ABSTRACT

Solanum paniculatum is a plant species widespread throughout tropical America, especially in the Brazilian Savanna region. It is used in Brazil for culinary purposes and in folk medicine to treat liver and gastric dysfunctions, as well as hangovers. Fractionation of the ethanolic extracts (70%) from aerial parts (leaves and twigs) of *S. paniculatum* (Solanaceae) led to the isolation of the two new saponins (22*R*, 23*S*, 25*R*)-3β, 6α, 23-trihydroxy-5α-spirostane 6-O-β-D-xylopyranosyl-(1''''$\rightarrow$3''')-O-[β-D-quinovopyranosyl(1'''$\rightarrow$2')]-O-[α-L-rhamnopyranosyl(1''$\rightarrow$3')]-O-β-D-quinovopyranoside (**1**) and diosgenin 3-O-β-D-glucopyranosyl(1''$\rightarrow$6')-O-β-D-glucopyranoside (**2**) together with four know compounds: caffeic acid (**3**), diosgenin β-D-glucopiranoside (**4**), rutin (**5**), and quercetin 3-O-α-L-rhamnopyranosyl (1'''$\rightarrow$6'')-O-β-D-galactopyranoside (**6**). The structures of these compounds were elucidated by extensive use of 1D and 2D NMR experiments along with ESI-MS analyses. Different doses (31.25 - 500 mg/kg) of ethanolic extract of leaves from *S. paniculatum* were evaluated against gastric ulcer induced by ethanol in rats. The lower dose of extract able to promote antiulcer effect was 125 mg/kg. The treatment with *S. paniculatum* by oral route was able to decrease gastric lesion area and also reduced levels of myeloperoxidase (MPO) in the gastric mucosa. Our results revels for the first time, steroidal saponins from *S. paniculatum* and the antiulcer effect of this species at this lower dose.

Keywords: Solanaceae, *Solanum paniculatum*, steroidal saponins, antiulcer activity

1. Introduction

The *Solanum* genus contains ca. 1500 species distributed all over the world. Plants in the genus are distributed in the tropical and subtropical regions and an estimated 1000-1100 species of the genus are found in South America regions (Willis, 1980). Due to the large number of species in the genus, the family was named Solanaceae (Kanada et al., 2012).

Many species of the genus are known for its economic importance, such as tomato (*S. lycopersicum*), egg-plant (*S. melongena*) and potato (*S. tuberosum*), and some are used in folk and traditional medicine, like as *S. americanum,* ("maria-pretinha"), used in the treatment of gastric ulcer, bladder spasm, joint pains, and as an effective vermifuge (Lorenzi, 2002).

Recents phytochemical studies of the *Solanum* species report the occurrence of flavonoids (Kanada et al., 2012), amides (Kanada et al., 2012), steroids (Kanada et al., 2012), lignans (Pinto et al., 2013), steroidal saponins (Kanada et al., 2012; Manase et al., 2012; Li et al., 2014; Chou et al., 2012; Chang et al., 2013), and steroidal alkaloids (Pinto et al., 2013; Zhang et al., 2013; Miranda et al., 2013). As part of our ongoing research on bioactive compounds from Brazilian plants for develop of the phytotherapics, we were investigated the potencial of the species *Solanum paniculatum*.

S. paniculatum, known popularly either as *jurubeba, jurupeba, jubeba* or *juna*, is a neotropical weed of very common occurrence in Brazil, Paraguay, Bolivia, and Argentina, used in folk medicine and for culinary purposes. This plant species is used in Brazilian folk medicine as a tonic, antifever agent, bitter, and eupeptic to treat liver and gastric dysfunctions and for the manufacture of beverages and culinary purposes (Miranda et al., 2013; Agra et al., 2007; Mesia-Vela et al., 2002). The plant is a component of various pharmaceutical formulations including: syrups, infusions or decoctions, ethanolic extracts, and elixirs. Many steroidal compounds have been isolated from this species, specially glycoalkaloids and saponins (Siqueira & Macan, 1976; Schereiber et al., 1965; Ripperger et al., 1967a; Ripperger

et al., 1967b; Ripperger et al., 1968) evaluated the antiulcerogenic effect of extract from different part of *S. paniculatum* and the antisecretory effect was observed in fruits and root but not in leaves (Mesia-Vela et al., 2002). Thus, the present study has been conducted to isolated and identified some of the constituents of this species and assess the gastroprotective effect of the ethanolic extract from aerial parts of *Solanum paniculatum*.

2. Materials and methods

2.1. Materials and chemicals

^{1}H (500 MHz), ^{13}C (125 MHz), and 2D NMR spectra were obtained on a Varian INOVA 11,7 T and with TMS as an internal reference. Electrospray ionisation (ESI) mass spectra were acquired in the positive and negative ion mode on a LCQ FLEET instrument (Thermo scientific) equipped with an ion-trap mass analyser. Optical rotations were measured on a Jasco P-1020 polarimeter. Analytical HPLC was performed on a Jasco PU-2089 Plus with UV-DAD detector (model MD-2010 Plus), using a Phenomenex Luna C18(2) column (250 mm x 4.6 mm, 5 μm). Semipreparative HPLC was performed on a Varian with with UV-PDA ProStar 330 detector, using Phenomenex Luna C18(2) (250 x 10 mm, 10 μm) column. For column chromatography (CC) Sephadex LH-20 (GE-Healthcare Bio-Sciences AB, Uppsala, Sweden) was used as packing material. TLC was performed using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 PF254 plates. Spots on TLC plates were observed under UV light and by spraying the plates with anisaldehyde-H_2SO_4 reagent, followed by heating at 120 °C. High-pressure liquid chromatography (HPLC) solvents (TFA and methanol – both ≥ 99.9%) were HPLC grade and purchased from Tedia® (Fairfield, OH, USA). A Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare deionized water for all mobile phases. NMR spectra were measured using $(CD_3)_2SO$ (≥ 99,8%) and pyridine-d_5 (≥ 99,8%) as solvent purchased from Sigma-Aldrich™ (St. Louis, MO, USA). Other organic solvents

(Ethyl acetate, methanol, and chloroform) were of analytical grade and from Synthlab® (São Paulo, Brazil).

2.2. Plant material

Aerial parts of *S. paniculatum* were collected in Araraquara municipality (January 2010), São Paulo State, Brazil. A voucher specimen (BOTU 027535, BOTU 027536, BOTU 027537) of the plant was deposited at the Herbarium BOTU in Botucatu, São Paulo State, Brazil.

2.3. Extraction and isolation of the compounds

The dried and ground twigs (66.0 g) were percolated with ethanol (70%). The ethanolic extract (5.8 g) was lyophilized and chromatographed (2.7 g) on Sephadex LH-20 column (67.0 x 3.0 cm), using MeOH/H_2O (7:3) as eluent. The fraction 48 (108.0 mg) was subjected to EFS-C18, using gradient H_2O (100%) to MeOH (100%), resulting in 19.0 mg of **1**. The fraction 57 (350.0 mg) was chromatographed in HPLC (reverse phase C-18 - 250 x 10 mm, 10 μm, eluent: MeOH/H_2O (25:75) + 0,1% TFA, flow 2 mL.min^{-1}, λ = 254 nm), resulting in 25.0 mg of **3**. The leaves (1.0 Kg) were dried, ground and percolated with ethanol (70%). The ethanolic extract (282.7 g) was lyophilized and chromatographed (3.4 g) on Sephadex LH-20 column (67.0 x 3.0 cm), using MeOH/H_2O (7:3) as eluent. The fractions 32 (73.4 mg), 38 (129.0 mg), 70 (36.5 mg) and 77 (81.0 mg) yielding the compounds **2**, **4**, **5**, and **5**+**6**, respectively.

*(22R, 23S, 25R)-3β, 6α, 23-trihydroxy-5α-spirostane 6-O-β-D-xylopyranosyl-(1''''→3'')-O-[β-D-quinovopyranosyl(1''→2')]-O-[α-L-rhamnopyranosyl(1''→3')]-O-β-D-quinovopyranoside (**1**)*

Yellow amorphous solid; $[\alpha]_D^{24}$ +90 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR (pyridine-d_5), see Tables 1, 2, 3 and 4; ESIMS *m*/*z* (rel. int.): 1041 $[M + Na]^+$ (10), 1056 $[M+K]^+$ (2) and 1027 [M+Na-

Me]$^+$ (25) (calcd for $C_{50}H_{82}O_{21}$), others peaks *m/z* 594 [M+H-Xyl-Qui-Rha]$^+$ (2), 749 [M+Na-Me-Xyl-Qui]$^+$ (7) and 763 [M+Na-Xyl-Qui]$^+$ (5); ESI-MS/MS of *m/z* 764 (70) result in *m/z* 617 [M+Na-Xyl-Qui-Rha]$^+$ (100).

*3-O-β-D-glucopyranosyl(1"→6')-O-β-D-glucopyranoside (**2**)*

Yellow amorphous solid; $[\alpha]_D^{24}$ -20 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR (pyridine-d_5), see Tables 1, 2, 3 and 4; ESIMS *m/z* 739 [M+H]$^+$ (13), 761 [M + Na]$^+$ (40) and 777 [M+K]$^+$ (20) (calcd for $C_{39}H_{62}O_{13}$), others peaks *m/z* 577 [M+H-Gly]$^+$ (4); ESI-MS/MS of *m/z* 762 (54) result in *m/z* 599 [M+H+Na-Gly]$^+$ (44).

*Caffeic acid (**3**)*

Yellow amorphous solid; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.42 (d, J = 16.0 Hz, H-8), 7.04 (d, J = 1.5 Hz, H-2), 6.97 (dd, J = 1.5 and 8.0 Hz, H-5), 6.77 (d, J = 8.0 Hz, H-6) and 6.16 (d, J = 16.0 Hz, H-7). ^{13}C NMR (125 MHz, DMSO-d_6): 165.8 (C-9), 148.3 (C-3), 145.5 (C-7), 144.9 (C-4), 125.6 (C-1), 121.4 (C-6), 115.7 (C-5), 114.7 (C-2) and 114.3 (C-8). HRTOF-ESIMS *m/z* 179.0364 [M-H]$^-$ (calcd. $C_9H_8O_4$: *m/z* 179.0349).

*Diosgenin β-D-glucopiranoside (**4**)*

Yellow amorphous solid; $[\alpha]_D^{24}$ -12 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR (pyridine-d_5), see Tables 1, 2, 3 and 4; ESIMS *m/z* 600 [M+H+Na]$^+$ and 616 [M+H+K]$^+$ (calcd for $C_{33}H_{49}O_8$).

*Rutin (**5**)*

Yellow amorphous solid; ^{1}H and ^{13}C NMR (DMSO-d_6) data were compared with the information in the literature (Agrawal, 1989), and it was identified as rutin; ESIMS *m/z* 609 $[M-H]^-$ (calcd for $C_{27}H_{30}O_{16}$).

*Quercetin 3-O-α-L-rhamnopyranosyl (1”’→6”)-O-β-D-galactopyranoside (**6**)*

Yellow amorphous solid; ^{1}H and ^{13}C NMR (DMSO-d_6) data were compared with the information in the literature (Agrawal, 1989), and it was identified as quercetin 3-O-α-L-rhamnopyranosyl (1”’→6”)-O-β-D-galactopyranoside; ESIMS *m/z* 634 $[M+Na+H]^+$ (calcd for $C_{27}H_{30}O_{16}$).

2.4. Gastric ulcer model induced by ethanol

Male Wistar (200-250 g) were randomly divided into groups (n=7) received oral treatment with saline (negative control), carbenoxolone 100 mg/Kg (positive control) or 70% ethanolic extratct (70%) of leaves of *S. paniculatum* at doses of 31.25, 62.5, 125, 250 and 500 mg/Kg, to obtain dose-response curve. We also included a group Sham, in which the animals were subjected to the same procedure as the other groups but without the use of agents that induced injury. This Sham group was used to determined only biochemical parameters (total glutathione and myeloperoxidase) because the gastric lesion area was absent in this group. The administration was done in a fixed volume of 10 mL/Kg. After one hour after treatment, 1mL of ethanol 99.8% was orally administered and after one hour, all animals are killed and the stomachs examined for obtaining the area (mm^2) of gastric lesions using the program AvSoft BioView (Robert et al., 1979).

2.5. Quantification of total glutathione (GSH)

Strips stored in the experiment of gastric ulcer induced by ethanol were weighed and stored in 1mL of 5% trichloroacetic acid (TCA). The total glutathione content of the stomach was determined using the substance 5,5 'dithio-bis (2-nitrobenzoic acid) (DTNB) (Anderson,

1985). The enzymatic reaction consists of 200µL of sample protein containing 2 mg/mL, 0.2 M phosphate buffer (pH 8.0), 0.5 mM DTNB (2 mg in 10 mL of 1% sodium citrate) in a final volume of 2 mL. The absorbance was determined at 412 nm using a spectrophotometer. The concentration of total glutathione was expressed using the extinction coefficient of 13.6 mM.

2.6. Activity of Myeloperoxidase (MPO)

Myeloperoxidase is an enzyme found in azurophilic granules of neutrophils, serving as a biochemical marker of granulocyte infiltration (Souza et al., 2004). The MPO activity is proportional to the amount of neutrophils in the mucosa. Strips stored the model of gastric ulcer ethanol were weighed. We used the reaction HTAB buffer (0.5% in 50 mM sodium phosphate buffer, pH 6.0) that acts as lysing detergent granules of neutrophils which contains the enzyme, this being released. The enzyme activity was determined by following the reaction kinetics of the enzyme with hydrogen peroxide in a reaction buffer, where one unit of MPO determined and 1 nmol/minute capable of degrading hydrogen peroxide at 25 °C (Krawisz et al., 1984). The absorbance was determined at 450 nm using a spectrophotometer.

2.7. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and subjected to analysis of variance (ANOVA) with post-hoc Tukey and Dunnet tests, with 5% significance level, using the GraphPad Prism 5 Demo.

3. Results and discussion

The leaves and twigs of *S. paniculatum* were percolated with ethanol (70%) and each extract was fractionated over Sephadex LH-20 column. The obtained fractions from twigs were chromatographed by EFS-C18 to yield a new compound **1**and reversed-phase HPLC to yield compound **3**. The fractions from leaves yield a new compound **2**, along with the known compounds **4**, **5** and **6** (see Experimental section).

Compound **1**, obtained as an yellow amorphous solid, showed a major ion peaks at *m/z* 1041 $[M + Na]^+$, 1056 $[M+K]^+$ and 1027 $[M+Na-Me]^+$ (calcd for $C_{50}H_{82}O_{21}$) in the positive ESIMS mass spectrum. The mass spectra present others important peaks at *m/z* 594 [M+H-Xyl-Qui-Rha]$^+$, 749 [M+Na-Me-Xyl-Qui]$^+$ and 763 [M+Na-Xyl-Qui]$^+$. The MS/MS of *m/z* 764 result in *m/z* 617 [M+Na-Xyl-Qui-146]$^+$, corresponding to the loss of a rhamnose unit.

The ^{1}H NMR spectrum of **1** showed signals for two tertiary methyl groups at δ_H 0.86 (3H, br s, H-18) and 0.83 (3H, br s, H-19), five secondary methyl groups at δ_H 1.52 (3H, d, *J* = 7.5 Hz, H-27), 1.55 (3H, d, *J* = 7.0 Hz, H-21), 1.58 (3H, d, *J* = 6.0 Hz, H-6"'), 1.63 (3H, d, *J* = 6.0 Hz, H-6') and 1.70 (3H, d, *J* = 6.0 Hz, H-6"), four protons attached to the anomeric carbons at δ_H 4.76 (1H, d, *J* = 8.0 Hz, H-1'), 4.83 (1H, d, *J* = 7.5 Hz, H-1"'), 5.25 (1H, d, *J* = 7.5 Hz, H-1"") and 6.28 (1H, br d, *J* = 1.5 Hz, H-1"). The ^{13}C, DEPT 90° and 135° NMR data (in pyridine-d5) showed the presence of 50 carbons divided into three quaternary, 30 methine, 10 methylene, and seven methyl carbons, with 11 carbon resonances being attributable to a *β*-D-xylopyranosyl-(1""→3"')-*β*-D-quinovopyranosyl moiety by comparing those already reported sugar residues in Solanolactoside B (Lu et al., 2011) and 12 carbon resonances being attributable to a *α*-L-rhamnopyranosyl(1"→3')-*β*-D-quinovopyranoside as listed in Tables 3 and 4. The resonances of the remaining 27 carbons originating the steroidal nucleous were identical to (22R, 23S, 25R)-3*β*, 6*α*, 23-trihydroxy-5*α* -spirostane, the aglycone of torvoside C isolated from *Solanum torvum* (Yahara et al., 1996) and (22R, 23S, 25R)-3*β*, 6*α*, 23-trihydroxy-5*α*-espirostane 6-O-*β*-D-xylopyranosyl-(1→3)-O-*β*-D-quinovopyranoside isolated from *Solanum surattense* (Lu et al., 2011). The signals at δ_C 70.9 (C-3) and 79.7 (C-6) corresponding the substituent 3*β*-OH and carbinolic carbon, respectively.

The attachments of sugar chain were deduced from the ROESY-1D and *g*HMBC experiments (Figure 2). The long range correlations between H-1' [δ_H 4.76 (1H, d, *J* = 8.0

197 Hz)] of the quinovopyranosyl' unit with C-6 (δ_C 79.7) of the aglycone, H-1'' [δ_H 6.28 (1H, br
198 d, J = 1.5 Hz)] of rhamnopyranosyl with C-3' (δ_C 83.6) of the quinovopyranosyl' unit, and H-
199 1'''' [δ_H 5.25 (1H, d, J = 7.5 Hz, H-1'''')]of the xylopyronosyl with C-3''' (δ_C 87.8) of the
200 quinovopyranosyl'' unit were observed (see Figure 2). Those results indicated that the
201 quinovopyranosyl' unit was linked to C-6 of the aglycone, the rhamnopyranosyl unit being
202 linked to C-3' of the quinovopyranosyl' moiety, and the xylopyranosyl unit was linked to C-
203 3''' of the quinovopyranosyl'' moiety. The linkage of quinovopyranosyl'' unit at C-2 of the
204 quinovopyranosyl' was supported by the ROESY-1D correlations between δ_H 4.76 (Qui' H-
205 1') and δ_H 4.07 (Qui'' H-4''), and δ_H 4.83 (Qui'' H-1'') and δ_H 6.28 (Rha H-1'') (Figure 2).
206 A β-anomeric configurations of the D-quinovopyranosyl', D-quinovopyranosyl'' and
207 D-xylopyranosyl moieties were indicated by large $^3J_{H\text{-}1,H\text{-}2}$ values of the coupling constants of
208 8.0, 7.5 and 7.5 Hz, respectively and ^{13}C-NMR spectroscopic data (Yahara et al., 1996). The
209 α-anomeric configuration of the L-rhamnopyranosyl unit was deduced from their C-3'' (δ_C
210 71.2) and C-5'' (δ_C 70.2) resonance (Lu et al., 2011; Yahara et al., 1996).
211 On the basis of this evidence, this compound was identified as (22R, 23S, 25R)-3β,
212 6α, 23-trihydroxy-5α-spirostane 6-O-β-D-xylopyranosyl-(1''''$\rightarrow$3''')-O-[β-D-
213 quinovopyranosyl(1'''$\rightarrow$2')]-O-[α-L-rhamnopyranosyl(1''$\rightarrow$3')]-O-β-D-quinovopyranoside, a
214 new steroidal saponin.
215 Compound **2**, obtained as an yellow amorphous solid, showed a major ion peaks at *m/z*
216 739 $[M + H]^+$, 761 $[M + Na]^+$ and 777 $[M+K]^+$ (calcd for $C_{39}H_{62}O_{13}$) in the positive ESIMS
217 mass spectrum. The mass spectra present other important peak at *m/z* 577 $[M+H\text{-}Glc]^+$. The
218 MS/MS of *m/z* 762 $[M+Na+H]^+$ result in *m/z* 599 $[M+Na+H\text{-}163]^+$, corresponding to the loss
219 of a glucose unit.

The ^{1}H NMR spectrum of **2** showed signals for four methyl groups at δ_H 0.82 (3H, br s, H-18), 0.95 (3H, s, H-19), 1.13 (3H, d, J = 7.0 Hz, H-21) and 1.08 (3H, d, J = 7.0 Hz, H-27) and two anomeric protons at δ_H 4.83 (1H, br d, J = 8.0 Hz, H-1') and 5.18 (1H, d, J = 8.0 Hz, H-1''). The ^{13}C, DEPT 90° and 135° NMR data (in pyridine-d5) showed the presence of 39 carbons divided into four quaternary, 22 methine, eight methylene, and four methyl carbons. The characteristics quaternary C atoms resonance at δ_C 109.7 (C-22) and 140.0 (C-5), indicate the presence of a steroidal skeleton. The 27-methyl group in its axial position (*S*-configuration at C-25) was deduced by the resonances of protons and carbons at C-25 (δ_H 1.60/δ_C 27.5), C-26 (δ_H 3.36, 4.06/δ_C 65.1) and C-27 (δ_H 1.08, d, J = 7.0 Hz/δ_C 16.3), in comparison with literature data (Agrawal et al., 1995; Han et al., 1999). In the *g*HMBC experiment, the methyl proton at δ_H 0.95 (Me-19) showed long range correlations with the carbon at δ_C 140.0 (C-5), and the olefinic proton at δ_H 5.41 (H-6) with the carbon at δ_C 37.4 (C-10). This evidence indicated the presence of a double bond ($\Delta^{5,6}$) in the B ring. From these results and by comparison with spectral data reported in the literature (Agrawal et al., 1995; Han et al., 1999), the aglycone of **2** was identified as (25*S*)-spirost-5-ene (diosgenin).

Additionally, the ^{1}H NMR spectrum of **2** displayed two signals for protons attached to the anomeric carbons at δ_H 4.83 (1H, br d, J = 8.0 Hz, H-1', Glc') and 5.18 (1H, d, J = 8.0 Hz, H-1'', Glc'') which gave correlations in the *g*HSQC spectrum with signals at δ_C 102.9, and 105.5, respectively, indicating the presence of two sugar units. The evaluation of chemical shifts and spin-spin couplings obtained from the 2D NMR spectra allowed the identification of two β-glucopyranosyl units. The relatively large ^{3}J value (8.0 Hz) of the anomeric proton signals of Glc units indicated a β-anomeric orientation. The saccharidic chain linked at the C-3 position of the aglycone was deduced by *g*HMBC correlations between δ_H 4.83 (Glc' H-1') and δ_C 73.9 (C-3), and between δ_H 5.18 (Glc'' H-1'') and δ_C 70.1 (Glc' C-6') (see Figure 3).

Thus, the structure of **2** was elucidated as diosgenin 3-O-β-D-glucopyranosyl(1''→6')-O-β-D-glucopyranoside.

The 70% ethanolic extract from the leaves of *S. paniculatum* showed significant gastroprotective effect at all doses tested, except the lower doses of 31.25 mg/Kg. It was observed that the effect was dose-dependent, with the effective dose (62.5 mg/Kg) had significantly gastroprotection 38.6% compared to saline (p<0.01), mean area injury of 142.6 mm², while the vehicle had an average of 232.4 mm² (Figure 4a). Doses of 125, 250 and 500 mg/Kg inhibited ulcer formation by 43.4, 67.6 and 71.8%, respectively. There was no statistically significant difference between the positive control (carbenoxolone 100 mg/Kg) and the doses of 250 and 500 mg/Kg from this ethanolic extract.

It was observed depletion in the levels of total glutathione present in samples of gastric tissue from animals treated with ethanolic extract (70%) from the leaves of *S. paniculatum* at different doses tested and no significant difference was observed in relation to vehicle (p<0.05). All test groups presents statistically differences from group treated with carbenoxolone (p <0.001) (Figure 4b). This result indicates that leaf extract of this species not exert gastroprotection with participation of the glutathione pathway.

However the ethanolic extract (70%) from the leaves of *S. paniculatum* was able to reduces significantly levels of myeloperoxidase from gastric tissue at doses of 62,5 until 250 mg/kg (p<0.05) (Figure 4a). Doses of 62.5, 125, 250 and 500 mg/Kg decreased the MPO levels compared to vehicle (p<0.001) 50.3, 78.1, 91.3 and 91.4% respectively, and the doses of 250 and 500 mg/Kg were more effective in reducing the group carbenoxolone (p<0.01), which decreased by 71.0% compared to saline group.

The results show that the ethanolic extract (70%) from the leaves of *S. paniculatum* (EESPL) is able to protect the gastric mucosa to damage caused by absolute alcohol. The model of gastric ulcers induced by ethanol is classical to evaluate antiulcer activity of a drug

to be tested. These agent lesive influences in several protective and lesive factors of mucosa, reducing mucus barrier and the local blood flow, and generate ROS and increase levels of MPO (Lopez et al., 1996).[27] Therefore, the gastroprotection effected by ethanolic extract of *S. paniculatum* may be due to the reduction of lesive factors and/or increased gastroprotective factors, such as mucous, bicarbonate, prostaglandins, NO and glutathione.

Among the factors gastroprotective, the ethanolic extract of leaves from *S. paniculatum* may act by increasing the levels of antioxidants cytoprotective agents which are already present in the mucosa, but in small quantities, such as glutathione (Thomas, 2000; Pavlick et al., 2002). However, the extract showed no activity in this way, which does not discard the possibility that this species has antioxidant effect by other mechanisms, which will be investigated in the future. However, when it comes to mitigating the damaging factors, extract significantly reduced levels of MPO, an enzyme pro-oxidant indicator lesion, which is released by neutrophils/aggregates mucosal lesions, showing that this species acts battling ROS front of an inflammatory response generated by a lesive agent (Guha et al., 2009). The results present in this study could be add better productive value to this species. Previus study realized from shown that this species only characterized the antiulcerogenic effects from the roots and fruit at highest doses (1000 mg/kg) and this study characterized the antiulcer effect from leaves at lower doses (from the dose of 250 mg/kg, by oral route) (Mesia-Vela et al., 2002).

4. Conclusion

Six compounds were identified in the aerial parts (leaves and twigs) of *S. paniculatum*, including two new steroidal saponins. This study showed that aerial parts of *S. paniculatum* are rich source in steroidal saponins and flavonoids that are certainly responsible for the biological properties of this plant. The results obtained here complements the current

294 knowledge about *S. paniculatum* composition and confirm that this byproduct contains
295 diverse phytochemicals, among which saponins and flavonoids predominate, which in turn
296 could offer interesting potential applications in the food and pharmaceutical industries.
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Figure Captions

Figure 1. Chemical constituents isolated from *Solanum paniculatum*.

Figure 2. Key ROESY-1D and *g*HMBC correlations of compound **1**.

Figure 3. Important *g*HMBC correlations observed for **2**.

Figure 4. Gastroprotective effect of ethanolic extratct (70%) of leaves from *S. paniculatum* administered orally against the ethanol-induced ulcers (a) overall glutathione levels (b) and myeloperoxidase (c) present in the sample of gastric tissue. Results were expressed as mean ± SEM, with subsequent Dunnet test (comparison with the negative control, saline) and Tukey test (comparison between the groups), with *$p<0.05$; ** $p<0.01$; *** $p<0.001$.