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RESEARCH ARTICLE

Antiprotozoal screening of the Cuban native plant *Scutellaria havanensis*

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ABSTRACT

Context: *Scutellaria havanensis* Jacq. (Lamiaceae) is a native medicinal herb with a history of use in Cuba.
Objective: This study screens the antiprotozoal activity of *S. havanensis*.
Materials and methods: Chloroform and methanol extracts from leaves and stems were evaluated *in vitro* at doses between 0.015 and 200 μg/mL against protozoan parasites: *Plasmodium berghei*, *Trichomonas vaginalis* and *Leishmania amazonensis*. Chloroform and methanol extracts were characterized by GC/MS. Cytotoxicity against mouse peritoneal macrophages was tested in parallel.
Results: *Scutellaria havanensis* extracts exhibited IC_{50} values between 7.7 and 32.2 μg/mL against trophozoites of *P. berghei* and *T. vaginalis*; while the extracts were inactive against *L. amazonensis* promastigotes. Trichomonicidal activity of methanol extract exhibited the best selectivity but chloroform extract showed the highest antiplasmodial, trichomonicidal and cytotoxic activity. The majority of compounds in the chloroform extract were hydroxy and/or methoxyflavones (77.96%), in particular, wogonin (48.27%). In methanol extract, wogonin (5.89%) was detected. Trichomonicidal effect of wogonin was moderate (IC_{50} = 56 μM) and unspecific with respect to macrophages (SI = 2). On the contrary, antiplasmodial activity of wogonin were particularly active (IC_{50} = 15 μM) demonstrating a higher selectivity index (SI = 7.4).
Conclusions: Wogonin is an active principle compound of the chloroform extract of *S. havanensis* against *P. berghei* and *T. vaginalis* trophozoites, whereas the methanol extract of *S. havanensis* should be investigated more deeply as a trichomonicide. Our findings suggest that wogonin is potentially useful for the development of antimalarial alternative treatments.

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KEYWORDS
Plasmodium berghei; *Trichomonas vaginalis*; *Leishmania amazonensis*; wogonin

Introduction

Scutellaria havanensis Jacq. (Lamiaceae) is a medicinal plant with origins in the Caribbean and distributed in the northern area of this region. Cuban ethnobotany reports its use to treat inflammation and chronic spleen affectations that follow intermittent fevers (Roig 1974). To our knowledge, there are currently no pharmacological studies reported for this native species, but there are several reports of antitumour (Kim et al. 2014), anti-inflammatory (Kim et al. 2014; Muluye et al. 2014), neuroprotective (Gaire et al. 2014), and antimicrobial activities (Muluye et al. 2014) reported for other *Scutellaria* species and their flavonoids. Moreover, there is limited research on the antiparasitic potential of *Scutellaria* extracts; including antitoxoplasma (Yang et al. 2012) and antitrypanosomal activities (Mamadalieva et al. 2011). However, several flavonoids in *Scutellaria* species have shown antitrypanosomal activity (Mamadalieva et al. 2011).

In this study, the chloroform and methanol extracts of *S. havanensis* aerial parts were evaluated against protozoa *Plasmodium berghei*, *Trichomonas vaginalis* and *Leishmania amazonensis* whereas cytotoxicity against peritoneal macrophages was tested in parallel.

Materials and methods

Plant material

The aerial parts (leaves and stems) of *S. havanensis* were collected in October 2011 from the National Botanic Garden (NBG), Havana, Cuba. The sample was identified by botanist Angela Leyva (PhD. Head of NBG). A voucher specimen was deposited in the NBG herbarium (087485-HAJB).

Extracts preparation

Fresh aerial parts of *S. havanensis* (50 g) were extracted with methanol or chloroform (250 mL) for 15 min (twice) in an ultrasonic bath (35 kHz, Bioblok Scientific, Germany). After filtering the extracts, the filtrates were pooled and concentrated under vacuum at 40 °C until dry. The concentrates were weighed and transferred to an air tight sample bottle and stored at −20 °C until further analysis. The yields of the extracts were 4.5% and 29.5%, respectively, for the chloroform and methanol extraction of the starting fresh material.

Gas chromatography-mass spectrometry (GC-MS) analysis

Samples of the chloroform or methanol extract (5.0 mg) were accurately weighed into a 2 mL vial, then 0.15 mL of *N*-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were added, the vial was tightly capped, heated at 80 °C for 1 h and 0.5 μL were analyzed by GC-MS.

An Agilent GC 6890N equipped with a mass selective detector 5975 B inert and a split-splitless injector, in splitless mode, was used (Agilent, Palo Alto, CA). Separations were made on a HP-5Ms fused-silica capillary column (30 m × 0.25 mm), with a

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film thickness of 0.25 µm Df (Agilent, Avondale, PA). The GC oven temperature was kept at 60 °C for 2 min and programed to 200 °C at a rate of 20 °C/min, then from 200 °C to 300 °C at a rate of 8 °C/min and kept constant at 300 °C for 5 min. The injection and source temperatures were 320 °C and 250 °C, respectively. MS interface temperature was 250 °C. Electron ionization/mass spectrometry (EI/MS) spectrum was taken at 70 eV. The mass spectrum was continuously acquired from 35 to 800 *m/z* with 3.12 scan/s in full scan mode. Peak identification was achieved by computer matching mass spectra against commercial libraries (National Institute of Standards and Technology (NIST) 2011 GC/MS), as well as MS literature data (Tashmatov et al. 2011; Tayarani-Najarani et al. 2011).

Wogonin extraction and analysis

Fresh aerial parts of *S. havanensis* (50 g) were extracted by the maceration method, using diethyl ether (250 mL) for 48 h. After extract filtering, the solvent was evaporated at room temperature to yield only yellow needles in high percent (5–8%). The purity of the yellow needles was evaluated by GC-MS and chemical structure elucidated with all spectroscopic techniques available as previously demonstrated: ultraviolet (UV), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) (Marrero et al. 2015).

Products preparation for biological test

Scutellaria extracts and wogonin were dissolved in dimethyl sulfoxide (DMSO, BDH, England) at 20 mg/mL. Chloroquine sulphate (Sigma, St. Louis, MO) dissolved in sterile distilled water at 1 µg/mL, amphothericin B (AmB, IMEFA, Cuba) at a 2 mg/mL in sterile distilled water and metronidazol (Sigma, St. Louis, MO) at 3.4 mg/mL in DMSO were used as standards.

Parasites cultures

Plasmodium berghei ANKA strain was maintained by successive passages on BALB/c mice: healthy mice were infected by intraperitoneal route with 10^6 red blood cells (RBC) from parasitized mice.

Leishmania amazonensis parasites (MHOM/77BR/LTB0016) were routinely isolated from mouse lesions and maintained as promastigotes at 26 °C in Schneider's medium (SIGMA, St. Louis, MO) containing 10% heat-inactivated foetal bovine serum (SIGMA, St. Louis, MO), 100 µg of streptomycin/mL and 100 U penicillin/mL.

The isolate T-H7 of *T. vaginalis* was axenized from a symptomatic woman following the methodology described by Rojas et al. (2004). Parasites were cultured in TYI (Diamond et al. 1978) supplemented with heat-inactivated bovine serum at a final concentration of 10%, under anaerobic conditions, at 37 °C. Cultures at exponential phase were used for the experiments.

Laboratory animals

Female BALB/c mice, with a body weight of approximately 20–22 g, were obtained from The National Center of Laboratory Animals Production (CENPALAB). The maintenance and care of mice was followed according to guidelines from Ethics Committee for the Human Use of Laboratory Animals at the Institute of Tropical Medicine 'Pedro Kourí' in accordance with Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes (Official Journal of the European Union L 276). The temperature and humidity were controlled, with 12 h light/dark cycle and they were allowed water and food *ad libitum*.

Antiplasmodial assay

The assay was performed in 96-well culture plates and the schizont counts were used to calculate the inhibition percent in relation to DMSO-treated controls. Animals with 0.5–1% parasitemia and 70% of ring forms were selected for culture. An RBC suspension at HCT 2% in RPMI culture media containing calf foetal serum (20%) was prepared. Serial double dilutions of products and DMSO in 100 µL RPMI were done. Equal volume of RBC suspension was added to obtain a product concentration range between 0.0078–100 µg/mL. After 18–20 h of incubation at 36 °C in a candle jar, thick RBC films of each well were stained with Giemsa (Quimefa, Cuba). At least 200 parasites were examined by film using a microscope (Carl Zeiss, Germany). Activity was expressed as concentration, which inhibits 50% of schizonts formation (medium inhibitory concentration or IC_{50}).

Trichomonicidal assay

The screening assay for trichomonicidal activity was performed in 96-well culture plates and 3-[4,5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT) (SIGMA, St. Louis, MO) colorimetric assay was used to detect viable parasites and to calculate the inhibition percent in relation with DMSO-treated controls (Sariego et al. 2014). Briefly, 50×10^3 parasites in 198 µL of TYI culture medium were incubated with 2 µL of pre-diluted test substance or solvent (DMSO) during 46 h, in a candle jar, at 37 °C. Furthermore, the culture medium was replaced with a medium containing the same substances to TYI, except for ascorbic acid and L-cysteine. MTT was dissolved in distilled water at a concentration of 5 mg/mL, sterilized by filtration, and 20 µL were added to the wells of the microtiter plate. After 2 h of incubation at 37 °C, the plate was centrifuged (5 min, 800 *g*), and the supernatant medium was aspirated from the wells, as completely as possible, without disturbing the formazan crystals on the plastic surface. Then, DMSO (100 µL) was added to each well, the plates were shaked for 1 min and immediately the optical density was measured using a spectrophotometer Sirio S Reader, 2.4-0 (Italy), at 560 nm, using 630 nm as the reference wavelength. In each experiment, nontreated and solvent controls were included. Several products concentrations between 0.152 and 200 µg/mL were assayed. Activity was expressed as concentration that reduces 50% of control absorbance (medium inhibitory concentration or IC_{50}).

Antipromastigote assay

As for the trichomonicidal assay, this test was also performed in 96-well culture plates and MTT was used to detect viable parasites and to calculate the inhibition percent in relation to DMSO-treated controls. Exponentially growing cells (4×10^5 promastigotes/mL, 199 µL) in Schneider's medium were distributed in each well. Product (1 µL) was added to plates at a final product concentration between 0.015 and 200 µg/mL, and then incubated at 28 °C. After 3 days of exposure, the parasites were additionally

Table 1. Antiprotozoal activity and cytotoxicity of *S. havanensis* extracts and wogonin.

	IC_{50} ± SD (μg/mL)			CC_{50} ± SD (μg/mL)
Product	*P. berghei*	*T. vaginalis*	*L. amazonensis*	Peritoneal macrophages
Methanol extract	32.2 ± 2.8	20.4 ± 2.7	126.4 ± 7.9	149.6 ± 9.1
Chloroform extract	7.7 ± 0.6	12.9 ± 3.2	>200	35.8 ± 3.3
Wogonin	4.3 ± 0.8 [15 μM][a]	16.1 ± 2.3 [56 μM][a]	>200	31.7 ± 0.9 [110 μM][a]
Chloroquine	0.016 ± 0.02 [0.031 μM]	–	–	>200 [>387.6]
Amphotericin B	–	–	0.026 ± 0.003 [0.028 μM]	6.9 ± 0.6 [7.466 μM]
Metronidazol	–	0.62 ± 0.05 [3.62 μM]	–	>200 [1168 μM]

IC_{50}: medium inhibitory concentration; CC_{50}: medium cytotoxic concentration; SD: standard deviation.
[a]Concentration at μg/mL was converted to μM using 284 g/mol as wogonin molecular mass (Marrero et al. 2015).

incubated for 4 h with MTT under the same conditions. Later, the supernatant medium was aspirated, dissolved in DMSO (100 μL) and the optical density was measured at 560 nm, using 630 nm as the reference wavelength (Sladowski et al. 1993). In each experiment, nontreated and solvent controls were included. Activity was expressed as concentration that reduces 50% of control absorbance (medium inhibitory concentration or IC_{50}).

Cytotoxicity assay

Resident macrophages were collected from peritoneal cavities of normal BALB/c mice in ice-cold RPMI 1640 medium (SIGMA, St. Louis, Mo) supplemented with antibiotics, and seeded at 3×10^4 cells/well. The cells were incubated at 37 °C for 2 h in 5% CO_2. Nonadherent cells were removed and then, dilutions of the extracts were added to 200 μL medium at 10% HFBS and antibiotics. The macrophages were treated by six concentrations of the product ranging from 12.5 to 200 μg/mL for 3 days. Macrophages treated with 1 μL DMSO were included as controls. The cytotoxicity was determined using the MTT colorimetric assay. In this case, 15 μL of MTT solution was added to each well. After incubation for additional 3 h, the formazan crystals were dissolved by the addition of 100 μL DMSO and the optical density was determined at 560 nm and a reference wavelength of 630 nm. Cytotoxicity was expressed as concentration that reduces 50% of control absorbance (medium cytotoxic concentration or CC_{50}).

Statistical analyses

The IC_{50} and CC_{50} values were determined from the concentration–response curves. The results were expressed as average and standard deviation of three assays. The selectivity index (SI) was calculated as the ratio of the CC_{50} to the IC_{50}.

Criteria of activity

Extracts IC_{50} values should certainly be below 100 μg/mL (Cos et al. 2006; Batista et al. 2009) and selectivity index of >4 (Al Musayeib et al. 2013). For pure compounds, the IC_{50} value below 20 μM is considered as having good activity (Batista et al. 2009).

Results

Scutellaria havanensis extracts exhibited IC_{50} values under 40 μg/mL against *P. berghei* and *T. vaginalis*; while against *L. amazonensis* promastigotes were inactive (Table 1).

In the aerial part chloroform extract of *S. havanensis*, 74 compounds were detected by GC/MS and 69 were completely identified (Table 2). Flavonoids such as wogonin, dihydrowogonin, baicalein, 5,2-dihidroxy-6,7,8-trimethoxyflavone, chrysophanic acid, neobaicalein, 5,6,3′ -trihydroxy-3,7,4′-trimethoxyflavone and chalcones represent the majority of compounds (85.49%). In addition, fatty acids (C6:0–C34:0), sterols likes β-sitosterol followed by stigmasterol and campesterol, triterpenoids (β- and α-amyrine, oleanolic and ursolic acid and its derivatives) were identified. Sesquiterpenoids such as β-caryophyllene, caryophyllene oxide, β-humulene and α-copaene were also detected herein. In methanol extract 33 compounds were detected. Several sugars (>64.27%) and wogonin (5.89%) were the most represented (Table 3).

Wogonin exhibited good antiplasmodial activity, CC_{50} and IC_{50} close to the chloroform extract values (Table 1). Selectivity indexes of *S. havanensis* extracts and wogonin are shown in Table 4. The higher values were observed for wogonin against *P. berghei* and for the methanol extract of *S. havanensis* against *T. vaginalis*.

Drugs used as standards showed IC_{50}, CC_{50} and SI values in the expected range (Tables 1 and 4).

Discussion

The chloroform extract of *Scutellaria havanensis* showed the highest anti-plasmodial, trichomonicidal and cytotoxic activity. A similar result was obtained by Mamadalieva et al. (2011) with *S. immaculate* and *S. ramosissima* chloroform extracts against *Trypanosoma brucei brucei* and cancer cells.

More than 295 compounds have been isolated from *Scutellaria* species, among them flavonoids and diterpenes are the most represented (Shang et al. 2010). Several flavonoids have been identified as active principle compounds in *Scutellaria* aerial part chloroform extracts (Lin & Shieh 1996; Mamadalieva et al. 2011). Baicalin, baicalein and wogonin were considered as anti-inflammatory principle compounds in *S. rivularis* extract (Lin & Shieh 1996); whereas chrysin showed the most potent antitrypanosomal activity compared with other flavonoids identified in *S. immaculate* and *S. ramosissima* chloroform extracts (Mamadalieva et al. 2011). The high content of hydroxy and/or methoxyflavones (77.96%), particularly, wogonin (48.27%) in the chloroform extract of *S. havanensis* aerial part is remarkable, confirming the observations of Marrero et al. (2015) and suggesting its antiprotozoal activity.

Even though the phytochemical screening of the methanol extract of *S. havanensis* revealed the presence of flavonoids, alkaloids, amine groups, sugars, quinones and resins (Marrero et al. 2012), GC/MS detected mostly sugars and the flavonoid wogonin. Due to the high polarity of this extract a more detailed composition analysis should be done by HPLC/MS in the future.

In *S. immaculata,* only chloroform extracted wogonin (Mamadalieva et al. 2011). In contrast, wogonin was identified in chloroform and methanol extract of *S. ramosissima* although at

Table 2. Chloroform extract composition of *S. havanensis* aerial parts by GC-MS.

Compound	Rt (min)	%	Compound	Rt (min)	%
2,3-Butanediol	7.03	0.04	Trihydroxymethoxychalcone	19.71	0.03
1,3-Butanediol	7.37	0.10	Dihydrowogonin	19.94	11.34
Hexanoic acid	7.44	0.01	Docosanoic acid	20.06	0.08
Benzoic acid	9.05	0.04	5,2-Dihydroxy-6,7,8-trimethoxyflavone	20.23	7.14
Glycerol	9.28	0.33	Flavonoid NI	20.28	1.02
2-Hydroxyheptanoic acid	9.68	0.02	Flavonoid NI	20.33	0.23
Nonanoic acid	9.86	0.02	1-Tetracosanol	20.96	0.06
α-Copaene	10.09	0.10	Chrysophanic acid	21.38	4.63
β-Caryophyllene	10.42	0.46	Wogonin (5,7-dihydroxy-8-methoxyflavone)	21.92	48.27
Decanoic acid	10.51	0.16	Baicalein (5,6,7-trihydroxyflavone)	22.23	7.20
α-Humulene	10.65	0.07	Flavonoid NI	22.31	0.57
β-Selinene	10.87	0.07	Trihydroxymethoxychalcone	22.46	0.55
Salicilic acid	10.94	0.14	Neobaicalein	22.66	1.82
Ethyl decanoate	11.37	0.03	5,6,3'-Trihydroxy-3,7,4'-Trimethoxiflavone	22.97	1.44
Caryophyllene oxide	11.50	0.01	Dihydroxytetramethoxychalcone	23.16	0.1
Lauric acid	11.76	2.16	Hexacosanoic acid	23.36	0.12
Vanillic acid	12.52	0.02	Flavonoid NI	23.59	0.29
Azelaic acid	12.76	0.27	1-Octacosanol	24.18	0.59
Neophytadiene	13.06	0.17	α-Tocopherol	24.34	0.23
Myristic acid	13.13	0.85	Cholesterol	24.47	0.05
Neophytadiene (D)	13.39	0.1	Hexacosylacetate	24.71	0.12
Pentadecanoic acid	13.89	0.03	Octacosanoic acid	24.89	0.30
p-Coumaric acid	13.91	0.04	Campesterol	25.29	0.16
Palmitoleic acid	14.51	0.06	Stigmasterol	25.52	0.37
Palmitoleic acid	14.72	1.03	1-Triacontanol	25.65	0.23
Palmitic acid	15.57	0.03	β-Sitosterol	25.95	0.32
1-Octadecanol	15.67	0.02	β-Amyrine	26.09	0.11
Linoleic acid	16.20	0.19	α-Amyrine + triacontanoic acid	26.37	0.18
Oleic + linolenic acid	16.24	0.85	Betulinic acid (D)	26.42	0.2
Stearic acid	16.46	0.29	Oleanolic acid (D)	26.66	0.11
Tricosane	16.94	0.02	Ursolic acid (D)	26.97	0.09
Nonadecanoic acid	17.35	0.01	1-Dotriacontanol	27.15	0.09
Dioctyladipate	17.88	0.08	Oleanolic acid	27.86	0.08
Eicosanoic acid	18.25	0.04	Dotriacontanoic acid	28.03	0.15
2′,4′,6′-Trihydroxychalcone	19.14	0.11	Ursolic acid	28.30	0.06
1-Hydroxy-trimethoxyflavone	19.33	0.79	2-Pentatriacontanone	29.04	0.06
Flavonoid NI	19.48	2.08	Tetratriacontanoic acid	30.24	0.09

Rt: retention time; (D): derivative, NI: nonidentified

Table 3. Methanol extract composition of *S. havanensis* aerial parts by GC-MS.<8>

Compound	Rt (min)	%	Compound	Rt (min)	%
Glycerol	9.256	5.57	Arabinose	14.542	0.46
Butanedioic acid	9.521	0.84	D-gluconic acid	14.620	0.39
Glyceric acid	9.683	0.56	Hexadecanoic acid	14.682	1.67
D-Erythronic acid .gamma.-lactone	10.411	0.15	*myo*-Inositol	15.379	1.18
Malic acid	10.745	0.75	Octadecanoic acid	16.420	0.25
Erythritol	10.896	0.23	2-*O*-Glycerol-α-d-galactopyranoside	17.430	0.69
Pyroglutamic acid	10.991	0.13	Uridine	18.512	0.34
2,3,4-Trihydroxybutyric acid	11.129	0.29	Wogonin	21.713	5.89
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone	11.722	0.35	Wogonin isomer	21.897	0.29
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone (isomer)	12.741	0.81			
D-(-)-Fructofuranose (isomer 2)	12.962	8.27			
D-(-)-Fructofuranose (isomer 2)	13.019	15.15			
D-(-)-Fructofuranose (isomer 1)	13.102	1.43			
D-(+)-Talofuranose (isomer 2)	13.240	1.59			
D-Galactose,	13.384	1.94			
Gulonic acid, 1,4-lactone	13.566	1.13			
D-(-)-Tagatose	13.611	1.42			
D-Mannopyranose	13.655	11.78			
α-D-Allopyranose	13.743	8.710			
Unknown	13.870	0.88			
D-Sorbitol	13.997	2.43			
L-Atrose	14.289	2.253			
β-D-Glucopyranose	14.348	20.37			
Deoxiinositol	14.445	1.68			

Rt: retention time; (D): derivative, NI: nonidentified.

different concentrations (Mamadalieva et al. 2011). A similar result was observed in *S. havanensis*, the methanol extract GC/MS revealed the presence of wogonin but in lower proportion than in chloroform extract (eight times less).

Wogonin (Figure 1) has been widely investigated for its antioxidant, anti-inflammatory and anticancer activities (Talbi et al. 2014). Besides, some studies describing its antimicrobial activity have been developed (Schrader 2010). However, antiprotozoal

Table 4. Selectivity index of *S. havanensis* antiprotozoal activity.

	Selectivity index	
Product	*P. berghei*	*T. vaginalis*
Methanol extract	4.6	7.3
Chloroform extract	4.6	2.8
Wogonin	7.4	2.0
Chloroquine	>12500	–
Metronidazol	–	322.6

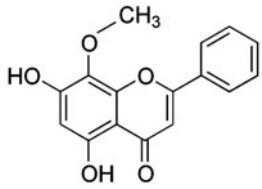

Figure 1. Wogonin structure.

activity of wogonin had not been explored. The flavonoid exhibited CC_{50} and IC_{50} close to the chloroform extract values, demonstrating that wogonin could be the main compound responsible for the extract's activity, but suggesting also the presence of other active metabolites and/or wogonin activity promoters in the extract. In fact, baicalein, the predominant compound in the extract after wogonin and dihydrowogonin, is a bioactive trihydroxyflavone known by its significative antitumour (Kim et al. 2014), antibacterial (Jang et al. 2014) and anti-*Candida* (Serpa et al. 2012) activities, therefore, an antiprotozoal effect could be also exerted. Chalcones are present in the chloroform extract of *S. havanensis* at lower proportions but are known to possess antiplasmodial properties (Kumar et al. 2013). Other compounds in the extract as ursolic acid (Al Musayeib et al. 2013) and C_{18} fatty acids as oleic acid (Carballeira, 2008) also have demonstrated antiplasmodial activity.

Relevant antiprotozoal efficacy must have a selectivity index of at least 10 (Soh & Benoit-Vical 2007). Following those criteria, low selectivity indexes of antiprotozoal activities were observed for the extracts and for wogonin. This suggests that the antiprotozoal activities exhibited by *S. havanensis* extracts and wogonin are probably due to unspecific cytotoxicity rather than activity against specific parasite targets.

Selectivity index of methanol extract for trichomonicidal activity was three times higher than the chloroform extract and wogonin, suggesting the presence of less potent but more specific trichomonicidal compounds in the methanol extract. Wogonin activity against *P. berghei* exhibited the highest selectivity index. This result demonstrates that wogonin preferably inhibits *P. berghei* multiplication respect to mammalian cells and suggests that no other compound in the extracts is substantially contributing to antiplasmodial activity.

Malaria disease is characterized by intermittent fevers with spleen disorders (WHO 2014). For that reason, ethnomedical reports of *S. havanensis* in Cuba (Roig 1974) lead us to consider its antimalarial use. In that case, antiplasmodial activity of *S. havanensis* extracts and wogonin validated the plant folkloric use. Further studies confirming the activity against *P. falciparum in vitro* and *P. berghei in vivo* should be explored (Benoit-Vical 2005).

Several drugs combinations include polyphenols that potentiate anti-infective activity, diminish toxicity and reduce the probability of resistance (Bhattacharya et al. 2009; Aditya et al. 2012). Particularly, baicalein improved efficacy of albendazol against eosinophilic meningitis induced by *Angiostrongylus cantonensis* in mice (He et al. 2011). Wogonin was much less active than reference drugs but should be tested in combination to explore its probably trichomonicidal and antiplasmodial favouring effects which could potentiate standard treatments. In other way, obtaining synthetic derivatives of wogonin with a more potent antiprotozoal activity cannot be ruled out.

Conclusions

In conclusion, leaves and stems of *S. havanensis* are characterized by a substantial accumulation of flavonoids. The methanol and chloroform extracts of *S. havanensis* possess antiplasmodial and trichomonicidal activities. Wogonin was identified as a major active principle compound of the chloroform extract of *S. havanensis* against *P. berghei* and *T. vaginalis* protozoa and only its antiplasmodial effect was specific with respect to mammalian cells. Our findings suggest that wogonin is potentially useful for the development of antimalarial alternatives, whereas methanol extract of *S. havanensis* should be investigated further for trichomonicidal activity.

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Disclosure statement

Authors declare no conflict of interest.

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