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Chemical constituents and leishmanicidal activity from leaves of *Kielmeyera variabilis*

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Abstract: Many phenolic compounds such as xanthones, quinones and coumarins have been isolated from *Kielmeyera* species; however the presence of flavonoids have been showed in other genera in the Calophylleae tribe as *Caraipa, Mesua* and *Calophyllum*. Six known glycosidic flavonoids: quercetin 3-β-*O*-galactopyranoside (**1**), quercetin 3-β-*O*-glucopyranoside (**2**), quercetin 3-*O*-α-rhamnoside (**3**), luteolin 6-*C*-β-glucopyranoside (**4**), isovitexin (**5**), kaempferol 3-*O*-α-rhamnoside (**6**) and one triterpene, lupenone (**7**) were isolated, for the first time, from organic crude extract of *Kielmeyera variabilis* Mart. & Zucc., Calophyllaceae, leaves. The crude organic extract from *K. variabilis* leaves exhibited 95% of leishmanidal activity at 20 µg/mL on amastigote-like form of *Leishmania (Leishmania) amazonensis in vitro* model and only compound **3** showed 40-45% of growth inhibition at concentration ranging from 0.78 to 20 µg/mL. In addition, quercetin 3-*O*-α-rhamnoside (quercitrin) was found to be the major metabolite. Our results and previous reports suggest that synergistic effects of flavonoid glycosides are the cause of significant leishmanidal activity of the crude organic extract from *K. variabilis* leaves.

Article

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Introduction

Kielmeyera variabilis Mart. & Zucc., Calophyllaceae, is a shrub or tree that reaches up to 6 m in height and is endemic to the Cerrado (Bittrich, 2010), the savanna-like vegetation of Central-Brazil. It is popularly known as "pau-santo", "rosa-do-campo" or "folha-santa". Methanol extract obtained from *K. variabilis* stems showed molluscicidal activity against *Biomphalaria glabrata* (12.5 ppm) (Pinheiro et al., 2003a) and activity against *Staphylococcus aureus* (3.0 µg/mL) and *Bacillus subtilis* (1.95 µg/mL). A phytochemical study of this species led to the isolation of three xanthones (assiguxanthone-B, kielcorin and 1,3,5,6-tetrahydroxy-2-prenylxanthone) and an organic acid (2,5-dihydroxy benzoic acid), being assiguxanthone-B active against *B. subtilis* (25 µg/mL) (Pinheiro et al., 2003b).

The occurrence of coumarins as phenylcoumarins (Gramacho et al., 1999), prenylated 4-*n*-propylcoumarins (Cruz et al., 1998a,b; Scio et al., 2003) and xanthones (Garcia Cortez et al., 1998, 2002) are frequently reported as isolated compounds from *Kielmeyera* genus. An osajaxanthone, isolated from a stem extract of *Kielmeyera coriaceae* showed protection against infection by cercariae of *Schistosoma mansoni* (Lopes et al., 1977) and other ones have exhibited antifungal activity against the pathogenic plant fungi *Cladosporium cucumerinum* and *Candida albicans* (Garcia Cortez et al., 1998). Extracts of leaves from *K. coriacea* have demonstrated anxiolytic activity after oral treatment with 120 mg/kg body weight per day for thirty days (Audi et al., 2002). Ethanolic extract from stem of *K. coriacea* at a dose of 60 mg/kg body weight and its dichloromethane semi-pure fraction at dose of 6 mg/kg body weight showed similar antidepressant-like effects after 45 days of oral treatment in Male Wistar rats (Martins et al., 2004, 2006). Hexane and dichloromethane extracts from different parts of this species have anticancer activity against SF-295 (brain); HCT-8 (human colon carcinoma); MDA-MB-435 (melanome) and HL-60 (leukemia) with IC50 values ranging from 3.0 to 34.9 µg/mL (Mesquita et al., 2009). Recently, Sobral et al. (2009) demonstrated antibacterial activity against *Micrococcus luteus* (CIM 7.8 µg/mL), *Bacillus subitilis*

(CIM 15.6 µg/mL), *Staphylococcus aureus* (CIM 31.2 µg/mL) and *Streptococcus mutans* (CIM 31.2 µg/mL) of the dichloromethane extract from trunk of *Kielmeyera cuspidata*.

In this work we describe for the first time the isolation of one triterpene and the occurrence of flavonoids, including *O*-flavonoids and *C*-flavonoids, from leaves extract of *K. variabilis*. In addition, we suggested that quercetin 3-*O*-α-rhamnoside is among the leishmanicidal compounds from crude extract.

Materials and Methods

Plant material

Leaves of *Kielmeyera variabilis* Mart. & Zucc., Calophyllaceae, were collected in Brumadinho, Minas Gerais, Brazil. A voucher specimen was identified and deposited by Dr. Marcos E. G. Sobral in the Herbarium BHCB, at Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas (Stehmann, number 2646) in Belo Horizonte-MG.

General procedures

The solvents were removed using a SpeedVac® vacuum centrifuge (ThermoSavant SPD SC250, Holbrook, NY, USA). Thin-layer chromatographic (TLC) analyses were conducted on pre-coated commercial silica gel G-60/F_{254} (0.25 mm, Merck, Darmstadt, Germany) plates. They were eluted in a pre-saturated chamber using solvents mixtures in different proportions of: a) chloroform:methanol:water (65:50:5); b) hexane:ethyl acetate (70:30) or c) ethyl acetate:methanol:formic acid:glacial acetic acid:water (100:11:11:26). The spots were visualized under visible, UV light at 254 nm and 360 nm, and after spraying the plate with mixture (1:1) of ethanolic solutions of vanillin (1%) and sulfuric acid (10%). Medium-pressure liquid chromatography (MPLC) was performed using an LC8 pump (Shimadzu, Kyoto, Japan), Büchi columns n° 19676 and n° 19674 for reverse (RP-18, 25-40 mesh) and normal (silica gel 60, 25-40 mesh) phases, respectively, at flow rates of 20 mL/min and detection at λ 220 nm and 254 nm. Semi-preparative purifications were performed using Shim-pack® C18 column (5 µm, 20x250 mm, *i.d.*), mixtures of MeOH:H_2O as eluents, at flow rates of 10 mL/min and detection at λ 220 and 254 nm using a Shimadzu chromatographic system (Shimadzu, Kyoto, Japan) equipped with an LC6AD pump and UV-visible dual-wavelength detector (SPD10A). Analytical RP-HPLC analyses were developed in a HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC6AD pump and a SPD M-10A VP Diode Array Detector. Analyses were carried out by Shim-pack® C18 column (5 µm, 4.6x250 mm *i.d.*) eluted with gradient of MeOH/H_2O from 10% to 100% of MeOH at a flow rate of 1 mL/min. Gel Permeation Chromatography (GPC) was carried out using glass columns filled with Sephadex LH-20™ (GE Healthcare, U.S.A.) gel and MeOH as mobile phase. NMR (nuclear magnetic resonance) spectra were obtained using a Brucker DRX 400 spectrometer at 400 MHz with the pulse programs provided by the manufacturer. The substances were dissolved in perdeuterated solvents containing 0.1% tetramethylsilane as the internal chemical shift standard. UV spectra (200-500 nm) were obtained by Shimadzu SPDM-84 10A VP Diode Array Detector from HPLC system (Shimadzu, Kyoto, Japan). Electrospray ionization mass spectrometry (ESI-MS) were performed using a Bruke maXis ETD (Bruker, DE). Solutions of the compounds at 200 µg/mL in ACN:H_2O (1:1) were infused at 3 µL/min with 0.1% formic acid in the negative mass spectra acquired over a range between *m/z* 50-1500 daltons. The instrument was operated under the following conditions: end plate offset, -500 V; capillary voltage, 4500 V; nebulizer pressure, 0.4 bar; dry gas (nitrogen) flow rate, 4.0 L/min; dry temperature, 180 °C; ISCID energy, 0-20 eV; hexapole RF, 600 Vpp; collision energy, 0-30 eV; collision RF, 600 Vpp; transfer time, 30-100 µs.

Extraction and isolation

Leaves (62 g) of *K. variabilis* were extracted with a dichloromethane and MeOH mixture (1:1) at room temperature. The solution was concentrated under vacuum in a rotary evaporator at temperatures below 45 °C, and the residual solvent was removed in a vacuum centrifuge at 40 °C. Organic crude extract (3 g) was suspended in MeOH:H_2O (1:1) and extracted with hexane. This procedure afforded a Hex soluble layer (Hex, 0.6 g) and an aqueous soluble layer (Aq, 1.8 g) fractions. Solvents from fractions were dried using a vacuum centrifuge. The Aq fraction was subjected to reverse-phase MPLC separation using increasing amounts of 10% aq. MeOH to produce 31 fractions. Fractions 15 (MeOH:H_2O; 1:1) and 17 (MeOH:H_2O 70:30) yielded a mixture of compounds **1** and **2** (15 mg) and a pure compound **3** (200 mg), respectively. Fraction 13 (MeOH:H_2O; 1:1) was purified on semi-preparative, using a gradient of MeOH:H_2O (MeOH, 10-100% in 40 min) to obtain **4** (3 mg). Fraction 14 (MeOH; 100%) (45 mg) was purified by GPC to afford **5** (7 mg). Fraction 18 (MeOH:H_2O; 60:40) (49 mg) was submitted to semi-preparative HPLC, using a Shim-pack® C18 column and eluted with mixtures of MeOH:H_2O (50:50→70:30 in 30 min; 50:40→60:40 in 2 min; 60:40 in 8 min) to obtain **6** (3 mg). The Hex fraction was subjected to a normal MPLC, developed with petroleum ether containing increasing amounts of EtOAc to yield seventeen fractions. Fraction 12 (210 mg) was purified over silica gel column, eluted with Hex-EtOAc (97:3), and then isolated by GPC to yield **7** (110 mg).

Quercetin 3-β-*O*-galactopyranoside or hyperoside (**1**) UV λ_{max}^{MeOH}/nm: 256, 356; NMR ^{1}H (400 MHz, CD_3OD): δH 7.82 (1H, *d*, *J* 2.0 Hz H-2'), 7.62 (1H, *dd*, *J* 8.6 and 1.1 Hz, H-6') 6.89 (1H, *dd*, *J* 8.6 and 2.0 Hz, H-5'), 6.43 (1H, *d*, *J* 2.0 Hz, H-8), 6.23 (1H, *d*, *J* 2.0 Hz, H-6), 5.26 (1H, *d*, *J* 7.6 Hz, H-1"), 3.86 (1H, *m*, H-4"), 3.81(1H, *dd, J* 9.5 and 8.0 Hz, H-2"), 3.65 (2H, *dd*, *J* 11.2 and 6.0 Hz, H-6"), 3.56 (1H, *m*, H-3"), 3.49 (1H, *t, J* 6.0 Hz, H-5"). NMR ^{13}C (100 MHz, CD_3OD): δC 179.61 (C-4) 166.13 (C-7), 163.19 (C-5), 158.92 (C-2), 158.56 (C-9), 150.06 (C-4'), 146.12 (C-3'), 135.66 (C-3), 123.34 (C-1'), 123.16 (C-6'), 117.92 (C-2'), 116.39 (C-5'), 105.66 (C-10), 105.16 (C-1"), 100.07 (C-6), 94.91 (C-8), 77.37 (C-5"), 75.18 (C-3"), 73.25 (C-2"), 70.12(C-4"), 62.10 (C-6"). ESI-MS *m/z* negative mode: 463.0788 $[M-H]^-$, MS^2 [300.0216].

Quercetin 3-β-*O*-glucopyranoside or isoquercitrin (**2**) UV λ_{max}^{MeOH}/nm: 256, 356; NMR ^{1}H (400 MHz, CD_3OD): δH 7.62 (1H, *dd, J* 8.6 and 1.1 Hz, H-6') 7.73 (1H, *d, J* 2.0 Hz H-2'), 6.89 (1H, *dd, J* 8.6 and 1.8 Hz, H-5'), 6.43 (1H, *d, J* 2.0 Hz, H-8), 6.23 (1H, *d, J* 2.0 Hz, H-6), 5.35 (1H, *d, J* 7.2 Hz, H-1"), 3.72 (2H, *dd, J* 12.0 and 2.2 Hz, H-6"a), 3.58 (m, H-6"b), 3.47 (1H, *m*, H-2"), 3.43 (1H, *m*, H-5"), 3.35 (1H, *m*, H-4"), 3.24 (1H, *ddd, J* 9.0, 5.0 and 2.0 Hz, H-3"). NMR ^{13}C (100 MHz, CD_3OD): δC 179.57 (C-4), 166.07 (C-7), 163.19 (C-5), 158.79 (C-2), 158.53 (C-9), 149.97 (C-4'), 146.12 (C-3'), 135.65 (C-3), 123.33 (C-1'), 123.17 (C-6'), 117.76 (C-2'), 116.31 (C-5'), 105.66 (C-10), 104.10 (C-1"), 100.07 (C-6), 94.91 (C-8), 78.63 (C-5"), 78.26 (C-3"), 75.87 (C-2"), 71.41(C-4"), 62.68 (C-6"). ESI-MS *m/z* negative mode: 463.0788 $[M-H]^-$, MS^2 [300.0216].

Quercetin 3-*O*-α-rhamnoside or quercitrin (**3**) UV λ_{max}^{MeOH}/nm: 256, 350; NMR ^{1}H (400 MHz, CD_3OD): δH 7.34 (1H, *dd, J* 8.3 and 2.0 Hz, H-2'), 7.30 (1H, *dd, J* 8.3 and 2.0 Hz, H-6'), 6.93 (1H, *d*, *J* 8.3 Hz, H-5'), 6.36 (1H, *d*, *J* 2.0, H-8), 6.19 (1H, *d,* J 2.0 Hz, H-6), 5.34 (1H, *d*, *J* 1.3 Hz, H-1"), 4.21 (1H, *dd*, *J* 3.0 and 1.5, H-5"), 3.74 (1H, *dd*, *J* 9.4 and 3.2 Hz, H-3"), 3.42 (1H, *dq J* 9.4 and 6.1 Hz, H-2"), 3.32 (1H, *dd*, *J* 9.3 and 2.0, 4"), 0.93 (3H, *d*, *J* 6.1 Hz, H-6"). NMR ^{13}C (100 MHz, CD_3OD): δC 179.65 (C-4), 165.95 (C-7), 163.21 (C-5), 159.32 (C-2), 158.54 (C-9), 149.80 (C-4'), 146.41 (C-3'), 136.24 (C-3), 123.00 (C-1'), 122.87 (C-6'), 116.96 (C-2'), 116.38 (C-5'), 105.89 (C-10), 103.55 (C-1"), 99.85 (C-6), 94.74 (C-8), 73.27 (C-4"), 72.14 (C-3"), 72.03 (C-2"), 71.90 (C-5"), 17.64 (C-6"). ESI-MS *m/z* negative mode: 447.0885 $[M-H]^-$, MS^2 [300.0248].

Luteolin 6-*C*-β-D-glucopyranoside or isoorientin (**4**) UV λ_{max}^{MeOH}/nm: 269, 339; NMR ^{1}H (400 MHz, CD_3OD): δH 7.39 (1H, *dd*, *J* 8.6 and 2.3 Hz, H-6'), 7.36 (1H, *s*, H-2'), 6.91 (1H, *d*, *J* 8.6 and 1.4 Hz, H-5'), 6.56 (1H, *s*, H-3), 6.50 (1H, *s*, H-8), 4.91 (1H, *d*, *J* 9.8 Hz H-1"), 4.16 (1H, *t* like, *J* 9.5 Hz, H-2"), 3.88 (1H, *dd*, *J* 12.0 and 2.0 Hz, H-6"), 3.74 (1H, *dd*, *J* 12.0 and 5.2 Hz , H-6"), 3.50 (2H, *m*, H-4"), 3.49 (1H, *m*, H-3"), 3.47 (1H, *m*, H-5"). NMR ^{13}C (100 MHz, CD_3OD): δC 184.20 (C-4), 166.46 (C-2), 165.10 (C-7), 162.19 (C-5), 158.89 (C-9), 151.22 (C-4'), 147.22 (C-3'), 123.74 (C-1'), 120.48 (C-6'), 116.96 (C-5'), 114.32 (C-2'), 109.36 (C-6), 105.37 (C-10), 104.10 (C-3), 95.35 (C-8), 82.78 (C-5"), 80.29 (C-3"), 75.47 (C-1"), 72.77 (C-2"), 71.94 (C-4"), 63.01 (C-6"). ESI-MS *m/z* negative mode: 431.0866 $[M-H]^-$, MS^2 [311.0480].

Isovitexin (**5**) UV λ_{max}^{MeOH}/nm: 270, 337; NMR ^{1}H (400 MHz, CD_3OD): δH 7.91 (1H, *dd*, *J* 8.8 Hz, H-2' and H-6'), 6.99 (1H, *d*, *J* 8.8 Hz, H-3' and H-5'), 6.68 (1H, *s*, H-3), 6.59 (1H, *s*, H-8), 4.92 (1H, *d*, *J* 10.0 Hz, H-1"), 4.15 (1H, *m*, H-2"), 3.90 (1H, *dd*, *J* 12.0 and 2.0 Hz, H-6"'b), 3.76 (1H, *d*, *J* 12.0 and 7.0 Hz, H-6'"a), 3.47 (2H, *m*, H-3" and H-4"), 3.41 (1H, *m*, H-5"). NMR ^{13}C (100 MHz, CD_3OD): δC 184.05 (C-4), 166.13 (C-2), 165.23 (C-7), 162.91 (C-9), 162.15 (C-4'), 158.77 (C-5), 2x 129.67 (C-2' and C-6'), 123.27 (C-1'), 2x 117.27 (C-3' and C-5'), 109.58 (C-6), 105.27 (C-10), 104.17 (C-3), 95.48 (C-8), 82.78 (C-5"), 80.31 (C-3"), 75.38 (C-1"), 72.65 (C-2"), 71.92 (C-4"), 62.95 (C-6"). ESI-MS *m/z* negative mode: 431.0910 $[M-H]^-$, MS_2 [341.0611], MS_2 [311.0511].

Kaempferol 3-*O*-α-rhamnoside (**6**) UV λ_{max}^{MeOH}/nm: 264, 344; NMR ^{1}H (400 MHz, DMSO-d_6): δH 7.75 (1H, *d*, *J* 8.8 Hz, H-2' and H-6'), 6.96 (1H, *d*, *J* 8.8 Hz, H-3' and H-5'), 6.38 (1H, *d*, *J* 2.0, H-8), 6.27 (1H, *d*, *J* 2.0 Hz, H-6), 5.51 (1H, *br s*, H-1"), 4.26 (1H, *dd*, *J* 3.0 and 1.2 Hz, H-2"), 3.71 (1H, *dd*, *J* 9.0 and 3.2 Hz, H-3"), 3.36 (1H, *m*, Hz, 4"), 3.30 (1H, *m*, H-5"), 0.96 (3H, *d*, *J* 6.0 Hz, H-6"). NMR ^{13}C (100 MHz, CD_3OD): δC 179.00 (C-4), 164.38 (C-7), 162.10 (C-5), 160.22 (C-4), 157.71 (C-2), 157.15 (C-9), 134.88 (C-3), 130.75 (C-2' and C-6'), 121.42 (C-1'), 2x 115.82 (C-3' and C-5'), 105.16 (C-10), 101.72 (C-1"), 99.29 (C-6), 94.10 (C-8), 72.50 (C-4"), 71.66 (C-3"), 70.52 (C-2"), 70.47 (C-5"), 17.44 (C-6"). ESI-MS *m/z* negative mode: 431.0873 $[M-H]^-$, MS^2, MS^2 [285.0331].

Lupenone (7) NMR ^{1}H (400 MHz, $CDCl_3$): δH 4.69 (br s, H-29), 4.57 (*br s*, H-29), 2.42 (*m*, H-2), 2.38 (*m*, H-19), 1.69, 1.89 (*m*, H-1), 1.68 (H-30 and H-13), 1.46 (*m*, H-6), 1.42 (*m*, H-7), 1.38, 1.48 (*m*, H-16), 1.37 (*m*, H-9 and H-18), 1.32, 1.91 (*m*, H-21), 1.31 (*m*, H-5), 1.26, 1.40 (*m*, H-11), 1.19, 1.38 (*m*, H-22), 1.07 (*s*, H-25 and H-23 and H-26), 1.07, 1.69 (*m*, H-12), 1.03, 1.69 (*m*, H-15), 1.01 (*s*, 3H, H-24), 0.95 (*s*, 3H, H-27), 0.93 (*s*, 3H, H-25), 0.79 (*s*, 3H, H-28). NMR ^{13}C (100 MHz, $CDCl_3$): δC 218.31 (C-3), 150.91 (C-20), 109.41

(C-29), 54.94 (C-5), 49.81 (C-9), 48.26 (C-18), 47.98 (C-19), 47.36 (C-4), 43.01 (C-14), 42.91 (C-17), 40.80 (C-),39.99 (C-22), 39.63 (C-1), 38.19 (C-13), 36.90 (C-10), 35.54 (C-16), 34.18 (C-2), 33.58 (C-7), 29.73 (C-21), 27.45 (C-15), 26.66 (C-23), 25.17 (C-12), 21.49 (C-11), 21.05 (C-24), 19.70 (C-6), 19.33 (C-30), 18.03 (C-28), 15.99 (C-25), 15.80 (C-26), 14.50 (C-27).

Assays with Leishmania (Leishmania) amazonensis

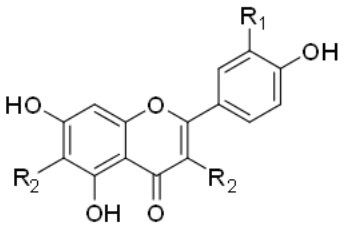
Leishmanicidal activity of the isolated compounds was determined against amastigote-like forms as previously described by Cota et al. (2008a). Briefly: promastigotes of *Leishmania* (L.) *amazonensis* (strain IFLA/BR/196/PH-8) were obtained from lesions of infected hamsters. The parasites were grown at 26 ºC in pH 7.2 Schneider's medium, and then stimulated to differentiate into the amastigote forms by rising the temperature (32 ºC), and lowering the pH (6.0) of the Schneider's medium. After seven days under these conditions, 90% of the promastigotes were transformed into amastigote-like forms, verified by means of microscope, and then used in the bioassays. Amastigote density was adjusted to $1x10^8$ parasites per mL, and 90 µL added to each well of 96-well plates. Solutions at 200 µg/mL in DMSO (1% in water) were performed for each fraction or compound and then ten microliters of the solution were added to each well of 96-well plates. The plates were incubated at 32 ºC for 72 h, and then the cells viability was determined using the MTT (methyl thiazolyl tetrazolium) assay (Teixeira et al., 2002). The results were expressed as percent inhibition in relation to the controls without drug. Amphotericin B at 0.2 µg/mL (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Results and Discussion

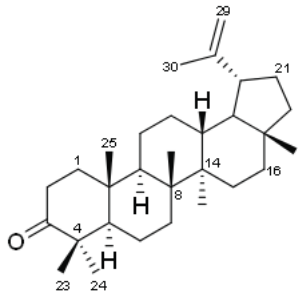
The structures of isolated compounds were identified by a combination of spectroscopic methods (MS, 1 D NMR and 2D NMR) and comparisons with the literature data as a mixture of quercetin 3-β-*O*-galactopyranoside or hyperoside (**1**) and quercetin 3-β-*O*-glucopyranoside or isoquercitrin (1:1) (**2**) (Almeida et al., 1998; Costa et al., 2007; Ohguchi et al., 2010), quercetin 3-*O*-α-rhamnoside or quercitrin (**3**) (Zhong et al., 1997), luteolin 6-*C*-β-glucopyranoside or orientin (**4**) (Li et al., 2009), apigenin-6-*C*-glucoside or isovitexin (**5**) (Leong et al., 2010), kaempferol 3-*O*-α-rhamnoside (**6**) (Fossen et al., 1999) and lupenone (**7**) (Prachayasittikul et al., 2010), respectively. Characterization of the sugar moieties from the mixture of quercetin 3-β-galactopyranoside (**1**) and quercetin 3-β-glucopyranoside (**2**) was performed according Cota et al. 2008b.

In this work, we isolated six flavonoids (**1-6**) and one lupane triterpene (**7**) for the first time from *Kielmeyera variabilis* Mart. & Zucc., Calophyllaceae. Triterpenes with different carbon skeletons that include lupeol, friedelin, β-amyrin and the sterol β-sitosterol, were found in *K. rugosa* Choisy (Nogueira et al., 2008), *K. lathrophyton* Saddi (Cruz et al., 2001), *K. candidissima* (Ferreira et al., 1972), *K. speciosa* A. St.-Hil. (Gottlieb et al., 1970) and *K. rugosa* Choisy (Nogueira et al., 2008), respectively. Indeed, Garcia-Cortez et al. (1998) obtained a mixture of 27-*O*-*cis* and trans-*p*-coumaroylcylicodiscic acid, lupeol derivated triterpenes from a dichloromethane extract of *K. coriaceae* leaves. Our chemical study on the aqueous-soluble fraction resulted in the isolation of glycosilated flavonoids, where two of them are *C*-flavonoids (**4-5**). The major compound from Aq soluble fraction in *K. variabilis* was found to be quercitrin (**3**) what suggests that this species is a good source of this flavonoid.

Many phenolic compounds such as xanthones (Pinheiro et al., 2003a; Sobral et al., 2009), quinones (Corrêa et al., 1970) and coumarins (Cruz et al., 1998b) have been isolated from *Kielmeyera* species. Despite 4-alkyl and 4-phenyl coumarins having been identified as the major constituents of some species that occur mainly



1 R_1=OH; R_2=O- -gal; R_3=H
2 R_1=OH; R_2=O- -glc; R_3=H
3 R_1=OH; R_2=O- -rham; R_3=H
4 R_1=OH; R_2=H; R_3=C- -glc
5 R_1=R_2=H; R_3=C- -glc
6 R_1=R_3=H; R_2=O- -rham



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in the cerrado (Gottlieb et al., 1969; Gottlieb & Stefani, 1970) and restinga (Cruz et al., 2002; Nogueira et al., 2008) and xanthones from species collected in the atlantic forest (Garcia-Cortez et al.,1998; Pinheiro et al., 2003a), *K. variabil*is did not demonstrate the same pattern as observed by previous studies. The presence of flavonoids in this species is in agreement with previous reports from other genera in the Calophylleae tribe as *Caraipa* (Silveira et al., 2010), *Mesua* (Banerji & Chowdhury, 1993) and *Calophyllum* (Cechinel et al., 2009). It is known that temperature and UV radiation can up-regulate key genes (Christie & Jenkins, 1996) that give plants the ability to offset the excessive absorption of UV radiation by accumulating UV-filtering secondary metabolites such as flavonoids (Cuadra et al., 1997). The chemotaxonomic applicability of phenolic compounds from *Kielmeyera* genus as markers needs to be properly evaluated, considering more members of the genus, their distribution and environmental influence.

The crude organic extract from *K. variabilis* leaves exhibited 95% of leishmanidal activity at 20 µg/mL on amastigote-like form of *Leishmania amazonensis*. From isolated compounds **1**-**7**, only quercitrin was able to inhibit the growth amastigote-like form of *L. amazonensi*s, but it was not dose-dependent in this assay (0.78-20 µg/mL, 40-45%). The major compound from Aq soluble fraction in *K. variabil*is was found to be quercitrin what suggests that this species is a good source of this flavonoid. The potent antileishmanial activity of quercetrin was demonstrated by *in vitro* bioguided fractionation of *Kalanchoe pinnata* (Muzitano et al., 2006a). In addition, previous study showed that oral administration of 16 mg/kg body weight of quercetin 3-*O*-α-L-arabinopyranosyl (1→2)-α-L-rhamnopyranoside or quercetrin or quercetin for 30 days in BALB/c mice suppressed parasite burden by 65%, 57%, and 76% in the lesion from infected ear compared with the untreated mice and similar to intraperitoneal pentostam (62%) (Muzitano et al., 2006b). These biological results and our *in vitro* studies are good evidence that synergistic effects of flavonoid glycosides are the cause of significant leishmanidal activity of crude organic extract from *K. variabilis* leaves.

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