Original Article

Antispasmodic activity from *Serjania caracasana* fractions and their safety

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ABSTRACT

In a previous study, we reported the antispasmodic and gastroprotective effects of the *Serjania caracasana* (Jacq.) Willd., Sapindaceae, extract. In the present study, we evaluated the LD_{50}, hemolytic and antispasmodic activities of its fractions and characterized its major constituents by isolation and GC–MS. The animals showed non-toxic symptoms with oral doses up to 2000 mg/kg, suggesting a safe oral administration. Furthermore, a low hemolytic activity was detected for the saponin fraction. Antispasmodic activity of the fractions was evaluated through carbachol-induced contractions in rat ileum. The hexane fraction was the most potent (IC_{50} 68.4 ± 5.9 µg/ml) followed by the dichloromethane fraction (IC_{50} 161.3.4 ± 40.7 µg/ml). Butanol fraction was the less effective (IC_{50} 219.8 ± 60.3 µg/ml). The phytochemical study of the *S. caracasana* fractions afforded the isolation of friedelin, β-amyrin, allantoin and quercitrin. This is the first time that the presence of allantoin and quercitrin in the *Serjania* genus has been reported. Among the isolated compounds and those characterized by GC–MS, β-amyrin and β-sitosterol were present in the most active fractions, hexane and dichloromethane, and they may be related to its antispasmodic activity. In addition, spathulenol was only found in the hexane fraction and its presence might justify the highest antispasmodic activity observed for this fraction.

Introduction

Serjania caracasana (Jacq.) Willd., Sapindaceae, is used for weaving baskets and rustic ropes, mostly because it is considered ichthyotoxic. This belief comes from its similarity with *S. lethalis*, which is known to contain hemolytic saponins (Teixeira et al., 1984). In fact, all previous publications about *S. caracasana* (Aragão and Valle, 1973; Xavier and Mors, 1975; Cordeiro and Valle, 1975; Maia-Braggio et al., 1978) used *S. lethalis* instead, as it was later confirmed (Teixeira et al., 1984).

Some *Serjania* species are used in folk medicine such as *S. comata* (rheumatism), *S. lethalis* (kidney pain, anti-inflammatory), *S. erecta* (gastric pain, anti-inflammatory), *S. marginata* (gastric pain) and *S. triquetra* (diuretic) (Chávez and Delgado, 1994; Guarin-Neto et al., 2000; Agra et al., 2008; Périco et al., 2015). Based on these popular uses, our group has previously demonstrated that the *S. caracasana* hydroethanolic extract showed a gastroprotective effect by inhibiting gastric ulcer induction and an *in vitro* antispasmodic activity (Silva et al., 2012).

In this study, we further evaluated the antispasmodic activity to identify the compounds responsible for this effect. The extract safety was initially evaluated through the determination of its median lethal dose (LD_{50}) and the search for hemolytic saponins in the butanol fraction.

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Materials and methods

Chemical materials

Standard sample of saponins of *Quillaja saponaria* was a kind courtesy of Prof. Dr. Carmen Queiroga. The citrated bovine blood was purchased at the USP Veterinary Hospital (USP-Brazil). Silica gel 60 F_{254} TLC aluminum sheets were purchased from Merck Company. All others reagents and solvents were analytical grade.

Plant material

The aerial parts of *Serjania caracasana* (Jacq.) Willd., Sapindaceae, were collected at the base of Pico do Jabre (7°15′34.27″ S, 37°23′8.53″ W), Paraiba, Brazil during fructification period (June, 2009) by Dr. Josean F. Tavares (UFPB). The plant material was identified by Prof. Dr. Maria de Fátima Agra (UFPB). A voucher specimen (No. M.F. Agra et al., 6963) was deposited in the Herbarium Prof. Lauro Pires Xavier (JPB), at the same University.

Phytochemical analysis

Spectroscopic properties of the isolated compounds

NMR analysis was performed in an Agilent INOVA-500 (500 MHz) spectrometer. High resolution mass spectra were performed on a MicroTOF LC mass spectrometer from Bruker Daltonics and IR spectra was recorded with a Bomem instrument. The structures of the isolated compounds were determined by ^{1}H and ^{13}C NMR spectroscopy, using one and two-dimensional techniques, together with IR and HRMS data by comparison with data previously published for these compounds.

Gas chromatography–mass spectrometry (GC–MS) analysis

The hexane and CH_2Cl_2 fractions had their chemical composition analyzed by gas chromatography–mass spectrometry (GC/MS), performed using a Shimadzu GCMS-QP2010 Ultra system, with a fused silica capillary column coated with 5% polyphenylsiloxane/95% dimethyl polysiloxane (Rxi®-5 ms, 10 m × 0.10 mm ID × 0.10 μm film thickness), electron ionization system operating at 70 eV with an interface temperature of 260 °C, scan time of 0.1 scans/s and acquisition mass range of 35.0–500.0 Da. For the GC analysis, the injection temperature was set at 250 °C, the oven temperature program was 100 °C for 1 min, 100–290 °C at 15 °C/min, maintaining 290 °C for 15 min with helium as a carrier gas (0.42 ml/min). The compound identification was performed by comparing the mass spectra with the Wiley library and literature data (Adams, 2007) together with the co-injection of standards. The relative composition was obtained from the electronic integration measurements using flame ionization detection (300 °C) without taking into account relative response factors, in the same conditions described above.

Extract preparation

The powdered air-dried aerial parts of *S. caracasana* (1916 g) were extracted exhaustively with 96% aqueous ethanol solution at room temperature. The combined extracts were filtered and concentrated under reduced pressure at 40 °C affording a hydroethanolic extract (202.84 g; 10.6% extraction yield) (EtOH).

Extract fractionation and compound isolation

The EtOH extract (200 g) was suspended in MeOH–H_2O (70:30, v/v) mixture, and it was subsequently fractioned with hexane, CH_2Cl_2 and BuOH. Each solvent fraction was then evaporated to dryness under reduced pressure to give hexane (31.53 g; 15.8%),

CH_2Cl_2 (21.9 g; 10.96%) and BuOH (44.50 g; 22.2%) fractions. The CH_2Cl_2 and BuOH fractions were separately subjected to CC on silica gel using step gradients of hexane–EtOAc and EtOAc–MeOH. Column fractions that presented only one major compound were resubmitted to the same chromatographic separation to obtain the purified compounds. CH_2Cl_2 fraction yielded four compounds (friedelin (**1**), β-amyrin (**2**), stigmasterol and β-sitosterol), while BuOH fraction afforded one compound (β-sitosterol glucoside). A remaining part of the BuOH fraction was suspended with 300 ml of MeOH. The solution was filtered and insoluble part reserved. Diethyl ether was carefully added into the solution until some precipitation started, and then it was placed in the freezer (−22 °C for 24 h). The precipitate formed was removed by filtration, and reserved. This process was repeated twice. The pooled precipitates were solubilized with MeOH and concentrated under reduced pressure at 40 °C, to give BuOH-1 fraction. The remaining organic phase was evaporated under reduced pressure to dryness to give BuOH-2 fraction. The BuOH-2 fraction was subjected to CC on silica gel using step gradients of $CHCl_3$–MeOH and after fixed solvent system ($CHCl_3$–acetone–formic acid, 75:16.5:8.5 (v/v)) (Ikeda et al., 1991) to obtain 27 fractions combined according to their TLC profiles into fifteen major fractions (Fr1–Fr15). From fraction Fr7 obtained allantoin (**3**) as precipitate after solvent drying. Fraction Fr8 was purified by preparative TLC ($CHCl_3$–MeOH, 80:20 (v/v)) to give quercitrin (**4**).

Friedelin (**1**). 5.6 mg, 0.03% yield; amorphous powder; IR ν_{max} (KBr) cm^{-1}: 3000, 2848, 1714; ^{1}H NMR (500 MHz, CDCl$_3$): 1.16 (3H, *s*, H-28), 1.03 (3H, *s*, H-27), 0.99 (3H, *s*, H-26), 0.98 (3H, *s*, H-30), 0.93 (3H, *s*, H-29), 0.86 (3H, *sl ap*, H-23), 0.85 (3H, *s*, H-25), 0.70 (3H, *s*, H-24). ^{13}C NMR data were consistent with those previously reported (Akihisa et al., 1992).

β-Amyrin (**2**). 169.8 mg, 1.05% yield; white powder; IR ν_{max} (KBr) cm^{-1}: 3299, 2946, 2852, 1034; ^{1}H NMR (500 MHz, CDCl$_3$): 5.16 (1H, *t*, *J* = 3.6 Hz, H-12), 3.20 (1H, *dd*, *J* = 11.15, 4.5, H-3), 1.11 (3H, *s*, H-27), 0.97 (3H, *s*, H-23), 0.94 (3H, *s*, H-26), 0.91 (3H, *s*, H-24), 0.80 (3H, *s*, H-28), 0.76 (3H, *s*, H-25). ^{13}C NMR data were consistent with those previously reported (Dias et al., 2011).

Stigmasterol. 37.8 mg, 0.24% yield; white powder; ^{1}H NMR (500 MHz; CDCl$_3$): 5.33 (1H, *sl*, H-6), 5.13 (1H, *dd*, H-23), 5.00 (1H, *dd*, H-22), 3.50 (2H, *m*, H-3). ^{13}C NMR data were consistent with those previously reported (Kojima et al., 1990).

β-Sitosterol. 21.4 mg, 0.13% yield; white powder; ^{1}H NMR (500 MHz; CDCl$_3$): 5.33 (1H, *sl*, H-6), 3.50 (2H, *m*, H-3). ^{13}C NMR data were consistent with those previously reported (Kojima et al., 1990).

β-Sitosterol glucoside. 3.2 mg, 0.04% yield; amorphous powder; ^{1}H NMR (500 MHz, Py-d$_5$): 5.32 (1H, *sl*, H-6), 5.04 (1H, *d*, H-1′), 4.55 (1H, *m ap*, H-6′), 4.28 (1H, *s ap*, H-4′), 3.99 (1H, *m*, H-3), 2.70/2.45 (1H, *m*, H-4), 0.98 (3H, *d*, H-21), 0.94 (3H, *sl*, H-19), 0.91 (3H, *s ap*, H-26), 0.87 (3H, *d ap*, H-29), 0.84 (3H, *d*, H-27), 0.64 (3H, *s*, H-18). ^{13}C NMR data were consistent with those previously reported (Kojima et al., 1990).

Allantoin (**3**). 6.5 mg, 0.08% yield; colorless crystal; IR ν_{max} (KBr) cm^{-1}: 3439, 3344, 3224, 3063, 1782, 1659; ^{1}H NMR (500 MHz, DMSO-d$_6$): 10.51 (1H, *br s*, H-1), 8.03 (1H, *s*, H-4), 6.91 (1H, *d*, *J* = 13.5 Hz, H-3), 5.78 (2H, *s*, H-8), 5.24 (1H, *d*, *J* = 13.5 Hz, H-6). ^{13}C NMR data were consistent with those previously reported (Sripathi et al., 2011). EI-HRMS (negative-ion mode) *m/z*: 157.03722 [M−H]$^-$ ($C_4H_6N_4O_3$ requires 157.0315).

Quercitrin (**4**). 30.9 mg, 0.41% yield; yellow powder; ^{1}H NMR (500 MHz, CD$_3$OD): 7.34 (1H, *s ap*, H-2′), 7.30 (1H, *d ap*, *J* = 7.74 Hz, H-6′), 6.91 (1H, *d*, *J* = 7.74 Hz, H-5′), 6.36 (1H, *s*, H-6), 6.19 (1H, *s*, H-8), 5.34 (1H, *s ap*, H-1″), 0.94 (1H, *d*, *J* = 5.85 Hz, H-6″). ^{13}C NMR data were consistent with those previously reported (Shi et al., 2010).

Toxicological assays

Median lethal dose (LD50)

Male Swiss mice (35–40 g) were separated in three groups of five animals for each treatment ($n = 5$). Animals from each experimental and control groups were hosted in cages maintained at constant room temperature ($22 \pm 1\,°C$) and subjected to a 12/12 h light/dark cycle with access to food and water *ad libitum*. Procedures involving animals and their care were performed in conformity with OECD 420 (2001), adopted in our laboratory, and in compliance with current internationally accepted instructions for the care of laboratory animals and ethical guidelines. Furthermore, clearance for conducting the study was obtained from the Ethics Committee of Animal Use of the Nove de Julho University (approval # AN 0003/11).

In order to determine the oral median lethal dose (LD_{50}) of the EtOH extract, this test followed the method described for Miller and Tainter (1944) with same modifications. The control group received the vehicle (0.1% Tween 20 in distilled water). Two doses (1000 and 2000 mg/kg, in a volume of 1 ml/g) were given orally, by gavage, to two groups of mice for the determination of LD_{50}. The animals were observed during the first 180 min after the treatment and after 24, 48 and 72 h for any toxic signs and symptoms.

Qualitative assay of hemolytic saponins

The qualitative hemolytic activity of the saponins was tested with bovine blood reagent as described by Sharma et al. (2012) with some modifications. Briefly, aliquots of 1 ml of citrated bovine blood were washed three times with 9 ml of saline (0.9%; w/v NaCl) followed by centrifugation at $180 \times g$ for 5 min. The cell suspension was finally prepared by diluting the pellet to 3% (v/v) in saline solution to obtain the blood reagent. Also, to visualize the saponins a comparative spray reagent (Liebermann–Burchard reagent) was prepared (Wagner and Bladt, 1996).

The assay was performed with a 10 µl aliquot of saponins from *Quillaja saponaria* (SQ) and BuOH fraction (conc. 20 mg/ml, solubilized in MeOH–H_2O, 70:30 (v/v)) applied in TLC plates which were eluted with the solvent system $CHCl_3$–MeOH–TFA 0.5% (60:40:5 (v/v)) to 6.5 cm from the origin. After the elution, the TLC plates were air dried.

One of the developed TLC plates was immersed for 20 s in a glass dish containing the 3% blood reagent freshly prepared. After this time, the TLC plate was removed and held vertically for 30 s, and subsequently immersed in saline for 30 s. Finally, this TLC plate was held vertically for complete drying and further visualization of the hemolytic spots. The hemolytic spots were compared with the developed TLC sprayed with Liebermann–Burchard reagent (Sharma et al., 2012).

Quantitative assay of hemolytic saponins

The quantitative hemolytic activity of the saponins was evaluated with a bovine blood cell suspension by turbidimetry as described by Xie et al. (2008) with some modifications. Briefly, aliquots of 10 ml of blood were washed three times with saline solution by centrifugation at $180 \times g$ for 2 min. The cell suspension was finally prepared by diluting the pellet to 5% (v/v) in saline solution.

The SQ and BuOH fraction samples were solubilized initially in saline to 25 mg/ml. For the assay, 180 µl of freshly prepared 5% blood cell suspension was mixed with 20 µl of sample or control solution. The sample concentration ranged from 820.0 to 16.4 µg/ml for BuOH fraction, and from 500.0 to 5 µg/ml for SQ. The microplate was incubated for 30 min at $37\,°C$ and centrifuged at $70 \times g$ for 10 min. An aliquot of each supernatant (75 µl) was transferred to a flat-bottom microplate and the free hemoglobin was measured at 540 nm (Silveira et al., 2011). Saline and SQ (100 µg/ml) were considered as minimal and maximal hemolytic controls. The concentration inducing 50% of maximum hemolysis

(HC_{50}) was calculated by non-liner regression (GraphPad Prism 5.01). Each experiment included triplicates for each concentration. The results of the quantitative hemolytic activity are presented as mean $\pm$ SD and the other results as mean $\pm$ SEM.

Pharmacological assay

Antispasmodic activity on ileum isolated rat

Ileum strips were isolated from rat following the methodology described by Walker and Wilson (1979) and suspended in organ baths (5 ml) containing modified Krebs physiological solution, consisting of (mmol/l): NaCl 117.0; KCl 4.7; $NaH_2PO_4·H_2O$ 1.2; $MgSO_4·7H_2O$ 1.3; $CaCl_2·2H_2O$ 2.5; $NaHCO_3$ 25.0; glucose 11.0; pH 7.4 (Sun and Benishin, 1994). Furthermore, the clearance for conducting the study was obtained from the Ethics Committee of Animal Use of the Federal University of São Paulo (approval # CEUA 4195060514/14).

The hexane, CH_2Cl_2 and BuOH fractions were dissolved initially in 0.01% Cremophor EL and diluted in MilliQ water to obtain the stock solution of 10 mg/ml which was stored at $0\,°C$.

The tissues were maintained under 1 g tension, bubbled continuously with O_2 at $37\,°C$. They were attached to force isometric transducers and connected to a data system AQCD (AVS Projetos, Brazil). Following control contractions with KCl (40 mmol/l) or carbachol (1 µmol/l), and washing with a fresh Krebs solution, tissue strips were exposed to concentration gradient ranging from 500 to 9 µg/ml of hexane, CH_2Cl_2 or BuOH fractions for 15 min (Walker and Wilson, 1979), then stimulated again with the previous referred concentration of carbachol. The antispasmodic activity of the samples was expressed as maximum contraction (E_{max} value) obtained in the presence of the distinct fraction relative to the maximum contraction in their absence (control). The concentration of fraction that reduces to 50% a maximal response for an agonist (IC_{50}) values were determined from individual concentration–response curves by non-linear regression (Jenkinson et al., 1995).

Statistical analysis

Statistical significance between control and experimental group was evaluated using either Student's *t*-test or one-way ANOVA following Dunnett's Multiple Comparison Test. Data were considered significant when $p < 0.05$.

Results and discussion

Median lethal dose (LD50)

In our earlier study, we observed the *S. caracasana* EtOH extract significantly inhibited KCl pre-contracted ileum, indicating an interesting *in vitro* antispasmodic activity (Silva et al., 2012). However, due to the possible toxicity to mammals that could be related to the presence of hemolytic saponins, as reported for other *Serjania* species, we decided to perform some preliminary safety assays. The oral acute toxicity from S. *caracasana* crude extract was assessed by its LD_{50}. In this assay, two oral doses (1000 and 2000 mg/kg) did not produce any visible signs of toxic symptoms 72 h after receiving the extract. Therefore, no further evaluation was performed and the LD_{50} was estimated as higher than 2000 mg/kg. This LD_{50} value places the extract in the Category 5 of the United Nations Globally Harmonized System for toxicity hazards, which leads this extract to be considered as safe after oral acute exposure. Additional animal tests with substances in this category range are strongly discouraged and should only be considered when there is a direct relevance for protecting human health (Bulgheroni et al., 2009). Similar results were found for the chloroform extract from *S. erecta*
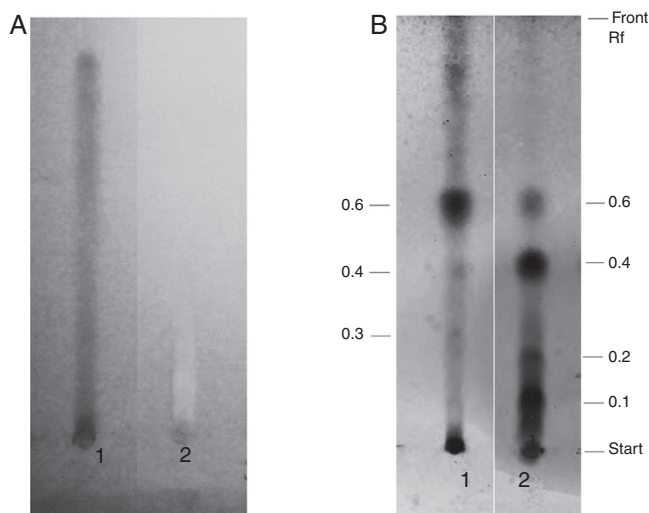
Fig. 1. TLC of BuOH fraction of *Serjania caracasana* aerial parts (1) and of rich fraction saponins of *Quillaja saponaria* (SQ) (2). Solvent system: chloroform–methanol–trifluoroacetic acid aqueous solution 0.5% (60:40:5, v/v). Detection: blood reagent 3% (A); Liebermann–Burchard reagent (B).

and hydroethanolic extract from *S. marginata*, where mice treated with a single oral dose of 5000 mg/kg had no signs of toxic effects in an acute toxicity study (Arruda et al., 2009; Périco et al., 2015).

Qualitative and quantitative assays of hemolytic saponins

Despite the low acute toxicity of *S. caracasana* EtOH extract, the presence of hemolytic saponins in *Serjania* extracts should be investigated. These compounds are considered harmful not only because of their acute toxic effects, as reported to the saponins isolated from *S. lethalis*, serjanosides A, B and C, that showed toxic acute effects in rats and mice (Teixeira et al., 1984). Also, chronic administration of saponins in animals is known to affect their growth or altering their palatability and, consequently, food consumption or altering the digestion process and absorption (Oleszek, 1996). The TLC chromatogram of the *S. caracasana* BuOH fraction sprayed with Liebermann–Burchard reagent (Fig. 1B) showed the presence of three main purple zones, characteristic of saponins, at R_f's 0.3, 0.4 and 0.6, with the latter as the major component. The comparison of this TLC profile with that obtained after spraying with the Blood reagent (Fig. 1A) indicated that the observed saponins were not able to cause hemolysis in the TLC assay in the concentration tested. In contrast, the positive control (SQ) showed some white spots (R_f = 0.1 and 0.2) against a pink background (Fig. 1A),

characteristic of hemolysis. However, SQ also presented two other compounds that were non-hemolytic compounds (R_f = 0.4 and 0.6), typically saponins for Liebermann–Burchard reagent (Fig. 1B).

Similarly, in the quantitative hemolytic activity, the BuOH fraction exhibited a low hemolysis rate (∼2%) until the maximum concentration tested (820 µg/ml) when compared with the positive control (SQ) (100% at 100 µg/ml) (Fig. 2). Once at the maximum concentration tested the hemolysis rates were under 50% of hemolytic activity, the BuOH fraction HC_{50} value was estimated as >1000 µg/ml. The positive control (SQ) showed high hemolytic activity (HC_{50} = 24.03 ± 1.03 µg/ml) under the same conditions. Thus, different than would be expected for an ichthyotoxic species, our results indicated that *S. caracasana* saponins could be considered as non-hemolytic.

Antispasmodic activity on ileum isolated rat

As *S. caracasana* demonstrated low toxicity and almost no hemolytic effect, we decided to investigate which extract fraction would be responsible for the antispasmodic activity previously reported (Silva et al., 2012).

The antispasmodic activity was analyzed for the *S. caracasana* hexane, CH_2Cl_2 and BuOH fractions. Fig. 3(A)–(C) shows the overall inhibitory effect of these fractions on rat ileum contractions. The hexane fraction significantly inhibited the maximum ileum contractions at the concentrations of 27, 81, 243 and 500 µg/ml (Fig. 3A), presenting the respective amplitude decrease values of 66.1 ± 7.1, 47.5 ± 3.8, 24.5 ± 2.4 and 23.5 ± 7.3% (Fig. 3A), while for the CH_2Cl_2 fraction a significant inhibition started at the contractions of 81, 243 and 500 µg/ml, with E_{max} values of 62.7 ± 12.6, 47.7 ± 7.9 and 20.2 ± 3.7% respectively (Fig. 3B). In contrast, the BuOH fraction was only able to significantly reduce the intensity of the contractions at 243 and 500 µg/ml with respective E_{max} of 44.9 ± 13.1 and 25.8 ± 5.5% (Fig. 3C).

These results indicated that the antispasmodic activity is distributed in all *S. caracasana* fractions. However, the *n*-hexane fraction was more potent than the crude EtOH extract, that was 46% at the concentration of 81 µg/ml (Silva et al., 2012). The CH_2Cl_2 fraction was less active showing similar results to those obtained with the crude extract, and the BuOH fraction showed even less activity than the extract. Thus, all the fractions were interesting to search for natural antispasmodic compounds.

Phytochemical analysis

In order to determine the possible compounds responsible for the antispasmodic activity, CH_2Cl_2 and BuOH fractions were submitted to column chromatography. From these fractions, we could
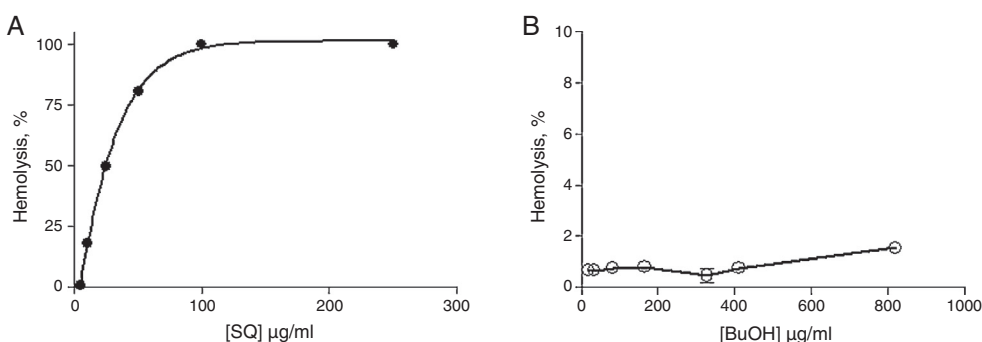

Fig. 2. Hemolytic activity of the enriched saponin fraction from *Quillaja saponaria* (SQ) (A) and of the BuOH fraction of *Serjania caracasana* aerial parts (B).
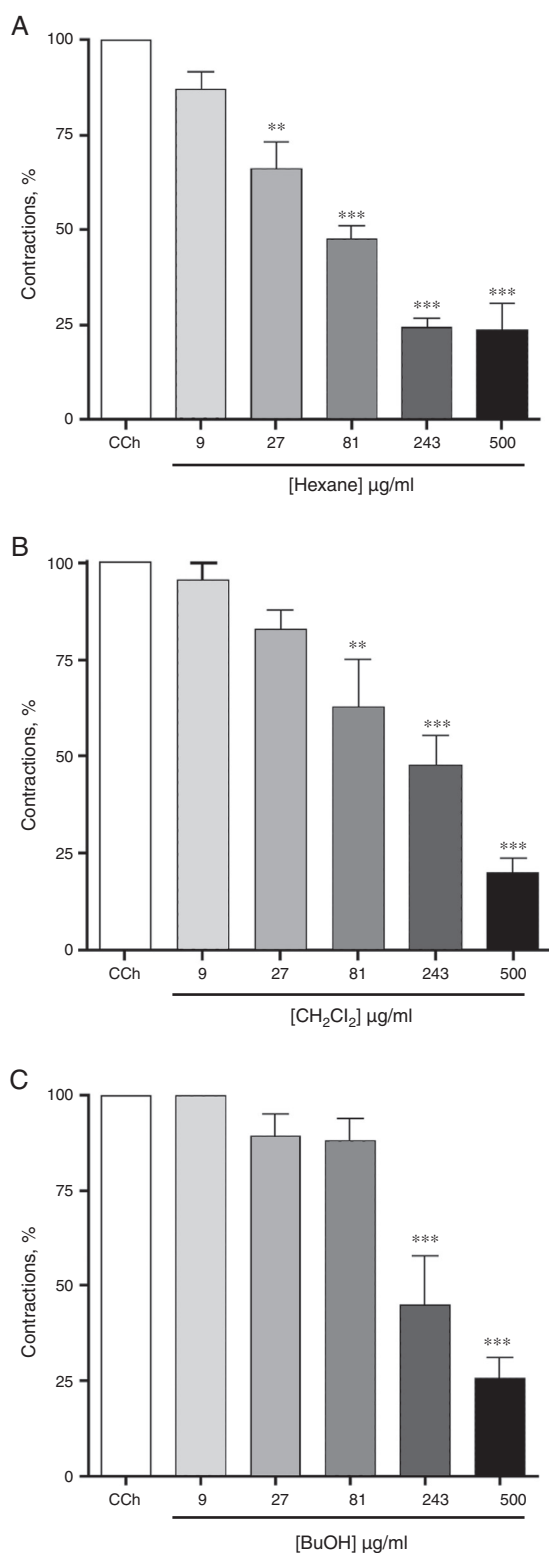
Fig. 3. Effect of hexane (A), CH$_2$Cl$_2$ (B) or BuOH (C) fractions of *Serjania caracasana* aerial parts on pre-contracted ileum by carbachol 1 μM (*n* = 3). One-way ANOVA followed by Dunnett's Multiple Comparison Test: **$p < 0.01$; ***$p < 0.001$ (control × fraction).

Table 1
Compounds characterized in the hexane and CH$_2$Cl$_2$ fractions of *Serjania caracasana* aerial parts.

Compound	Hexane fraction		CH$_2$Cl$_2$ fraction	
	RT (min)	%	RT (min)	%
Spathulenol (**5**)	5.8	4.2	–	–
6,10,14-Trimethyl-2-pentadecanone	7.3	1.7	7.3	1.8
Methyl palmitate	7.7	1.7	7.7	2.7
Ethyl palmitate	8.1	6.2	8.1	8.6
β-Sitosterol	14.7	1.2	14.7	2.1
β-Amyrin (**2**)	15.1	60.0	15.1	51.0
Total		75.0		66.2
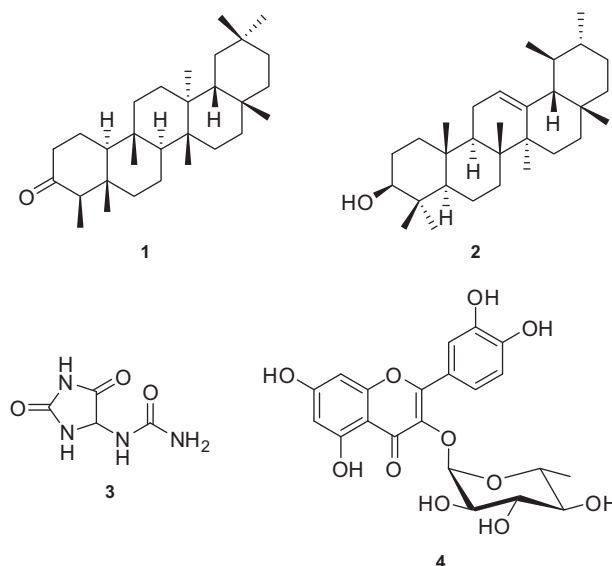
Additionally, the two most active fractions, hexane and CH$_2$Cl$_2$, had their chemical composition compared by GC–MS, indicating a similar chemical composition for these fractions (Fig. 4). The mass spectra obtained allowed to characterize six compounds (Table 1) representing 79.8% and 66.6% of the total of compounds detected in the GC–MS. The two fractions showed the same major components, β-amyrin (**2**) (60.0% and 51.0%) and ethyl palmitate (6.2% and 8.6%), respectively for hexane and CH$_2$Cl$_2$ fractions. Additionally, in the hexane fraction was found spathulenol (**5**) in a representative quantity (4.2%). This sesquiterpene is a common essential oil component in several plant species, it has shown an antispasmodic activity in uterus rings contraction model induced by KCl (Perez-Hernandez et al., 2008). The presence of spathulenol exclusively in the hexane fraction might justify the highest antispasmodic activity observed in comparison to the other fractions.
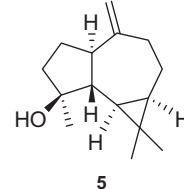
In a recent study, Coutinho et al. (2015) reported the seed fatty-acid compositions for sixteen Sapindaceae species. *Serjania* species, including *S. caracasana*, presented high levels eicosenoic acid and other palmitic acid esters. In our study, palmitic acid methyl and ethyl esters were found in both hexane and CH$_2$Cl$_2$ fractions. Friedelin (**1**) and β-amyrin (**2**), together with other triterpenes, are typically isolated within the genus *Serjania*, such as the presence of **1** and **2** reported in *S. salzmanniana* (Barbosa-Filho et al., 1988). In this study, we detected for the first time in a *Serjania* species quercitrin (**4**), allantoin (**3**) and spathulenol (**5**).

isolate and characterize seven components, two oleanane triterpenes: friedelin (**1**) and β-amyrin (**2**), three steroids: stigmasterol, β-sitosterol and β-sitosterol glucoside, one urate derivative: allantoin (**3**) and one flavonol: quercitrin (**4**).
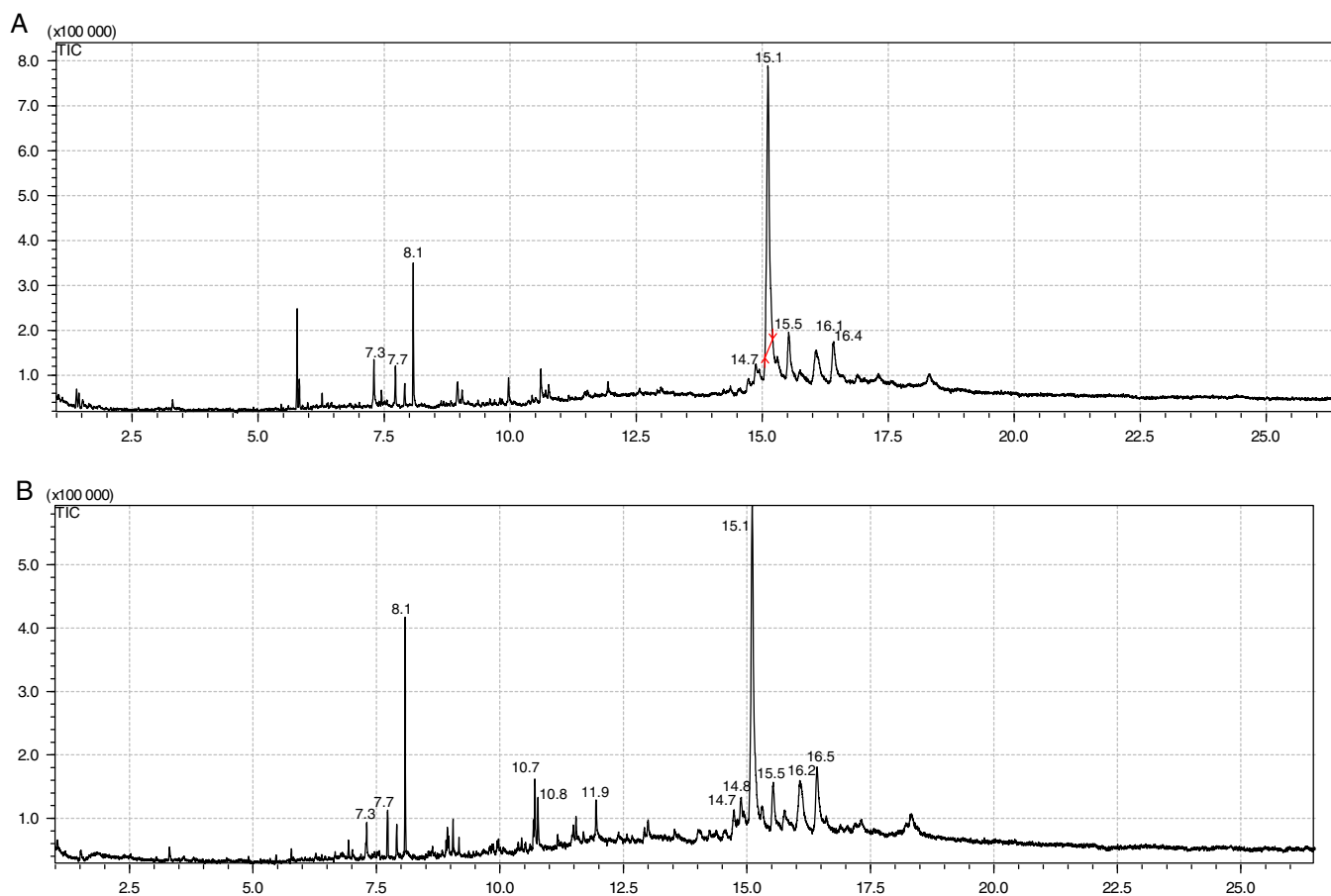
Fig. 4. GC–MS chromatogram of hexane (A) and CH$_2$Cl$_2$ (B) fractions of *Serjania caracasana* aerial parts.

According to our results, *S. caracasana* saponins and quercitrin are not the main metabolites responsible for the antispasmodic effect observed, but they may contribute to the overall effect observed in the crude extract. In similar studies, it has been observed that the fractions enriched with saponins and flavonol derivatives, including quercitrin, showed significant antispasmodic activity in the acetylcholine model (Trute et al., 1997).

In conclusion, our findings demonstrated that although of *S. caracasana* is considered an ichthyotoxic species, this property, if present, cannot be related to the presence of hemolytic saponins. The toxicity for mammal species was not confirmed by the acute toxicity test, requiring complimentary studies on the subject. In addition, future studies should be carried out with the isolated compounds to verify their spasmolytic activity.

Authors' contributions

FLS (Ph.D. student) contributed by conducting the phytochemical laboratory work, the hemolytic evaluation and drafting the paper. JLVS contributed by conducting the pharmacological assays, teaching JMS and LSAM in the conducting of the pharmacological assays and by critical reading of the manuscript. VLAN supervised the pharmacological work and contributed to critical reading of the manuscript. MY contributed with the characterizing of many isolated secondary metabolites. PHV contributed determining the EI-HRMS of allantoin. MNE supervised the laboratory work and contributed to critical reading of the manuscript. JMBF designed the phytochemical work and contributed guiding and supporting the plant collection and the laboratory work. PRHM contributed supporting and designing the phytochemical work and the hemolytic

assays, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical responsibilities

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

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