**ABSTRACT:** *Pyrostegia venusta* is usually found in the secondary growth of the Atlantic forests, and in the Brazilian Savanna. Flowers and leaves of this plant are used in folk remedies for treating a wide variety of healthy conditions, this way is important evaluate its safety and antioxidant potential for this applications. For this, was made a ethanolic extract from its flowers and analyzed with toxicological, genotoxicity and antioxidant tests, the toxicological analysis was made by reproductive toxicity in rats and clatogenicity/aneugenicity in human lymphocytes. The genotoxicity was studied by micronucleus test mice bone marrow. The antimutagenic test in root cells of *Allium cepa*, the antioxidant assays used was DPPH, FRAP, Lipid Peroxidation and REM, beyond of that the extract was analyzed in HPLC showing the profile of its compounds. The toxicological analysis showed that *P. venusta* has no negative significant effect on reproductive and cellular level. The micronucleus test in mouse bone marrow, the extract protected cells from cyclophosphamide, mutagenic compound, in a similar way. The *A. cepa* test showed that the extract reduced chromosomal disorders formations. The antioxidant activity of extract was significant, except in REM test. The phytochemical analysis showed the presence of flavonoids compounds. *P. venusta* extract does not present reproductive toxicity and genotoxic effects. However, the extract of this species showed antigenotoxic and antioxidant potential, possibly due to the different flavonoid compounds present in its extract.

**KEYWORDS:** Reproductive toxicology. Cytotoxicity. Oxidation. Mutagenesis. Flavonoids. Phenol compounds.
The flowers of P. venusta were collected from specimen of remaining Savanna vegetation near the campus of UNESP/Assis, SP, Brazil (22°32'26"S and 50°22'31"N and 22°32'18"S; 50°22'47"N) and a copy was taxonomically identified at the Herbarium of the Forestry Institute of Assis, São Paulo, Brazil (voucher specimen: SPSF-40207). The collection was made in a sustainable manner and without causing environmental impact to the specie collected.

Preparation of plant material in the laboratory, production and storage of ethanolic extract

Flowers of P. venusta were selected and dried with airflow at a temperature of 40 °C, and then crushed and pulverized to prepare the ethanolic crude extract. The mechanical extraction of the powdered plant under stirring with ethanol PA at 1:10 (w:v) was carried out over a 24 h period and repeated twice. After obtaining the extract, the sample was taken to a rotary evaporator to remove all of the alcohol, and the resulting concentrated extract was taken to the drying chamber to obtain a dry extract.

Evaluation of reproductive toxicity in rats

Wistar adult female rats that were 12 weeks of age and weighed 250 g were kept at the Faculty of Sciences and Letters (UNESP- Assis, SP, Brazil), and maintained under controlled conditions of temperature (23 ± 1 °C) and lighting. (12L, 12D photoperiod, lights switched on at 7 a.m.). Tap water and commercial chow were supplied ad libitum. The experimental protocol followed the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and was approved by the Ethical Committee for Animals Use (Permit number: 002/2010).

Females were weighed and randomly distributed in two groups (n = 14/group), either a control group (given 0.5 mL distilled water) or the experimental group (given P. venusta, 100 mg/Kg body weight (bw) of ethanolic extract that was diluted in 0.5 mL distilled water). The rats of each group received the treatment by via an oral (gavage), single dose daily, during 20 consecutive days.

During the experiment, animals were given a cytological examination (vaginal swabs), according (MARCONDES et al., 2002). The time collection was fixed at 7 a.m. Each slide was analyzed under a light microscope (Olympus CX31 RBSFA, Japan).

The consumption of water and food were registered daily. After the treatment period, the females in estrus phase of the estrous cycle...
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At the end of the treatment period, other 7 females of each group were mated with untreated males. After mating, the presence of sperm in the morning vaginal smear was indicative of fertilization and was considered as day one of gestation (GD1). Pregnant females were kept in individual cages. On the 19th gestational day (GD19) a laparotomy was performed after an intramuscular administration of ketamine (40 mg/kg bw) and xylazine (20 mg/kg bw), and records were obtained for the number of implantations, the number of corpora lutea, the litter size, the litter weight and the number of resorptions. Copulation rate (number of females with sperm in the smear/number of mated females in the group) x 100). Fertility rate (number of pregnant females/number of copulated females) x 100). Pre-implantation loss rate (number of corpora lutea - number of implants/number of corpora lutea) x 100) and post-implantation loss rate (number of implants – number of fetuses/number of implants) x 100) were calculated as described (DAMASCENO, 2008; VIEL et al., 2017). The evaluation/cell count of MN (micronucleus) was recorded for 1,000 bi-nucleated cells (500 per replicate/blade). The slides were examined in a blind condition. For each individual in the sample two blades were selected and MN (micronucleus) was recorded for 1,000 bi-nucleated cells (500 per replicate/blade). The evaluation/cell count of MN

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alcohol. Blood was collected from the antecubital vein between 7:00 and 10:00 am in heparinized tubes and under sterile conditions. The material was processed within 4 h of collection.

Lymphocyte isolation of whole blood

Whole blood collected was homogenized by inversion and diluted with saline RPMI with unsupplemented culture for experiments performed in cell culture in the proportion of 1:1 (v/v). The diluted blood (7 mL) was added slowly into a centrifuge tube containing 3 mL of Ficoll-Paque TM Plus and centrifuged for 30 min at 1,000 rpm and refrigerated at 4 °C. Then, the lymphocyte cloud was removed with a Pasteur pipette and washed 2 times with RPMI 1640 medium.

Culture of human lymphocytes

The culture was performed according (TITENKO-HOLLAND et al., 1997; FAREED; AFZAL; SIDDIQUE, 2011) with an initial density of 10⁶ cells in 2 mL of culture medium. Then, the lymphocytes were cultured. Culture medium consisted of RPMI 1640 supplemented with 15% fetal bovine serum, 2 mM of L-glutamine, 100 units mL⁻¹ of penicillin, 100-µg/mL of streptomycin and 1.5% phytohemagglutinin.

Treatment conditions

For each individual 5 culture vials were mounted followed by 8 h of incubation at 37 °C with the addition ethanolic extract of P. venusta at a concentration of 50, 100 or 200 mgL⁻¹. Two culture flasks were reserved for the positive control (50 mL⁻¹ of cyclophosphamide) and the negative control (without a test substance).

Micronucleus assay

After 48 h of incubation, 0.2 mL of cytochalasin B was added to 5mL of medium to prevent cytokinesis of the dividing cells. After 72 h the culture was stopped with 0.5 mL of methanol-acetic acid fixative (3:1) for 5 min at room temperature. The material was centrifuged at 800 rpm, discarding the supernatant. Next, 5 mL of fixative was added with stirring, and then the material was centrifuged. This procedure was repeated 3 to 4 times until the precipitate remained clean. The material was dripped onto slides, allowed to dry at room temperature, and then stained with Giemsa at a ratio of 1:3 in phosphate buffer (pH = 6.8) for 5 to 8 min.

The slides were examined in a blind condition. For each individual in the sample two blades were selected and MN (micronucleus) was recorded for 1,000 bi-nucleated cells (500 per replicate/blade). The evaluation/cell count of MN
Toxicological, genotoxic… followed the criteria according (FENECH et al., 2003), such that the chromatin structure and similar refractions between nucleus were evaluated, the cytoplasm was well preserved, the staining intensity was similar between nucleus, the definition of the nuclear contours were oval or round in shape, there were no links to the main nucleus, and the diameter was between 1/3 and 1/16 of the average diameter of the main core corresponding to 1/256 and 1/9 of an area of a nucleus of a main bi-nuclear cell.

Significant differences between the controls and treated samples were determined with the Fisher exact test for MN frequencies and with the Chi-square test.

**Antimutagenic assay**

**Micronucleus test in mice bone marrow**

Antigenotoxic effects of the extract of *P. venusta* on cyclophosphamide-induced micronucleus in mice was tested using 7-week to 12-week old male Swiss albino mice (Mus musculus Rodentia, Muridae) weighing 25-35 g. The mice were provided by the central bioterium (Instituto de Biociências/UNESP/Botucatu) and acclimatized in cages at 24 ± 1 °C under a 12 h light period for one week. During acclimatization and throughout the experiments the mice had free access to standard granulated chow and drinking water. Each cage contained 5 mice that were randomly assigned to 1 of the 4 following groups: 1) a negative control group that was given distilled water by oral gavage; 2) a positive control group that was given a single intraperitoneal injection of the equivalent of 0.2 mg per 100 g of body weight (bw) of cyclophosphamide (CAS n.50-18-0; Endoxan, Baxter Oncology Gmb, Germany) dissolved in distilled water; a extract group that was given the equivalent of 50 mg, 100 mg or 200 mg per kg bw during 7 days by oral gavage. However, on the seventh day, the mice also received the same treatment as the positive control group.

All mice were killed by cervical dislocation on day 8. This study conforms to the relevant Brazilian guidelines regarding the ethical use of live animals. Genotoxic effects were evaluated in the mouse bone marrow by the micronucleus test (BAESSE et al., 2015). Immediately after sacrifice the mice both femurs were removed from each mouse and the bone marrow flushed out into centrifuge tubes containing 2 mL of fetal calf serum at 1,000 revolutions per min for 10 min, after which the supernatant was discarded and the pellet resuspended in a drop of serum, and a smear was made on a clean slide. The smear was air-dried, fixed with absolute methanol for 5 min, then air-dried and either stored at room temperature or directly stained for 5 min with a freshly prepared working solution of Giemsa stain diluted 1:1 v/v in 0.06 M of sodium phosphate buffer and 0.06 M of potassium phosphate buffer (both at pH 6.8). After staining, the slides were rinsed in distilled water, dried at room temperature and scored for micronucleus according to the criteria of Krishna and Hayashi (2000) using 100X magnification and a Carl Zeiss optical microscope. We scanned 2000 polychromatic erythrocytes (PCE) per mouse and recorded the number of micronucleated PCE (MNPCE).

To compare the frequencies of MNPCE and normal PCE between treated and control groups the results were expressed as the mean ± standard deviation and analyzed statistically using the nonparametric Mann-Whitney U-test with the significance level set at α = 0.05. The statistical analyses were carried out using the SPSS 12.0 statistical package for PCs (SPSS, Chicago, IL).

**Antimutagenic test in root cells of *Allium cepa***

Onion bulbs (*A. cepa* L., 2n = 16) were obtained commercially in Assis, São Paulo, Brazil. They were cleaned and the outer scales were removed, leaving the ring intact with primordial roots. The bulbs were used for the bioassay according to standard procedures (BABATUNDE; BAKARE, 2006). The growth of the roots was used a culture solution (Hoagland's solution). The bulbs were kept suspended in a 100-mL beaker leaving the ring of roots in contact with the solution, changed every 24 h for a period of 72 h, and maintained at a photoperiod (18 h/6h light/dark) and temperature (22 ± 2 °C) in controlled chamber B.O.D. Bulbs with roots approximately 2 cm were used in the experiment.

To evaluate the mitotic index-MI, micronucleus-MN and induction of chromosomal aberrations-CA (c-metaphase; chromosome delay; chromatoidal bridge; aberrant telophase; nucleolus outside the nucleus; amorphous nucleus), 6 onion bulbs were exposed to each concentration of ethanolic extract (0.1, 5 and 10 mg/mL) of *P. venusta*. Mineral water and positive control solution of MMS (methyl methanesulfonate) at 10 mg/L was used for the negative control, as described (CARITÁ; MARIN-MORALES, 2008). At the end of 48 h of exposure and 24 h recovery in culture solution, the roots of treated and control bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v). These were hydrolyzed in 1 N of HCl at 60 °C for 8 min, after which they were rinsed in distilled water. The roots were stained with acetic carmine for 10 min, the tips were...
Toxicological, genotoxic… removed and the roots carefully crushed between slide and coverslip were sealed, as suggested (AKINBORO; BAKARE, 2007). Five slides were prepared for each treatment and controls were analyzed at 1000x magnification. The mitotic index was calculated on the number of dividing cells per 1000 cells observed (FISKESJÖ, 1985; DE CAMPOS VENTURA-CAMARGO; MARIN-MORALES, 2016). The frequency was calculated based on the number of aberrant cells and micronucleus per total cells analyzed for each treatment and controls (BAKARE et al., 2000). The MI, CA e MN obtained were compared with the controls and statistically analyzed using the Kruskal-Wallis test (p <0.05), as described (BAKARE et al., 2000).

**Antioxidant potential**

**DPPH radical scavenging activity**

The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA) radical scavenging activity was determined according to the methodology proposed (HELM et al., 2008). The dry ethanolic extract of each sample was dissolved in ethanol (75%) at different concentrations (25, 50, 75, 100, 250, 500 and 1,000 µg/mL) and then mixed with 5 mL of DPPH solution (1.5 x 10-4 M). The extract reacted with the DPPH radical for a period of 30 min in a low luminosity, and then they were submitted to the UV-Vis spectrophotometer (model: SP220, BIOSPECTRO, Brazil) at 517 nm wavelength. The calculation of the antioxidant activity was performed according to the following formula:

\[
\text{Scavenging effect (I%) = } \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control, and \( A_{\text{sample}} \) is the absorbance of sample, with extract. Triplicates were made to the analyses

**Ferric reducing antioxidant power (FRAP test)**

The FRAP test was performed as previously described (MANIAN et al., 2008) with some modifications. Up to 2.7 mL of FRAP reagent, freshly prepared was mixed with 270 µl of distilled water and 90 µl of each sample. Then, this mixture was maintained in water bath at 37 °C for 30 min. The FRAP reagent contained 2.5 mL of 10 mM of TPZ solution in 40 mM of HCl, plus 2.5 of 20 mM of FeCl\(_2\)-6H\(_2\)O, plus 25 mL of 0.3-M acate buffer (pH 3.6). Readings was performed at the absorption maximum (595 nm). Solutions of known Trolox concentration were used for calibration. The final results were expressed as micromole Trolox equivalents (TE) per grams of extract.

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**Lipid peroxidation test**

For the lipid peroxidation test a source of homogenized egg yolk lipid was used according to the method proposed (PULIDO et al., 2000). In 5 mL of DPPH solution (1.5 x 10-4 M) in buffer TBA (Thioarbituric acid) was added to the samples. After this period, 300 µL was removed from each tube and transferred to Eppendorf tubes. Then, 600 µL of TCA (trichloroacetic acid) at 15% was added to the Eppendorf tubes, and the tubes were centrifuged at 10,000 g at 4 °C. Then, 500 µL of supernatant from each reaction was transferred to Eppendorf tubes followed by the addition of 500 µL of 0.67% TBA in Milli-Q water. The final mixture was heated in a water bath for 30 min. Afterwards the absorbance was measured at 532 nm.

The antioxidant activity was calculated as the percentage of inhibition of lipid peroxidation, based on the following equation: Inhibition of lipid peroxidation (%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100

Where \( A_{\text{control}} \) is the absorbance of the control, and \( A_{\text{sample}} \) is the absorbance of sample. The concentration of the extract (mg.mL\(^{-1}\)) required to inhibit 50% of the lipid peroxidation (IC50) was calculated. The TMP standard curve was prepared from an aqueous solution at a concentration of 2.0 nmol.mL\(^{-1}\). Aliquots of 30, 60, 120 and 180 µL of TMP solution are transferred to glass tubes and the volume was adjusted to 500 µL with distilled water. The solutions were prepared in triplicate.

**Relative electrophoresis mobility (REM) test**

REM was adapted (HSIEH et al., 2005), bovine serum albumin (BSA, 2 mg/ml) was diluted in PBS (10 mM, pH 7.4) and incubated with Cu\(^{2+}\) (2 mM) at 37 °C for 24 h in the presence or absence of the herbal ethanolic extract (1000 µg/mL). \( H_2O_2 \) (0.25 mM) was used as a positive control for oxidation. Electrophoresis of BSA was performed using polyacrylamide gels (SDS-PAGE). Running gel solution was comprised of 12% acrylamide, and the stacking gel was 5% acrylamide. Proteins were stained with 0.25% Coomassie Blue R-250. The results were
Toxicological, genotoxic… expressed in the REM in mm using that of native BSA as the base.

**Phytochemical compounds**

**Total phenols and flavonoids quantification**

The quantification of total phenols and flavonoids was performed for extract diluted in ethanol concentrations of 25, 50, 75, 100, 250, 500, 1,000, 3,000, 5,000 and 10,000 µg/mL-1. For the determination of total phenols, the Folin-Ciocalteu method was performed. For each 0.5 mL of extract at the different concentrations 5 mL of distilled water was added in addition to 0.25 mL of Folin-Ciocalteu Reagent. After 3 min, 1 mL of saturated Na2CO3 solution was added at 10% and the mixture was stored for 1 h. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer (model: SP220, BIOSPECTRO, Brazil). All tests were performed in triplicate and the results were expressed in mg of gallic acid equivalent (AGE) per gram of extract.

Total flavonoid quantification of the extract was determined based on the UV-Vis spectrophotometer and the samples were prepared as described (TODA, 2005), based on the flavonoids complexation with AlCl3. An aliquot of 250 µL of extract at the different concentrations was mixed with 1.25 mL distilled water and 75 µL NaNO2 solution at 5%. After 6 min, a 150 µL AlCl3/H2O solution at 10% was added. After 5 min, 0.5 mL of a 1 M NaOH solution was added and then the total volume was adjusted by adding 2.5 mL of distilled water. The samples were shaken in a vortex mixer and the absorbance was measured at 510 nm. All tests were performed in triplicate and the results were expressed in mg of rutin equivalent (RE) per gram of extract.

**Analysis in HPLC**

The fingerprint of the crude ethanolic extract of the flowers of *P. venusta* was analyzed using HPLC-ESI-IT-MS (Accela High Speed LC from Thermo Scientific®, coupled to an Accela Thermo Scientific® LCQ Fleet with Ion Trap 3D and ionization by electrospray (Column Phenomenex® Luna C18 (2)). The conditions for HPLC were a 250 mm x 4.6 mm x 5 µm column; a mobile phase with water ultra-pure + formic acid 0.1% (A) and methanol + formic acid 0.1% (B); a gradient of 25% of A to 100% of B in 80 min; an injection volume of 20.0 µL; a column temperature of 25 °C; a flow ratio of 0.8 mL.min⁻¹, λ= 254 nm. FIA-ESI-IT-MSn was in the negative mode.

**RESULTS**

**Evaluation of reproductive toxicity**

There was no significant difference (p>0.05) in the water and food consumption and ovarian weight between the two groups (Table 1). The females treated with *P. venusta* had a significant increase (p<0.05) in uterine weight compared to the control females. The estrous cycle was regular during the treatment period, being that the number of estrus phase was similar (p>0.05) in the control and experimental groups (5.0 ± 0.0 and 5.0 ± 0.50, respectively).

| Parameters                          | Control group (n=7) | Experimental group (n=7) |
|-------------------------------------|---------------------|--------------------------|
| Water consumption¹ (mL)             | 241.05 ± 30.94      | 244.21 ± 25.18           |
| Food consumption² (g)               | 128.00 ± 29.50      | 125.00 ± 29.50           |
| Ovaries weight¹ (g%)                | 0.017 ± 0.007       | 0.021 ± 0.004            |
| Uterus weight¹ (g%)                 | 0.193 ± 0.048       | 0.245 ± 0.037*           |

¹Values express as the mean ± standard deviation. t-Student test. ²Values express as the median ± interquartile deviation. Mann-Whitney test. *p<0.05 in comparison between the groups.

The ovarian histological structure showed that in the control (Figure 1A) and experimental (Figure 1B) groups, there were several corpora lutea and health follicles inserted into a fibrocellular stroma. Comparing the groups, there was no significant difference (p>0.05) in the number of corpora lutea (control, 3.0 ± 3.0 and experimental, 2.5 ± 4.0), growth follicles (control, 2.0 ± 2.0 and experimental, 2.0 ± 1.2), antral follicles (control: 2.0 ± 2.2 and experimental: 2.0 ± 2.0) and atretic follicles (control: 5.0 ± 4.0 and experimental, 5.0 ± 3.0). Data are expressed as the median and interquartile deviation. The uterus of untreated females (Figure 1C) presented with endometrium composed of loose connective tissue, with many leukocytes scattered throughout the interstitium. Nevertheless, in the females treated with *P. venusta* (Figure 1D), the endometrial stroma exhibited a predominantly fibrous appearance, with leukocytes located mainly in the subepithelial region. In this group, there was an increase in the tissular vascularization and...
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Moreover, a significant decrease (p<0.05) in luminal epithelium height and thickness of the endometrial stroma and perimetrium was observed in the group that received the extract (experimental vs. control: 29.3 ± 8.7 vs. 32.3 ± 9.0 µm; 256.2 ± 269.0 vs. 424.6 ±

Figure 1. Photomicrographs of the ovarian tissue (A and B) and uterine tissue (C and D). In A and B, observe the similarity of ovaries in the two groups, with corpora lutea (CL) and health follicles (F) in stroma. In C, observe the glands (g) in the endometrium (E). In D, are shows glands (g) and blood vessels (v) inserted in endometrium (E) of fibrous aspect and leukocytes (L) in subepithelial region. Hematoxylin-eosin. Bars = 100 µm.

Table 2 shows that there was no effect of treatment with *P. venusta* extract in maternal and fetal parameters. No external morphologic anomaly was observed in the fetuses of the experimental group.

Table 2. Evaluation of maternal and fetal parameters.

| Parameters                        | Control group (n=7) | Experimental group (n=7) |
|-----------------------------------|--------------------|-------------------------|
| Copulation rate (%)               | 100                | 100                     |
| Fertility rate (%)                | 71.4               | 71.4                    |
| Body weight of dams (g)           | 344.0 ± 36.0       | 350.0 ± 15.0            |
| Gravid uterus weight (g)          | 44.9 ± 10.0        | 43.0 ± 7.4              |
| Placentas weight (g)              | 5.7 ± 0.9          | 5.9 ± 0.8               |
| Litter weight (g)                 | 21.9 ± 5.4         | 22.2 ± 3.6              |
| Size litter                       | 13 ± 4             | 12 ± 2                  |
| Pre-implantation loss (%)         | 0                  | 0                       |
| Post-implantation loss (%)        | 0                  | 0                       |

Values are expressed as median ± interquartile deviation. Mann-Whitney test. None statistical difference between the groups (p>0.05).
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Clatogenicity/aneugenicity in human lymphocytes

Table 3 shows the micronucleus frequency in bi-nucleated lymphocytes. There were no significant differences between extract treatments, the different concentrations, and the negative control group. Compared to the positive control, the extract-treated groups showed a significant difference; the PC presented the highest frequency of micronucleated bi-nucleated lymphocytes (26.17 ± 2.14), as the group treated with 200 mg.Kg\(^{-1}\) bw (highest concentration) showed a frequency of 7.83 ± 2.64.

Table 3. Micronucleus frequency in peripheral blood lymphocytes treated with different concentrations of \(P.\) _venusta\) (50mg/Kg - E50, 100mg/Kg - E100, 200mg/Kg - E200).

| Treatments | Number of individuals | MN per 1000 binucleated lymphocytes |
|------------|-----------------------|-------------------------------------|
| NC         | 6                     | 5.83±2.40a                          |
| E50        | 6                     | 9.33±1.75a                          |
| E100       | 6                     | 8.33±2.06a                          |
| E200       | 6                     | 7.83±2.64a                          |
| PC         | 6                     | 26.17±2.14b                         |

Means followed by same letter in a column not differ significantly by Mann-Whitney and U-test (\(\alpha=0.05\)). NC= negative control; PC= positive control (cyclophosphamide).

Antimutagenic assay

Micronucleus test in mice bone marrow

Table 4 shows the results of a micronucleus test in mice bone marrow. The groups treated with different concentrations of extract and cyclophosphamide presented a superior rate of MNPCEs in relation to negative control (NC = 50.0); however compared to positive control group (PC) treated with only with cyclophosphamide, the extract-treated groups showed a significant decrease in the frequency of MNPCEs. The different treatments with the extracts showed no significant difference between them.

Table 4. Frequency of erythrocytes polychromatics micronuclei (MNPCEs) of bone marrow cells of Swiss mice in experimental groups treated with hydroethanolic extract of \(P.\) _venusta\) (NC=negative control was distilled water; Animals were treated with the following doses of extract: 50mg/Kg bw (E50), 100mg/Kg bw (E100) and 200mg/Kg bw (E200) or 2mg/Kg bw of cyclophosphamide (PC)).

| Treatments   | Number of animals | MNPCE per 2000 PCE |
|--------------|-------------------|--------------------|
| NC           | 5                 | 50,0               |
| E50 + PC     | 5                 | 89,0               |
| E100 + PC    | 5                 | 76,0               |
| E200 + PC    | 5                 | 84,0               |
| PC           | 5                 | 286                |

Means followed by same letter in a column not differ significantly by Mann-Whitney and U-test (\(\alpha=0.05\)).

Antimutagenic test in root cells of \(Allium\) _cepa_

The \(Allium\) _cepa_ test results are shown in Table 5. There was a significant decrease in the micronucleus frequency for roots treated with 5.0 mg.mL\(^{-1}\) + MMS (0.44 ± 2.41) compared to other treatments (0.5 mg.mL\(^{-1}\) + MMS = 13.8 ± 5.89 and 1.0 mg.mL\(^{-1}\) + MMS = 10.2 ± 3.63) and controls (0.10 ± 1.22 = NC and PC = 16.4 ± 2.30), revealing a dose-dependent profile. Increasing extract concentrations resulted in a significant decrease in total chromosomal disorders between the two higher concentrations (0.5 mg.mL\(^{-1}\) MMS + = 2.92 ± 0.90, 1 mg.mL\(^{-1}\) MMS + = 3.52 ± 0.66 and 5 mg.mL\(^{-1}\) MMS + = 2.60 ± 0.51). Compared to controls sample the results were higher than the NC (1.72 ± 0.59) and lower than the PC (5.10 ± 1.21).
### Table 5

Total cells with micronuclei and chromosomal disorders in meristem tissue of *Allium cepa* root under different *P. venusta* extract concentrations (Pv) with Metilmetanosulfonato (MMS), negative control (NC) and positive control (PC).

| Treatment (mg/mL) | Micronuclei  | c-Metaphase | Chromossomic delay | Cromossomic bridge | Telophaso Aberrant | Nucleoli out the Nucleus | Amorphous nucleus | Total chromosomal aberrations |
|-------------------|--------------|-------------|--------------------|--------------------|-------------------|--------------------------|------------------|-----------------------------|
| NC                | 01.0 ± 1.22a | 5.0 ± 3.16  | 3.8 ± 0.45         | 1.8 ± 0.45         | 0.8 ± 0.8         | 03.8 ± 3.27              | 02.0 ± 2.55      | 1.72 ± 0.59a                |
| Pv(0.5)+MMS(10)   | 13.8 ± 5.89b | 3.6 ± 2.97  | 0.8 ± 0.84         | 5.2 ± 0.45         | 2.0 ± 1.41        | 12.2 ± 6.87              | 05.4 ± 1.82      | 2.92 ± 0.90b                |
| Pv(1.0)+MMS(10)   | 10.2 ± 3.63b | 3.6 ± 1.52  | 3.0 ± 1.87         | 3.4 ± 3.00         | 4.0 ± 0.70        | 11.6 ± 3.51              | 09.6 ± 4.83      | 3.52 ± 0.66b                |
| Pv(5.0)+MMS(10)   | 04.4 ± 2.41c | 3.0 ± 1.22  | 2.4 ± 1.52         | 3.6 ± 1.67         | 2.4 ± 1.82        | 07.6 ± 1.82              | 07.0 ± 3.54      | 4.60 ± 0.51b                |
| PC                | 16.4 ± 2.30d | 5.6 ± 1.34  | 2.8 ± 1.92         | 3.2 ± 2.68         | 2.6 ± 1.52        | 15.0 ± 3.54              | 21.8 ± 6.38      | 5.10 ± 1.21c                |

* Equal letters to no significant differences (p≥0.05).
Antioxidant potential and total phenols and flavonoids DPPH, FRAP and TBARS, total phenols and flavonoids compounds

Table 6 presents the results of the antioxidant tests (DPPH, FRAP and TBARS) and quantification of total phenols and flavonoids in the \textit{P. venusta} extract. For these tests revealed a dose-dependency for the antioxidant activity and phenolic content and total flavonoid. The EC50 was calculated for the DPPH and TBARS tests, resulting in concentrations of, respectively, 131.63 µg and 1.038 mg. The FRAP test found 154.00 µM of TE per extract gram in the extract concentration of 1000 µg/mL.

Table 6. Antioxidant activity, total phenols and flavonoids of \textit{P. venusta} extract.

| Concentration (µg.mL\(^{-1}\)) | DPPH (%)\(^{a}\) | FRAP\(^{b}\) | TBARS (%)\(^{c}\) | Total Phenols | Total Flavonoids |
|-------------------------------|-----------------|--------------|-----------------|---------------|----------------|
| 100                           | 48.90           | 54.92        | 6.78            | 8.42          | 40.50          |
| 250                           | 61.43           | 98.96        | 14.56           | 21.40         | 65.34          |
| 500                           | 63.23           | 138.00       | 23.90           | 37.64         | 113.25         |
| 1000                          | 65.62           | 154.00       | 38.34           | 51.92         | 190.52         |

\(^{a}\)Average values standard deviation of triplicates for test cleaning of DPPH radical scavenging activity; \(^{b}\)FRAP: ferric reducing power; \(^{c}\)lipoperoxidation inhibition percentage (TBARS (%)).

Relative electrophoresis mobility (REM) test

The electrophoresis gel revealed that there was an extensive BSA protein fragmentation resulting from the combined activity of Cu\(^{2+}\) and the extract (Figure 2, lane 3), and fragmentation was much higher compared to the positive control with only Cu\(^{2+}\) and hydrogen peroxide in the acting protein (Figure 2, lane 2) and with only Cu\(^{2+}\) and BSA (Figure 2, lane 1).

![Figure 2](image)

**Figure 2.** Effect of hydroethanolic extract of \textit{P. venusta} on migration of BSA with PAGE (Incubation period was 24 hours) in oxidative condition. A: Native BSA; 1: BSA with Cu\(^{2+}\); 2: BSA with Cu\(^{2+}\) and H\(_2\)O\(_2\); 3: BSA with Cu\(^{2+}\)/H\(_2\)O\(_2\) and hydroethanolic extract (1000 µg/mL).

Phytochemical analysis using HPL

The analysis of the HPLC-PDA chromatogram of \textit{P. venusta} showed two main peaks at 4.11 min and 31.37 min. Based on the \(m/z\) values, UV spectra and comparison with the literature, we can conclude that peak 2 is quinic acid, and peak 3 is the flavonoid diglycoside. Furthermore, we have propose that peak 1 is a myo-inositol derivative (3).
DISCUSSION

*P. venusta* is widely used in popular culture for treating various diseases (ALTOÉ et al., 2014). There is promising research that aims to treat cancer using *P. venusta* (FIGUEIREDO et al., 2014). Moreover, the flowers and roots extract of this plant contain significant amounts of phytochemicals with antioxidant properties (VELOSO; SILVA, 2010). Thus, the use of this species could possibly expand to include applications in the pharmaceutical and food industry; but for this expansion, and for its current use, it is necessary to establish its safety.

There are several side effects that may be caused by the chemical compounds found in medicinal plants. Among them include changes in water consumption, diet and body weight, and clinical symptoms of piloerection, changes in behavior, tremors, convulsions and even death (FIGUEIREDO et al., 2014). In studies of the antitumor properties associated with phenolic compounds such as flavonoids, (FERNANDES et al., 2010) there was no change in water and food consumption of Wistar rats treated with a phenolic compound concentration of 50 mg.kg$^{-1}$, as in the present study, treatment of Wistar adult female rats with *P. venusta* did not cause any clinical signs of toxicity.

Female rats treated with isoflavones at concentrations of 3.3 mg/100 mL and 100 mg/100 mL showed a relative weight of the ovaries that was similar to the control group. But the relative uterine weight was higher in the group treated with isoflavones at 100 mg/100 mL compared to groups treated with 3.3 mg/100 mL and controls (IKAKI et al., 2008). In addition, rats treated with both flavonoid concentrations showed no changes in the estrous cycle or in the number of estrus phase in relationship to the control group. In the present study, the same result was obtained with *P. venusta*, which has a high content of flavonoids. The increase in uterine weight observed in this study probably occurred due to discrete changes observed in the endometrium. Currently, no other report is found in the literature on the effects of *P. venusta* extract on the weight and structure of these reproductive organs.

The *P. venusta* chemical constituents did not promote ovarian toxicity, as gonadal tissue showed similar characteristics to that observed in the control group. A study performed (ROMERO et al., 2008) showed that rats treated with *Ginkgo biloba* (120 mg.Kg$^{-1}$), which is rich in flavonoids, showed no histopathological change in ovarian tissue. In a experiment with *Tabebuia avellanedae* (ipe-purple), which is in the same Bignoniaceae family as *P. venusta*, (KUNTZE et al., 2012) examined, in rats, the cicatrizant activity of a topical ointment consisting of plant species extracts. Kuntze et al. (2012) highlighted the importance of flavonoids in tissue healing because the phenolic compound promotes neovascularization and
fibroplasia inhibition in inflammatory process, contributing to increased oxygenation and rapid recovery of the injured tissue. This study indicated that the *P. venusta* flavonoids may have contributed to the fibrous endometrial characteristics and the vasodilation observed in the uterus of female rats. These characteristics did not affect endometrial implantation of the egg or embryo development, indicating that the plant does not present toxicity to the embryo or the fetus.

The micronucleus assay with lymphocytes can be efficiently used to measure the cytotoxic and genotoxic effects of different compounds as reactive oxygen species by ionizing radiation (FENECH et al., 2003). Regarding the *P. venusta* clatogenicity/aneugenicity to human lymphocytes, the result was negative, thus it can be considered that the species studied shows low genotoxicity in respect of the formation of the MN of lymphocytes analyzed, which supports the safety of using this species. In cancer patients there is a higher frequency of MN, and there is a correlation between genotoxic agents such as ionizing radiation, cancer on set and MN (GREENROD; FENECH, 2003).

In the micronucleus test in mouse bone marrow, *P. venusta* extract reduced the chances of genetic alterations in the presence of mutagenic compounds. The extract of this species, reduced the formation of micronucleated polychromatic erythrocytes in relation to the controls that showed the mutagen activity of cyclophosphamide. This reduction was similar in all tested concentrations, making this species promising for use in preventing genomic damage because MN in polychromatic erythrocytes (PCE) of mouse bone marrow is a very sensitive measure of the damage caused by chemical mutagens (FENECH, 2002).

Tests of *A. cepa* have a high level of sensitivity in mammals, and thus is suitable to verify chromosome damage and disorders of the mitotic cycle, cytotoxicity, and genotoxicity to a wide variety of compounds, from heavy metals to aromatic compounds (AKINBORO; BAKARE, 2007). In the present study, the results for total chromosomal disorders were similar to those shown for the micronucleus test in mice bone marrow. The number of chromosomal disorders after treatment was lower than that of the positive control. In the same test, regarding the number of micronuclei, unexpectedly, lower concentrations were more effective by inhibiting the formation of micronuclei in the cells analyzed, and the highest contraction (5 mg/mL) had a less significant effect. Nonetheless, these results also confirm the results of the previous experiment on the micronucleus of the mouse bone marrow.

In the DPPH assay, the values achieved with *P. venusta* extract were lower than the values reported previously (ALTOÉ et al., 2014) for *P. venusta* extracts, where the results showed a 94.6% antioxidant activity. The reducing capability of the extract is similar to that described (ROY et al., 2011) in a FRAP test for the extract of *P. venusta* flowers. The results of the FRAP test were lower than that for other species. For example, the value found for the carambola residue was 510.3-µMol/g of dry weight (PEREIRA et al., 2014). In a study conducted by Guo et al. (2003) the reducing power found for the red grape was equivalent to 670.5-µMol.g⁻¹ of dry weight; however the level of the reducing能力 of *P. venusta* is still significant, and it can be correlated with the phenolic compounds dosage ($r^2 = 0.9587$). Thus, the test results of FRAP and DPPH confirm that this species has the potential to protect the body from free radicals.

In the work (SHUI; LEONG, 2006) the results obtained with the cyan-carvone, at a concentration of 7.2-µg/mL caused a 72.1% inhibition of lipid peroxidation. The study, (SHUI; LEONG, 2006) examined different flavonoids at a concentration of 15 µM, and quercetin reached more than 60% inhibition lipid peroxidation and rutin showed 8% inhibition. Because they are single compounds, the results obtained using *P. venusta* extract can be considered high, and it is possible to isolate individual compounds. The value of $r^2$ (0.988) resulting from the correlation between the dosage of compounds and the ability to inhibit lipid peroxidation show a possible dependence.

In the relative mobility test in an electrophoresis gel in the presence of Cu²⁺ and H₂O₂, the extract induced more intense activity that fragmented the BSA protein. Thus, the most prominent action shown in the gel was that the tested extract can present oxidative action under certain conditions. In the work (COSTA et al., 2013), antioxidants tested differently in a similar oxidation system. The antioxidant with the best results was glutathione, which almost completely inhibited oxidation, but only at higher concentrations.

A phytochemical analysis indicated the likely presence of quinic acid, a flavonoid diglycoside and myo-inositol. Similarly, (ROY et al., 2011) was identified these same classes of compounds in extracts from the flowers and roots of *P. venusta*, and also confirmed the antioxidant potential of these compounds.
CONCLUSION

*P. venusta* extract does not present reproductive toxicity and genotoxic effects. However, the extract of this species showed antigenotoxic and antioxidant potential, possibly due to the different flavonoid compounds present in its extract.

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REFERENCES

ALTOÉ, T. D. P.; AMORIM, G. M.; GOMES, J. V. D.; BORGES, A. S.; VALADÃO, I. C.; SILVA, I. V.; RANGEL, L. B. D. A.; VIEIRA, P. C.; JAMAL, C. M.; KITAGAWA, R. R.; BORGES, W. S. *In vitro* antioxidant and cell viability of *Pyrostegia venusta* (Ker Gawl.) Miers). *Orbital: The Electronic Journal of Chemistry*, Mato Grosso do Sul, v. 6, n. 4, p. 215-222, 2014.

AKINBORO, A.; BAKARE, A. A. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *Journal of Ethnopharmacology*, South Africa, v. 112, p. 470-475, 2007. https://doi.org/10.1016/j.jep.2007.04.014

BABATUNDE, B. B.; BAKARE, A. A. Genotoxicity screening of wastewaters from Agbara industrial estate, Nigeria evaluated with the Allium test. *Pollution Research*, Germany, v. 25, p. 227-232, 2006.

BAESSE, C. Q.; TOLENTINO, V. C. M.; SILVA, A. M.; SILVA, A. A.; FERREIRA, G. A.; PANIAGO, L. P. M.; NEPOMUCENO, J. C.; MELO, C. Micronucleus as biomarker of genotoxicity in birds from Brazilian Cerrado. *Ecotoxicology and Environmental Safety*, United States, v. 115, p. 223-228, 2015. https://doi.org/10.1016/j.ecoenv.2015.02.024

BAKARE, A. A.; MOSURO, A. A.; OSIBANJO, O. Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa*(L.). *Journal of Environmental Biology*, India, v. 21, p. 263-271, 2000.

CARITÁ, R.; MARIN-MORALES, M. A. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*, United Kingdom, v. 72, p. 722-725, 2008. https://doi.org/10.1016/j.chemosphere.2008.03.056
COSTA, D. A.; OLIVEIRA, G. A. L. D.; SOUSA, D. P. D.; FREITAS, R. M. Avaliação do potencial antioxidante in vitro do composto ciano-carvona. Revista de Ciências Farmacêuticas Básica e Aplicada, Araraquara, v. 33, p. 567-575, 2013.

DAMASCENO, D. C. Anomalias Congênitas - Estudos Experimentais. 1. ed. Belo Horizonte: Coopmed, 2008. 102 p.

DE CAMPOS VENTURA-CAMARGO, B.; MARIN-MORALES, M. A. Micronuclei and chromosome aberrations derived from the action of Atrazine herbicide in Allium cepa meristematic cells. SDRP Journal of Earth Sciences & Environmental Studies, United States, v. 1, n. 1, p. 1-7, 2016.

FAREED, M.; AFZAL, M.; SIDIQUE, Y. H. Micronucleus investigation in buccal mucosal cells among pan masala/gutkha chewers and its relevance for oral cancer. Biology and Medicine, India, v. 3, p. 8-15, 2011.

FENECH, M.; CHANG, W. P.; KIRSCH-VOLDERS, M.; HOLLAND, N.; BONASSI, S.; ZEIGER, E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, China, v. 534, n. 1-2, p. 65-75, 2003.

FENECH, M. Chromosomal biomarkers of genomic instability relevant to cancer. Drug Discovery Today, Netherlands, v. 7, p. 1128-1137, 2002. https://doi.org/10.1016/S1359-6446(02)02502-3

FERREIRA, D. T.; ALVARES, P. S. M.; HOUGHTON, P. J.; BRAZ-FILHO, R. Chemical constituents from roots of Pyrostegia venusta and considerations about its medicinal importance. Química Nova, São Paulo, v. 23, p. 42-46, 2000. https://doi.org/10.1590/S0100-40422000000100010

FERNANDES, E. S.; PINTO, R. M.; REIS, J. E. P.; GUERRA, M. O.; PETERS, V. M. Effects of Ginkgo biloba extract on the embryo-fetal development in Wistar rats. Birth Defects Research (Part B): Developmental and Reproductive Toxicology, United States, v. 89, p. 133-138, 2010.

FERNANDES, E. S.; PINTO, R. M.; REIS, J. E. P.; GUERRA, M. O.; PETERS, V. M. Effects of Ginkgo biloba extract on the embryo-fetal development in Wistar rats. Birth Defects Research (Part B): Developmental and Reproductive Toxicology, United States, v. 89, p. 133-138, 2010.

FERREIRA, F. I. C.; VARGAS, V. M. F. Mutagenicity of medicinal plant extracts in Salmonella/microsome assay. Phytotherapy Research, London, v. 13, p. 397-400, 1999. https://doi.org/10.1002/(SICI)1099-1573(199908/09)13:5<397::AID-PTR473>3.0.CO;2-

FIGUEIREDO, C. R.; MATSUO, A. L.; PEREIRA, F. V.; RABAÇA, A. N.; FARIAS, C. F.; GIROLA, N.; SILVA, R. M. Pyrostegia venusta heptane extract containing saturated aliphatic hydrocarbons induces apoptosis on B16F10-Nex2 melanoma cells and displays antitumor activity in vivo. Pharmacognosy Magazine, Philadelphia, v. 10, n. 38, p. 363-368, 2014. https://doi.org/10.4103/0973-1296.133284

FISKESJÖ G. The Allium test as a standard in environmental monitoring. Hereditas, Germany, v. 102, p. 99-112, 1985. https://doi.org/10.1111/j.1601-5223.1985.tb00471.x

GREENROD, W.; FENECH, M. The principal phenolic and alcoholic components of wine protect human lymphocytes against hydrogen peroxide- and ionizing radiation-induced DNA damage in vitro. Mutagenesis, United Kingdom, v. 18, p. 119-126, 2003. https://doi.org/10.1093/mutage/18.2.119

GUO, C.; YANG, J.; WEI, J.; LI, Y.; XU, J.; JIANG, Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research, Netherlands, v. 23, n. 12, p. 1719-1726, 2003. https://doi.org/10.1016/j.nutres.2003.08.005

HELM, P. A.; GEWURTZ, S. B.; WHITTE, D. M.; MARVIN, C. H.; FISK, A. T.; TOMY, G. T. Occurrence and biomagnification of polychlorinated naphthalenes and non- and mono-ortho PCBs in Lake Ontario sediment and biota. Environmental Science & Technology, United State, v. 42, p. 1024-1031, 2008.
HSIEH, C. L.; YEN, G. C.; CHEN, H. Y. Antioxidant activities of phenolic acids on ultraviolet radiation-induced erythrocyte and low density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry*, Netherlands, v. 53, p. 6151-6155, 2005. https://doi.org/10.1021/jf050707a

IKAKI, J.; NAGY, T.; LYRA, T.; GONÇALVES, I. D.; QUINCOCES, J. A.; PARDI, P. C. Estudo dos efeitos de compostos fenólicos com atividade antitumoral na ingestão de água, ração e na variação de peso de ratos Wistar. In: *I Jornada de Iniciação Científica e Tecnológica*, São Paulo: Uniban, 2008.

KRISHNA, G.; HAYASHI, M. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, China, v. 455, p. 155-166, 2000.

KUNTZE, L. B.; KONDO, A. K.; BEZERRA, B. T. S.; PINTO, T.; CAMARGO, I. C. C. Estudo comparativo dos efeitos do extrato de *Ginkgo biloba* L. e *Panax ginseng* CA Meyer na reprodução de ratos machos e fêmeas Wistar. *Revista Brasileira de Plantas Medicinais*, Botucatu, v. 14, p. 34-42, 2012.

MAGALHÃES, E. A.; JÚNIOR, G. S.; DE CAMPOS, T. A.; SILVA, L. P.; SILVA, R. M. Avaliação do potencial genotóxico do extrato bruto de *Pyrostegia venusta* (Ker Gawl.) Miers, Bignoneaceae, em medula óssea de camundongos. *Brazilian Journal of Pharmacognosy*, Curitiba, v. 20, p. 65-69, 2010. https://doi.org/10.1590/S0102-695X2010000100014

MANIAN, R.; ANUSUYA, N.; SIDDHURAJU, P.; MANIAN, S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chemistry*, Netherlands, v. 107, p. 1000-1007, 2008. https://doi.org/10.1016/j.foodchem.2007.09.008

MARCONDES, F. K.; BIANCHI, F. J.; TANNO, A. P. Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian Journal of Biology*, São Paulo, v. 62, p. 609-614, 2002. https://doi.org/10.1590/S1519-69842002000400008

MOHD-FUAT, A. R.; KOEI, E. A.; ALLAN, G. G. Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. *Tropical Biomedicine*, Malaysia, v. 24, p. 49-59. 2007.

PEDERSEN, T.; PETERS, H. Proposal for a classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility*, United Kingdom, v. 17, p. 555-557, 1968. https://doi.org/10.1530/jrf.0.0170555

PEREIRA, A. M. S.; HERNANDES, C.; PEREIRA, S. I.; BERTONI, B. W.; FRANÇA, S. C.; PEREIRA, P. S.; TALEB-CONTINI, S. H. Evaluation of anticanudial and antioxidant activities of phenolic compounds from *Pyrostegia venusta* (Ker Gawl.) Miers. *Chemico-Biological Interactions*, Netherlands, v. 224, p. 136-141, 2014. https://doi.org/10.1016/j.cbi.2014.10.023

PLOWCHALCK, D. R.; SMITH, B. J.; MATTISON, C. R. Assessment of toxicity to the ovary using follicle quantitation and morphometrics. In: HEINDEL, J.; CHAPIN, R. E. (Ed.). Methods in Toxicology. Female Reproductive Toxicology. San Diego: Academic Press Inc., 1993. p. 57-68.

POOL, A. A review of the genus *Pyrostegia* (Bignoniaceae). *Annals of the Missouri Botanical Garden*, United States, n. 95, p. 495-510, 2008.

PULIDO, R.; BRAVO, L.; SAURA-CALIXTO, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, Netherlands, v. 48, p. 3396-3402, 2000. https://doi.org/10.1021/jf9913458

ROMERO, V.; DELA CRUZ, C.; PEREIRA, O. Reproductive and toxicological effects of isoflavones on female offspring of rats exposed during pregnancy. *Animal Reproduction*, Netherlands, v. 5, p. 83-89, 2008.
ROY, P.; AMDEKAR, S.; KUMAR, A.; SINGH, V. Preliminary study of the antioxidant properties of flowers and roots of Pyrostegia venusta (Ker Gawl) Miers. BMC Complementary and Alternative Medicine, London, v. 11, p. 69-77, 2011. https://doi.org/10.1186/1472-6882-11-69

ROY, P.; AMDEKAR, S.; KUMAR, A.; SINGH, R.; SHARMA, P.; SINGH, V. In vivo antioxidative property, antimicrobial and wound healing activity of flower extracts of Pyrostegia venusta (Ker Gawl) Miers. Journal of Ethnopharmacology, South Africa, v. 140, n. 1, p. 186-192, 2012. https://doi.org/10.1016/j.jep.2012.01.008

SANTOS, M. D.; BLATT, C. T. T. Teor de flavonóides e fenóis totais em folhas de Pyrostegia venusta Miers. de mata e de cerrado. Brazilian Journal of Botany, São Paulo, v. 21, p. 135-140, 1998. https://doi.org/10.1590/S0100-84041998000200004

SHUI, G.; LEONG, L. P. Residue from star fruit as valuable resource for functional food ingredients and antioxidant nutraceuticals. Food Chemistry, Netherlands, v. 97, p. 277-284, 2006. https://doi.org/10.1016/j.foodchem.2005.03.048

SOUZA, M. B.; SILVA JÚNIOR, J. O. C.; BARBOSA, W. L. R.; VALÉRIO, E. S.; LIMA, A. M.; ARAÚJO, M. H.; MUZITANO, M. F.; NAKAMURA, C. V.; MELLO, J. C. P.; TEIXEIRA, F. M. Pyrostegia venusta (Ker Gawl.) Miers Crude Extract and Fractions: Prevention of Dental Biofilm Formation and Immunomodulatory Capacity. Pharmacognosy Magazine, India, v. 12, n. Suppl 2, p. S218-S222, 2016.

TITENKO-HOLLAND, N.; WINDHAM, G.; KOLACHANA, P.; REINISCH, F.; PARVATHAM, S.; OSORIO, A. M.; SMITH, M. T. Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: a study of malathion-exposed workers. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, China, v. 388, p. 85-95, 1997.

TODA, S. Antioxidative effects of polyphenols in leaves of Houttuynia cordata on protein fragmentation by copper-hydrogen peroxide in vitro. Journal of Medicinal Food, United States, v. 8, p. 266-268, 2005. https://doi.org/10.1089/jmf.2005.8.266

VELOSO, H. J. F.; SILVA, A. A. M. Prevalência e fatores associados à obesidade abdominal e ao excesso de peso em adultos maranhenses. Revista Brasileira de Epidemiologia, São Paulo, v. 13, p. 400-412, 2010. https://doi.org/10.1590/S1415-790X2010000300004

VIEL, A. M.; PEREIRA, A. R.; NERES, W. E.; DOS SANTOS, L.; OLIVA NETO, P.; SOUZA, E. B.; SILVA, R. M. G.; CAMARGO, I. C. C. Effect of Agave sisalana Perrine extract on the ovarian and uterine tissues and fetal parameters: comparative interventional study. International Journal of Public Health, Switzerland, v. 3, n. 5, p. 129-138, 2017.