Moreover, the hydrogen at δ 6.66 (H-3) as well as the methoxyl hydrogens at δ 3.60 and δ 3.81 showed the long-range ^{1}H-^{13}C correlations with the same carbons at δ 144.3 (C-1) and δ 153.4 (C-2). It showed a strong correlation with the carbon at δ 144.3 and a weak correlation with de carbon at δ 153.4 revealing $^{3}J_{H,C}$ and $^{2}J_{H,C}$ correlations, respectively. The presence of a proaporphine moiety was also established on basis of long-range ^{1}H-^{13}C correlation of the hydrogens at δ 7.06 and δ 6.93 (H-8 and H-12) with the carbon at δ 186.7 (C-10). The overall analysis of 1D and 2D NMR experiments enabled its complete and unambiguous ^{1}H and ^{13}C NMR chemical shift assignments (Table 4).

Among the compounds found in *A. pickelli*, the aporphinoids alkaloids liriodenine (**8**), anonaine (**11**), and asimilobine (**12**),[20,22,26-30] are

Table 4. NMR data (400 and 100 MHz for ^{1}H and ^{13}C, respectively) for the proaporphine alkaloid **19**

Position	Stepharine (**19**)	
	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]
1	144.3, C	
2	153.4, C	
3	112.7, CH	6.66 *s*
3a	128.8, C	
$4_{pseudoeq}$	26.1, CH_2	2.79 *m*
$4_{pseudoax}$		2.79 *m*
$5_{pseudoeq}$	44.8, CH_2	3.44 *ddd* (12.7; 6.6; 2.3)
$5_{pseudoax}$		3.14 *ddd* (12.7; 10.3; 6.6)
6a	57.9, CH	4.30 *dd* (10.8; 6.3)
6b	134.7, C	
$7_{pseudoeq}$	48.2, CH_2	2.41 *dd* (12.1; 6.3)
$7_{pseudoax}$		2.21 *dd* (12.1; 10.8)
8	150.8, CH	7.06 *dd* (10.0; 2.9)
9	127.6, CH	6.30 *dd* (10.0; 1.9)
10	186.7, C	
11	128.4, CH	6.42 *dd* (10.0; 1.9)
12	154.3, CH	6.93 *dd* (10.0; 2.9)
12a	51.4, C	
12b	137.0, C	
H_3CO-1	61.4, CH_3	3.60 s
H_3CO-2	56.6, CH_3	3.81 *s*

[a]The experiments were acquired at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$. [b] Multiplicities determined by DEPT 135, HSQC e HMBC experiments.

the most representative of the family Annonaceae, once they are found in most genera of this family being considered as chemotaxonomic markers.[13,20,21] On the other hand, the lignans eudesmin (**5**), magnolin (**6**), and yangambin (**7**) have been found only in the genus *Annona*. Therefore, this finding is very important from the chemotaxonomic point of view, once this species belonging to the genus *Rollinia* was recently reclassified as *Annona*.[1] Thus, these lignans may be useful as chemotaxonomic markers of the genus.

The hexane and methanol crude extracts, as well as the alkaloidal and neutral fractions from methanol extract showed antifungal and antibacterial activities (Table 5). Therefore, the major compounds eudesmin (**5**), magnolin (**6**), yangambin (**7**), discretamine (**14**), and stepholidine (**15**), mixture of β-sitosterol (**2**), stigmasterol (**3**) and campesterol (**4**), and β-sitostenone (**1**) were also evaluated. Among them, only the lignans and the tetrahydroprotoberberine alkaloid discretamine (**14**) showed significant activities (Table 5). The alkaloids liriodenine (**8**), anonaine (**11**) and asimilobine (**12**) has been previously investigated.[6-8] The lignans were active against *C. albicans* (ATCC 10231 and ATCC 1023), all with MIC of 125 μg mL^{-1}. Eudesmin (**5**) and magnolin (**6**) were also active against *C. dubliniensis* (ATCC 778157), while only eudesmin (**5**) was active against *C. tropicalis* (ATCC 157 and CT), both with MIC of 250 μg mL^{-1} (Table 5). The tetrahydroprotoberberine alkaloid, discretamine (**14**) was active against *C. parapsilosis* (ATCC 22019) and *C. dubliniensis* (ATCC 778157) with MIC of 125 μg mL^{-1}. On the other hand, all the steroids were inactive until 500 μg mL^{-1}. MIC values lower than 100 μg mL^{-1} were considered as good activity, between 100 and 1000 μg mL^{-1} moderate activity and small activity to MIC value higher than 1000 μg mL^{-1} for extracts. On the other hand, for pure compounds MIC value lower than 100 μg mL^{-1} was considered as good activity, between 100 and 500 μg mL^{-1} moderate activity and small activity to value higher than 500 μg mL^{-1}.[25]

Among crude extracts evaluated, methanol shows the high antioxidant activity which ORAC of 1925.82 μmol of TE g^{-1} (Table 5). After acid-base extraction, the antioxidant activity was revealed only in the alkaloidal fraction with ORAC value of 4545.04 μmol of TE g^{-1}. In a previous work, the aporphinoid alkaloids liriodenine (**8**), anonaine (**11**), and asimilobine (**12**) were evaluated on ORAC assay and asimilobine (**12**) was the most active. In this work the tetrahydroprotoberberine alkaloids discretamine (**14**) and stepholidine (**15**) were investigated, although only discretamine was active with ORAC of 2.10 trolox equivalents relative (Table 5). According to these results, the antioxidant activity of the methanol extract can be attributed in part to the alkaloids asimilobine (**12**) and discretamine (**14**). Those extracts and pure compounds that shown ORAC values higher than 1000.00 μmol of TE g^{-1} and 1.00 Trolox equivalent relative were considered to have good antioxidant capacity.[5,23]

CONCLUSION

This is the first phytochemical and biological investigation of the stem bark of *A. pickelii*. The lignans found in this species support their recent reclassification from *Rollinia* to *Annona*. In this way, the alkaloids liriodenine, anonaine, and asimilobine support that *A.*

Table 5. Biological activities of the crude extracts, fractions and pure compounds

Microorganisms	Antifungal and antibacterial activities expressed as MIC[a] (μg mL^{-1})									
	Hexane extract	Methanolic extract	Alkaloidal fraction	Neutral fraction	Eudesmin (5)	Magnolin (6)	Yangambin (7)	Discretamine (14)	Stepholidine (15)	Positive controls[b]
Bacillus subtilis (Bs)[d]	250	250	500	500	-	-	-	-	-	50
Candida albicans (ATCC 10231)[c]	5000	5000	500	1000	125	125	125	>500	>500	12.5
Candida albicans (ATCC 1023)[c]	5000	5000	1000	1000	125	125	125	>500	-	12.5
Candida parapsilosis (ATCC 22019)[c]	5000	500	500	500	-	-	-	125	-	12.5
Candida tropicalis (ATCC 157)[c]	5000	5000	5000	1000	250	500	-	>500	-	12.5
Candida tropicalis (CT)[d]	5000	5000	5000	1000	250	-	-	>500	-	12.5
Candida glabrata (ATCC 30070)[c]	5000	5000	250	1000	-	-	-	-	-	12.5
Candida dubliniensis (ATCC 778157)[c]	5000	5000	1000	1000	250	250	-	125	-	12.5
Enterobacter aerogenes (Ea)[d]	500	500	250	500	-	-	-	-	-	50
Escherichia coli (ATCC 10538)[c]	> 5000	> 5000	1000	> 5000	-	-	-	-	-	50
Escherichia coli (ATCC 10799)[c]	1000	1000	1000	1000	-	-	-	-	-	50
Proteus vulgaris (Pv)[d]	500	1000	500	500	500	-	-	>500	-	50
Pseudomonas aeruginosa (ATCC 27853)[c]	> 5000	1000	1000	1000	-	-	-	-	-	850
Staphylococcus aureus (ATCC 14458)[c]	500	500	500	500	500	500	500	-	-	25
Staphylococcus aureus (ATCC 6538)[c]	> 5000	> 5000	500	> 5000	500	500	500	-	-	25
Staphylococcus epidermidis (ATCC 1228)[c]	1000	500	1000	500	-	-	500	>500	-	50
Staphylococcus epidermidis (6epi)[d]	1000	500	1000	500	-	-	500	>500	-	50
Antioxidant activity (ORAC-FL)										
	352.73 (3.60)[e]	1925.82 (1.37)[e]	4545.04 (2.46)[e]	862.60 (7.01)[e]	-	-	-	2.10 (1.35)[f]	0.78 (1.57)[f]	*

[a]MIC (minimum inhibitory concentration with 100% of microorganism inhibition) in μg mL^{-1}; [b]positive controls (chloramphenicol for bacteria strains and ketoconazole for yeast strains); [c]standard strain; [d]field strain. [e]ORAC data expressed as μmol of TE g^{-1} of extract or fraction, mean (%RSD, relative standard deviation) of triplicate assays; [f]ORAC data expressed as Trolox equivalent relative, mean (%RSD, relative standard deviation) of triplicate assays; *Positive controls (Caffeic acid: **2.85 (1.15)^{f}**, quercetin: **5.60 (1.20)^{f}** and isoquercitrin: **5.10 (1.40)^{f}**).

pickelii is a typically species of the family Annonaceae. Significant antifungal and antioxidant activities were found to the lignans and the alkaloids from *A. pickelii*, and then it can be a promising source for bioactive compounds. In addition, several NMR data for the alkaloids were reviewed and are unequivocally described in this work.

SUPPLEMENTARY MATERIAL

NMR data including spectra and correlation maps for compounds **11**-**19** are available free of charge at http://quimicanova.sbq.org.br as PDF file.

ACKNOWLEDGMENTS

The authors are grateful to FAPITEC/SE (Editais # 07/2009 and 10/2009), CNPq, CAPES, FINEP, UFPR and FAPESP for financial support and fellowships, and Professor Dr. Ana Paula do Nascimento Prata for botanical identification.

REFERENCES

1. Chatrou, L. W.; Pirie, M. D.; Erkens, R. H. J.; Couvreur, T. L. P.; Neubig, K. M.; Abbott JR.; Mols, J. B.; Maas, J. W.; Saunders, R. M. K.; Chase, M.W.; *Bot. J. Linn. Soc.* **2012**, *169*, 5.
2. Corrêa, M. P.; *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*, IBDF, Rio de Janeiro, RJ, 1984.
3. Stévigny, C.; Bailly, C.; Quetin-Leclercq, J.; *Curr. Med. Chem.: Anti-Cancer Agents* **2005**, *5*, 173.
4. Alali, F. Q; Liu X.-X.; McLaughlin, J. L; *J. Nat. Prod.* **1999**, *62*, 504.
5. Costa, E. V.; Dutra, L. M.; Salvador, M. J.; Ribeiro, L. H. G.; Gadelha, F. R.; Carvalho, J. E.; *Nat. Prod. Res*. **2013**, *27*, 997.
6. Costa, E. V.; Da Cruz, P. E. O.; Lourenço, C. C.; Moraes, V. R. S.; Nogueira, P. C. L.; Salvador, M. J.; *Nat. Prod. Res*. **2013**, *27*, 1002.
7. Costa, E. V.; Dutra, L. M.; De Jesus, H. C. R.; Nogueira, P. C. L.; Moraes, V. R. S.; Salvador, M. J.; Cavalcanti, S. C. H.; Dos Santos, R. L. C.; Prata, A. P. N.; *Nat. Prod. Commun.* **2011**, *6*, 907.
8. Costa, E. V.; Pinheiro, M. L. B.; Silva, J. R. A.; Maia, B. H. L. N. S.; Duarte, M. C. T.; Amaral, A. C. F.; Machado, G. M. C.; Leon, L. L.; *Quim. Nova* **2009**, *32*, 78.
9. Costa, E. V.; Pinheiro, M. L. B.; Souza, A. D. L.; Barison, A.; Campos, F.R.; Valdez, R. H.; Ueda-Nakamura, T.; Filho, B. P. D.; Nakamura, C. V.; *Molecules* **2011**, *16*, 9714.
10. Siqueira, C. A. T.; Oliani, J.; Sartoratto, A.; Queiroga, C. L.; Moreno, P. R. H.; Reimão, J. Q.; Tempone, A. G.; Fischer, D. C. H.; *Rev. Bras. Farmacogn.* **2011**, *21*, 33.
11. Costa, E. V.; Marques, F. A.; Pinheiro, M. L. B.; Braga, R. M.; Delarmelina, C.; Duarte, M.C.T.; Ruiz, A. L. T. G.; Carvalho, J. E.; Maia, B. H. L. N. S.; *J. Braz. Chem. Soc.* **2011**, *22*, 1111.
12. Chavan, M. J.; Wakte, P. S.; Shinde, D. B.; *Inflammopharmacology* **2011**, *19*, 111.
13. Dutra, L. M.; Costa, E. V.; Moraes, V. R. S.; Nogueira, P. C. L.; Vendramin, M. E.; Barison, A.; Prata, A. P. N.; *Biochem. Syst. Ecol.* **2012**, *41*, 115.
14. De Mesquita, L. M.; Roque, N. F.; Quintana, L. M. B.; Paulo, M. D. Q.; Barbosa Filho, J. M.; *Biochem. Syst. Ecol.* **1988**, *16*, 379.
15. Costa, E. V.; Pinheiro, M. L. B.; Xavier, C. M.; Silva, J. R. A.; Amaral, A. C. F.; Souza, A. D. L.; Barison, A.; Campos, F. R.; Ferreira, A. G.; Machado, G. M. C.; Leon, L. L. P. J.; *J. Nat. Prod.* **2006**, *69*, 292.
16. Facundo, V. A.; Polli, A. R.; Rodrigues, R. V.; Militão, J. S. L. T.; Stabelli, R. G.; Cardoso, C. T.; *Acta Amazonica* **2008**, *38*, 733.
17. Della Greca, M.; Monaco, P.; Previtera, L.; *J. Nat. Prod.* **1990**, *53*, 1430.
18. Batista, A. N. L.; Batista Junior, J. M.; López, S. N.; Furlan, M.; Cavalheiro, A. J.; Silva, D. H. S.; Bolzani, V. S.; Nunomura, S. M.; Yoshida, M.; *Quim. Nova* **2010**, *33*, 321.
19. Ahmed, A. A.; Mahmoud, A. A.; Ali, E. T.; Tzakou, O.; Couladis, M.; Mabry, T.J.; Gáti, T.; Tóth, G.; *Phytochemistry* **2002**, *59*, 851.
20. Xue, L.; Shi, L.; Han, Y.; Xia, C.; Huynh, H. V.; Li, F. *Dalton Trans.* **2011**, *40*, 7632.
21. Leboeuf, M.; Cavé, A.; Bhaumik, P. K.; Mukherjee, B.; Mukherjee, R.; *Phytochemistry* **1982**, *21*, 2783.
22. Da Cruz, P. E. O.; Costa, E. V.; Moraes, V. R. S.; Nogueira, P. C. L.; Vendramin, M. E.; Barison, A.; Ferreira, A. G.; Prata, A. P. N.; *Biochem. Syst. Ecol.* **2011**, *39*, 872.
23. Salvador, M. J.; Ferreira, E.O.; Pral, E. M. F.; Alfieri, S. C.; Albuquerque, S.; Ito, I. Y.; Dias, D. A.; *Phytomedicine* **2002**, *9*, 566.
24. Ou, B.; Hampsch-Woodill, M.; Prior, R.L.; *J. Agric. Food Chem.* **2001**, *49*, 4619.
25. Salvador, M. J.; Ferreira, E. O.; Mertens-Talcott, S. U.; Castro, W. V.; Butterweck, V.; Derendorf, H.; Dias, D. A.; *Z. Naturforsch., C: J. Biosci.* **2006**, *61*, 19.
26. Guinaudeau, H.; Leboeuf, M.; Cavé, A.; *Lloydia* **1975**, *38*, 275.
27. Guinaudeau, H.; Leboeuf, M.; Cavé, A.; *J. Nat. Prod.* **1979**, *42*, 325.
28. Guinaudeau, H.; Leboeuf, M.; Cavé, A.; *J. Nat. Prod.* **1983**, *46*, 761.
29. Guinaudeau, H.; Leboeuf, M.; Cavé, A.; *J. Nat. Prod.* **1988**, *51*, 389.
30. Guinaudeau, H.; Leboeuf, M.; Cavé, A.; *J. Nat. Prod.* **1994**, 57, 1033.