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Evaluation of triterpenes isolated from stems of *Pouteria macahensis* T. D. Penn. against *Proteus mirabilis*

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ABSTRACT

This article presents the results from a phytochemical study on an acetonic extract from the stems of *Pouteria macahensis* T.D. Penn., an endemic species of the Brazilian Atlantic Rainforest. By means of the bioautography method, this extract was evaluated against *Acinetobacter baummanii*, *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus epidermidis* bacteria, of which only the *P. mirabilis* was found to be susceptible. The acetonic extract was chromatographed on a silica gel column, eluted with hexane and ethanol, producing 14 groups, allowing the isolation of the following compounds: friedelin, 3β-friedelinol and 3β-erythrodiol linoleate, arachidate and linolenate. Their structures were elucidated based on data of IR, ^{1}H NMR, ^{13}C NMR, including DEPT-135, HSQC and HMBC techniques. The minimum inhibitory concentrations of the compounds tested against *P. mirabilis* growth, were 500.0 μg mL^{-1} for friedelin and erythrodiol and 600.0 μg mL^{-1} for 3β-friedelinol and esterified 3β-erythrodiol. Based on these results, the potential of these compounds to inhibit the growth of this bacterium was confirmed.

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1. Introduction

Several sources of natural products have been described by ethnobotanical and ethnopharmacological scientific reports giving support to the new drugs development. Such reports show essential oils, extracts and natural compounds as therapeutic resources and molecule models distributed in different ecological hotspots (Costa-Lotufo et al., 2010; Camilo et al., 2017; Mohammadhosseini et al., 2017; Wansi et al., 2018; Mohammadhosseini et al., 2019; Wansi et al., 2019). The biodiversity of ecological niches is a chemical storage not fully explored and it harbors possible and diverse scientific and technological applications in the areas of economy, agrobusiness and pharmaceutical industry. The Atlantic Rainforest Biome (Brazil), characterized by the great biodiversity of flora and fauna (Galindo-Leal and Câmara, 2003; Schaffer and Campanili, 2010), contains endemic plant species not yet phytochemically and biologically studied and therefore represents an important source of bioactive compounds. Within this context, the search for alternatives to combat the multidrug resistant bacteria has long been of great interest for researchers. The bacteria resistance to current antibiotics is a threat to the benefits brought by the discovery of these drugs and technological advances in the pharmaceutical industry, which makes this problem a constant challenge (Pearson and Schaffer, 2015). In this context, what stands out is the gram-negative *Proteus mirabilis*, which causes urinary infections, especially in patients with urinary system illnesses. This bacterium, in addition to presenting unknown mechanisms of pathogenicity (Rathera et al., 2017), was also included by the World Health Organization (WHO) as presenting a multidrug resistance to antibiotics. In the flora of the Atlantic Rainforest, it is estimated that around 8,000 plant species are endemic (Amorim et al., 2009), some of which belong to the Sapotaceae family. This family consists of approximately 1,250 species distributed in 53 genera, 11 of which (about 450 species) are located in neotropical regions. The

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Pouteria Aublet and *Chrysophyllum* L. genera are the most widely found around the world, with about 330 and 80 species, respectively (Pennington, 2004). In the Atlantic Rainforest, there are reports of 34 species of *Pouteria*, 19 of which are at risk of extinction and 18 of which are endemic (Amorim et al., 2009). *Pouteria macahensis* T. D. Penn is endemic of the county of Itacaré, in the state of Bahia, Brazil. In the Brazilian traditional medicine, there are reports of the use of *Pouteria* species in the treatment of diarrhea, vomiting, inflammation, fever, ulcers, skin rashes, nausea, back pain, diabetes and the stimulation of lactation (Souza et al., 2012). Moreover, reports have shown the isolation of triterpenes (pentacyclic triterpenes and steroids) and flavonoids (flavonol, flavonone and flavanol) in the *Pouteria* genus (Silva et al., 2009). In the literature, phytochemical studies with the fruits of the *Pouteria* species are more common. Studies have been conducted on *P. sapota* (Jacq.) H. E. Moore et Stern (Ma et al., 2004; Torres-Rodrigues et al., 2011; Gulyás-Fekete et al., 2013; Turcsi et al., 2015), *P. viridis* (Pittier) Cronquist (Ma et al., 2004), *P. obovata* (R. Br.) Pierre (Dini, 2011), *P. campechiana* (Kunth) Baehni (Ma et al., 2004) and *P. caimito* (Ruiz & Pav.) Radlk. (Pelliccari et al., 1962), with reports mainly focusing on the isolation of flavonoids. The isolation of glycosylate flavonoids have been illustrated in studies conducted on the leaves of *P. campechiana* (Hernandez et al., 2008) and *P. torta* (Mart.) Radlk species (Costa et al., 2014). Triterpenes were also found in the study of branches and stem bark of *P. torta* (Che et al., 1980), *P. venosa* (Mart.) Baehni (Montenegro et al., 2006) and *P. caimito* species (Ardon and Nakano, 1973). The present study aimed to isolate and identify the compounds of the acetonic extract from the stems of *P. macahensis* and evaluate its antibacterial property. To the best of our knowledge, this is the first phytochemical-based study from the stems of this herbal species.

2. Experimental

2.1. General experimental procedures

Silica gel 60 (0.063-200 mm) was used for column chromatography (CC), silica gel 60 (0.040-0.063 mm) for flash column chromatography and silica gel 60G for thin layer chromatography (TLC). Infrared spectra (IR) were taken on a KBr pellet in a Perkin Elmer ®400 (Massachusetts, USA). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in a Bruker DRX 400 (Ettlingen, Germany), using TMS as an internal standard and $CDCl_3$ as a solvent. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired under standard conditions. Two-dimensional inverse hydrogen detected heteronuclear shift correlation spectra were obtained by the HMQC pulse sequence [^{1}J(C,H)]. Two-dimensional inverse hydrogen detected heteronuclear long-range correlation experiments were carried out with HMBC pulse sequence [^{n}J(CH); n=D2 and 3]. The gas chromatography (GC) analyses were carried out on a Varian Saturn 3800 (Califonia, USA) equipped with an FID detector using a carbowax capillary column (30 m × 0.25 mm × 0.25 µm) and helium 5.0 purity as a carrier gas having a flow rate of 1.0 mL min^{-1} (10 psi). The injection temperature was set at 220 °C and the detector at 240 °C. The analyses were performed by injecting 1 µL of 10% (w/v) solution in $CHCl_3$, manual injection, in the split mode 1:10. The initial temperature of the column was 60 °C, raised by 5 °C min^{-1} until reaching 200 °C; this temperature was then maintained for 5 minutes. To identify the fatty materials, a mixture of Fatty Acid Methyl Ester (FAME) and C_8-C_{22} (certified by Supelco-USA; catalog 47801-Lot: LC00126) was used. The transesterification of the triterpene mixture was performed according to the methodology proposed by Mendes et al. (1999).

2.2. Plant Material

P. macahensis T. D. Penn was collected in September 2015, on the banks of the Rio de Contas, in the Municipality of Itacaré, Bahia, Brazil (coordinates: 14°15'53.0"S 38°59'59.6"W), area at an altitude of 2900 mm. The species was identified by Luiz A. Mattos and José L. da Paixão, logged under the voucher number 21.426 and deposited at the Herbarium of the Universidade Estadual de Santa Cruz (UESC). The stems were separated and dried in the shade at room temperature (26-28 °C). Thereafter, the stems were ground in knife mill type Willye 30 mesh and packed in airtight plastic bottles at room temperature.

Fig. 1. Representation of the *Pouteria macahensis*: (A) Photograph; (B) Localization of the species.

2.3. Extraction and fractionation

The stems (1,500 g) were submitted to exhaustive extractions with acetone to produce the extract (PMA, 144.08 g). PMA (60 g) was submitted to a silica gel CC (1,300 g) using the hexane and ethanol solvents eluted successively in increasing order of polarity in various combinations: 100:0; 95:5; 90:10; 80:20; 70:30; 50:50; 30:70 and 0:100 v/v, respectively; resulting in 39 fractions. The grouping by TLC led to the obtaining of 14 groups of fractions (G1-G14). Group G-3 (4.42 g) was treated with acetone, resulting in a solid, containing the mixture of the compounds (**1**) and (**2**), 18.9 mg. Group G-4 (4.5 g) was solubilized with MeOH/H2O (7:3) leading to the separation of a solid A (83.0 mg). The rest of the material was partitioned with hexane. Solid A was then treated with acetone and recrystallized in ethanol, yielding the compound (**2**), 26.8 mg. The hexane phase, after concentration, resulted in a solid (3.1 g). This, upon being treated with acetone, yielding a solid B (6.8 mg), which, after comparison with TLC, proved to be identical to compound (**2**). The remaining material (2.1 g) was submitted to CC flash and eluated with hexane/ CH_2Cl_2, leading to 7 fractions (GS1-GS7). Group GS5 (103.6 mg) was submitted to separation by preparative thin-layer chromatography (Silica gel Vetec® 60G, thickness 0.75 mm). The solid was then recrystallized in acetone, affording the mixture of the compounds (**3a-c**), 33.8 mg. Compounds (**3a-c**), 12.0 mg, were submitted to transesterification reaction with MeOH/MeONa, leading to the obtaining 6.0 mg of compound (**4**). **Friedelin (1) and 3β-friedelinol (2):** m.p. 260-263 °C, IR ν_{max} (KBr): 3472, 2925, 2854 1465, 1363, 1705; ^{1}H NMR ($CDCl_3$) δ: 0.86 (s), 0.95 (s), 0,97 (s), 0.98 (s), 0.99 (br), 1.02 (s), 1.10 (s), 1,17 (s), 1.13 (s), 1.17 (s), 1.18 (s), 1.90 (m), 2.47 (m), 3.82 (br); ^{13}C NMR ($CDCl_3$) δ: 6.70 (C-23), 11.66 (C-23), 14.53 (C-24), 15.85 (C-1), 16.33 (C-24), 17.50 (C-7), 17.82 (C-25), 18.17 (C-25), 18.53 (C-27), 20.01 (C-26), 22.17 (C-1), 28.07 (C-20), 29.93 (C-17), 30.56 (C-12), 31.69 (C-29), 31.71 (C-30), 32.00 (C-28), 32.25 (C-21), 32.76 (C-15), 34.90 (C-29), 34.91 (C-30), 35.02 (C-16), 35.26 (C-19), 35.38 (C-11), 35.51 (C-16), 36.02 (C-2), 37.05 (C-9), 37.85 (C-5), 38.29 (C-14), 39.18 (C-22), 39.60 (C-13), 41.40 (C-2), 41.19 (C-6), 41.76 (C-18), 42.78 (C-5), 49.30 (C-4), 53.14 (C-8), 58.10 (C-4), 59.37 (C-10), 61.42 (C-10), 72.14 (C-3), 212.85 (C-3). **3β-friedelinol (2):** m.p. 246-248 °C, IR ν_{max} (KBr): 3470, 2852, 1450, 1363, 1465, 1368; 1H NMR ($CDCl_3$) δ: 0.86 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-23), 0,97 (3H, s, CH_3-29), 0.98 (3H, s, CH_3-26), 0.99 (3H, d, *J* =6.8 Hz, CH_3-27), 1.02 (3H, s, CH_3-30), 1.10 (3H, s, CH_3-24), 1,17 (3H, s, CH_3-28), 1.25 -1.99 (H-C (*n*-alkyl), 3,73 (1H, br, CH-3); ^{13}C NMR (CDCl3) δ: 11.70 (C-23), 15.83 (C-1), 15.88 (C-24), 16.43 (C-7), 17.60 (C-25), 18.27 (C-27), 20.13 (C-26), 28.19 (C-20), 29.71 (C-17), 30.05 (C-12), 30.67 (C-30), 31.81 (C-28), 32.11 (C-15), 32.37 (C-21), 32.86 (C-29), 35.03 (C-19), 35.37 (C-11), 35.60 (C-16), 36.12 (C-2), 37.15 (C-9), 37.91 (C-5), 38.41 (C-13), 39.30 (C-22), 39.71 (C-14), 41.81 (C-6), 42.88 (C-18), 49.29 (C-8), 53.24 (C-4), 61.45 (C-10), 72.56 (C-3). **3β-Erythrodiol linoate, arachidate and linolenoate (3a-c):** m.p. 119-121 °C; IR ν_{max} (KBr): 3472, 2925, 2855, 1732, 1465, 1363; ^{1}H NMR ($CDCl_3$) δ: 0.86 (6H, s, CH_3-29 and CH_3-30), 0.87 (3H, s, CH_3-24), 0.89 (3H, s, CH_3-23), 0.94 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-26), 1,16 (3H, s, CH_3-27), 1.26 (nH, s, CH_2), 1.55-1.98 (nH, m, H-C (*n*-alkyl), 2.28 (2H, t, J =7.4 Hz, H-2'), 3.21 (1H, d, *J* =10.9 Hz, H-28a), 3.54 (1H, d, *J* = 10.9 Hz, H-28b), 4.50 (1H, m, H-3), 5,19 (1H, t, *J* =3.2 Hz, H-12), 5.29 (1H, s, H); ^{13}C NMR ($CDCl_3$) δ: 14.09 (C-20'), 14.09 (C-18'), 15.56 (C-24); 15.56 (C-25), 16.75 (C-26); 18.25 (C-6), 22.04 (C-16), 23.54 (C-11), 23.58 (C-30), 23.60 (C-2), 25.18 (C-3'), 25.56 (C-15), 25.90 (C-27), 28.05 (C-23), 29.14 - 29.76 (Cn; CH2), 31.04 (C-20), 31.04 (C-22), 32.54 (C-7), 33.17 (C-29), 34.10 (C-21), 34.90 (C-2'), 36.84 (C-10), 36.96 (C-17), 37.77 (C-4), 38.29 (C-1), 39.83 (C-8), 41.75 (C-14), 42.36 (C-18), 46.44 (C-19), 47.52 (C-9), 55.28 (C-5), 69.71 (C-28), 80.55 (C-3), 122.31 (C-12), 144.23 (C-13), 173.67 (C -1'). **Erythrodiol (4):** m.p. 228-230 °C; IR ν_{max} (KBr): 3492; 2925; 2855; 1465; 1352; ^{1}H NMR ($CDCl_3$) δ: 0.86 (6H, s, CH3-29 and CH3-30), 0.87 (3H, s, CH3-24), 0.89 (3H, s, CH3-23), 0.94 (3H, s, CH3-25), 0.95 (3H, s, CH_3-26), 1,16 (3H, s, CH3-27), 1.55-1.98 (nH, m, H-C(*n*-alkyl), 3.21 (1H, d, *J* =10.9 Hz, H-28a); 3.54 (1H, d, *J* =10.9 Hz, H-28b), 5.17 (1H, t, *J* =3.2 Hz, H-12), 5.29 (1H, br, H-9); ^{13}C NMR ($CDCl_3$) δ: 15.6 (C-24), 16.7 (C-25), 16.8 (C-26), 18.2 (C-6), 22.0 (C-16), 23.5 (C-11), 23.5 (C-2), 23.6 (C-30), 25.5 (C-15), 25.9 (C-27), 28.0 (C-23), 31.0 (C-22), 31.0 (C-20), 32.5 (C-7), 34.1 (C-29), 34.1 (C-21), 36.8 (C-10), 36.9 (C-17), 37.7 (C-4), 38.2 (C-1), 39.8 (C-8), 41.7 (C-14), 42.3 (C-18), 46.4 (C-19), 47.5 (C-9), 55.2 (C-5), 69.8 (C-28), 80.5 (C-3), 122.3 (C-12), 144.2 (C-13).

2.5. Antibacterial tests

2.5.1. Bioauthography

The *Acinetobacter baummanii* (ATCC 19606), *Proteus mirabilis* (ATCC 15920), *Escherichia coli* (EPEC CDC 086H35) and *Staphylococcus epidermidis* (ATCC 12228) bacteria were obtained from FIOCRUZ-INCQ, Rio de Janeiro, Brazil. In a 50 mL polypropylene tube, a suspension of each microorganism prepared in 3 mL of Brain Heart Infusion (BHI) at 1 on the McFarland scale was added to 20 mL of nutrient agar at 45 ºC and 150 µL of 2,3,5-triphenyltetrazolium chloride dye. Approximately, 1,000 µL of this mixture was distributed over a silica gel chromatography plate, containing extract spots. After 24 hours at 37 °C, the inhibitory halo was measured in triplicate. Chloramphenicol and pure solvents were used as positive controls. The inhibition halo was measured with ruler and positive results were considered when absence of bacterial growth was seen beyond the extract spot (values equal to or above 5 mm).

2.5.2. Minimum inhibitory concentration

To assess the potential antibacterial activity, the isolated compounds were tested quantitatively against *P. mirabilis*. It is important to note that friedelin was isolated as a mixture; however, the availability of this pure compound in our laboratory allowed it to be tested. Compounds were initially solubilized in sterile 10% dimethylsulfoxide (DMSO) in distilled water at 1,200 µg mL^{-1}. The microdilution technique used followed the Clinical and Laboratory Standard Institute (CLSI, 2008), with adaptation. Briefly, 90 µL of the compounds, at concentrations from 75.0 to 600.0 µg mL^{-1} diluted in BHI were distributed onto 96-well plates, later adding 10 µL of *P. mirabilis* at 0.5 on the McFarland scale which corresponds to 1.5×10^8 colony forming units (CFU/mL) of bacteria, totaling a volume of 100 µL in each orifice. The plates were incubated for 24 h at 37 ºC. Next, 20 µL of resazurin (0.01% final concentration) were added to each orifice to determine the MIC and 10 µL of the samples referent to each concentration were transferred to the Petri plates containing nutrient agar to determine the minimum bactericidal concentration (MBC). The tests were carried out in triplicate and repeated three times. Chloramphenicol at a concentration of 50.0 µg mL^{-1}, microorganism tests, BHI broth, test solutions without bacteria and broth only with the bacteria were used as controls.

3. Results and Discussion

3.1. Identification of compounds

From the extract, the following triterpene mixture was isolated and identified: (**1-2**) and (**3a-c**), Fig. 1. Compound (**4**) was obtained as a byproduct of the compound's hydrolysis of (**3a-c**). Their structures were elucidated through spectra analyses, including IR, NMR (1D and 2D experiments) and compared with the data available in the literature. The structural elucidations of the mixture of triterpenes (**1**) and (**2**) were based on the analysis of the spectra together with the literature data for friedelin (Fulgentius et al., 1990) and 3β-friedelinol (Salazar et al., 2000), in addition to the comparison of TLC with authentic samples.

Compound (**2**), an amorphous white solid, was also isolated in pure form; the elucidation of its structure was conducted by comparing the spectral data with the literature for 3β-friedelinol (Salazar et al., 2000), in addition to the comparison of TLC with authentic samples.

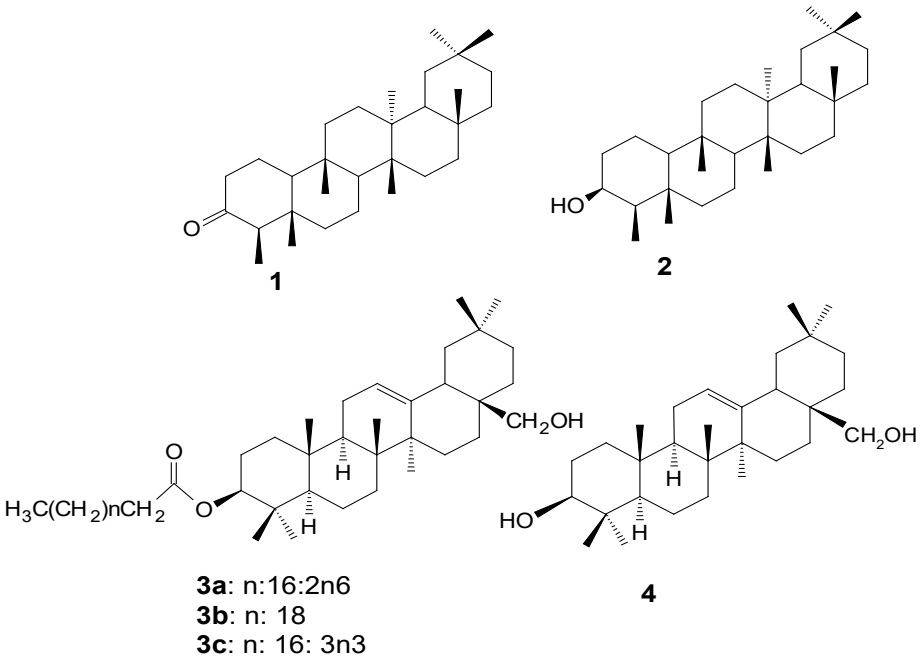

Fig. 2. Chemical structure of pentacyclic triterpenes isolated from the acetonic extract of *P. macahensis*.

The mixture of compounds (**3a-c**) was identified by IR, ^1H and ^{13}C NMR, DEPT-135, HSQC and HMBC spectra analyses. The IR spectrum displayed a strong absorption at 3472 cm^{-1} (νOH), 2925 and 2855 cm^{-1} (νCH), 1732 cm^{-1} (νC=O) and medium absorptions at 1465 cm^{-1} and 1363 cm^{-1} (δCH). In the ^1H NMR spectrum, six signals were observed in δ_H: 0.86, 0.87, 0.89, 0.94, 0.95 and 1.16, characteristic of the methyl groups in triterpenes.

The presence of signals at δ_H 1.26 (nCH_2, s); δ_H 2.28 (2H, t, J =7.4 Hz, CH_2 neighboring the ester group) and δH 4.5 (1H, m) referent to the oxymethinic hydrogen, suggest the presence of the pentacyclic triterpene substituted at the C-3 position. The signals observed at δ_H 3.54 (1H, d, J =10.9 Hz), at δ_H 3.21 (1H, d, J =10.9 Hz) and at δ_H 5.19 (1H, br), characteristic of olefin hydrogen, compared with data from the literature for pentacyclic triterpenes (Mahato and Kandu, 1994) suggest the presence of the olean-12-ene nucleus. The spectrum of ^{13}C NMR presented 45 signals of carbon, associated with the DEPT-135 experiment, allowing for the attribution of 8 signals referent to the CH_3, 20 to CH_2, 5 to CH and 8 to non-hydrogenated carbons, while the signal at δ_C 173.67 was referent to ester carbonyl. The presence of signals at δ_C 122.31 (CH) and δ_C 144.23 (non-hydrogenated carbon) suggest the olean-12-ene nucleus (Mahato and Kundu, 1994). The ^{1}H-^{13}C correlations were confirmed by the HSQC experiment. The ^{1}H-^{13}C correlations in the HMBC for the compounds (**3a-c**) confirmed the localization of the double bond position between carbon C-12 and C-13 through the cross-peak identified at δ_H 5.19(H-12)/(δC 47.52(C-9), δ_H 5.19(H-12)/δC 23.54(C-11) and δ_H 5.19(H-12)/δC2.36 (C-18) (Fig. 2) and the presence cross-peak at δC 69.71(C-28)/δH 3.54 (H-28b) and δ_C 69.71(C-28)/δ_H 3.21(H-28a), indicating the structure of the erythrodiol triterpene (Wang et al., 2009) for the compound. In this case, a long chain ester, probably located at the C-3 position. The position of the oxymethylene carbon at C-28 was proven by means of the cross-peak at δ_H 3.53(1H-28a)/δ_C 22.03(C-16) and δ_H 3.21 (1H-28b)/(δ_C 31.04 (C-22). The correlation of δ_H 4.50 (C-3)/δ_C 173.67 (C1') confirmed the esterification of erythrodiol at the C-3 position. To characterize the fatty acid chain, the mixture of the compounds (**3a-c**) was submitted to transesterification reaction with MeOH/MeONa. The CG-FID analysis of the chloroform phase indicated the presence of methyl esters of the linoleate, arachidate and linolenate. Thus, the compounds (**3a-c**) were identified as a mixture of the 3β-erythrodiol linoleate, arachidate and linolenate. To the best of our knowledge, this is the first report of this mixture of compounds in this genus. Other such derivatives of esterified triterpenes have been reported in this genus: acetates of α and β-amyrin, lupeol, taraxerol and erythrodiol palmitate (Silva et al., 2009).

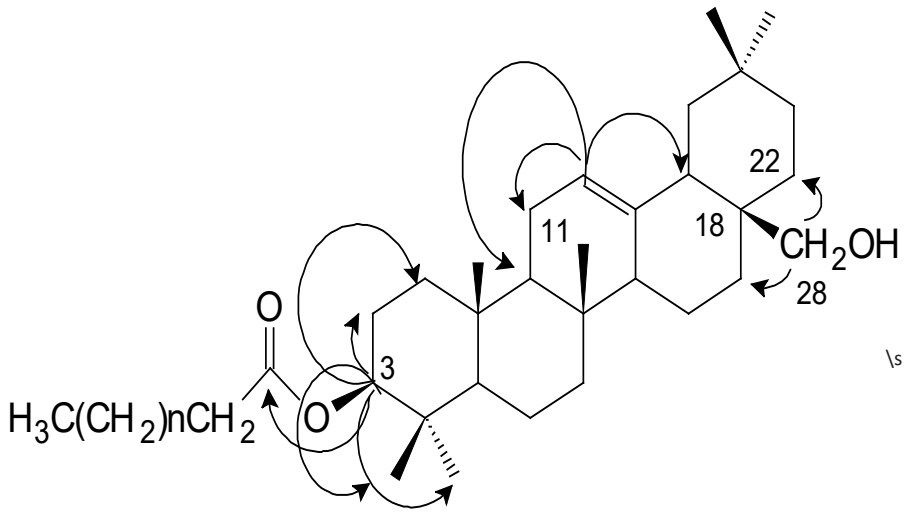

Fig. 3. Main HMBC correlations (H→ C) observed for compund **3**.

The aqueous phase of the transesterification reaction produced a white solid compound (**4**). The structural elucidation of this compound was supported by the IR, ^{1}H and ^{13}C NMR and DEPT-135 analyses and was compared to that reported in the literature for erythrodiol (Wang et al., 2009). The ^{1}H-^{13}C correlations were confirmed by the HSQC experiment. Few phytochemical studies of the stems of *Pouteria* species are reported in the literature. In 1973, Ardon and Nakano (1973) reported the isolation of β-Sitosterol and taraxerol for *P. caimito*. Che et al. (1980) reported 3α and 3β-amyrin acetate, betulinic acid and ursolic acid for *P. torta* and Montenegro et al. (2006) reported espinasterol, ursolic acid, taraxerol, 3β,19α,23-trihydroxyurs-12-en-28-oic acid and 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid for *P. venosa*.

3.2. Antibacterial tests

The acetonic extract was evaluated using the bioautography method against *Acinetobacter baummanii*, *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus epidermidis* bacteria. However, a positive response was only observed with the *P. mirabilis*. Subsequently, the isolated and identified compounds were submitted to quantification by microdilution technique in order to establish the minimal concentration which inhibits the growth of *P. mirabilis*. The obtained results from quantitative technique revealed a bacteriostatic activity of all compounds at 500.0 or 600.0 μg mL^{-1} (Table 1) being the best results for friedelin and erytrodiol.

Table 1
Minimum inhibitory concentration of the triterpenes against *P. mirabilis*.

Num.	Compounds	MIC (μg mL^{-1})
1	Friedelin	500.0
2	3β-Friedelinol	600.0
3	Esterified erythrodiol[a]	600.0
4	Erythrodiol	500.0
Control	Chloramphenicol [b]	50.0

[a] Linoleate, arachidate and linolenoate; [b] Positive control. Test performed in triplicate

Few studies report on the activity of isolated compounds against *P. mirabilis*. The ethanol extract of *Lithrea molleoides* (Vell), from Argentina, exhibited an activity when it came into contact with this microorganism, showing an MIC of 2,000 to 8,000 μg mL^{-1} and the compound(*Z*,*Z*)-5-(trideca-4',7'-dienyl)-resorcinol, which presented an MIC of 4,000 μg mL^{-1} (Carpinella et al., 2011). Viswanathan et al. (2012) reported the activity for this bacteria both for the methanolic extracts and the chloroform of the leaves of *Jatropha tanjorensis* J.L.Ellis & Saroja, from India as well as the activity of friedelin, using the agar diffusion method. The extracts tested at concentrations of 50.0, 25.0 and 12.5 μg mL $^{-1}$ presented inhibition halos of 24, 22 and 19 mm for the methanolic extract and 23, 22 and 10 mm for chloroform, respectively. Friedelin in the concentrations of 10.5 and 2.5 μg mL^{-1} presented an inhibition halo, which differed from the present study's results. For the other compounds, to the best of our knowledge, no tests have been performed with *P. mirabilis*.

4. Concluding remarks

P. macahensis represents a promising source of pentacyclic triterpenes **1** to **3** and in this research the evaluation of the biological activity of some pure known triterpenes against *P. mirabilis*, for the first time. The present study contributes to the chemical knowledge of the *Pouteria* genus and offers perspectives for a more in-depth study of the molecules that act against bacterial resistance, especially against multidrug resistant bacteria, such as *P. mirabilis*.

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Supplementary material

GC-FID profile of the transesterification reaction of the compounds **3a-c** available as electronic supplementary material.

Conflict of interest

The authors declare that there is no conflict of interest.

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