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Original Article

Phytochemical study of *Pilosocereus pachycladus* and antibiotic-resistance modifying activity of syringaldehyde

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ABSTRACT

Pilosocereus pachycladus F. Ritter, Cactaceae, popularly known as "facheiro", is used as food and traditional medicine in Brazilian caatinga ecoregion. The plant is used to treat prostate inflammation and urinary infection. The present work reports the first secondary metabolites isolated from *P. pachycladus*. Therefore, the isolated compound 4-hydroxy-3,5-dimethoxy benzaldehyde (syringaldehyde) was evaluated as modulator of *Staphylococcus aureus* pump efflux-mediated antibiotic resistance. The isolation of compounds was performed using chromatographic techniques and the structural elucidation was carried out by spectroscopic methods. In order to evaluate syringaldehyde ability to modulate *S. aureus* antibiotic resistance, its minimum inhibitory concentrations (μg/ml) was first determinate, then, the tested antibiotics minimum inhibitory concentrations were determined in the presence of the syringaldehyde in a sub-inhibitory concentration. The chromatographic procedures led to isolation of twelve compounds from *P. pachycladus* including fatty acids, steroids, chlorophyll derivatives, phenolics and a lignan. The syringaldehyde did not show any antibacterial activity at 256 μg/ml against *S. aureus*. On the other hand the compound was able to reduce the antibiotic concentration (tetracycline, norfloxacin, ethidium bromide) required to inhibit the growth of drug-resistant bacteria, showing the ability of syringaldehyde of inhibiting the efflux pump on these bacteria.

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Introduction

The Cactaceae family consists of about 127 genera and 1405 species (Hunt et al., 2006). Brazil is considered the third largest center of diversity of this family where the caatinga ecoregion comprises 58 species (Zappi et al., 2010).

The cacti are plants that show special morpho-physiological characteristics with several anatomical variations that ensure their capacity to retain water and to survive in hot and arid environments (Hernández-Hernández et al., 2011). Cacti are used as supplemental food for ruminants and in drought times the species are also used as food for human, mainly in preparation of flour and cakes (Cavalcanti and Resende, 2013; Maciel et al., 2016).

Pilosocereus pachycladus F. Ritter is a perennial shrub popularly known in Brazil as "facheiro" (Abud et al., 2010). It plays important role in rural communities due the use of this species to make biscuits, cakes and candies. Therefore, local people mention the use of its roots to treat prostatic inflammation, and the stem decoction is used against urinary infections, flu and furuncle (Lucena et al., 2013; Agra et al., 2008). Accordingly, the antimicrobial activity of Cactaceae species has been previously reported (Sanchez et al.,

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2014). In fact, plant extracts and compounds have been studied aiming to discover new bactericides, bacteriostatic and bacterial resistance modulator active products. This fact has gain attention especially in recent years, when the raising of bacterial resistance has become a serious public health concerning to almost all antibacterial agents. Among the resistant strains studied, the main mechanism of bacterial resistance is the presence of efflux pumps in bacterial cell membrane, which allow the leaving of antibiotics from the cell (Piddock, 2006).

Considering the great importance of Cactaceae family, this study reports the first phytochemical study of *P. pachycladus* and the evaluation of the isolated compound 4-hydroxy-3,5-dimethoxy benzaldehyde (syringaldehyde) as modulator of *Staphylococcus aureus* pump efflux-mediated antibiotic resistance.

Materials and methods

Instruments and reagents

Isolated compounds were identified by 1D and 2D NMR analysis (^{1}H 500 MHz, ^{13}C 125 MHz, Mercury Varian and ^{1}H 200 MHz, ^{13}C 50 MHz, Mercury Varian) using deuterated solvents and infrared (IR) Perkin-Elmer FT-IR-1750 model using KBr discs; medium pressure liquid chromatography (MPLC) was carried out using a BUCHI (Switzerland) Pump Manager C-615. High pressure liquid chromatography (HPLC) was performed using a Shimadzu HPLC with detector diode array.

Plant material

The plant material of *Pilosocereus pachycladus* F. Ritter, Cactaceae, was collected in Boa Vista (Paraíba, Brazil), in November 2010. The botanical identification was carried out by Prof. Dr. Leonardo Pessoa Felix (CCA/UFPB) and a voucher specimen (19544) was deposited in the Herbarium Prof. Jaime Coelho de Morais (CCA/UFPB).

Extraction, fractionation and isolation of compounds

Fresh plant material of the stems, fruit and flowers of *P. pachycladus* were dried separately in an oven with circulating air at 40 °C for 72 h and each the material was ground in a mechanical mill, providing 4.934 g, 2.884 g and 10.022 g of powder, respectively. The powder of the stem, fruit and the flowers were separately submitted to maceration in ethanol (EtOH) (95%) for 72 h at room temperature. The obtained ethanolic extracts were concentrated with a rotatory evaporator yielding 205.58 g of crude ethanol extract of stem (CES), 206.69 g of fruit (CEFR) and 253.53 g (CEFL) of flowers.

The crude ethanolic extract of stem (CES) was solubilized using EtOH:H_2O (7:3) and the obtained solution was sequentially partitioned in separation funnel using hexane, dichloromethane, ethyl acetate (EtOAc) and *n*-butanol. The obtained solutions were concentrated in rotatory evaporator, yielding 33.18 g of the hexane phase, 16.70 g of the dichloromethane phase, 6.11 g of ethyl acetate phase and 29.34 g of *n*-butanol phase and the hydroalcoholic phase (90.20 g).

A portion of the hexane phase of the CES (10.5 g) was subjected to chromatographic column (Column 1) using silica gel and the solvents hexane, dichloromethane and methanol, pure or in mixtures increasing polarity, resulting in 124 fractions. The obtained fractions were concentrated and analyzed by thin layer chromatography (TLC). The fraction 31/59 (0.7883 g) was chromatographed at the same conditions, which were combined after analysis by TLC. The sub-fraction 1/33 (0.151 g) was again chromatographed following the same method to obtain a pure white amorphous solid (39 mg), coded as compound **1**.

The fraction 81/113 (0.72 g) from column 1 was chromatographed in silica column using hexane, CH_2Cl_2 and MeOH and provided a pure white precipitate encoded as compound **2** (250 mg).

The fraction 114/124 (0.90 g) was chromatographed in silica flash column using hexane, EtOAc and MeOH. From this column 73 fractions were collected and combined by TLC. The combined fractions 22/24 and 41/46 (dark green solids) were pure when analyzed by TLC, being labeled as compound **3** (20 mg) and compound **4** (40 mg), respectively.

The fraction 60/80 (1.28 g) from column 1 was submitted to silica column using as eluents hexane, CH_2Cl_2 and MeOH. The column yielded 52 fractions analyzed and combined by TLC. The combined fractions 08/16 provided 600 mg of pure white crystals (compound **5**).

An aliquot of dichloromethane phase of the CES (10.07 g) was submitted to vacuum liquid chromatography (VLC I), using silica gel in a Büchner funnel with porous plate. The following gradient mixture of solvents were used: hexane:CH_2Cl_2 (7:3), CH_2Cl_2 (100%), CH_2Cl_2:MeOH (9:1), CH_2Cl_2:MeOH (8:2), CH_2Cl_2:MeOH (7:3), CH_2Cl_2:MeOH (1:1) and EtOAc:MeOH (1:1). The fractions were concentrated under reduced pressure to obtain: 0.019 g, hexane:CH_2Cl_2 (7:3); 0.162 g, CH_2Cl_2; 8 g, CH_2Cl_2:MeOH (9:1); 2.239 g, CH_2Cl_2:MeOH (8:2); 0.244 g, CH_2Cl_2:MeOH (7:3), 0.400 g, CH_2Cl_2:MeOH (1:1) and 0.220 g, EtOAc:MeOH (1:1).

The fraction CH_2Cl_2 (0.162 g) from VLC I was subjected to MPLC using silica flash as stationary phase and hexane, CH_2Cl_2 and methanol as mobile phase (pure or binary mixtures) obtaining fifty fractions analyzed and combined by TLC. The fraction 14/18 showed pure yellow crystals being labeled as compound **6** (25 mg).

The fraction CH_2Cl_2:MeOH (9:1) (7.9139 g) from VLC I was chromatographed in silica column using hexane, CH_2Cl_2 and methanol (pure or binary mixtures). This procedure resulted in a pure white amorphous solid, named as compound **7** (32 mg).

The CEFR was partitioned in separation funnel using the same method described for CES. From the partition the following phases were obtained: 87.13 g of the hexane phase, 20.14 g of the dichloromethane phase, 4.69 g of ethyl acetate phase, 41.83 g of *n*-butanolic phase, and 16.76 g of hydroalcoholic phase. The dichloromethane phase of the CEE (10 g) was submitted to VLC (VLC II) using hexane, CH_2Cl_2 and MeOH alone or mixtures in gradient wise. The obtained fractions were concentrated in rotary evaporator.

The fraction CH_2Cl_2:MeOH (9:1) (3.4 g) from VLC II was subjected to silica column chromatography using hexane, CH_2Cl_2 and MeOH. The combined fractions 6/12 (0.95 g) by from this column chromatography were rechromatographed following the same method resulting in 33 fractions. The fraction 12 showed white crystals (12 mg), named as compound **8**.

The ethyl acetate phase of CEFR (4.69 g) was chromatographed in column of Sephadex LH-20 eluted with methanol yielding 37 fractions. The combined fractions 4/14 were rechromatographed using the same method. An aliquot (0.100 g) of the obtained sub-fractions 4/10, was injected at a concentration of 5 mg/ml in an HPLC (λ 269 nm) using a C18 column and gradient method H_2O:MeOH (5:95) by 1 h. The chromatogram showed three peaks. The samples were collected and analyzed. The second peak collected was named as compound **9** (6 mg) (white solid).

The CEFL (253 g) was partitioned in separation funnel using the same method described for CES. From the partition the following phases were obtained: 20 g of the hexane phase, 33 g of the dichloromethane phase, 0.5 g of ethyl acetate phase, 5 g of *n*-butanol phase and 90 g of hydroalcoholic phase.

An aliquot of hexane phase of CEFL (10 g) was submitted to column chromatography using hexane, EtOAc and MeOH pure or in gradient mixtures. From this column, 82 fractions collected of 20 ml were collected. The sub-fraction 36/42 showed a precipitate and a supernatant. The supernatant (0.20 g) was chromatographed following the same methodology purifying transparent crystals, labeled as compound **10** (46 mg). The precipitate was washed with hexane and named as compound **11** (18 mg). The combined fraction 56/60 (0.065 g) from CEFL hexane phase showed a precipitate (white crystals). It was washed with hexane to yield the compound 12 (25 mg).

Minimum inhibitory concentration and modulation of bacterial resistance

The antibiotics norfloxacin, erythromycin and tetracycline were obtained from Sigma Chemical Co. (USA). The antibiotic solutions were prepared according to the guidelines of CLSI (2005). Stock solutions of 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) were prepared using DMSO and at the highest concentration after dilution in the broth (4%) caused no bacterial growth inhibition.

The minimum inhibitory concentrations (MIC) of the antibiotics and the tested compound syringaldehyde were determined in BHI by the microdilution assay using a suspension of *ca.* 10^5 cfu/ml and a drug concentration range of 256-0.5 μg/ml (two-fold serial dilutions). The MIC was defined as the lowest concentration at which no bacterial growth was observed. For the evaluation of syringaldehyde as modulator of antibiotic resistance, the MIC of the antibiotics were determined in the presence of the compound in a sub-inhibitory concentration (Falcão-Silva et al., 2009).

The *S. aureus* strains used were: SA 1199B, which over expresses the *norA* gene encoding the NorA fluoroquinolones (such as norfloxacin) efflux protein; RN4220 harboring plasmid pUL5054, which carries the gene encoding the MsrA macrolide (such as erythromycin) efflux protein; and IS-58, which possesses the TetK tetracycline efflux protein. The strains, kindly provided by professor Simon Gibbons (University College London, UK), were maintained in blood agar base (Laboratórios Difco Ltda., Brazil) slants and, prior to use, the cells were grown overnight at 37 °C in brain heart infusion broth (BHI – Laboratórios Difco Ltda., Brazil).

Results

By using typical chromatographic procedures and spectroscopic methods (IR, NMR ^{1}H and ^{13}C) 13 compounds were identified, including fatty acids, steroids, chlorophyll derivatives, phenolics and a lignan.

Compound **1**: IR (KBr, cm^{-1}): 3401, 2948, 2868. NMR ^{1}H (δ, CD_3OD, 200 MHz): 3.63 (t, J = 6.56 Hz, H-1), 1.24–1.69 (m), 0.87 (t, J = 6.26 Hz, H-10). NMR ^{13}C (δ, CD_3OD, 50 MHz): 63.09 (C-1); 32.77 (C-2), 31.92 (C-4), 29.70, 29.61, 29.42, 29.36, 25.72 (C-3), 22.69, 14.13 (C-10).

Compound **2**: MS (m/z): M$^-$ 255.1; IR (KBr, cm^{-1}): 2918, 2849, 1771, 1448, 1305. NMR ^{1}H (δ, $CDCl_3$, 200 MHz): 2.34 (t, J = 7.46 Hz, H-2), 1.60 (q, J = 7.35 Hz, H-15), 1.25 (bs), 0.87 (t, J = 6.44 Hz, H-16). NMR ^{13}C (δ, $CDCl_3$, 50 MHz): 180.29 (C-1), 34.07 (C-2), 31.92 (C-4), 29.69 (C-5, C-12, C-13, C-14), 29.59 (C-11), 29.43 (C-6), 29.36 (C-7), 29.24 (C-8), 29.05 (C-9, C-10), 24.65 (C-3), 22.69 (C-15), 14.13 (C-16).

Compound **6**: IR (KBr, cm^{-1}): 1670, 1514, 1423, 1172, 1038. NMR ^{1}H (δ, $CDCl_3$, 200 MHz): 9,92 (s, H-7), 7.26 (s, H-2/H-6), 4.07 (s, OCH_3 C-3/C-5). NMR ^{13}C (δ, $CDCl_3$, 50 MHz): 190.78 (C-7), 147.29 (C-3/C-5), 140.75 (C-4), 129.30 (C-1), 106.63 (C-2/C-6), 56.43 (OCH_3 C-3/OCH_3 C-5).

Compound **8**: IR (KBr, cm^{-1}): 3354, 1655, 1597, 1516, 1126, 1026. NMR ^{1}H (δ, C_3D_6O, 500 MHz): 7.13 (2H, d, J = 8.6 Hz, H-2/H-6), 6.78 (2H, d, J = 8.6 Hz, H-3/H-5), 3.50 (s, 2H, H-7). NMR ^{13}C (δ, C_3D_6O, 100 MHz): 173.29 (C-8), 157.14 (C-4), 131.15 (C-2/C-6), 126.50 (C-1), 115.91 (C-3/C-5), 40.50 (C-7).

Compound **9**: IR (KBr, cm^{-1}): 3450, 1450, 1160. NMR ^{1}H and ^{13}C: Table 1.

Minimum inhibitory concentration and modulation of bacterial resistance

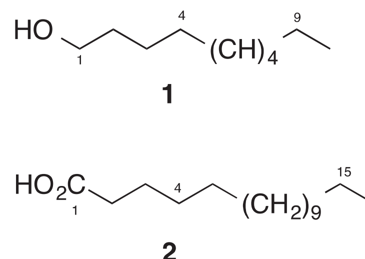
The syringaldehyde isolated from *P. pachycladus* stems did not show any antibacterial activity at 256 μg/ml against all tested strains of *S. aureus* (MIC used ≥ 512 μg/ml). When the compound was added in the growth medium at 128 μg/ml (MIC ≤ ¼), a 32 times reduction in MIC was observed for tetracycline, a dramatic reduction in the MIC of the antibiotic. The MIC of norfloxacin was reduced by two times and the MIC of erythromycin did not decrease (Table 2).

Discussion

Structural elucidation

The IR spectrum (KBr) of compound **1** showed absorptions at 2948 and 2868 cm^{-1} indicating the presence of sp^3 carbon; and a broad band at 3401 cm^{-1} characteristic of O—H. ^{1}H NMR spectrum of **1** showed one terminal methyl as a triplet at δ_H 0.87 (3H, J = 6.26 Hz), multiplets for eight methylene hydrogens (δ_H 1.24–1.69) and one methylene group linked with hydroxyl (δ_H 3.63 (t), J = 6.56 Hz). The ^{13}C NMR spectrum of **1** presented eight methylene carbons (δ_C 32.77, 25.72, 31.92, 29.70, 29.61, 29.42, 29.36, and 22.69), one signal in upfield for one methyl carbon (δ_C 14.13) and one carbon at δ_C 63.09 bonded to oxygen. The spectral data and comparison with literature data (Pavia et al., 2010) allowed to identify the compound 1 as the aliphatic alcohol decanol, produced by plants with ecological relevance by acting as growth regulator and commercially used as pesticide (Arn and Acree, 1998; EFSA, 2010).

The IR spectrum (KBr) of compound **2** showed absorptions at 2918 and 2849 cm^{-1} (C-sp^3); an intense band at 1771 cm^{-1} (C=O), and bands at 1448 and 1305 cm^{-1} (C=C). The ^{1}H NMR spectrum of **2** showed one terminal methyl at δ_H 0.87 (3H, t, J = 6.44 Hz), signals for thirteen methylene hydrogens (δ_H 1.25–1.60) as well as a signal for another methylene proton (H-2) next to a carbonyl (δ_H 2.34, t, J = 7.46 Hz). The ^{13}C NMR spectrum of **2** exhibited one carboxyl group (δ_C 180.29), signals to fourteen methylene carbons (δ_H 34.07, 31.92, 29.69, 29.59, 29.69, 29.43, 29.24, 29.36, 29.05, 24.65, 22.69), and one methyl carbon (δ_H 14.13). The mass spectrum (EI/MS) showed the molecular ion peak (M$^-$ m/z) 255.1, confirming the identity of compound **2** as palmitic acid (Bang et al., 2002), a fatty acid widely produced and previously reported from many species of Cactaceae (López-Cervantes et al., 2011; Mayworm et al., 1998).

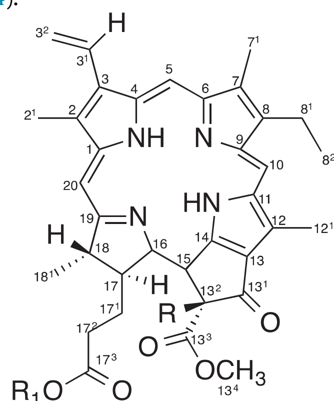


Compounds **3** and **4** were identified by spectral data and comparison with literature data as the chlorophyll deriva-

Table 1
^{1}H and ^{13}C NMR (C_2D_6OS, 500 and 125 MHz) spectral data of **9**.

	HMQC		HMBC	
	δ_C	δ_H	^2J	^3J
1/1′	132.49	–		
3/3′	145.26	–		
4/4′	144.76	–		
CH				
2/2′	113.75	6.72 (*s*)		C-4/C-6/C-7/C-4′/C-6′/C-7′
5/5′	115.46	6.67 (*d*, *J* = 7.82 Hz)		C-1/C-3/C-1′/C-3′
6/6′	117.24	6.59 (*d*, *J* = 7.82 Hz)		C-4/C-2/C-7/C-4′/C-2′/C-7′
7/7′	85.19	4.52 (*d*, *J* = 2.88 Hz)	C-8/C-8′	C-8′/C-9/C-9′/C-2/C-6/C-2′/C-6′
8/8′	53.70	2.93 (sl)	–	–
CH_2				
9/9′	70.58	4.07 (*dd*, *J* = 8.34 and 6.32 Hz) 3.67 (*dd*, *J* = 8.34 and 2.20 Hz)	C-8	C-7/C8′/C-7′

tives phaeophytin a and the 13^2-hydroxy-(13^2-*S*)-phaeophytin a (Schwikkard et al., 1998; Jerz et al., 2007; Teles et al., 2014; Brito-Filho et al., 2014).



3 R=H; R_1=phytyl ester
4 R=OH; R_1= phytyl ester
phytyl ester = $C_{20}H_{39}$

The analysis of the spectral data and comparison with literature (Kojima et al., 1990; Liu et al., 2011) permitted to identify the substances 5, 7, 10, 11 and 12 as known steroids and their glucosyl derivatives. The compound 5 was identified as β-sitosterol; compound 7 as sitosterol-3-*O*-β-D-glucopyranoside; the compound 10 was defines as a mixture of β-sitosterol and stigmasterol; compound 11 was found to be a mixture of β-sitosterol and ergosterol; and the compound 12 was identified as a mixture of sitosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranosoide. They have been previously reported from other Cactaceaes such as *Pilosocereus gounellei* (Maciel et al., 2016). Steroids are widely spread in plants. Their biological relevance is related to their role composing the vegetable membrane and cell wall, and as precursors of vitamin D. They have been shown to possess anti-inflammatory properties, and many of them are being reported as effective agents to prevent cardiovascular diseases (Silva et al., 2012).

Compound **6** was obtained as yellow crystals. The IR absorptions indicated the peaks of aldehyde carbonyl (1670 cm^{-1}), C–O (1038–1172 cm^{-1}) and aromatic bonds (1514 cm^{-1} and 1423 cm^{1}). The ^{1}H NMR spectrum presented three singlets: one deshielded proton at δ_H 9.92 (1H) (s, H-7); the second one indicating the presence of two equivalent methoxy groups at δ 4.07 (6H) (s, OCH3 C-3/C-5) and the third one in the aromatic zone at δ 7.26 (2H) (s, H-2/H-6). The ^{13}C NMR spectrum showed signals compatible with a 1,3,4,5-tetrasubstituted aromatic ring with chemical and magnetic equivalence. The substituents and positions are: hydroxyl at C-4 (δ_C 140.75), methoxyl at positions 3 and 5 (δ_C 147.29), and aldehyde group at C-1 (δ_C 129.30). The aldehyde carbonyl was found at δ_C 190.78 (C-7), two methine carbons at δ_C 106.63 (C-2/C-6) and a doubled signal for two methoxy groups at δ_C 56.43 (OCH_3 C-3/OCH_3 C-5).

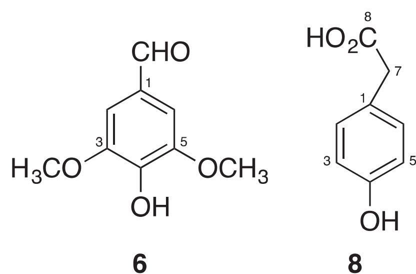
The spectral and literature data (Kim et al., 2003) led to identify the compound **6** a**s** 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde), a phenolic constituent of the lignin cell wall of plants. The lignin is responsible for mechanical support and vascular elements of plants, furthermore, the lignin is the source from which syringaldehyde can be obtained through enzimatic oxidation reactions (Aguiar and Ferraz, 2011; Ibrahim et al., 2012).

The IR spectrum of compound **8** showed absorptions indicating the presence of the following groups: carbonyl (1655 cm^{-1}), C–O (1026–1126 cm^{-1}), aromatic bonds (1597 and 1516 cm^{-1}) and hydroxyl (3354 cm^{-1}). The ^{1}H NMR spectrum presented one singlet at δ_H 3.50 (s, 2H) and two doublets at δ 7.13 (2H) and δ_H 6.78 (2H) and *J* = 8.6 Hz, suggesting and AA′BB′ substituted ring. The ^{13}C NMR spectrum of 8a revealed 6 signals, from which 3 are non-hydrogenated and a carbonyl at δ_C 173.29 (C-8) characteristic of carboxylic acid. Additionally, one deshielded aromatic carbon was found at δ_C 157.14 (C-4). A couple of intense signals at δ_C 131.15 (C-2/C-6) and δ_C 115.91 (C-3/C-5) confirmed the AA′BB′ system and the peak at δ_C 40.50 was attributed to the methylene carbon (C-7). The structure was confirmed by the correlations observed at HMBC spectrum, identifying the compound 8 as 4-hydroxyphenylacetic

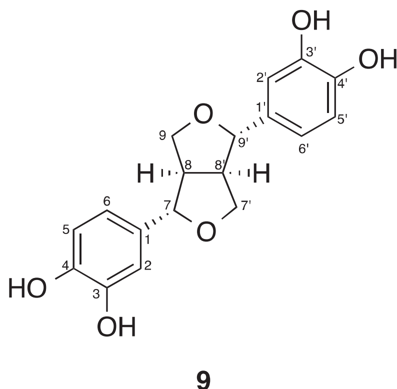
Table 2
MIC values (μg/ml) of test strains to antibiotics in the absence and presence of 4-hydroxy-3,5-dimethoxy benzaldehyde (syringaldehyde) – 128 μg/ml.

Lineage/Antibiotic	Antibiotic isolated	Antibiotic + syringaldehyde (1/4 MIC)
IS-58/Tetracycline	64	4 (32×)
RN-4220/Erythromycin	≥256	>256
AS-1199B/Norfloxacin	128	64 (2×)

acid (p-hydroxyphenylacetic acid).



The IR spectrum of compound **9** showed absorptions suggesting the presence of aromatic bonds (1450–1160 cm^{-1}) and hydroxyl group (3450 cm^{-1}). The ^{1}H NMR spectrum showed aromatic absorptions indicating the presence of an ABX substituted ring. The following signals were observed: δ_H 6.67 (d, J = 7.82 Hz, H-5/H-5′) coupling *ortho* with δ_H 6.59 (d, J = 7.82 Hz, H-6/H-6′), a singlet at δ_H 6.72 (H-2/H-2′), an oxymethinic proton at δ_H 4.52 (d, J = 2.88 Hz, H-7/H-7′), oxymethylenic protons at δ_H 4.07 (dd, J = 8.34 and 6,32 Hz, H-9/H-9′) and δ_H 3.67 (dd, J = 8.34 and 2.20 Hz, H-9/H-9′), and a methine hydrogen at δ_H 2.93 (bs, H-8/H-8′). The ^{13}C NMR spectrum showed 9 peaks, characteristic of lignans: six aromatic carbons δ_C 145.26 (C-3/C-3′), δ_C 144.76 (C-4/C-4′), δ_C 132.49 (C-1/C-1′), δ_C 117.24 (C-6/C-6′), δ_C 115.46 (C-5/C-5′), δ_C 113.75 (C-2/C-2′); one oxymethinic carbono at δ_C 85,19 (C-7/C-7′), one oxymethylenic carbon δ_C 70,58 (C-9/C-9′), and one methinic at δ_C 53,70 (C-8/C-8′). The HSQC and HMBC data are showed in Table 1. The spectral data combined with literature data (Kamiya et al., 2004) allowed to identify the compound 9 as 3,3′-bisdemethylpinoresinol, the first lignan isolated from species of Cactaceae family (Table 1).



Modulation of bacterial resistance

The syringaldehyde modulated the activity of antibiotics by reducing the concentration of antibiotic required to inhibit the growth of drug-resistant bacteria. The found activity may be related to its lipophilicity, a common feature of several inhibitors of efflux. This quality, as pointed out by Gibbons (2004), is important for the solubility in the bacterial membrane and for the interaction with efflux transporters. Thus, the results presented here indicate that syringaldehyde is a promising molecule to be used as antibiotic adjuvant.

Conclusions

The present study contributed to the chemotaxonomic knowledge of Cacteaceae family through the isolation and identification of twelve substances from *P. pachycladus*: an alcohol (decanol), two fatty acids, two chlorophyll derivatives (pheophytin a and hydroxy-pheophytin a), five steroids, one phenolic aldehyde (syringaldehyde), a phenolic acid (p-hydroxyphenylacetic acid) and a lignan (3,3′-bisdemethylpinoresinol). The compounds syringaldehyde and 3,3′-bisdemethylpinoresinol are being reported for the first time from species of Cacteaceae family. Additionally, the substance syringaldehyde was showed to be a promising molecule to reverse bacterial resistance mediated by efflux pump.

Authors' contributions

SGBF, JKSM, MMMSF, MDLF worked on the extraction, partition and chromatography procedures. YCFT, OSC, RBF, MFVS worked on spectroscopy and structural elucidation. PDF and LPF contributed in plant collection, identification and herbarium confection. JPSJ and ICSC worked on biological assays. All the authors have read the final manuscript and approved the submission.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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