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In vitro antileishmanial and antitrypanosomal activities of flavanones from *Baccharis retusa* DC. (Asteraceae)

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ABSTRACT

Leishmaniasis and Chagas' are parasitic protozoan diseases that affect the poorest population in the world, causing a high mortality and morbidity. As a result of highly toxic and long-term treatments, novel, safe and more efficacious drugs are essential. In this work, the CH_2Cl_2 phase from MeOH extract from the leaves of *Baccharis retusa* DC. (Asteraceae) was fractioned to afford two flavonoids: naringenin (**1**) and sakuranetin (**2**). These compounds were *in vitro* tested against *Leishmania* spp. promastigotes and amastigotes and *Trypanosoma cruzi* trypomastigotes and amastigotes. Compound **2** presented activity against *Leishmania* (*L.*) *amazonensis*, *Leishmania* (*V.*) *braziliensis*, *Leishmania* (*L.*) *major*, and *Leishmania* (*L.*) *chagasi* with IC_{50} values in the range between 43 and 52 μg/mL and against *T. cruzi* trypomastigotes (IC_{50} = 20.17 μg/mL). Despite of the chemical similarity, compound **1** did not show antiparasitic activity. Additionally, compound **2** was subjected to a methylation procedure to give sakuranetin-4′-methyl ether (**3**), which resulted in an inactive compound against both *Leishmania* spp. and *T. cruzi*. The obtained results indicated that the presence of one hydroxyl group at C-4′ associated to one methoxyl group at C-7 is important to the antiparasitic activity. Further drug design studies aiming derivatives could be a promising tool for the development of new therapeutic agents for Leishmaniasis and Chagas' disease.

1. Introduction

The genus *Baccharis*, one of the most important genera of Asteraceae family, is widespread throughout Central and South America (Nesom, 2000). Some of these species have been used in the folk medicine to the treatment of stomach and liver diseases, to reduce inflammatory processes and to cure ulcers and skin wounds (Melo et al., 2001; Korbes, 1995; Corrêa, 1984; Franco, 1995; Bandoni et al., 1978; Cortadi et al., 1999; Verdi et al., 2005). Previous chemical studies on *Baccharis* species have been carried out and several compounds such as terpenoids, tricotecenes, chromenes, and flavonoids were found (Bohlmann et al., 1979; Labbe et al., 1986; Zdero et al., 1989; Jarvis et al., 1991; Verdi et al., 2005; Grecco et al., 2010a). Additionally, several works demonstrated that some of these species have been sources of antileishmanial compounds, mainly phenolics and triterpenoids (Fournet et al., 1994; da Silva Filho et al., 2009; Zalewski et al., 2011).

Previous works have described that the *Baccharis uncinella* EtOH extract as well as its main derivatives (oleanolic/ursolic acids, pectolinaringenin, caffeic acid, and ferulic acid) showed activity against promastigotes and amastigotes forms of both *Leishmania* (*L.*) *amazonensis* and *Leishmania* (*V.*) *braziliensis* (Passero et al., 2011). It was also reported that 5,6,7-trihydroxy-4′-methoxyflavanone, an isolated derivative from MeOH extract of *Baccharis retusa*, showed activity against cutaneous species of *Leishmania* (Grecco et al., 2010b). In continuation with *B. retusa* investigation, the present study was undertaken to determine the effects of main compounds isolated from MeOH extract from the leaves of *B. retusa* against *Leishmania* spp. and *Trypanosoma cruzi*: naringenin (**1**) and sakuranetin (**2**). Additionally, sakuranetin-4′-methyl ether (**3**) was prepared after methylation of **2** and tested against the same parasites.

2. Materials and methods

2.1. General experimental procedures

Silica gel (Merck, 230–400 mesh) was used for column chromatographic separation, while silica gel 60 PF_{254} (Merck) was used

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for analytical TLC (0.25 mm). ^{1}H NMR and ^{13}C spectra were recorded, respectively, at 300 and 75 MHz in a Bruker DPX-300 spectrometer. $CDCl_3$ (Aldrich) was used as solvent and TMS (Aldrich) as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (J) in Hz. LREIMS were measured in a HP 5990/5988A mass spectrometer.

2.2. Plant material

Baccharis retusa DC. leaves were collected in Campos do Jordão, SP, Brazil, in October 2008 and were identified by Dr. Oriana A. Fávero. Voucher specimen has been deposited at Herbarium of Instituto de Botânica-SEMA, São Paulo, SP, Brazil.

2.3. Extraction and isolation

Dried and powdered leaves of *B. retusa* (460 g) were exhaustively defatted with hexane (at room temperature). Sequentially, the plant material was extracted with MeOH (six times at room temperature) affording 32 g of a syrupy green extract, after solvent remotion under reduced pressure. This extract was partitioned between MeOH:H_2O (1:2) and CH_2Cl_2. After evaporation under reduced pressure, the CH_2Cl_2 phase (13 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2 containing increasing amounts of EtOAc (up to 100%) and with EtOAc containing increasing amounts of MeOH (up to 100%), to give 8 fractions (A1–A8). Fraction A2 (3.6 g) was purified by silica gel column chromatography eluted with increasing amounts of EtOAc in CH_2Cl_2 (up to 100%) to afford 700 mg of **2**. Fraction A4 (590 mg) was subjected to silica gel column chromatography eluted with mixtures of CH_2Cl_2:EtOAc:MeOH in gradient form to give 12 fractions (B1–B12). Fraction B2 (142 mg) was purified by silica gel column chromatography eluted with CH_2Cl_2 containing increasing amounts of EtOAc (up to 100%) and with EtOAc containing increasing amounts of MeOH (up to 100%) to give 30 mg of **1**.

2.4. 5,7,4'-Trihydroxy-flavanone (naringenin-***1***)

^{1}H NMR (300 MHz, CD_3OD/DMSO-d_6) δ_H: 7.27 (d, J = 8.5 Hz, H-2'/H-6'), 6.82 (d, J = 8.5 Hz, H-3'/H-5'), 5.91 (s, H-6/H-8), 5.29 (dd, J = 12.9 and 2.8 Hz, H-2), 3.06 (dd, J = 17.1 and 12.9, H-3a), 2.68 (dd, J = 17.1 and 2.8 Hz, H-3b). ^{13}C NMR (75 MHz, CD_3OD/DMSO-d_6) δ_C: 197.3 (C-4), 167.9 (C-9), 164.9 (C-7), 164.3 (C-4'), 158.4 (C-5), 130.4 (C-1'), 130.4 (C-6), 128.7 (C-2'/C-6'), 116.2 (C-3'/C-5'), 103.2 (C-10), 96.7 (C-8), 80.0 (C-2), 43.8 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 272 (100), 254 (6), 229 (5), 207 (4), 179 (20), 166 (28), 153 (83), 120 (81), 107 (27), 91 (35), 69 (35). 32 (60).

2.5. 5,4'-Dihydroxy-7-methoxyflavanone (sakuranetin-***2***)

^{1}H NMR (300 MHz, CD_3OD) δ_H: 7.26 (d, J = 8.5 Hz, H-2'/H-6'), 6.83 (d, J = 8.5 Hz, H-3'/H-5'), 6.01 (s, H-6/H-8), 5.32 (dd, J = 13.0 and 3.0 Hz, H-2), 3.77 (s, OCH_3-7), 3.08 (dd, J = 17.2 and 13.0 Hz, H-3a), 2.73 (dd, J = 17.2 and 3.0 Hz, H-3b). ^{13}C NMR (75 MHz, CD_3OD) δ_C: 196.5 (C-4), 168.0 (C-4'), 163.6 (C-7), 163.0 (C-5), 157.4 (C-9), 129.0 (C-1'), 127.7 (C-2'/C-6'), 127.6 (C-6), 115.3 (C-3'/C-5'), 102.8 (C-10), 93.9 (C-8), 79.1 (C-2), 55.3 (OCH_3), 42.8 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 286 (67), 193 (33), 180 (39), 167 (100), 138 (24), 120 (44), 95 (38), 69 (25).

2.6. Preparation of sakuranetin-4'-methyl ether (***3***)

Compound **2** (100 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (10 mL) and subjected to a methylation reaction driven by phase transfer catalysis using methyl iodide (1.49 g, 9.9 mmol), potassium carbonate (1 g, 7.23 mmol), and cetyltrimethyl ammonium bromide (30 mg, 0.082 mmol). After 72 h at room temperature, the crude product was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent under reduced pressure followed by purification on CC using SiO_2 and n-hexane:EtOAc 3:2 as eluent afforded **3** (12 mg, 0.04 mmol).

2.7. 5-Hydroxy-7,4'-dimethoxyflavanone (sakuranetin 4'-methyl ether - ***3***)

^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 12.02 (s, OH-5), 7.37 (d, J = 8.7 Hz, H-1′/H-6′), 6.94 (d, J = 8.7 Hz, H-2′/H-5′), 6.06 (d, J = 2.3 Hz, H-6), 6.03 (d, J = 2.3 Hz, H-8), 5.35 (dd, J = 12.9 and 3.0 Hz, H-2), 3.82 (s, OCH_3-4′), 3.79 (s, OCH_3-7), 3.09 (dd, J = 17.1 and 13.0 Hz, H-3b), 2.77 (dd, J = 17.1 and 3.0 Hz, H-3a). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 196.0 (C-4), 168.0 (C-7), 164.1 (C-5), 162.9 (C-9), 160.0 (C-4′), 130.4 (C-1′), 127.7 (C-2′, 6′), 114.2 (C-3′, 5′), 103.1 (C-10), 95.1 (C-6), 94.2 (C-8), 79.0 (C-2), 55.7 (OCH_3-4′), 55.4 (OCH_3-7), 43.2 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 300 (46), 282 (3), 207 (6), 193 (18), 166 (11), 134 (83), 121 (73), 108 (12), 91 (37), 65 (17), 32 (100).

2.8. Bioassays procedures

BALB/c mice and Golden hamsters were supplied by the animal breeding facility at the Instituto Adolfo Lutz, São Paulo, and maintained in sterilized cages under a controlled environment, receiving water and food *ad libitum*. Animal procedures were performed with the approval of the Research Ethics Commission, in agreement with the Guide for the Care and Use of Laboratory Animals from the National Academy of Sciences.

2.9. Parasite maintenance

Isolated promastigotes of *L.* (*L.*) *amazonensis* (WHO/BR/00/LT0016), *L.* (*V.*) *braziliensis* (MHO/BR/75/M2903), *Leishmania (L.) chagasi* (MHOM/BR/1972/LD) and *Leishmania (L.) major* (MHOM/1L/80/Fredlin) were maintained in M-199 medium supplemented with 10% calf serum and 0.25% hemin at 24 °C. *L.* (*L.*) *chagasi* (MHOM/BR/1972/LD) was maintained in hamsters (*Mesocricetus auratus*). Amastigotes were harvested from spleens of infected hamsters by differential centrifugation (Stauber, 1958). *T. cruzi* trypomastigotes (Y strain) were maintained in LLC-MK2 (ATCC CCL 7) cells using RPMI-1640 medium supplemented with 2% calf serum at 37 °C.

2.10. Mammalian cells

Peritoneal macrophages were collected from the peritoneal cavity of female BALB/c mice by washing with RPMI-1640 without phenol red, supplemented with 10% fetal bovine serum. THP-1 (human monocytes ATCC number TIB-202) and kidney *Rhesus* monkey cells (LLC-MK2) were maintained in RPMI-1640 medium without phenol red and supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 humidified incubator (Corrêa et al., 2011).

2.11. Determination of the activity against Leishmania spp. – promastigotes

To determine the 50% inhibitory concentration (IC_{50} value) against *Leishmania* promastigotes, compounds **1–3** were previously dissolved in MeOH and diluted with M-199 medium in 96-well microplates. Promastigotes were counted in a Neubauer hemocytometer and seeded at 1 × 10^6/well with a final volume of 150 μL. Controls with MeOH and without drugs were also performed. Pentamidine was used as a standard drug. Top concentrations were 200 μg/mL for compounds **1–3** and 1.5 μg/mL for pentamidine

(based on dry weight) and the compounds were 2-fold serially diluted over seven concentrations. Each point was tested in duplicate. The plate was incubated for 24 h at 24 °C and the viability of promastigotes was verified by morphology in the light microscopy and by the MTT assay (Tempone et al., 2008). Briefly, MTT (5 mg/mL) was dissolved in PBS, sterilized through 0.22 mm membranes and added, 20 μL/well, for 4 h at 24 °C. Promastigotes were incubated without compounds and used as viability control. Formazan extraction was performed using 10% SDS for 18 h (80 μL/well) at 24 °C and the optical density (OD) was determined in a Multiskan MS (UNISCIENCE) at 550 nm. 100% viability was expressed based on the OD of control promastigotes, after normalization.

2.12. Determination of the activity against Leishmania spp. - intracellular amastigotes

Peritoneal macrophages were obtained as described previously and *L.* (*L.*) *chagasi* (MHOM/BR/1972/LD) amastigotes were obtained from spleens of infected hamsters by differential centrifugation. Peritoneal macrophages were seeded at 4×10^5 cells per well in 13-mm glass cover slips in 24-well microplates for 24 h at 37 °C in a 5% CO_2-humidified incubator. Amastigotes were added to macrophages at 10:1 ratio (amastigotes:macrophage) and incubated for 24 h. Test compounds were incubated for 120 h at the same conditions. Glucantime was used as standard drug. Top concentrations were 100 μg/mL for compounds **1–3** and 240 μg/mL for Glucantime (based on dry weight) and the compounds were 2-fold serially diluted over seven concentrations. Each point was tested in duplicate. Finally, glass cover slips were fixed with methanol, stained with Giemsa, and observed in a light microscope. The number of amastigotes/macrophage was determined by counting 500 macrophages in each of the duplicates (Yardley et al., 2010).

2.13. Determination of the activity against T. cruzi - trypomastigotes

Compounds **1–3** were dissolved in MeOH and diluted in RPMI-1640 medium to determine the 50% inhibitory concentration (IC_{50} value) as described above for the anti-leishmanial assay. Free trypomastigotes obtained from LLC-MK2 cultures were counted in a Neubauer hemocytometer and seeded at 1×10^6/well in 96-well microplates. Compounds **1–3** were incubated for 24 h at 37 °C in a 5% CO_2 humidified incubator. Benznidazole was used as standard drug. Top concentrations were 200 μg/mL for compounds **1–3** and 500 μg/mL for benznidazole (based on dry weight) and the compounds were 2-fold serially diluted seven concentrations. Each point was tested in duplicate. The viability of the trypomastigotes was verified by the MTT assay as described above (Tada et al., 1986; Lane et al., 1996).

2.14. Determination of the activity against T. cruzi – intracellular amastigotes

The effect on *T. cruzi* intracellular amastigotes was performed in macrophages previously infected with cell culture-derived trypomastigotes. Peritoneal macrophages were obtained as described above. Cell culture-derived trypomastigotes were added to macrophages at 10:1 ratio (parasites/macrophage) and incubated for 24 h. Compounds **1–3** were incubated with parasites for 72 h at 37 °C in a 5% CO_2 humidified incubator. Top concentrations were 100 μg/mL for compounds **1–3** and 500 μg/mL for benznidazole (based on dry weight) and 2-fold serially diluted over seven concentrations. Each point was tested in duplicate. In the end of the assay, the glass cover slips were fixed with methanol, stained with Giemsa and observed in a light microscope. The parasite burden was defined as the mean number infected macrophages out of 200 cells (Silva et al., 2007).

2.15. Determination of the cytotoxicity against mammalian cells

Rhesus monkey kidney cells (LLC-MK2) or THP-1 cells were seeded at 4×10^4 cells/well in 96-well microplates and incubated with compound **2** to the highest concentration of 500 μg/mL for 48 h at 37 °C in a 5% CO_2 humidified incubator. Top concentration was 500 μg/mL for compound **2** (based on dry weight), which was 2-fold serially diluted over seven concentrations. The viability of cells was determined by the MTT assay as described above. Glucantime, pentamidine, and benznidazole were used as standard drugs. Control cells were incubated in the presence of MeOH and without drugs.

2.16. Statistical analysis

The data obtained represent the mean and standard deviation of duplicate samples from two independent assays. The IC_{50} values were calculated using sigmoid dose–response curves in Graph Pad Prism 5.0 software, and the 95% confidence intervals are included in parentheses. The Mann–Whitney test was used for significance test (*P* value).

3. Results and discussion

Baccharis species have been the source of several anti-parasitic compounds, such as phenolic and terpenoids (da Silva Filho et al., 2009). Our previous works reported the isolation of anti-leishmanial triterpenes and phenolic derivatives from *B. uncinella* (Passero et al., 2011) as well as one flavonoid from *B. retusa* (Grecco et al., 2010b). As part of a continuous search, two flavanones (**1** and **2**) were isolated from *B. retusa* and characterized by analysis of its spectroscopic data. The ^{1}H NMR spectra of **1** and **2** showed signals at δ 5.29/5.32 (dd, J = 13.0 and 3.0 Hz), 3.06/3.08 (dd, J = 17.2 and 13.0 Hz), and at δ 2.68/2.73 (dd, J = 17.2 and 3.0 Hz), assigned, respectively, to H-2, H-3a, and H-3b (Danelutte et al., 2003). These spectra showed also doublets attributed to H-3′/H-5′ at δ 6.82/6.83 (J = 8.5 Hz, 2H) and to H-2′/H-6′ at δ 7.27/7.26 (J = 8.5 Hz, 2H) as well as one singlet assigned to H-6/H-8 at δ 5.91/6.01 (2H). Additionally, in the spectrum of **2**, was observed one singlet at δ 3.77 (3H), assigned to one methoxyl group linked to C-7. Comparatively, ^{13}C and DEPT 135° NMR spectra of **1** and **2** indicated the presence of carbonyl carbons at δ 197.3/196.5 (C-4), oxybenzylic carbons at δ 80.0/79.1 (C-2) and aliphatic carbons at δ 43.8/42.8 (C-3). In the spectrum of compound **2** was detected also an additional peak at δ 55.3, assigned to methoxyl group at C-4′. Remaining peaks at range δ94–168 were attributed to aromatic carbons C-5 to C-10 and C-1′ to C-6′. Comparison of spectroscopic data with those reported in the literature (Agrawal, 1989), allowed the identification of isolated compounds as 5,7,4′-trihydroxy-flavanone (naringenin, **1**) and 5,4′-dihydroxy-7-methoxy-flavanone (sakuranetin, **2**).

Aiming to discover preliminary structure–activity relationships, part of compound **2** was subjected to methylation procedures, affording **3**. The ^{1}H NMR spectrum of this compound showed similarities of that recorded to compound **2**, except to the presence of one additional peak at δ 3.82 (s, 3H), assigned to methoxyl group linked to C-4′. The position of this additional methyl group was also confirmed by LREIMS analysis due the fragments at *m/z* 166 ($C_8H_6O_4$) and 134 ($C_9H_{10}O$), formed due to retro-Diels–Alder rearrangement. Comparison with spectral data with those described in the literature (Agrawal, 1989; Silva et al., 2009), allowed the identification of **3** as 5-hydroxy-7,4′-dimethoxyflavanone (sakuranetin 4′-methyl ether).

Flavanones **1–3** were tested against Cutaneous Leishmaniasis species (*L.* (*V.*) *braziliensis*, *L.* (*L.*) *amazonensis*, and *L.* (*L.*) *major*) and against a Visceral Leishmaniasis specie (*L.* (*L.*) *chagasi*). As

Table 1
Antiparasitic (antileishmanial and antitrypanossomal) and cytotoxic effects of flavanones **1–3** and standard drugs.

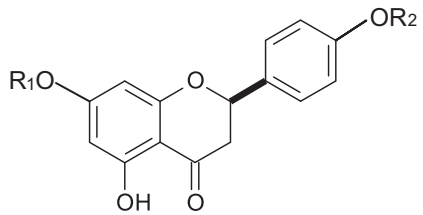
Cell	IC_{50} μg/mL (95% CI)			
	1	**2**	**3**	Standard drugs
L. (*L.*) *chagasi* promastigotes	na	38.41* (30.06–49.13)	265.6* (213.9–329.7)	0.17[a] (0.09–0.22)
L. (*L.*) *chagasi* amastigotes	na	43.66* (43.31–44.05)	na	22.07[b] (18.74–26.38)
L. (*L.*) *amazonensis* promastigotes	na	51.89* (39.31–69.98)	na	0.040[a] (0.039–0.088)
L. (*L.*) major promastigotes	na	52.60* (37.82–75.20)	na	0.24[a] (0.18–0.27)
L. (*V.*) *braziliensis* promastigotes	na	45.12* (37.01–55.33)	na	0.13[a] (0.09–0.16)
T. cruzi trypomastigotes	na	20.17* (18.80–21.73)	na	42.45[c] (39.08–50.43)
T. cruzi amastigotes	na	na	na	>50
THP-1 cytotoxicity	nd	39.50* (37.06–42.09)	nd	>100
LLC-MK2 cytotoxicity	nd	25.97* (21.44–30.70)	nd	>100

IC_{50} – 50% inhibitory concentration (μg/mL); 95% CI – 95% confidence interval; na – not active at 150 (promastigotes and trypomastigotes) or 300 μg/mL (amastigotes); nd – not determined; standard drugs.
[a] Pentamidine.
[b] Glucantime.
[c] Benznidazole.
* $P < 0.05$ was compared to standard drugs.

showed in Table 1, flavanone **1** was not active against *Leishmania* and *T. cruzi* parasites to the highest concentration. Conversely, flavanone **2** showed activity against *L.* (*L.*) *amazonensis*, *L.* (*V.*) *braziliensis*, and *L.* (*L.*) *major* with IC_{50} values of 51.89, 45.12, and 52.60 μg/mL, respectively. The obtained data also showed the anti-leishmanial activity of compound **2** against *L.* (L.) *chagasi* promastigotes, with an IC_{50} value of 38.41 μg/mL. By the MTT test, it was possible to confirm the leishmanicidal activity of compounds after 24 h incubation, resulting in 100% of death to the highest tested concentration. Considering the most relevant clinical form of the parasite, compound **2** eliminated *L.* (*L.*) *chagasi* intracellular amastigotes, with an IC_{50} value of 43.66 μg/mL after 120 h incubation. No toxicity to host cells could be detected as a result of a normal morphology of peritoneal macrophages observed by light microscopy. However, this compound showed a considerable toxicity to kidney cells LLC-MK2 and human monocytes THP-1 cells, with IC_{50} values of 25.97 and 39.50 μg/mL, respectively.

In order to evaluate the *in vitro* activity of the flavonoids **1–3** against *T. cruzi*, these compounds were evaluated against trypomastigotes forms. Compound **2** presented activity against *T. cruzi* trypomastigotes, with an IC_{50} value of 20.17 μg/mL, killing 100% of parasites after 24 h. As observed to *Leishmania* spp., compounds **1** and **3** did not show activity (Table 1) to the highest concentration. However, the obtained results to compound **2** demonstrates that this flavonoid is at least twofold more effective than the standard drug benznidazole, which resulted in an IC_{50} value of 47.50 μg/mL. Our results corroborate the anti-trypanosomal activity of sakuranetin (**2**) against *T. cruzi* trypomastigotes (Ribeiro et al., 1997) and describe for the first time the *in vitro* anti-leishmanial activity of this flavonoid.

Chemically, compounds **1** and **2** shows related structures (Fig. 1), differing only by the substituent on C-7 at ring A. Considering that compound **1** presents a hydroxyl group at C-7 while **2** was substituted to methoxyl group as well, the effect of an additional methylation at B ring of **2** was tested. Compound **3** (sakuranetin-4'-methyl ether) was prepared and assayed against the same parasites. The obtained results indicated that, similarly as observed to compound **1**, neither anti-leishmanial nor anti-trypanosomal activity was detected to compound **3**, suggesting that co-occurrence of methoxyl groups at C-7 and C-4′ led to a reduction in the anti-parasitic activity. Therefore, the occurrence of hydroxyl group at C-4′ associated to the presence methoxyl group at C-7 in related flavanones is crucial to anti-leishmanial or/and anti-trypanosomal activity.



1 $R_1 = R_2 = H$
2 $R_1 = CH_3$; $R_2 = H$
3 $R_1 = CH_3$; $R_2 = CH_3$

Fig. 1. Chemical structures of flavonoids **1–3**.

Numerous plant-derived natural products from different structural classes including various alkaloids, flavonoids, quinonoids, and terpenoids, have been investigated as antileishmanial and antitrypanosomal candidates. Several works report the antikinetoplastid activities of plant-derived natural products in the literature (Akendengue et al., 1999; Fournet and Muñoz, 2002; Salem and Werbovetz, 2006). Given the activities of these agents, natural products are a potentially rich source of drug candidates and leads against leishmaniasis and trypanosomiasis (Salem and Werbovetz, 2006).

4. Conclusion

Flavonoids **1–3** were tested *in vitro* against *Leishmania* spp. promastigotes and amastigotes and *Trypanosoma cruzi* trypomastigotes and amastigotes. Compound **2** presented activity against *L.* (*L.*) *amazonensis*, *L.* (*V.*) *braziliensis*, *L.* (*L.*) *major*, and *L.* (*L.*) *chagasi* and against *T. cruzi* trypomastigotes while compound **1** did not show antiparasitic activity. Compound **2** was subjected to a methylation procedure to give sakuranetin-4'-methyl ether (**3**), which resulted in an inactive compound against both *Leishmania* spp. and *T. cruzi*. The obtained results indicated that the presence of one hydroxyl group at C-4' associated to one methoxyl group at C-7 in related flavonoids is important to the antiparasitic activity.

In conclusion, the present study provided useful information about the structural features required for the anti-protozoan activity of these flavonoids. This information could be used as a tool for the design of novel and more efficacious agents against Leishmaniasis and Chagas' disease.

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