

Full Length Research Paper

Chemical characterization and toxicological analyses of hydroalcoholic extracts from the stem and leaves of mangabeira (*Hancornia speciosa* Gomes) as a guide for the development of green cosmetics

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The increasing interest in green cosmetics has a major influence on the cosmetics market, whether due to the incorporation of the functionalities of the extracts, or the substitution of synthetic ingredients with natural ones. Yet, the natural substances found in those products must be evaluated for their chemical characterization and toxicity. This study determined the chemical profile and assessed the toxicity of stem (S) and leaf (L) extracts of *Hancornia speciosa* Gomes obtained by Soxhlet apparatus (SOX) and ultrasonic-assisted extraction (US). Hydroethanolic (70%) extracts were prepared from the stem and leaves of *H. speciosa* resulting in Soxhlet stem extract, ultrasound stem extract, Soxhlet leaf extract, ultrasound leaf extract. The chemical characterization of the extracts was performed by phytochemical screening and the compounds were identified by GC-MS analyses. The toxicity test was analyzed at concentrations of 50, 250 and 500 µg/ml, using the *Allium cepa* and *Artemia salina* tests. The phytochemical analysis identified flavonoids, tannins and saponins while the GC-MS analysis found lupeol, hentriacontane, undecanoic acid, Friedelan-3-one and a potentially toxic substance known as oxalic acid. The toxicity test showed low genotoxic and antimutagenic indexes in the extracts of *H. speciosa*. The findings suggest that the safest extracts in cosmetic preparations were Soxhlet stem extract and Soxhlet leaf extract, while the most toxic extract was ultrasound leaf extract.

Key words: Chemical profile, mangaba tree, toxicity, extracts, green cosmetics

INTRODUCTION

Brazil has an extensive territory with distinctive vegetation, different climatic conditions and it holds an

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invaluable biodiversity. The state of Tocantins is in the transition region between the Cerrado and the Amazon forest (Becerra et al., 2009), which presents distinct environmental conditions, especially regarding temperature and humidity. This wide diversity results in a vast number of plant species (Calixto, 2003), and several are popularly used by indigenous and quilombola communities for therapeutic purposes (Souza et al., 2020). Mangabeira (*Hancornia speciosa* Gomes) is an example of a species from this region (Sousa et al., 2007). While this plant is well-known for its appreciated fruit flavor (Almeida et al., 2016), other parts of the plant are used in folk medicine, prompting further investigation into their biological activities.

Among the biological properties presented by *H. speciosa* described in the literature, the antioxidant activity stands out as promising for the development of cosmetics (Santos et al., 2016), while studies in the bio-prospecting area of mangabeira for cosmetic purposes are innovative.

The use of plant extracts in green cosmetics has become economically relevant in the market (Amberg and Fogarassy, 2019; Jaini et al., 2019), as observed in *Aloe vera* extract used in moisturizings (Saraf et al., 2010) and *Vitis vinifera* extract used in antioxidants cosmetics (Surini et al., 2018). Many consumers choose this type of product considering, mainly, health care (Cervellon and Carey, 2011). The chemical characteristics of extracts, which can vary depending on the extraction method and extractor solvent, must be considered when developing green cosmetics (Ma et al., 2019). Therefore, the monitoring of the extraction process must be accomplished in order to ensure that the desired substances are in adequate concentrations and that unwanted substances are not extracted.

In the process of developing a new formulation that is bioactive, it is also necessary to perform toxicological tests to evaluate the safety of new compounds. Cosmetics companies are currently adapting to consumer demands for sustainable cosmetics. Hence, plant models, such as the *Allium cepa* system or arthropods, such as *Artemia salina*, have shown a viable alternative to replace tests with vertebrate animals (Kumari et al., 2011; Maiti et al., 2016; Viega et al., 2019).

This work aimed to identify the compounds present in *H. speciosa* leaves and stem extracts obtained by different extraction methods, as well as to test the toxicity of these extracts for further use in cosmetic formulations.

MATERIALS AND METHODS

Plant material

The leaves and stem of *H. speciosa* were collected in the city of Palmas, State of Tocantins, Brazil, in August 2018, in the morning period, in the geographical coordinates 10° 18'00 "S, 48° 31'41" W. The SISGen (<https://sisgen.gov.br/>) was registered under number

A8853F4 and the specimen voucher was produced and deposited by the herbarium of the Universidade Estadual do Tocantins (UNITINS), under registration number 7278.

Preparation of extracts

The leaves and stems were oven dried at 40°C for 3 days until constant weight. Thereafter, parts of the plant were separately powdered in a Willey knife mill (FT Fortinox STAR 50), in mesh 20 and stored in amber flasks. The stem and leaf extracts were obtained by hot extraction, using Soxhlet apparatus (SOX) and cold, using ultrasonic-assisted extraction (US), called S_SOX, S_US, L_SOX and L_US, respectively.

The extractions were performed according to the method proposed by Nile et al. (2017), with modifications. The hot extraction was performed using 5 g of the powder from each part of the plant, with 200 ml of 70% ethanol, for five cycles. In cold extraction, 5 g of the powder from each part of the plant was mixed with 80 ml of 70% ethanol and immersed in an ultrasonic bath (USC1600, ultrasonic cleaner, frequency 40 kHz, 135 W), for a cycle of 1 h at room temperature. The process was repeated five times, combining the supernatants. The extracts were rota-evaporated (45°C) and then lyophilized in LIOTOP bench freeze dryer L101.

Phytochemical screening

Phytochemical screening of the extracts was performed with the freshly prepared extract, before proceeding with freezing and lyophilization. Phytochemical characterization was based on colorimetric and precipitation reactions, with tests for detecting flavonoids, tannins (Mouco et al., 2003) and saponins (Sociedade Brasileira de Farmacognosia, 2020).

Chemical characterization by Gas Chromatography - Mass Spectrometry (GC-MS)

The extracts obtained from the stem (S_SOX and S_US) and from the leaf (L_SOX and L_US) were chemically characterized by GC-MS. The GC-MS analyses were performed in a Shimadzu® chromatograph model QP2020 equipped with a ZB-5HT Inferno column (Phenomenex®), 0.25 µm thick, 0.25 mm in diameter, 30 m in length and column oven temperature of 60°C. Helium gas was used as a carrier with flow of 1.8 ml/min, pressure of 111.5 kPa, total flow of 13.8 ml/min, column flow of 1.8 ml/min and programming time of 58 min. MS specifications were ion source temperature and interface temperature set in 280°C, and solvent cut time 2.5 min. The identification of the compounds was performed by comparison with the mass spectra of the reference library, NIST 107 and Wiley 229. The percentage of the components was calculated using the peak area of each substance in the chromatogram.

Toxicity test against *Allium cepa*

The *A. cepa* assay was performed according to the method developed by Ribeiro et al. (2016), with modifications. Onion bulbs (n = 3; 39 units) were obtained commercially from a supermarket in Palmas - TO, Brazil. All the onions were apparently healthy and of similar size. Onion bulbs were grown in tap water at 25°C for 72 h to stimulate root growth in order to test the quality of the vegetable. Subsequently, the onions had their roots cut and 3 onions were randomly separated for the negative control and a group of 36

Table 1. Results obtained in phytochemical screening for flavonoids, tannins and saponins in the plant extracts of the stem (S) and leaf (L) of mangabeira (*H. speciosa*), obtained by ultrasonic-assisted extraction (US) and Soxhlet apparatus (SOX).

| Tests performed | S_SOX | S_US | L_SOX | L_US |
|------------------------------|-------|------|-------|------|
| Flavonoids | | | | |
| Shinoda | + | + | + | + |
| Ferric Chloride | - | - | + | + |
| Sodium Hydroxide | + | + | + | + |
| Tannins | | | | |
| Ferric chloride | + | + | + | + |
| Lead acetate | - | - | - | - |
| Copper acetate | + | + | + | + |
| Lead acetate and acetic acid | + | + | + | + |
| Saponins | | | | |
| Rosol | - | - | + | - |
| Mitchell | - | - | + | - |
| Rosenthalen | - | - | + | - |
| Sulfo-Vanilic | - | - | + | - |
| Liebermann-Burchard | - | - | + | - |

+: Positive, -: negative.

onions separated into 4 subgroups with 3 different concentrations (50, 250 and 500 µg/ml) of each extract (S_SOX, S_US, L_SOX, L_US), in triplicate. Each subgroup was placed with the bulb in contact with the different concentrations of each extract for 5 days.

After this period, the length of the roots was measured with a caliper (cm) and the number of roots was counted to assess the average root size. The Relative Growth Index (RGI) and the Growth Rate (GR%) was calculated according to Young et al. (2012). In the RGI analysis, values below 0.8 were classified as growth inhibition; values between 0.8 and 1.2 indicated no significant effect and values above 1.2 evidenced root growth stimulation.

Root fixation was performed in Carnoy (3:1) and staining in hematoxylin, according to Guerra and Souza (2002). After staining, the meristems were divided into small fragments, crushed, and then microscopic analysis was performed.

Analysis of cytotoxic potential

This was evaluated according to the method proposed by Bonciu et al. (2018). In each slide, 1000 meristematic cells of *A. cepa* were analyzed using an optical microscope (100x). The stages of cell division in mitosis, interphase, prophase, metaphase, anaphase and telophase were observed, in addition to cytological anomalies. The Mitotic Index (MI) was obtained from the count of mitotic cells in relation to the number of cells observed.

Toxicity test against *Artemia salina*

The *A. salina* assay was performed based on the method of Meyer et al. (1982), with modifications. *A. salina* eggs were added in artificial seawater (1 L of distilled water, 20 g of sea salt, pH 8), under lighting (20W) and temperature of 27°C until hatching. The dry extracts were diluted in artificial seawater in the concentrations of 50, 250 and 500 µg/ml and the blank was prepared only with

artificial seawater. About 10 ml of each solution was transferred to a test tube and 10 nauplii of *A. salina* were placed in each tube. After 24 h, the surviving nauplii were counted. The test was performed in triplicate.

Toxicity tests against *Allium cepa* and *Artemia salina*

The results found for the average growth of *A. cepa* roots in the different treatments showed statistically significant differences (p-value = 0.000) (Table 6). As the concentration of the extracts increased, there was an increase in toxicity, which can be observed by decreasing the size (cm) of the roots.

Statistical analysis

The experiments performed in triplicate had the results expressed as mean ± standard deviation. The data obtained were submitted to statistical analysis using the program SISVAR version 5.6 (Ferreira, 2019). ANOVA analysis of variance (p <0.05) was performed, and the Tukey test was applied.

RESULTS

Phytochemical screening

Phytochemical screening was performed on plant extracts of the mangabeira stem and leaf (*H. speciosa*), obtained by ultrasonic-assisted extraction and Soxhlet apparatus, in order to verify whether the extractive process was from a qualitative point of view, effective in the extraction of compounds of interest (Table 1).

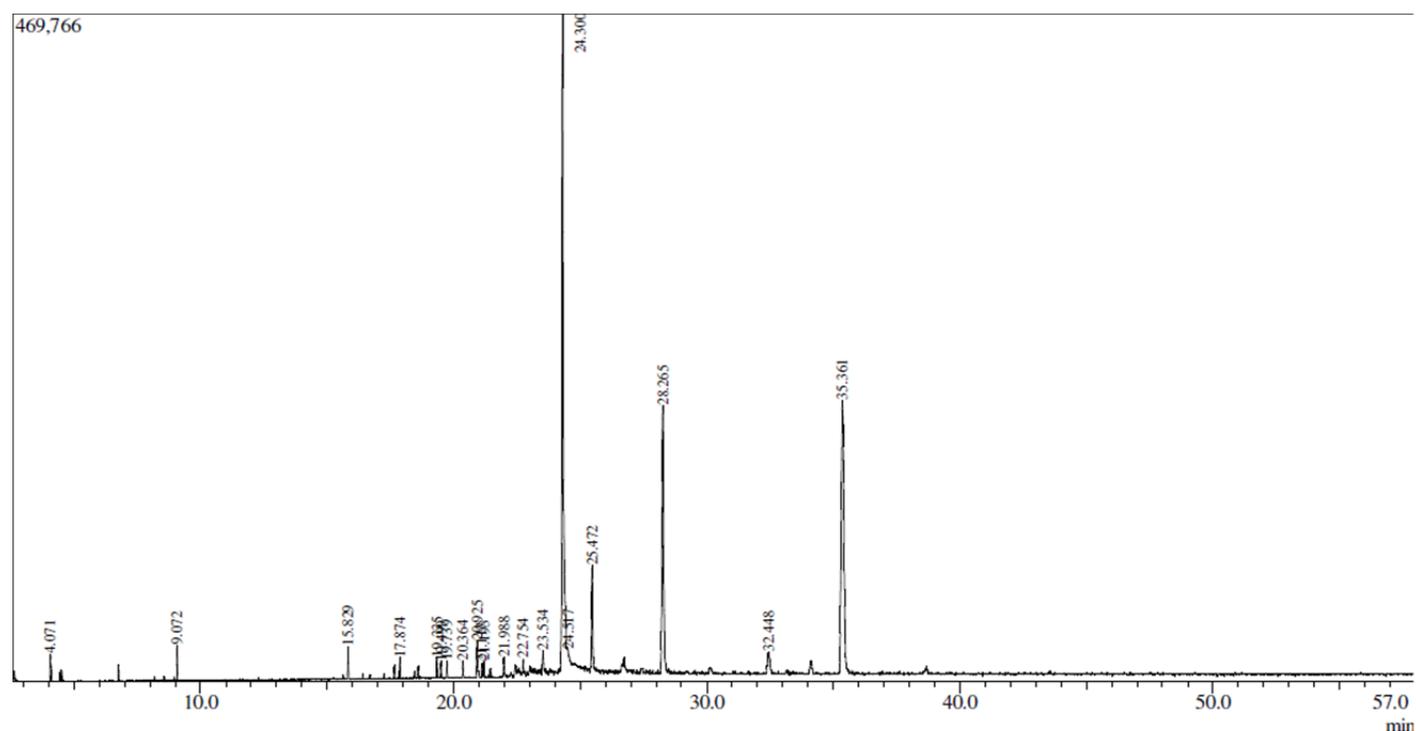


Figure 1. GC-MS analysis: chromatogram of the hydroalcoholic extract of the stem of *H. speciosa*, obtained by Soxhlet apparatus.

The results found in the tests for flavonoids showed, that the extraction process did not influence the qualitative profile of the analyzed extracts, so that regardless of the extraction method used, the results were qualitatively very similar. In the analysis of flavonoids, the stem extracts were positive for the Shinoda and sodium hydroxide test. Leaf extracts showed a positive result in all tests.

In the assay for qualitative identification of tannins, all extracts were positive in the ferric chloride, copper acetate, lead acetate and acetic acid assays.

As for the saponin reactions, L_SOX showed a higher saponin sign profile, since it showed positivity in the reactions of Rossol, Rosenthalen, Sulfo-Vanilic and Liebermann-Burchard, and an inconclusive result in the Mitchell reaction.

Chemical characterization by GC-MS

The chemical characterization of the hydroalcoholic extracts of the leaves and stem of *H. speciosa* obtained by ultrasonic-assisted extraction and Soxhlet apparatus was performed by GC-MS. The 20 compounds identified in the extracts are shown in Figures 1 to 4 and Tables 2 to 5.

In the stem extracts, when ultrasonic-assisted extraction

was used, the major compounds identified were Lupeol (49.64%) and Oxalic acid, bis (2-ethylhexyl) ester (8.01%), already in the extraction by Soxhlet apparatus, the compounds identified in the greatest amount were cis-11-eicosenamide (36.80%) and lupeol (29.07%).

In the leaf extracts, it was possible to observe that the major compound was hentriacontane, both in the extract obtained by ultrasonic-assisted extraction (41.18%) and for the extract obtained by Soxhlet apparatus (49.17%). Lupeol was also identified in the leaf; however, in a smaller quantity (21.61% in the extract obtained by ultrasonic-assisted extraction and 17.27% in the extract obtained by Soxhlet apparatus).

To a lesser extent, undecanoic acid, Friedelan-3-one and oxalic acid were identified. In addition, the extracts showed a large number of hydrocarbons, such as pentacosane and hentriacontane, which shows that the plant has compounds with a lipophilic characteristic.

When analyzing the results found for the average of the length of the roots (ARL, in cm), it was possible to verify significant difference for the studied samples ($p = 0.000$), being the greater length presented by the extract L_US 50 $\mu\text{g/ml}$ and the smaller by the extract L_US 500 $\mu\text{g/ml}$.

In the analysis of the RG, it was found that the lowest RGI was observed in the roots that were in contact with the L_US 500 $\mu\text{g/ml}$ extract. In all extracts, there was an inversely proportional pattern between the RGI and the

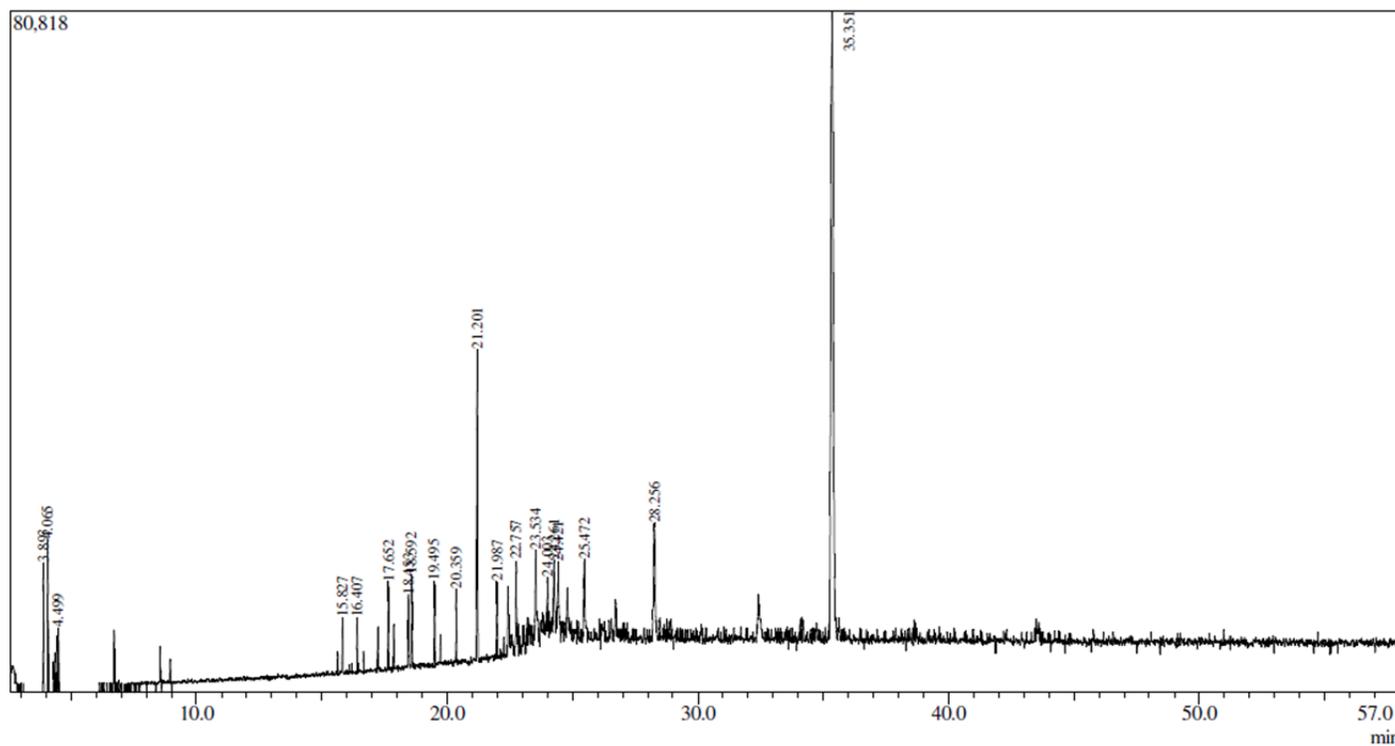


Figure 2. GC-MS analysis: chromatogram of the hydroalcoholic extract of the stem of *H. speciosa*, obtained by ultrasonic-assisted extraction.

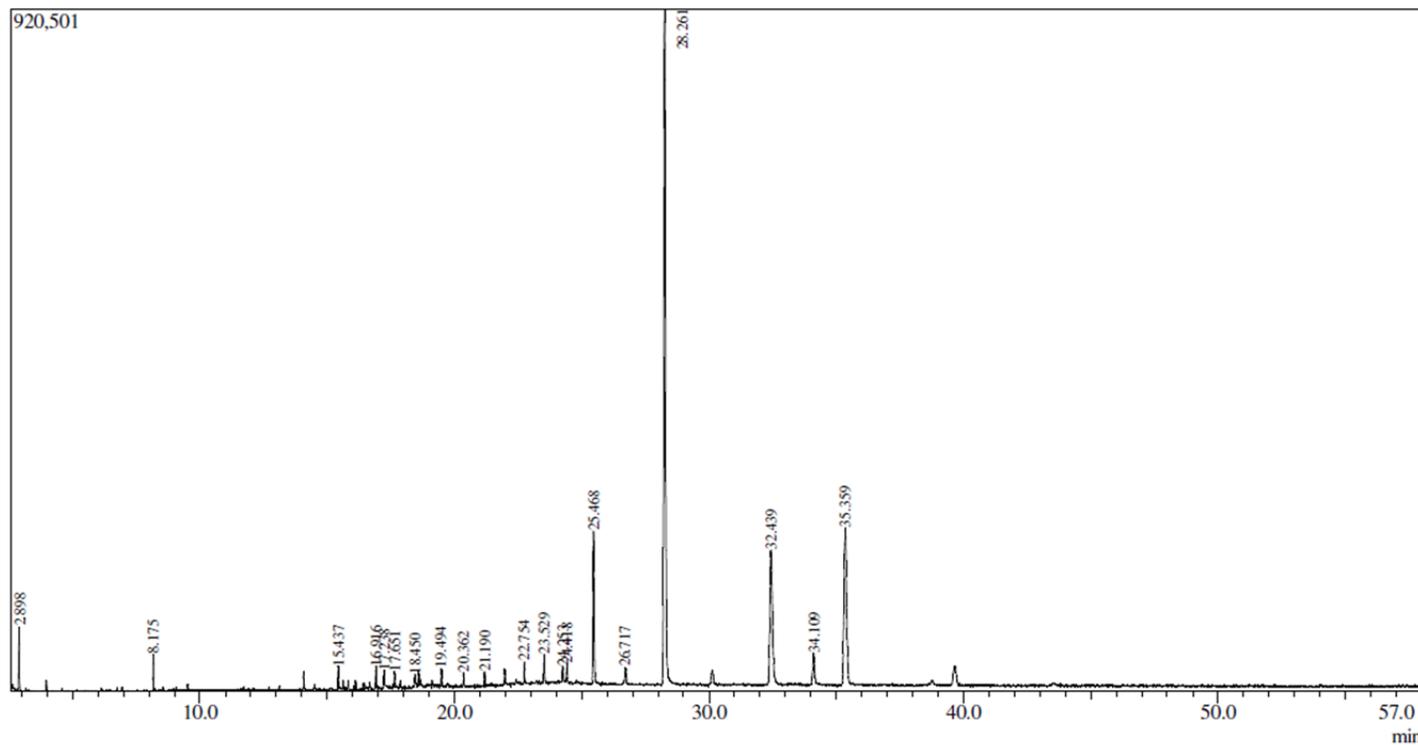


Figure 3. GC-MS analysis: chromatogram of the hydroalcoholic extract of the leaf of *H. speciosa*, obtained by Soxhlet apparatus.

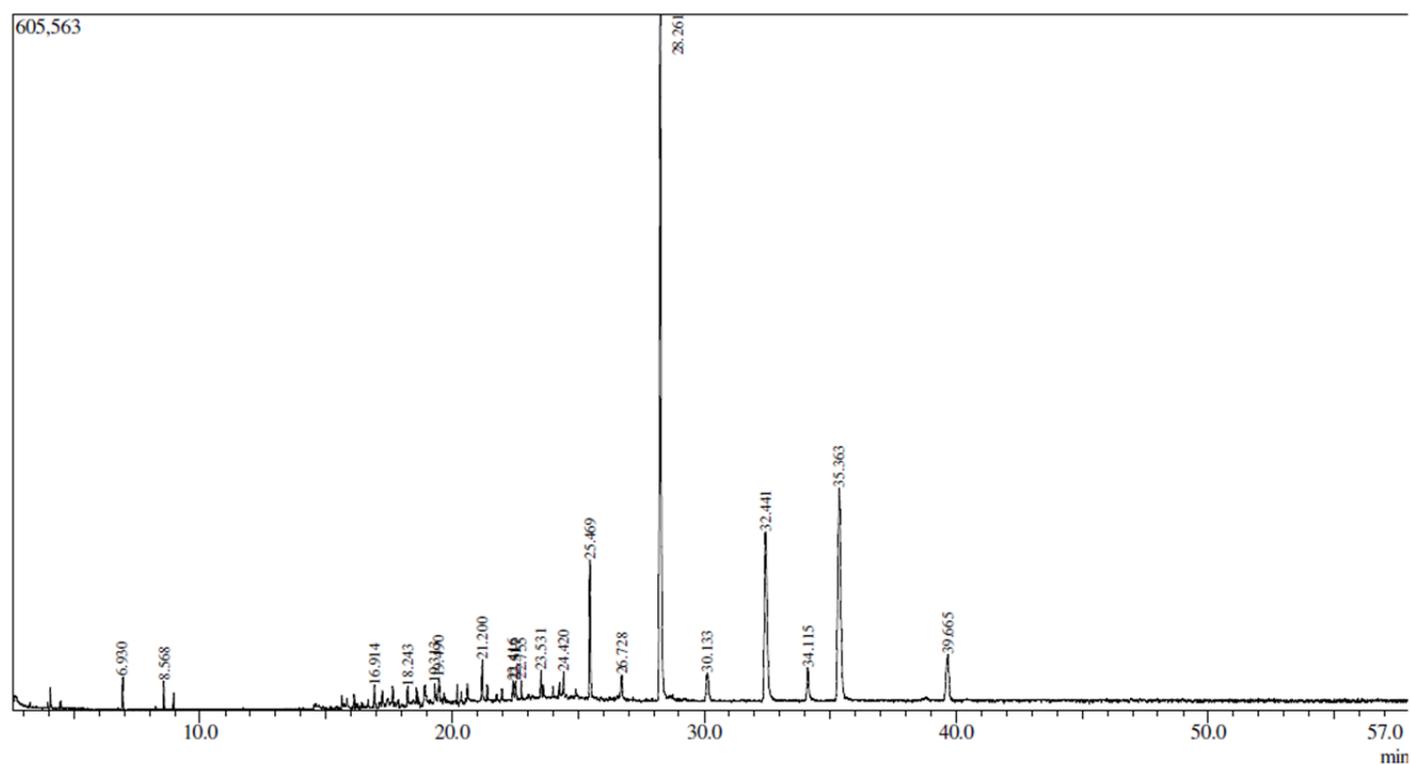


Figure 4. GC-MS analysis: chromatogram of the hydroalcoholic extract of the leaf of *H. speciosa*, obtained by ultrasonic-assisted extraction.

Table 2. Chemical compounds identified by GC-MS in the hydroalcoholic extract of the stem of *H. speciosa*, obtained by Soxhlet apparatus.

| Peak | Name | Retention Time | Area (%) |
|-------|------------------------------|----------------|----------|
| 1 | 2-Butenal, 3-methyl- | 4.071 | 1.12 |
| 2 | 1-Octene, 3,7-dimethyl- | 9.072 | 0.88 |
| 3 | 14-Heptadecenal | 15.829 | 0.86 |
| 4 | cis-3-Hexenylpyruvate | 17.874 | 0.48 |
| 5 | Butanamide, 3,3-dimethyl- | 19.335 | 0.72 |
| 6 | Acetyl valeryl | 19.499 | 0.48 |
| 7 | cis-3-Hexenylpyruvate | 19.739 | 0.41 |
| 8 | Dodecane, 1-iodine- | 20.364 | 0.51 |
| 9 | Undecanol-3 | 20.925 | 1.30 |
| 10 | 2-Propanol, 2-methyl- | 21.117 | 0.63 |
| 11 | Acetyl valeryl | 21.193 | 0.50 |
| 12 | Dodecane, 1-iodine- | 21.988 | 0.61 |
| 13 | Nonane, 1-iodine- | 22.754 | 0.39 |
| 14 | Dodecane, 1-iodine- | 23.534 | 0.73 |
| 15 | cis-11-Eicosenamide | 24.300 | 36.81 |
| 16 | Butane, 1-ethoxy- | 24.517 | 0.68 |
| 17 | 2-methyloctacosane | 25.472 | 4.42 |
| 18 | Hentriacontane | 28.265 | 17.53 |
| 19 | Borane, diethyl (decyloxy) - | 32.448 | 1.87 |
| 20 | Lupeol | 35.361 | 29.07 |
| Total | | | 100.00 |

Table 3. Chemical compounds identified by GC-MS in the hydroalcoholic extract of the stem of *H. speciosa*, obtained by ultrasonic-assisted extraction.

| Peak | Name | Retention Time | Area (%) |
|-------|---|----------------|----------|
| 1 | 2-Butenal, 3-methyl- | 3.893 | 3.21 |
| 2 | 2,3,3-Trimethyl-1-hexene | 4.065 | 4.67 |
| 3 | Cycloheptane, bromo- | 4.499 | 1.51 |
| 4 | Oxalic acid, butyl propyl ester | 15.827 | 1.26 |
| 5 | Phthalic acid, 4-bromophenyl heptyl ester | 16.407 | 1.15 |
| 6 | Acetyl valeryl | 17.652 | 2.01 |
| 7 | Oxalic acid, allyl heptyl ester | 18.453 | 1.80 |
| 8 | 3-Hexanone, 2,2-dimethyl- | 18.592 | 1.89 |
| 9 | 3-Hexanone, 2,4-dimethyl- | 19.495 | 2.00 |
| 10 | 3-Hexanone, 2,2-dimethyl- | 20.359 | 1.66 |
| 11 | Oxalic acid, bis (2-ethylhexyl) ester | 21.201 | 8.01 |
| 12 | 3-Hexanone, 2,2-dimethyl- | 21.987 | 2.04 |
| 13 | Acetyl valeryl | 22.757 | 3.54 |
| 14 | Nonane, 1-iodine- | 23.534 | 1.84 |
| 15 | 1-Butanol, 3-methyl-, propanoate | 24.003 | 1.77 |
| 16 | Acetic acid, trifluoro-, 2,2-dimethylpropyl | 24.261 | 2.06 |
| 17 | Borane, diethyl (decyloxy) - | 24.421 | 1.25 |
| 18 | Borane, diethyl (decyloxy) - | 25.472 | 2.45 |
| 19 | Sulfurous acid, 2-ethylhexyl hexyl ester | 28.256 | 6.23 |
| 20 | Lupeol | 35.351 | 49.64 |
| Total | | | 100.00 |

Table 4. Chemical compounds identified by GC-MS in the hydroalcoholic extract of the leaf of *H. speciosa*, obtained by Soxhlet apparatus.

| Peak | Name | Retention Time | Area (%) |
|-------|---|----------------|----------|
| 1 | 3-Buten-2-one, 3-methyl- | 2.898 | 1.58 |
| 2 | Methyl salicylate | 8.175 | 0.94 |
| 3 | Octane, 1,8-dibromo- | 15.437 | 0.57 |
| 4 | 1-Heptanol, 2,4-dimethyl-, | 16.916 | 0.63 |
| 5 | Undecanoic acid | 17.238 | 0.53 |
| 6 | Sulfurous acid, 2-ethylhexyl hexyl ester | 17.651 | 0.48 |
| 7 | 1-Decene, 8-methyl- | 18.450 | 0.49 |
| 8 | Borane, diethyl(decyloxy)- | 19.494 | 0.50 |
| 9 | Sulfurous acid, 2-ethylhexyl isohexyl ester | 20.362 | 0.49 |
| 10 | Sulfurous acid, 2-ethylhexyl hexyl ester | 21.190 | 0.48 |
| 11 | Borane, diethyl(decyloxy)- | 22.754 | 0.66 |
| 12 | Sulfurous acid, 2-ethylhexyl isohexyl ester | 23.529 | 0.88 |
| 13 | Carbonic acid, neopentyl cyclohexylmethy | 24.253 | 0.49 |
| 14 | Borane, diethyl(decyloxy)- | 24.418 | 0.74 |
| 15 | Hentriacontane | 25.468 | 7.02 |
| 16 | Borane, diethyl(decyloxy)- | 26.717 | 0.73 |
| 17 | Hentriacontane | 28.261 | 49.17 |
| 18 | Pentacosane | 32.439 | 14.21 |
| 19 | 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a, | 34.109 | 2.14 |
| 20 | Lupeol | 35.359 | 17.27 |
| Total | | | 100.00 |

Table 5. Chemical compounds identified by GC-MS in the hydroalcoholic extract of the leaf of *H. speciosa*, obtained by ultrasonic-assisted extraction.

| Peak | Name | Retention Time | Area (%) |
|-------|--|----------------|----------|
| 1 | Phenylethyl Alcohol | 6.930 | 0.87 |
| 2 | 1,6-Octadiene, 3,7-dimethyl-, (S)- | 8.568 | 0.57 |
| 3 | Ether, 6-methylheptyl vinyl | 16.914 | 0.52 |
| 4 | 1,1'-Bicyclohexyl-2,2'-diol | 18.243 | 0.51 |
| 5 | 3-Nonyne | 19.313 | 0.74 |
| 6 | Borane, diethyl(decyloxy)- | 19.490 | 0.55 |
| 7 | Oxalic acid, 2-ethylhexyl hexyl ester | 21.200 | 1.17 |
| 8 | Butylaldehyde, 4-benzyloxy-4-[2,2,-dimeth | 22.416 | 0.53 |
| 9 | 2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxa | 22.517 | 0.69 |
| 10 | Borane, diethyl(decyloxy)- | 22.755 | 0.52 |
| 11 | Borane, diethyl(decyloxy)- | 23.531 | 0.70 |
| 12 | Borane, diethyl(decyloxy)- | 24.420 | 0.98 |
| 13 | 2-methyloctacosane | 25.469 | 5.58 |
| 14 | Borane, diethyl(decyloxy)- | 26.728 | 0.54 |
| 15 | Hentriacontane | 28.261 | 41.18 |
| 16 | Borane, diethyl(decyloxy)- | 30.133 | 1.54 |
| 17 | 2-methyloctacosane | 32.441 | 15.60 |
| 18 | Hop-22(29)-en-3.beta.-ol | 34.115 | 1.40 |
| 19 | Lupeol | 35.363 | 21.61 |
| 20 | Friedelan-3-one | 39.665 | 4.18 |
| Total | | | 100.00 |

concentrations of the extracts.

In the GR% analysis, the percentage of the growth rate was inversely proportional to the concentrations tested, probably because the higher the concentration, the lower the growth rate, so that the extracts with RGI <0.8 are statistically different from the other samples ($p < 0.5$).

From the microscopic analysis of the meristems, it was possible to observe cells in all phases of the mitotic process, the number of cells in the mitotic process being inversely proportional to the concentrations analyzed (Table 6). In addition, the number of mitotic cells confirms the RGI result, since the growth of the roots was lower when the MI was lower.

Genotoxicity and mutagenicity were observed in a low percentage in the analyzed extracts (Figures 5 and 6). The statistical analysis showed that only the extracts L_US 500 µg/ml, L_US 250 µg/ml and S_US 500 µg/ml differ significantly from the other samples, presenting significantly more chromosomal abnormalities. Among the anomalies found, it was possible to highlight a chromosomal bridge and delayed chromosome (Figure 7).

In *A. salina* test, the survival rate of *A. salina* nauplii was verified after 24 h of contact with the tested substances. The hydroalcoholic extracts did not present a high mortality rate (Figure 8), which made the calculation

of the IC₅₀% unfeasible from the tested concentrations.

However, even not reaching 50% of deaths of nauplii of *A. salina*, it was observed that, statistically ($p = 0.000$), the L_US 500 µg/ml extract presented the highest toxicity among the different concentrations of the analyzed extracts, followed by the extract S_US 500 µg/ml (Figure 8), confirming the results obtained in the other tests proposed in this work.

DISCUSSION

Phytochemical screening showed a similar profile for flavonoids in the tested extracts. In the analysis of tannins, the ferric chloride test showed a greenish color, which shows condensed or catechin tannins, such as catechin, while the reddish precipitate formed in the lead acetate and acetic acid test indicates gallic tannins. These results show a variation of tannins in the analyzed extracts. All extracts showed a negative result for the lead acetate test, which shows a possible absence of hydrolyzable tannins. In the saponin assay, the Liebermann-Burchard reaction is not exclusive to saponins, since it detects steroidal nuclei; thus, the positivity in this reaction may be related to cardiotonic glycosides.

Table 6. Average Root Length (ARL), Relative Growth Index (RGI) and Growth Rate (GR), Number of *A. cepa* mitotic cells and Mitotic Index (MI) observed in the different concentrations of stem (S) extracts and leaf (L) of *H. speciosa* submitted to extraction in Soxhlet apparatus (SOX) and ultrasonic-assisted extraction (US) and statistical analysis.

| Sample ($\mu\text{g/ml}$) | ARL \pm sd (cm) | RGI | GR (%) | I | P | M | A | T | MI |
|-----------------------------|---|------|--------|-----|----|----|----|----|-------------------------------|
| Control | 6.7 \pm 0.56 ^{AB} | 1 | 100 | 536 | 43 | 29 | 10 | 17 | 9.3 \pm 0.30 ^{ABC} |
| S_SOX 50 | 6.57 \pm 1.19 ^{AB} | 0.97 | 91.71 | 61 | 31 | 3 | 2 | 61 | 9.7 \pm 0.40 ^{ABC} |
| S_SOX 250 | 6.20 \pm 1.10 ^{ABC} | 0.93 | 91.35 | 43 | 27 | 9 | 15 | 43 | 9.4 \pm 0.40 ^{BC} |
| S_SOX 500 | 3.47 \pm 0.38 ^{DE} | 0.52 | 48.87 | 39 | 13 | 2 | 2 | 29 | 4.6 \pm 0.44 ^E |
| S_US 50 | 6.67 \pm 0.78 ^{AB} | 1 | 136.69 | 62 | 20 | 10 | 6 | 62 | 9.8 \pm 0.10 ^{ABC} |
| S_US 250 | 5.47 \pm 0.49 ^{ABCD} | 0.82 | 96.69 | 48 | 22 | 1 | 2 | 48 | 7.3 \pm 0.3 ^D |
| S_US 500 | 2.57 \pm 0.40 ^{EF} | 0.38 | 24.82 | 25 | 2 | 0 | 0 | 1 | 0.3 \pm 0.05 ^F |
| L_SOX 50 | 7.10 \pm 0.20 ^A | 1.07 | 206.63 | 586 | 65 | 31 | 7 | 2 | 10.5 \pm 0.7 ^{AB} |
| L_SOX 250 | 6.07 \pm 1.27 ^{ABCD} | 0.90 | 91.33 | 410 | 53 | 26 | 3 | 4 | 8.6 \pm 0.781 ^{CD} |
| L_SOX 500 | 3.53 \pm 2.23 ^{CDE} | 0.53 | 50.12 | 133 | 30 | 11 | 5 | 3 | 4.9 \pm 0.7 ^E |
| L_US 50 | 7.47 \pm 0.32 ^A | 1.12 | 93.73 | 324 | 72 | 25 | 5 | 6 | 10.8 \pm 0.361 ^A |
| L_US 250 | 4.03 \pm 0.49 ^{B^{CD}} | 0.61 | 12.75 | 68 | 32 | 15 | 5 | 2 | 5.4 \pm 0.361 ^E |
| L_US 500 | 0.6 \pm 0 ^F | 0.1 | 0.7 | 10 | 1 | 0 | 0 | 0 | 0.1 \pm 0 ^F |

I: Interphase, P: Prophase, M: Metaphase, A: Anaphase, T: Telophase, MI: Mitotic index. Means that do not share the same letter are significantly different ($p < 0.05$).sd: standard deviation

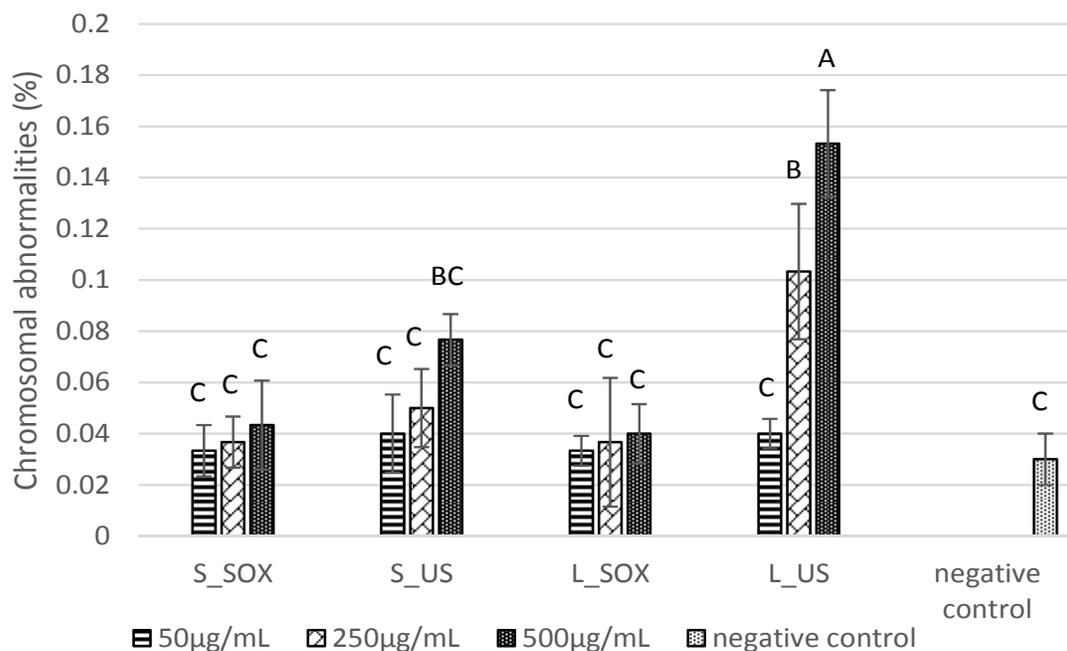


Figure 5. Genotoxicity profile observed at different concentrations of the extracts stem (S) and leaf (L) of *H. speciosa* obtained by extraction of the Soxhlet apparatus (SOX) and ultrasonic-assisted extraction (US) and statistical analysis. Means that do not share the same letter are significantly different ($p < 0.05$).

The presence of flavonoids and tannins (Table 1) indicates antioxidant activity of the extracts. Few studies have reported phytochemical screening of *H. speciosa*. Silva et al. (2010) found positive results for flavonoids, tannins and saponins. Santos et al. (2016) described the

high antioxidant potential of *H. speciosa* leaves as a result of the presence of phenolic compounds. In addition, Zaid and Ramahi (2019) described that these compounds have depigmenting and anti-aging activity. Thus, the incorporation of phenolic compounds of *H.*

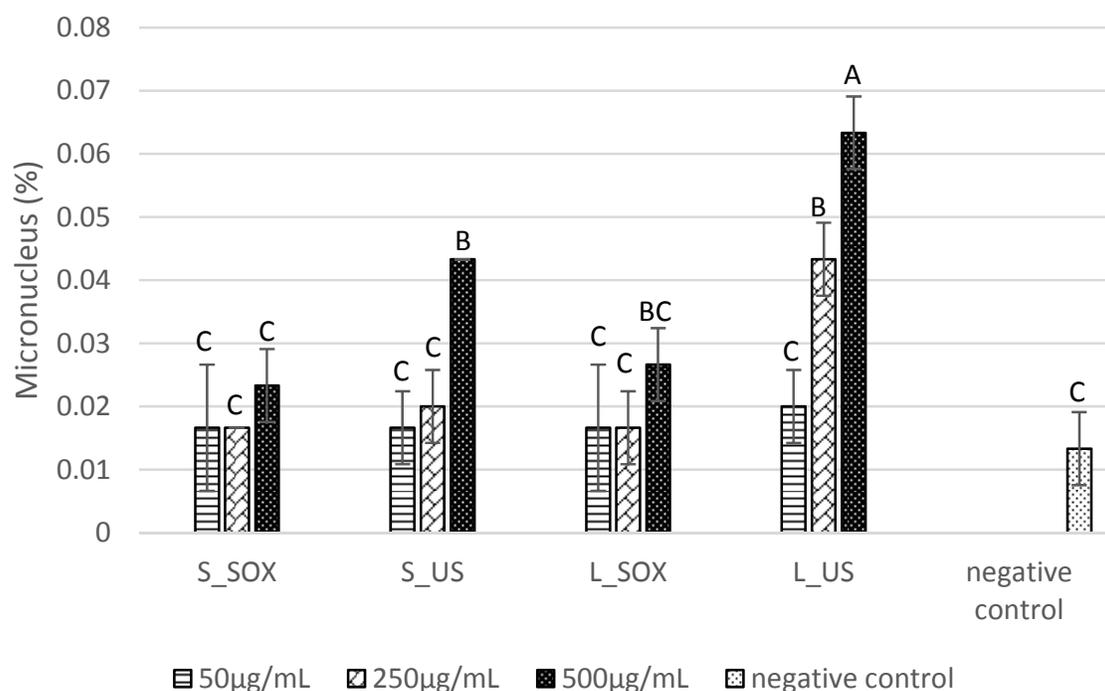


Figure 6. Mutagenicity profile observed at different concentrations of *H. speciosa* stem (S) and leaf (L) extracts obtained by extraction in Soxhlet apparatus (SOX) and ultrasonic-assisted extraction (US) and statistical analysis. Means that do not share the same letter are significantly different ($p < 0.05$).



Figure 7. Photomicrograph of anomalies chromosome found in meristem cells *A. cepa* after contact with hydroethanolic extract the stem and leaf *H. speciosa* obtained by the Soxhlet apparatus and ultrasonic-assisted extraction. a: Delayed chromosome, b: irregular metaphase, c: anaphase with chromosomal bridge.

speciosa in cosmetics has the potential to act as an antioxidant and anti-aging agents. The saponins found in L_SOX extract suggest the investigation of this extract in foaming cosmetics intended for cleaning purposes, such as shampoos, because saponins have the potential to replace the surfactants that are effectively used as cleaning agents in these cosmetics (Moghimi-pour et al., 2020).

The GC-MS analyses allowed the identification of several substances that can be used in cosmetology, with emphases on lupeol, hentriacontane, undecanoic acid, and Friedlan-3-one. The range of compounds extracted differed between the Soxhlet apparatus and ultrasonic assisted extraction. According to Hemwimol et al. (2006),

the differences in the methods, such as the temperature of the Soxhlet apparatus and the cavitation of the ultrasound, are responsible for the extraction of a diversity of molecules. Chemical analysis detected anti-aging and anti-acne substances.

Lupeol was identified in all extracts analyzed. According to Kwon et al. (2015), lupeol contains healing properties and contributes to the reduction of skin oiliness. Both of these mechanisms contribute to the anti-acne activity, making the L_SOX extract a strong candidate for the development of anti-acne cosmetics preferably in vehicles with a non-comedogenic aqueous base. Lupeol can also be useful in cosmetology in antioxidant-acting formulations due to its high ability to

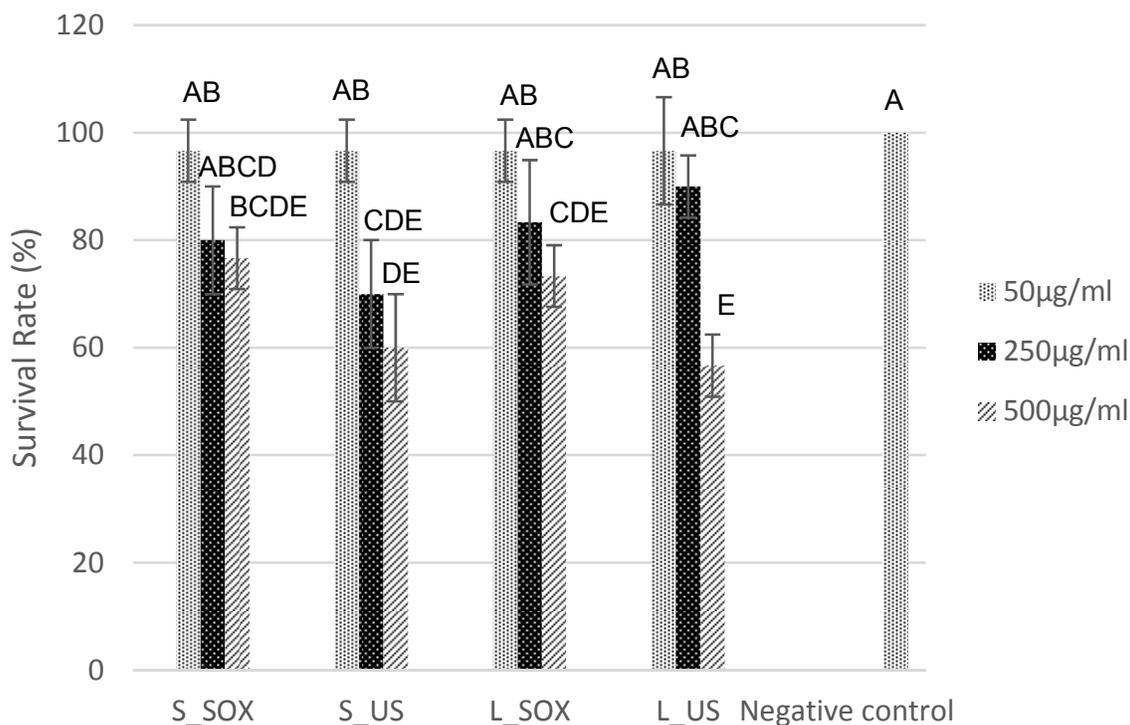


Figure 8. Percentage of survival of *A. salina* nauplii at different concentrations of the stem (S) and leaf (L) extracts of *H. speciosa* submitted to extraction in a Soxhlet apparatus (SOX) and ultrasonic-assisted extraction (US) and statistical analysis. Means that do not share the same letter are significantly different ($p < 0.05$).

eliminate the radical DPPH (Santiago and Mayor, 2014; Tchimene et al., 2016). This process guides its use in anti-aging cosmetics because the antioxidant activity is related to the delay in aging and the healing process (Beserra et al., 2018, 2019). For acne treatment, lupeol is used by inhibiting the expression of lipogenic molecules at the transcriptional level (Kwon et al., 2015). Derivative esters of lupeol have also shown regenerative properties of the skin, such as regeneration of thermal, chemical and radiation burns (Malinowska et al., 2019).

Lupeol has already been identified in the leaves (Oliveira et al., 2015; Pereira et al., 2015; Leite et al., 2020) and in the bark of *H. speciosa* (Oliveira et al., 2015; Leite et al., 2020), with activities attributed to acetylcholinesterase inhibitor and antidiabetic. Leite et al. (2020) suggested lupeol as a possible marker for this species in the analysis by GC-MS.

Hentriacontane was identified in the S_SOX, L_US and L_SOX extracts. This substance has anti-inflammatory activity (Khajuria et al., 2017; Kim et al., 2011), which can be interesting for inflammatory skin recovery mechanisms when exposed to environmental factors, such as ultraviolet radiation, for example.

Undecanoic acid was identified only in the L_SOX extract. Muthamil et al. (2018) reported that this substance has antifungal activity against *Candida ssp*

and can be used as a microbiological preservative. Studies must be performed to verify the potential of this substance to be used in cosmetic formulations, since a good microbiological preservative must, among other characteristics, be effective in low concentrations, broad spectrum of action and low toxicity (Thompson, 2009).

Friedelan-3-one was found in the L_US extract. It has potential for use in cosmetics because of the reported antimicrobial activity against several pathogens, including *Staphylococcus aureus* (Ichiko et al., 2016), an important pathogen involved in the development of acne (Delost et al., 2016). This substance also has anti-inflammatory activity by inhibiting some inflammatory mediators, such as complement system, histamine, prostaglandins and pro-inflammatory cytokines (Antonisamy et al., 2011).

It is necessary to consider the presence of oxalic acid in S_US and L_US extracts, as this substance is irritant to the skin, causing pain (Fu et al., 2006) and dermatitis (Brans et al., 2021), even at low concentrations. Brazil (RDC 03/12) and Mercosul prohibit oxalic acid in facial cosmetics, which makes impracticable the use of these extracts.

The presence of oxalic acid, hentriacontane and Friedlan-3-one for leaves and stem of *H. speciosa* Gomes were not found in the literature, being identified for the first time in this species.

Regarding toxicity in *A. cepa* (Table 6), S_US and L_US extracts, at a concentration of 500 µg/ml, showed a significantly lower mean root length reduction than the other samples, suggesting that the proliferation *A. cepa* meristems cell count was decreased in the presence of this extract, indicating antiproliferative activity. This can be seen by the low mitotic index presented, 0.1 ± 0.0 and 0.3 ± 0.05 , respectively, for leaf and stem. The results found in the *A. cepa* assay suggest that the toxicity presented is dose-dependent, since, with the increase in concentration, there was a decrease in the growth rate.

When analyzing the RGI, it can be seen that all extracts at a concentration of 500 µg/ml and L_US extract at a concentration of 250 µg/ml showed growth of less than 80% (0.7 to 61%) in relation to the negative control (1 or 100%), which indicates potentially antiproliferative effect due to the possible cytotoxicity presented by the plant. The L_US extract 500 µg/ml showed the lowest RGI (0.1). The other extracts presented RGI between 0.8 and 1.2, with no cytotoxic potential.

Studies testing the genotoxicity of plants in the Brazilian cerrado are still rare, so studies on the toxicity of leaf and stem extracts of *H. speciosa* in the *A. cepa* model that tested chromosomal abnormalities were not found in the literature. The extracts L_US 250 µg/ml and L_US 500 µg/ml differed from the others in the mutagenicity assay, with significantly more micronuclei (Figures 5 and 6). Similar results related to the low percentage of micronuclei and chromosomal abnormalities were found in the different latex concentrations of *H. speciosa* tested by Ribeiro et al. (2016). Therefore, according to this analysis, the extracts studied are unlikely to trigger cytotoxic, genotoxic and mutagenic processes, which is ideal for the cosmetic industry.

Low mortality of nauplii was observed in *A. salina* test (Figure 8). According to Rodríguez-Magaña et al. (2019), the high survival rate suggests that in these concentrations, the use of extracts does not appear to be potentially toxic.

It is still possible to verify that, both in the *A. cepa* test and in the *A. salina* test, S_SOX and L_SOX extracts showed lower toxicity, being more suitable for the production of cosmetics, while the extracts obtained by ultrasonic-assisted extraction were the most toxic among the tested extracts. The most pronounced toxicity of the S_US and L_US extracts in the *A. cepa* and *A. salina* assays may be related to the presence of the oxalic acid compound identified in the GC-MS assay.

Few studies have been performed in order to determine the toxicity of mangabeira extracts. Leite et al. (2020) also found low toxicity for *H. speciosa* leaf extracts; however, no studies have been found in the literature about the low toxicity of leaf and stem extracts in *A. cepa* models, emphasizing the importance of this study.

The results show that the extracts of the leaf and stem

of *H. speciosa* obtained by Soxhlet apparatus have greater application for cosmetology, validating the use of this plant from the Brazilian cerrado to produce of green cosmetics.

Conclusion

The presence of flavonoids, tannins and saponins traces a promising future for the use of extracts of this plant in cosmetics. The GC-MS analysis pointed to the presence of lupeol, hentriacontane, undecanoic acid and Friedelan-3-one, that can be used in cosmetics. Hentriacontane, undecanoic acid and Friedelan-3-one were identified for the first time in this plant. The presence of oxalic acid in ultrasonic-assisted extracts may be linked to their toxicity in test models. Thus, the safest extracts that can be used in cosmetic preparations are S_SOX and L_SOX, both at 50 and 250 µg/ml. Additional research is required to validate the use of these extracts in cosmetics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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