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Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds of *Dimorphandra gardneriana* and *Platymiscium floribundum*, native plants from Caatinga biome[1]

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ABSTRACT.- Vila-Nova N.S., Morais S.M., Falcão M.J.C., Bevilaqua C.M.L., Rondon F.C.M., Wilson M.E., Vieira I.G.P. & Andrade H.F. 2012. **Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds of *Dimorphandra gardneriana* and *Platymiscium floribundum*, native plants from Caatinga biome.** *Pesquisa Veterinária Brasileira* 32*(11):1164-1168.* Centro de Ciências e Tecnologia, Universidade Estadual do Ceará, Av. Paranjana 1700, Campus do Itaperi, Fortaleza, CE 60740-000, Brazil. E-mail: selenemaiademorais@gmail.com

In recent years, the Brazilian Health Ministry and the World Health Organization have supported research into new technologies that may contribute to the surveillance, new treatments, and control of visceral leishmaniasis within the country. In light of this, the aim of this study was to isolate compounds from plants of the Caatinga biome, and to investigate their toxicity against promastigote and amastigote forms of *Leishmania infantum chagasi*, the main responsible parasite for South American visceral leishmaniasis, and evaluate their ability to inhibit acetylcholinesterase enzyme (AChE). A screen assay using luciferase-expressing promastigote form and an *in situ* ELISA assay were used to measure the viability of promastigote and amastigote forms, respectively, after exposure to these substances. The MTT colorimetric assay was performed to determine the toxicity of these compounds in murine monocytic RAW 264.7 cell line. All compounds were tested *in vitro* for their anti-cholinesterase properties. A coumarin, scoparone, was isolated from *Platymiscium floribundum* stems, and the flavonoids rutin and quercetin were isolated from *Dimorphandra gardneriana* beans. These compounds were purified using silica gel column chromatography, eluted with organic solvents in mixtures of increasing polarity, and identified by spectral analysis. In the leishmanicidal assays, the compounds showed dose-dependent efficacy against the extracellular promastigote forms, with an EC_{50} for scoporone of 21.4µg/mL, quercetin and rutin 26 and 30.3µg/mL, respectively. The flavonoids presented comparable results to the positive control drug, amphotericin B, against the amastigote forms with EC_{50} for quercetin and rutin of 10.6 and 43.3µg/mL, respectively. All compounds inhibited AChE with inhibition zones varying from 0.8 to 0.6, indicating a possible mechanism of action for leishmacicidal activity.

INDEX TERMS: Leishmaniasis, *Dimorphandra gardneriana, Platymiscium floribundum*, scoparone, flavonoids, acetylcholinestarase inhibition, treatment.

RESUMO.- [**Atividade inibitória Leishmanicida e Colinesterásica de Compostos Fenólicos de *Dimorphandra gardneriana* e *Platymiscium fliribunbum*, plantas nativas do bioma Caatinga.**] Nos últimos anos, o Ministério da Saúde do Brasil e a Organização Mundial da Saúde tem apoiado a investigação de novas tecnologias que possam contribuir para a vigilância, novos tratamentos e controle da leishmaniose visceral no país. Assim, o objetivo deste trabalho foi isolar compostos de plantas do bioma Caatinga, e investigar a toxicidade destes compostos contra as formas promastigotas

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e amastigotas de *Leishmania infantum chagasi*, principal parasita responsável pela leishmaniose visceral na América do Sul, e avaliar a sua capacidade para inibir a enzima acetil-colinesterase (AChE). Após a exposição aos compostos em estudo, foram realizados testes utilizando a forma promastigota que expressa luciferase e ELISA *in situ* para medir a viabilidade das formas promastigotas e amastigota, respectivamente. O ensaio colorimétrico MTT foi realizado para determinar a toxicidade destas substâncias utilizando células monocíticas murina RAW 264.7. Todos os compostos foram testados *in vitro* para as sua propriedade anti-colinesterásica. Um cumarina, escoparona, foi isolada a partir de hastes de *Platymiscium floribundum*, e os flavonóides, rutina e quercetina, foram isolados a partir de grãos de *Dimorphandra gardneriana*. Estes compostos foram purificados, utilizando cromatografia em coluna gel eluída com solventes orgânicos em misturas de polaridade crescente, e identificados por análise espectral. Nos ensaios leishmanicidas, os compostos fenólicos mostraram eficácia contra as formas extracelulares promastigotas, com EC_{50} para escoporona de 21.4μg/mL e para quercetina e rutina 26 e 30.3μg/mL, respectivamente. Os flavonóides apresentaram resultados comparáveis à droga controle, a anfotericina B, contra as formas amastigotas com EC_{50} para quercetina e rutina de 10.6 e 43.3μg/mL, respectivamente. Os compostos inibiram a enzima AChE com halos de inibição variando de 0,8 a 0,6cm, indicando um possível mecanismo de ação para a atividade leishmanicida.

TERMOS DE INDEXAÇÃO: Leishmaniose, *Dimorphandra gardneriana, Platymiscium floribundum*, escoparona, flavonoides, inibição acetil-colinestarase, tratamento.

INTRODUCTION

Leishmaniasis is still one of the most neglected diseases in the world. During the last 10 years, many scientific studies involving this disease have been related to treatment strategies and led to a reduction in drug prices; however, the morbidity and mortality of this disease has continued to increase worldwide (WHO 2010). For more than 50 years, the traditional chemotherapy used to treat leishmaniasis has been based on the use of pentavalent antimonial drugs (Chan-Bacab & Pena-Rodriguez 2001). However, the toxicity of these agents and their side effects, along with the development of resistance and differences in strain sensitivity are challenges that must be overcome (Carvalho et al. 2000, Osório et al. 2007).

Antileishmanial drugs have different mechanisms of action. Antimonial drugs interfere with bioenergetic process of *Leishmania* amastigotes by inhibition of several proteins (Chan-Bacab & Pena-Rodriguez 2001). Miltefosine (hexadecylphosphocholine) is thought to perturb the microorganism cell membrane by interfering with the biosynthesis of the glycosyl phosphatidylinositol (GPI) membrane anchor. Miltefosine may also interfere with leishmania signal transduction (Croft et al. 2003). Amphotericin B interacts with sterols present in the *Leishmania* membrane, the drug binds with ergosterol damaging the cell permeability, allowing ions such as K^+ to escape from the cell (Ordonez-Gutierrez et al. 2007, Filippin & Souza 2006).

Choline is the precursor of phosphatidylcholine, a main component of *Leishmania* promastigote membranes (Wassef et al. 1985). Therefore, inhibition of choline formation may decrease *Leishmania* survival. This hypothesis can be tested by using inhibitors of the acetylcholinesterase enzyme (AChE), which catalyzes the hydrolysis of acetylcholine to choline and acetic acid, as leishmanicidal compounds. This may identify another mechanism of action for leishmanicidal activity.

As part of the search for new and better drugs with high feasibility and low toxicity, the Special Programme for Research and Training in Tropical Diseases of the World Health Organization (WHO) encourages research into plants for the treatment leishmaniasis (WHO 2009). In recent years, the Brazilian Health Ministry has supported research into laboratory diagnosis of leishmaniasis in humans and dogs, patient treatment, evaluation of the effectiveness of control strategies, and the development of new technologies that may contribute to the surveillance and control of visceral leishmaniasis in the country (Maia-Elkhoury et al. 2008). Several compounds from plants that have activity against *Leishmania* species have been reported in the literature (Chan-Bacab & Pena-Rodriguez 2001). These results encourage the investigation into other compounds present in the Brazilian flora.

Two plants native to the Caatinga biome of Northeastern Brazil, *Platymiscium floribundum* and *Dimorphandra gardneriana*, were selected for study, contain phenolic compounds, such as coumarins and flavonoids, with potential leishmanicidal and anticholinesterase activities, respectively. The aim of this work was to test the compound, scoparone, a coumarin isolated from *P. floribundum*, and the two flavonoids, rutin and quercetin, isolated from *D. gardneriana*, against *L. infantum chagasi* promastigotes and amastigotes and evaluate their ability to inhibit acetylcholinesterase.

MATERIALS AND METHODS

The heartwood and sapwood from the stems of *Platymiscium floribundum*, and the beans of *Dimorphandra gardneriana*, were collected in the city of Acarape and city of Crato, respectively, in the state of Ceará, Brazil. The aerial parts from both plants were deposited in the Prisco Bezerra Herbarium under the numbers 31052 and 32339, respectively.

For column chromatography, silica gel (d 0.063-0.200mm; 70-230 mesh) and Sephadex LH – 20 were used. Thin layer chromatography (TLC) was performed using silica gel 60 G-F-254 on glass plates. TLC plates were observed under an ultraviolet lamp (Vilbert Loumart, CN-15 LM model), with two wavelengths (312 and 365nm). Then, the plates were sprayed with 2.5% vanilin in perchloric acid in ethanol (1:1), heated in a 100°C oven, and, if necessary, saturated in an iodine chamber.

Plant heartwood (800g) was subjected to cold extraction using the organic solvents hexane, chloroform and ethanol. The solvents were eliminated using a rotary evaporator to obtain the, respective extracts. The chloroform fraction was subjected to silica gel column chromatography and was then eluted with mixtures of hexane, dichloromethane, ethyl acetate and methanol with increasing polarity. The fractions were collected and compared by TLC for the presence of a blue spot under UV light. The chemical structures of the isolated compounds were determined by spectroscopic analysis of the infrared spectra, recorded on a FT-IR PerkinElmer 1000 spectrophotometer. The values are expressed in cm^{-1}. Nuclear magnetic resonance spectra were recorded on a Bruker Avance DRX-500 spectrometer, in $CDCl_3$.

The beans of *D. gardneriana* (150 g) were added to a Soxhlet extractor and were extracted with hexane, ethyl acetate, methanol

and water. The solvents were concentrated in a rotary evaporator, and hexane (0.38g), ethyl acetate (4.47g), methanol (47.53g), and aqueous (8.49g) extracts were obtained. After analysis of the extracts by TLC, the ethyl acetate and methanol extracts were combined, and dispersed in 200mL of cold water. After stirring, the mixture was filtered, the resulting residue was washed with additional 100 mL of water, and after filtration, and the residue was dried in a 100ºC oven, yielding 18.0g of a yellow powder. This material was subjected to column chromatography on silica gel column, and was eluted with mixtures of hexane, dichloromethane, ethyl acetate and methanol with increasing polarity. The fractions were collected and compared by TLC. This method resulted in the purification of 12.42g of rutin. Spectral data were compared to those found in the literature (Agrawal & Bansal 1989).

The method of Pulley & Loesecke (1939), adapted by Kratkay & Tandy (1991), was used to prepare quercetin. A mixture of 0.9g of rutin and 9.0mL of a 3% sulfuric acid solution was added to a 25mL flask adapted with a reflux condenser. The reaction mixture was refluxed for 5 hours, cooled to 60ºC and filtered. The resulting quercetin was washed with hot water until a neutral pH was reached. The crude quercetin was dried, and then dissolved in 7.2mL of hot ethanol solution (78ºC) at 75ºGL. After the quercetin was completely dissolved, the solution was filtered and cooled to 25ºC for subsequent crystallization. The spectral data were compared to those found in the literature (Agrawal & Bansal 1989).

A strain of *L. infantum chagasi* expressing luciferase, called *Lic-luc*, was generated with the integrating vector pIR1SAT, provided by Dr. Steve Beverley, as described (Thalhofer et al. 2010). The promastigote form of *Lic-Luc* was cultured in hemoflagellate-modified MEM (HOMEM) medium, supplemented with 10% fetal bovine serum at 26°C. For the leishmanicidal assay, an adapted methodology from Tempone et al. 2005 was used. The compounds were dissolved in DMSO at a concentration of 0.2% and diluted with HOMEM medium in 96-well microplates. The assay was performed at concentrations of 100, 50, 25, 12.5 and 6.25μg/mL. Control wells contained DMSO or no additives. Each concentration was tested in triplicate in replicate experiments. Promastigotes were counted in a Neubauer hemocytometer and seeded at $1x10^6$/well. The plates were incubated at 26°C for 24 h, and the viability of the promastigotes, based on their morphology, was observed under a light microscope. Pentamidine (Sigma-Aldrich, St Louis, MO, USA) was used as the positive control drug. The optical density (OD) was determined in a Fluostar Omega (BMG Labtech) at 620nm.

The drug activity against amastigote was measured by a modified ELISA measuring the viability of *L. i. chagasi* infecting the murine macrophage RAW 264.7 cell line (ATCC TIB-71). Four x 10^5 RAW 264.7 cells in RPMI-1640, 10% fetal bovine serum, were added to each well of a 96-well plate, and incubated with *L. i. chagasi* promastigotes at a 1:10 (macrophage/promastigote) ratio at 37ºC, 5% CO_2 in an incubator for 72 h. An adapted methodology from Piazza et al. 1994 was used for the *in vitro* assay. Stock solutions of all of the compounds were prepared at a concentration of 0.2% in ethanol. An additional five concentrations were obtained by two-fold serial-dilution. The compound solutions were added to microplates containing the confluent layer of cells with amastigotes for 48 h at 37°C in a humidified 5% CO_2 incubator. Macrophages incubated without drugs were used as the control and, and amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) was used as the positive control drug at same concentrations as the other compounds. Murine macrophage RAW 264.7 cells were incubated with 0.01% saponin in PBSA, containing 1% bovine serum albumin, for 30 min. The wells were then blocked for 30 min with 5% nonfat milk (Nestlé) in PBS. In the next step, the cells were incubated in serum from a rabbit immunized with a saline extract of *L. i. chagasi* promastigotes, collected 30 days after the infection, at 37°C for 1 h. The serum employed was diluted 1:500 and pre-absorbed with 10% fetal calf serum (FCS) at 22°C for 1 h. The wells were washed three times with 0.05% Tween-20 in PBSA, and peroxidase-conjugated goat anti-rabbit IgG (Sigma Chemical Co.) diluted 1:5000 in 5% nonfat milk was added and incubated at 37°C for l h. After the wells were washed, *o*-phenylenediamine (0.4mg/mL) and 0.05% H_20_2 were added. The reaction was stopped by the addition of 1 M HC1. The plates were read at 492 nm in a Multiskan MS (UniScience) ELISA reader.

To test the toxicity of compounds for mammalian cells, murine RAW 264.7 macrophages were seeded at $4x10^4$/well in 96-well microplates and incubated at 37°C for 48 h in the presence of the compounds at a concentration of 120μg/mL. These compounds had previously been dissolved in 0.2% ethanol. The microplates were incubated for 48 h at 37°C in a humidified 5% CO_2 incubator. Control cells were incubated in the presence of DMSO, without drugs, and with the positive control drugs, amphotericin B and Pentamidine, at a concentration of 40 μg/mL and 100 μg/mL, respectively. The viability of the murine RAW 264.7 macrophages cells was determined using the MTT assay, as described previously by Vila-Nova et al. 2011. MTT at a concentration of 5mg/mL were added at 20 μg/mL in 96-well microplates and incubated at 37ºC for 4 h. Formazan extraction was performed using 10% SDS incubated at 24ºC for 18 h. The OD was determined in a Multiskan MS (UniScience) at 570nm. Light microscopy was used to observe the morphology of the cells and confirm the viability of the control group.

Inhibition of the AChE was evaluated by TLC in accordance with the methodology described by Ellman et al. 1961, later adapted by Rhee et al. 2001. The following solutions were prepared for this test: (1) 50mM Tris / HCl, pH 8 (buffer); (2) 1 mM Ellman's reagent, which was prepared by mixing 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and a buffer solution of acetylthiocholine iodide (ATCI); and (3) 1 mM ACTI. The lyophilized AChE enzyme was diluted in buffer solution (1) to obtain a 1000 U/mL enzyme solution. The compounds, in 5μL aliquots dissolved in $CHCl_3$ (4mg/mL), were applied to the TLC plates (DC-Alufolien, silica gel 60 F254, 0.2mm Merck). The plate was then sprayed with solutions (2) and (3). After 3 min, which is the time necessary for the solution to completely dry, the plate was sprayed with AChE (3 U/mL). After approximately 10 min, the appearance of white spots was observed, and the spot diameters were immediately measured. Physostigmine (Sigma-Aldrich, St Louis, MO, USA) was used as a positive control.

The 50% effective concentration (EC_{50}) values at the 95% confidence interval (95% CI) were calculated using a nonlinear regression curve. One-way ANOVA and comparative analysis between treatments was performed with Tukey's parametric post-test using the numbers of living promastigotes, amastigotes and/or murine RAW 264.7 cells, which were determined indirectly by optical density (OD), to represent the percentage of surviving cells after normalization. GrhaphPad Prism 4.0 statistical software was used for analyses.

RESULTS

The ^{13}C-NMR spectroscopic data of the compound isolated from *Platymiscium floribundum* revealed the presence of nine sp^2 carbons, among them one carbonyl (161.4 **δ**) and two methoxyl groups. In the ^1H-NMR spectrum, a cis-double bond at 6.23 **δ** and 7.59 **δ** (J=9.5 Hz) conjugated with a carbonyl is characteristic of a lactone ring of a coumarin. Comparison with literature data (Ma et al. 2006) confirmed the structure of 6,7-dimethoxycoumarin, named scoparone, for the compound isolated from *P. floribundum*. The NMR spectral data are shown in Table 1.

Table 1. NMR ^{1}H and ^{13}C data from 6,7-dimethoxycoumarin

C	dC	dH
2(C=O)	161,4	_
4ª	111,4	_
6	146,3	_
7	152,8	_
8ª	150,0	_
CH		
3	113,4	6,23 (d, J= 9,5 Hz)
4	143,3	7,59 (d, J= 9,5 Hz)
5	108,0	6,83 (s)
8	100,0	6,78 (s)
CH_3		
OCH_3	56,4	3,90 (s)
OCH_3	56,4	3,88 (s)

Table 2. Leishmanicidal activity against *Leishmania i. chagasi* and acetylcholinesterase inhibition activities of phenolic compounds scoparone, rutin and quercetin and standard drugs amphotericin B and Pentamidine

Compound (μg/mL)	*EC50 Promastigote (μg/mL)	*EC50 Amastigote (μg/mL)	AChE Inhibition(cm)
Scoparone	21.4b (16.9 – 27.2)	>100d	0.8
Rutin	30.3a (24.5 – 37.4)	43.35c (16.04 – 117.1)	0.6
Quercetin	26.0a (16.6 – 40.8)	10.64c (1.72 – 65.56)	0.6
Pentamidine	5.2a (0.06 – 403.8)	Nd	-
Amphotericin B	Nd	19.75c (6.3 – 62)	-
Physostigmine	Nd	Nd	0.9

The different letters in the columns show the statistical difference between the EC_{50} values ($p<0.05$) by Tukey´s test. 95% CI = 95% confidence interval; Nd = not determined. *Values indicate the effective concentration of a compound in μg/mL necessary to achieve 50% growth inhibition (EC_{50}).

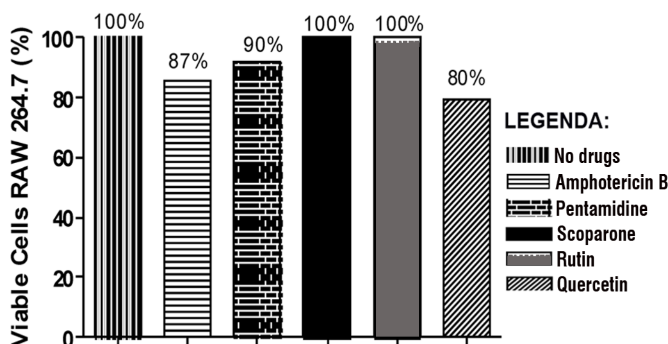

Fig.1. Toxicity of compounds at 120μg/mL on murine RAW 264.7 macrophage cells comparing with Amphotericin B and Pentamidine (40μg/mL and 100μg/mL, respectively). P<0.05.

To determine the 50% effective concentration (EC_{50}) of the phenolic compounds, promastigotes of *Leishmania i. chagasi* were incubated with the compound for 24 hours at 24°C. Leishmanicidal activity for the extracellular cells was observed for all compounds. Pentamidine was used as the control and was suitable for comparison with the compounds. Scoparone had no activity in the amastigote assay, and the flavonoids, rutin and quercetin, were not significantly different compared to the control, amphotericin B (Table 2).

The toxicity of compounds for mammalian cells was tested at a drug concentration that was higher than all EC_{50} levels for sensitive drugs in Table 2. Viability was measured using the MTT assay, which measures mitochondrial respiration. None of the compounds caused a significant decrease from control was measured with the MTT assay; i.e. none of compounds demonstrated toxicity at the concentration tested (Fig.1).

Scoparone, quercetin, and rutin, at a concentration of 2mg/mL, were tested for AChE inhibitory activity using Ellman's colorimetric method adapted by Reed on TLC plates. Scoparone had an inhibition zone of 0.8cm, which was similar to the inhibition zone of the control physostigmine (0.9cm). Quercetin and rutin were less active, with inhibition zones of 0.6cm (Table 2).

DISCUSSION

The list of compounds available to treat leishmaniasis is short and the expanding problems of parasite resistance and difficulty of delivering drugs to afflicted populations, demonstrate a critical need for efficacious and nontoxic drugs against leishmaniasis. Although plant extracts have been used by traditional healers, the active compounds are often unknown. In this study screened phenolic compounds as a coumarin (scoporone) isolated from *Platymiscium floribundum* and two flavonoids, rutin and quercetin, isolated from *Dimorphandra gardneriana,* showed potential leishmanicidal activities (Fig.1). The data suggested that all 3 compounds exhibit activity against the extracellular promastigote form of *L. i. chagasi*, and two of three had activity against the intracellular amastigote form.

The lactone groups present in coumarins are also present in the structures of Annonaceous acetogenins that show leishmanicidal activity (Vila-Nova et al. 2011). Annonaceous acetogenins have chains ranging from 37 to 35 carbon atoms, depending on the species from which they are derived. In this study, scoparone had an EC_{50} of 21.4μg/mL (95% CI=16.9-27.2) in the promastigote assay. In the AChE assay, the inhibition zone of scoparone was 0.8 cm, and was similar to the control, physostigmine (0.9cm), implying similar acetylcholinesterase inhibition characteristics. In a previous study, scoporone, was isolated from *Helietta apiculata* and tested against three other *Leishmania* promastigotes strains (*L. amazonensis, L. infantum* and *L. braziliensis*), and the results showed and EC_{50} greater than 50μg/mL (Ferreira et al. 2010). AChE inhibition was demonstrated for nine coumarins isolated from *Ferula szowitsiana*, with inhibition zones ranging from 6-8mm at a concentration of 4mg/mL (Santos et al. 2008), presenting results similar to those in this study.

Acetylcholinesterase inactivates acetylcholine by hydrolyzing it, into the acetyl and choline groups. This enzyme provides a mean of regulating the concentration of the active neurotransmitters (Soreq & Seidman 2001). Phosphatidylcholine (PC) is the most abundant phospholipid both in the surface membranes (14.9%) and the whole cell cytoplasm (51.6%) of *Leishmania*; however PC synthesis requires acquisition of the choline precursor from the host (Wassef et al. Dwyer 1985, Zuffrey & Mamoun 2002). Without sufficient host-derived choline, it is very likely that PC synthesis would be impaired and the *Leishmania* plasma membrane would be compromised. The AChE inhibitory activity of scoporone (0.8mm) indicates a possible action mechanism by disrupting the viability of leishmania's cell membranes. The metabolic pathways leading to the PC uptake and biosynthesis are likely to play critical roles in parasite development and survival, and may be a viable

target for antileishmanial chemotherapy (Zufferey & Mamoun 2002). The uptake of choline is highly specific and is inhibited by choline carrier inhibitors, including the antileishmanial phosphocholine analogs miltefosine and edelfosine and other choline analogs that have antimalarial and anti-cancer activities (Zuffrey & Mamoum 2002, Croft et al. 2003). We hypothesize that the coumarin and scoparone have mechanism of action acting on the same pathway as above compounds, resulting in a net negative effects on choline uptake by the parasite. Since the coumarin may act by the decreasing the amount of available choline for transport, one would hypothesize that these agents would have synergistic activity. It is possible that a combination of studied compounds with miltefosine would enable lowering the dose, and consequent toxicities, of both drugs.

Quercetin and rutin demonstrated leishmanicidal activity in both the promastigote and amastigote stages. The activity of these flavonoids was similar to that of Pentamidine and Amphotericin B, established agents used to treat leishmaniasis. These results correlate with another study that tested the activity of quercetin isolated from *Galphimia glauca* against *L. donovani* promastigotes, where the activity was evaluated by counting microscopically the number of live parasites. In that study, quercetin had an EC_{50} 29.5µg/mL against *L. donovani* promastigotes (Camacho et al. 2002). However, the leishmanicidal activity of 3-O-methyl-quercetin isolated from *Chromolaena hirsuta* against *L. amazonensis* had an EC_{50} of 87.8µg/mL (Taleb-Contini et al. 2004). Furthermore, in a third study, using quercetrin (glicosyl-quercetin) showed an EC_{50} of 1.0µg/mL against the amastigote form of *L. donovani* (Tasdemir et al. 2006).

These flavonoids are able to inhibit acetylcholine hydrolysis and interfere in the PC due to their low concentration of choline precursor from the host. Nevertheless, the leishmanicidal activity of these compounds may be related to their ability to chelate iron (Fe), depriving this essential nutrient from the intracellular amastigote forms (Sen et al. 2008, Fonseca-Silva et al. 2011).

CONCLUSIONS

The data provide evidence that Brazilian Caatinga biome is a rich source of leishmanicidal compounds.

In this study, scoparone, rutin and quercetin have shown activity against *Leishmania infantum chagasi* promastigote and amastigote forms, and act as AChE inhibitor.

This suggests a new mechanism of action for these leishmanicidal natural compounds.

These observations raise the possibility of further studies using these compounds in combination with existing clinically approved antileishmanial compounds to evaluate the synergism.

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