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Article

Phytochemical evaluation and antioxidant potential of *Echinodorus macrophyllus* extracts

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Belonging to the Alismataceae family, *Echinodorus macrophyllus*, known in Brazil as "chapéu de couro", is popular in the food industry, where it is used in teas and infusions. The objective of this study was to evaluate the active chemical compounds in the powder of *E. macrophyllus* leaves extracted by two different methods (Soxhlet [SXT] and ultrasound-assisted extraction [UAE]), quantify the total phenolic compound (TPC) and total flavonoid (TFC) content, and evaluate the antioxidant potential and larvicidal activity. The SXT extraction was the most efficient (6.05% yield). Analysis by thin-layer chromatography (TLC) and high-performance liquid chromatography with a diode array detector (HPLC-DAD) evidenced the presence of cinnamic acid derivatives, flavones, and flavanones in the extracts. The TPC was higher in the SXT extract (7.71 ± 0.05 µg GAE/mL). However, there was no significant difference in TFC. The SXT extract exhibited greater antioxidant potential according to the ferric reducing antioxidant power (FRAP) method (IC_{50} = 3.37 ± 0.45 µg/mL), while the UAE extract showed higher activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (IC_{50} = 42.16 ± 5.79 µg/mL). Both extracts were nontoxic to *Artemia salina*, suggesting the potential health benefits of this plant, which is rich in phenolic compounds and diverse pharmacological properties.

Keywords: Chapéu de couro. FRAP. Phenolic compounds. Soxhlet. Ultrasound.

INTRODUCTION

The biodiversity of native plant species in the Brazilian flora comprises over 45,000 species, many of which are used by the population for their medicinal potential. However, there are few studies has been conducted to characterize the pharmacological properties of these plants (Antunes, Arbo, Konrath, 2022). One such plant is *Echinodorus macrophyllus* (Kunth) Micheli, commonly known as Amazon sword, an aquatic plant of the Alismataceae family. In Brazil, it is known by various names including "chapéu-de-couro," "erva-do-brejo," "congonha-do-brejo," "chá-da-campanha," "chá-mineiro," "erva-de-pântano," and "erva-de-bugre", it is widely consumed as a soft drink and used as an ornamental plant (Barbosa *et al.*, 2013; Ferreira, Gonçalves, Ming, 2018). In Brazilian traditional medicine, its leaves are prepared by decoction or infusion and bottled as an anti-rheumatic; purifier; diuretic; or treatment of acute and chronic inflammatory conditions, infections, and respiratory and liver diseases (Da Silva *et al.*, 2016; Ferreira, Gonçalves, Ming, 2018).

The phytochemical characterizations of *E. macrophyllus* showed the presence of polyphenols, flavonoids, flavonols, diterpenes, triterpenes, sesquiterpenes, steroids, and xanthones (Fernandes *et al.*, 2021; Ferreira, Gonçalves, Ming, 2018). The aqueous extract of this plant exhibited an immunosuppressive effect on T cells in mice and demonstrated an antinociceptive activity mediated by both peripheral and central

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mechanisms, which was also observed for the hexane extract (Fernandes *et al.*, 2021). *In vivo* and *in vitro* studies with the ethanolic extract and the flavonoid-enriched fraction have confirmed its anti-inflammatory effects (Da Silva *et al.*, 2016). Chronic and sub chronic treatments with the ethanolic extract of *E. macrophyllus* leaves in mice revealed no mutagenic, genotoxic, or cytotoxic effects. However, it was observed that at high doses, the leaf infusion of this plant induces hepatotoxic and genotoxic effects in these animals (Antunes, Arbo, Konrath, 2022; Vaz *et al.*, 2016). Additionally, if grown in contaminated areas, *E. macrophyllus* may present higher toxicity due to the absorption of metals and metalloids for its nutrition (Barbosa *et al.*, 2013).

Another species from the Alismataceae family, prevalent in Brazil, and sharing botanical characteristics and medicinal properties similar to those of *E. macrophyllus*, is *E. grandiflorus*. A phytochemical characterization of this species demonstrated the presence of diterpenes and phenolic derivatives, as well as alkaloids, organic acids, glycosides, tannins, triterpenes, and steroids. In addition to its use in traditional medicine, *E. grandiflorus* and its extracts have pharmacological potential with anti-hypertensive, anti-inflammatory, and diuretic properties, proven by *in vivo* and *in vitro* studies described in the literature (Marques *et al.*, 2017). Secondary metabolites, mainly flavonoids and their glycosylated derivatives, have been associated with hypolipidemic, antioxidant, and anti-nitrosative properties, which can modulate local inflammatory processes, assisting in the fight against diseases such as atherosclerosis (Gasparotto *et al.*, 2019). Pereira *et al.* (2022), showed that the anti-*Zika* virus activity of *E. grandiflorus* is significant in infected SH-SY5Y neuronal cells, as it was able to reduce the viral load and cell death while maintaining cell viability. However, limited studies have evaluated the toxicological aspects of this species, and further chronic toxicological investigations are needed to establish the risks of excessive ingestion of preparations from this plant (Marques *et al.*, 2017).

The objective of this study was to evaluate the active chemical compounds in the powder of *E. macrophyllus* leaves extracted by two different methods (Soxhlet [SXT] and ultrasound-assisted extraction [UAE]), quantify the total phenolic compound (TPC) and total flavonoid (TFC) content, and evaluate the antioxidant potential and larvicidal activity.

MATERIAL AND METHODS

Echinodorus macrophyllus extraction

The powdered leaves of *E. macrophyllus* were purchased commercially from Mader Comercial e Importadora Química e Farmacêutica Ltda® (Capivari/SP/Brazil). The present study has access permission for the components of plant genetic heritage registered in the SisGen Platform (Registration A5E3BA2), in accordance with the Brazilian Biodiversity Law (13.123/2015).

The extracts were obtained using 30 g of dry powdered leaves extracted with 300 mL of 50% hydroethanolic solution (1 g/10 mL). Two different extraction methods were used: Soxhlet (SXT) or ultrasound-assisted extraction (UAE), allowing for a comparative analysis of their extraction potential. The plant material was extracted in SXT apparatus for 3 h consecutively with ethanol. In the UAE, the plant material was extracted with ethanol followed by 25 min of cavitation and 25 min of resting, consecutively for a period of 3 h. After the extraction process, both extracts were taken to the rotary evaporator at a temperature of 21 ºC to reduce the solvent volume, and then were placed in an oven for complete drying until mass stabilization is achieved.

Phytochemical screening

The SXT and UAE extracts were subjected to phytochemical screening to verify the presence of secondary metabolites: terpenes/steroids, saponins, coumarins/anthraquinones, flavonoids, alkaloids, and tannins (Matos, 2009; Silva, Miranda, Conceição, 2010). Specific reagents were used for each test (Table I). For the triterpene and steroid test, a small aliquot of the dried extract was added to test tube, while for other tests, 1 mL of the stock solution (10 mg of the extracts solubilized in 10 mL of 96% ethanol) was pipetted into the test tubes.

TABLE I - Secondary metabolites reaction test

Test	Metabolites	Reagents or Methods
1	Terpenes/steroids	Liebermann Burchard test (Sulfuric acid and glacial anhydride)
2	Saponins	Foam test
3	Coumarins/anthraquinones	NaOH 1 mol/L
4	Flavonoids	Sulfuric Acid
5	Alkaloids	Dragendorff Reagent A and B
6	Tannins	Ferric chloride

Thin-layer chromatography (TLC)

To confirm the phytochemical screening, the SXT and UAE extracts were analyzed by thin-layer chromatography (TLC), performed on Merck silica gel 60 F_{254} aluminum plates. The presence of coumarins, flavonoids, and alkaloids was assessed using eluents and developers as described in the literature (Wagner, Bladt, 1996). For the detection of phenolic acids and flavonoids, rutin, quercetin, and chlorogenic, caffeic, cinnamic, and gallic acids were used as standards (Sigma, St. Louis, USA). Compound determination was performed by comparing the retention factor (RF) of samples and standards, as well as with data from the literature (Wagner, Bladt, 1996).

High performance liquid chromatography with a diode array detector (HPLC-DAD) analysis

The HPLC-DAD analysis of extracts was performed using the UFLC Proeminence chromatographic system (Shimadzu, Kyoto, Japan). This system included a binary pump system (LC-20AD), DAD detector (SPD-M20A), autosampler (SIL 20AHT), communicator (CBM-20A) and degasser, controlled by LabSolutions software (version 1.25, Shimadzu, Kyoto, Japan). The column used was Kinetex C18 (5 µm, 100 mm x 2.1 mm, Phenomenex), eluated using methanol (solvent B) and a formic acid solution (0.1%). The gradient profile was: 0-8.5 min: 10-90% of B; 9-10 min: 90% of B and 10-11 min: 10% of B. Prior to injection, all samples (rutin, cinnamic acid, and extracts) were solubilized (1 mg/mL) and filtered through a 0.45 µm PTFE filter syringe. The injection volume was 10 µL of samples and the flow rate was 0.5 mL/min. Spectra were recorded in the range of 200 to 600 nm. The compounds were characterized by comparing their retention times and UV spectra with standards and relevant literature data (Da Silva *et al.*, 2016; Sakakibara *et al.*, 2003).

Total phenolic content (TPC)

The total phenolic content of *E. macrophyllus* extracts using Folin-Ciocalteu reagent was performed as described by Mano-Sousa *et al.* (2021), with adaptations. A volume of 2,250 µL of Folin-Ciocalteu solution (1:4 v:v) was added to 250 µL of the samples, followed by the addition of 250 µL of sodium carbonate solution. After vigorous agitation, the solutions were allowed to stand for 30 min at 25 °C, and protected from light. Absorbance was measured at 750 nm using a spectrophotometer (Thermo Scientific Genesys 10S, USA), with the sample blank, as well as the standard solution and the samples. Gallic acid was used as the reference compound, and the total phenolic content was expressed in micrograms of gallic acid equivalents (GAE) per milliliter (mL). All assays were performed in triplicate.

Total flavonoid content (TFC)

The total flavonoid content of *E. macrophyllus* extracts was estimated according to the aluminum chloride

method used by Da Costa *et al.* (2015), with adaptations. A volume of 1900 µL of ethanol (50%, v:v) was added to 100 µL of extracts, along with 500 µL of aluminum chloride solution. After stand for 30 min, the absorbance was measured at 425 nm using a spectrophotometer (Thermo Scientific Genesys 10S, USA). Quercetin was used as the reference substance to produce a standard curve, and the TFC was expressed as micrograms of quercetin equivalents (QE) per milliliter (mL). All tests were performed in triplicate.

DPPH scavenging activity

The radical scavenging ability of the extracts was evaluated based on reaction with DPPH, using the standards, butylhydroxytoluene (BHT) and ascorbic acid (AA) (Araújo *et al*., 2013). A solution of DPPH (0.002% w:v) in ethanol was prepared, to which 75 µL of each sample (1, 10, 100, 250, and 500 µg/mL) were added to the wells in a 96-well flat-bottom plate containing 150 µL of DPPH solution. Following incubation in the darkness at 25 °C for 30 min, the absorbance was measured at 517 nm using spectrophotometer (Biotek Power Wave XS2, USA), with ethanol serving for baseline correction. The absorbance was expressed as the percentage of inhibition and was calculated using the equation (Burda, Oleszek, 2001):

Antioxidant ability (%) = [1 - (Abs control - Abs sample)/Abs control] *100

Where Abs control = uptake of the DPPH radical in ethanol and Abs sample = uptake of the extract or fractions in ethanol + DPPH. The antioxidant activity results of all samples were expressed as EC_{50}, which was defined as the concentration in (µg/mL) of the sample required to inhibit the formation of DPPH radicals by 50%. All tests were performed in triplicate.

FRAP (Ferric Reducing Antioxidant Power) assay

The assessment of antioxidant activity by the FRAP method was performed according to Urrea-Victoria *et al.* (2016), with 96-well plate adaptation (Morais *et al.*, 2022).

Extracts and standards (BHT and AA) were solubilized in ethanol at concentrations of 2, 5, 10, 15, and 30 µg/mL, and subsequently, 60 µL of each sample were pipetted into a 96-well plate, along with 240 µL of FRAP reagent (0.3 M sodium acetate buffer, pH 3.6 + 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) + 20 mM ferric chloride). Control wells were prepared by replacing TPTZ with ethanol, and after 30 min in darkness at 25 °C, the absorbance was measured at 595 nm using a spectrophotometer. The percentage of antioxidant activity was calculated according to the equation (Lagouri, Alexandri, 2013):

Fe ion reduction (%) = [(Abs sample – Abs control)/ Abs sample] *100

Where Abs control = absorbance of FRAP with ethanol and Abs sample = absorbance of FRAP sample. The effective concentration for 50% reduction of Fe (III) ions (EC_{50}) of each sample was calculated and noted in µg/mL. All test were performed in triplicate.

Artemia salina larvicide bioassay

A. salina eggs (200 mg) were incubated in 400 mL of seawater under artificial light at 28°C, pH 7-8. After 48 h of incubation, the *metanaupli* were collected with a Pasteur pipette. All samples were dissolved in DMSO and serially diluted (125, 250, 500, and 1000 µg/mL) in saline water. To each set of tubes containing the samples, 10 nauplii were added. Negative controls containing saline water and varying concentrations of DMSO (0.025, 0.050, 0.1, and 0.2%), were included in each experiment, according to methodology by Pimenta *et al*. (2003). The number of survivors were counted after 24 h. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Two different methods were used to obtain *E. macrophyllus* extracts. In the UAE method, 484.95 mg of extract was obtained, whereas the SXT method yielding 907.55 mg of extract, resulting in a global yield of 1.62% and 6.05%, respectively. These finding suggest that the SXT extraction was more efficient than UAE extraction,

as evidenced by the higher global yield of *E. macrophyllus* extract.

The SXT method involves heating and employs cyclic percolation, distillation, and solvent reuse, rendering it an exhaustive, high-efficiency process ideal for extracting organic compounds, particularly those from plant metabolism (Souza, Gasparoti, De Paula, 2022). In contrast, the UAE method involves the use of ultrasound waves to facilitate the extraction process. This method typically relies on the cavitation effect generated by ultrasound waves to enhance the penetration of solvents into the plant material, thereby facilitating the extraction of bioactive compounds (Mano-Sousa *et al.*, 2022). These operational characteristics likely responsible for the substantial difference in yield between the UAE method (1.62%) and the SXT method (6.05%).

Phytochemical screening tests confirmed the presence of secondary metabolites in the UAE and SXT extracts. The flavonoid test yielded a positive result for both extracts, but terpenes, steroids, saponins, coumarins, anthraquinones, alkaloids, and tannins were absent in the evaluated extracts. TLC analysis further revealed the presence of flavonoids and phenolic acids.

In contrast to our study, Flor *et al.* (2011) obtained the fluid extract of *E. macrophyllus* by extracting the plant material *in natura* with a mixture of distilled water and ethanol for 6 h. The authors explored different drying and treatment processes for the extract, including autoclaving, freeze-drying, and microwave irradiation. In TLC analysis, yellow-green fluorescence was observed in all samples, indicating the presence of flavonoids (Flor *et al.*, 2011).

Numerous studies have investigated the phytochemical composition and biological properties of *E. macrophyllus*, often employing HPLC analysis, a common technique used to identify compounds in plants (Da Costa *et al.*, 2015; Mano-Sousa *et al.*, 2022; Marques *et al.*, 2017). Our study suggests the presence of phenolic compounds, such as cinnamic acid derivatives and flavonoids, in both SXT and UAE extracts (Figure 1). Specifically, cinnamic acid derivatives were observed in both extracts at 254 nm. UAE showed a greater number of peaks than SXT, although the presence of a peak with the same retention time as the co-injected standard (peak 5–10.03 min) was found in both extracts. In the evaluation of the UV spectra, it was possible to observe that the compounds detected in the UAE and SXT were similar to cinnamic acid derivatives (Sakakibara *et al.*, 2003), which was corroborated by the TLC results. The only non-corresponding or low-concentration peaks in the two extracts were 1 and 2 (retention time [RT] 7.61 and 8.39 min) with $\lambda_{max} \approx 281$ (sh) and 324 nm and ≈ 291 (sh) and 330 nm, respectively. Additionally, flavonoids and some cinnamic acid derivatives were detected at 350 nm (Figure 2). The peaks at RT 9.43 and 9.96 min in both extracts have similar UV spectra to those of flavones ($\lambda_{max} \approx 268$ and 345 nm), probably derived from luteolin or apigenin (Sakakibara *et al.*, 2003). There was no evidence of the presence of flavonols in either the TLC or the UV spectra. It was noted that the peak with RT 8.96 presents characteristics in the UV spectrum similar to those of chlorogenic acid ($\lambda_{max} \approx 307$ [sh] and 329 nm).

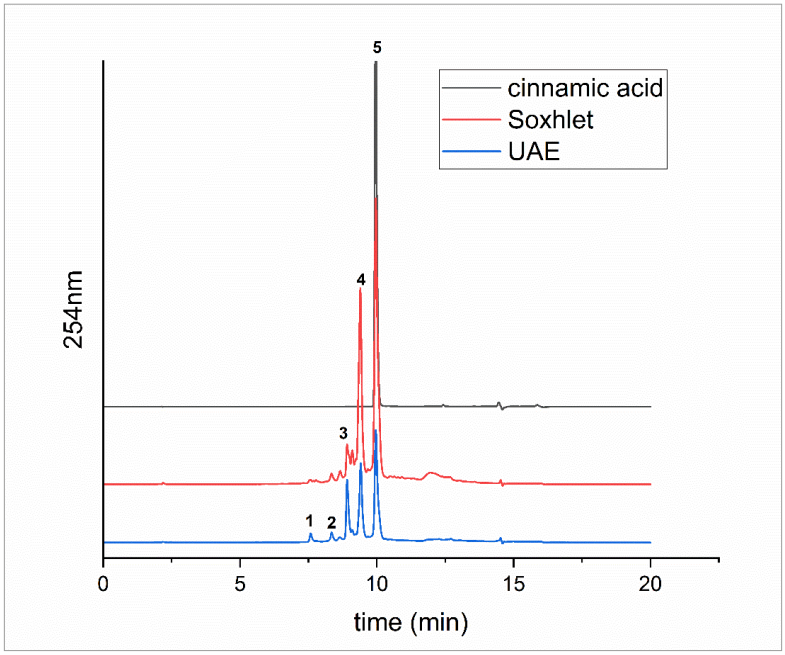

FIGURE 1 - Chromatographic profile of the *Echinodorus macrophyllus* extracts obtained by Soxhlet (SX) and ultrasound-assisted extraction (UAE) performed on HPLC-DAD at 254 nm.

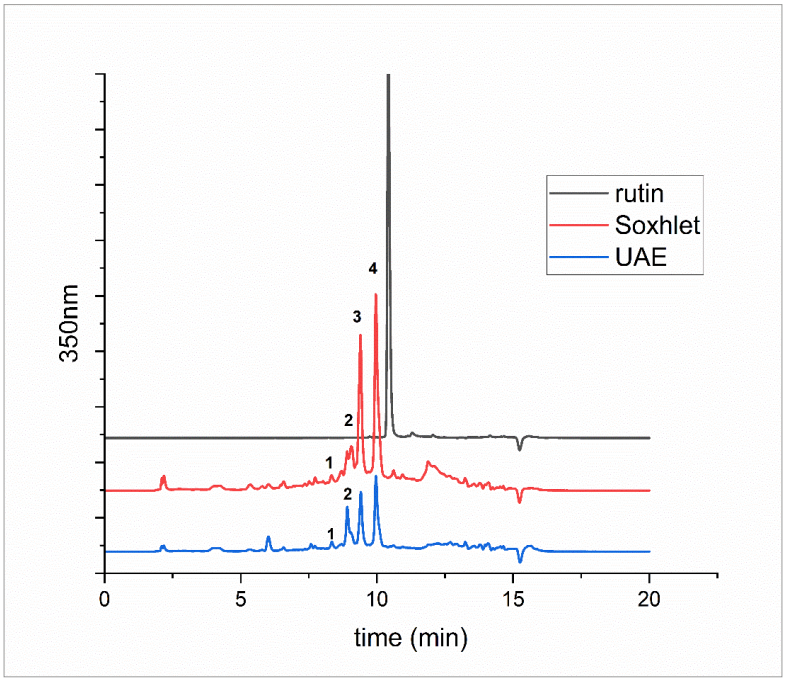

FIGURE 2 - Chromatographic profile of the *Echinodorus macrophyllus* extracts obtained by Soxhlet (SX) and ultrasound-assisted extraction (UAE) performed on HPLC-DAD at 350 nm.

Marques *et al.* (2017) reported the presence of various compounds in *E. grandiflorus*, including caffeic acid and phenolic acids, whereas Flor *et al.* (2011) characterized flavones and coumarin derivatives in *E. macrophyllus*. Our findings corroborate the literature data, rutin was absent in both extracts, as confirmed by HPLC analysis (Figure 2).

Lunardi *et al.* (2014) detected various phenolic compounds in different preparations of *E. grandiflorus* tea, including polyphenols, phenolic acids, chlorogenic acid, ferulic acid, flavonoids (vitexin), catechins, and xanthines (theobromine, theophylline, caffeine). Based on the UV spectra obtained from *E. macrophyllus* extracts, it is suggested that the extracts may contain ferulic acid, cinnamic acid, or caffeic acid derivatives.

Total phenolic compounds and flavonoids were quantified by spectrophotometry (Table II). The extracts obtained by both methods had similar levels of flavonoids, suggesting that the extraction technique did not significantly affect the content of these compounds in the extracts. However, the extract obtained by SXT showed about 1.5 times higher total phenolic compounds than that obtained by UAE, suggesting that the extraction technique can impact the content of phenolic compounds and thus antioxidant potential. In other studies, flavonoids were also found in *E. macrophyllus*, with 2.90% of flavonoids present in the plant *in natura*, and 33.50 mg equivalent of quercetin/g of leaves (Da Silva *et al.*, 2016; Flor *et al.*, 2011). The results obtained in our study verify the quality of the material used.

The antioxidant potential of the extracts of *E. macrophyllus* was evaluated using DPPH and FRAP assays. The extracts demonstrated high antioxidant potential (Table II); however, the IC_{50} values were different. The ethanol extract of UAE exhibited a lower IC_{50} in the DPPH assay, indicating a higher ability to donate electrons and neutralize free radicals. In contrast, the extract obtained by SXT had a lower IC_{50} in the FRAP assay, indicating a greater ability to reduce ferric ions.

The results obtained by DPPH assay indicate the ability of *E. macrophyllus* to eliminate oxidants, which is likely related to the presence of phenolic compounds, particularly flavonoids. Flavonoids are responsible for therapeutic activities, such as anti-inflammatory action (Santos *et al.*, 2021). Kobayashi *et al*. (2000) reported the presence of flavonoids, diterpenes, and tannins in *E. macrophyllus* leaf extracts. These compounds are known to not only have antioxidant potential but also to assist in inhibiting the enzyme systems that carry free radicals (Kobayashi *et al.*, 2000; Santos *et al.*, 2021).

TABLE II - DPPH scavenging activity, FRAP potential, IC_{50} values for the antioxidant activity, total flavonoid, and total phenolic content of the two methods of ethanol extraction of *Echinodorus macrophyllus*

Samples (µg/mL)	**SXT**	**UAE**	[1]**BHT**	[2]**AA**
DPPH-scavenging activity (%)				
1	33.86 ± 0.37[a]	34.36 ± 1.83[a]	18.50 ± 0.65	36.11 ± 0.34
10	36.83 ± 1.62[ab]	35.97 ± 0.57[ab]	25.90 ± 0.64	82.57 ± 0.23
100	48.35 ± 1.34[ab]	53.06 ± 0.22[ab]	86.00 ± 0.56	90.87 ± 0.32
250	68.17 ± 0.57[ab]	74.24 ± 0.21[ab]	91.40 ± 0.28	95.80 ± 0.43
500	89.72 ± 0.22[ab]	86.87 ± 0.22[ab]	94.02 ± 0.51	99.82 ± 0.58
EC_{50}	105.73 ± 15.42[ab]	42.16 ± 5.79 [ab]	16.36 ± 1.63	1.62 ± 0.25
FRAP activity (%)				
2	37.56 ± 1.37[ab]	16.45 ± 1.94[ab]	70.35 ± 0.82	77.10 ± 0.89
5	59.22 ± 0.27[ab]	26.07 ± 0.50[ab]	85.97 ± 0.63	93.17 ± 0.42
10	73.19 ± 0.56[ab]	38.30 ± 1.05[ab]	91.38 ± 0.32	96.48 ± 0.19
15	79.63 ± 0.46[ab]	49.13 ± 0.20[ab]	93.49 ± 0.18	100.00 ± 0.00
30	87.44 ± 0.28[ab]	64.68 ± 0.06[ab]	95.32 ± 0.10	100.00 ±0.00
EC_{50}	3.37 ± 0.45[ab]	15.95 ± 1.62[ab]	1.62 ± 0.25	0.76 ± 0.04
Total Flavonoid and Total Phenolic Content				
TFC[3]	0.34 ± 0.03	0.32 ± 0.02	-	-
TPC[4]	7.71 ± 0.05	5.07 ± 0.30	-	-

SXT: Soxhlet; UAE: Ultrasound-Assisted Extraction; BHT: 2,6-di-tert-butyl-4-methylphenol; AA: ascorbic acid. [1]EC_{50}: concentration (in µg/mL) of samples required to inhibit the formation of DPPH radicals by 50%. [2]EC_{50}: concentration (in µg/mL) of samples required to reduce [Fe (III)(2,4,6-tripyridyl-s-triazine)]$^{3+}$ by 50%. [3]TFC: Total Flavonoid Content: results expressed as µg of quercetin equivalents/mL of extract or fraction. [4]TPC: Total Phenolic Content: results expressed as µg of gallic acid equivalents/mL of extract or fraction. Each value in the table is the mean ± standard deviation (n = 3), (-) not determined. [a]$p < 0.05$ compared with BHT; [b]$p < 0.05$ compared with AA.

In addition, Garcia *et al*. (2016) studied the species *E. grandiflorus* and quantified the flavonoids (6.01 ± 0.18% w/w), indicating that they are possibly the main constituents responsible for the antioxidant potential of this species. Similarly, Prando *et al.* (2015) found that a flavonoid concentration of 102 ± 43 µg/mL was needed to capture 50% of DPPH radicals by *E. grandiflorus*. Comparison of these results with those obtained in this study using *E. macrophyllus* extracts revealed a similar antioxidant capacity of the SXT extract (105.73 µg/mL). However, the UAE extract exhibited a greater reduction of free radicals (42.16 ± 5.79 µg/mL), suggesting that the extraction method and this species (*E. macrophyllus*) may have contributed to the increase in radical scavenging.

Franco *et al*. (2018) evaluated the antioxidant and anti-glycation potential of several medicinal plants, as well as their ability to inhibit digestive enzymes related to type 2 diabetes mellitus. The results showed that, in general, the ethanolic extracts showed a higher antioxidant capacity than the hexanoic extracts. Specifically, the ethanolic extract of *E. grandiflorus* showed an antioxidant capacity greater than 75% in the DPPH test, which was similar to that of the ascorbic acid standard. However, none of the extracts exhibited antioxidant capacities

compared to ascorbic acid in the FRAP method (1714.0 ± 7.4 µmol Trolox eq/g). In this test, *E. grandiflorus* showed a result of 90.3 ± 2.3 µmol Trolox eq/g, whereas *Bauhinia forficata* Link and *Camellia sinensis* (L.) Kuntze showed the higher results, with 600.2 ± 19.7 and 761.3 ± 13.6 µmol Trolox eq/g, respectively.

Phenolic acids, such as chlorogenic acid and caffeic acid, and flavonoids, such as luteolin, apigenin, and rutin, possess antioxidant and anti-inflammatory properties. Chlorogenic acid, as well as the flavonoids luteolin and apigenin, has demonstrated the ability to reduce inflammation and pain in animal models of arthritis and osteoarthritis, as well as neuroprotective activity (Tajik *et al*., 2017; Yao *et al*., 2019). *In vivo* and *in vitro* studies have demonstrated the anticarcinogenic activity of caffeic acid, attributed to its antioxidant and pro-oxidant capacity (Espíndola *et al*., 2019). Luteolin has been associated with an anticancer effect by inhibiting cell proliferation and inducing apoptosis in tumor cells (Yao *et al*., 2019). Similarly, apigenin has been linked to antimicrobial and anticancer activity (Wang *et al*., 2019). Rutin is a flavonoid used in the treatment of varicose veins and chronic venous insufficiency due to its ability to improve blood circulation (Kawabata, Mukai Ishisaka, 2015).

In an acute cytotoxicity test using *Artemia salina*, there was no mortality at concentrations of 125, 250, and 500 µg/mL of either extract. At a concentration of 1000 µg/mL, SXT promoted a low mortality rate (8.33%), while for UAE, mortality was zero. These results confirm the low toxicity of the *E. macrophyllus* extract, consistent with literature findings. Vaz *et al.* (2016) evaluated the toxicological, genotoxic, mutagenic, and apoptotic potential of the ethanol extract of *E. macrophyllus* (at doses of 500, 1000, and 2000 mg/kg b.w.) in an *in vivo* assay with Wistar rats. The results showed no acute lethality or signs of acute toxicity (LD_{50} > 2000 mg/kg b.w.). The extract did not exhibit mutagenic or genotoxic activity in treated animals and did not induce apoptosis in the liver or kidney. However, it is important to note that the plant should be cultivated in non-contaminated areas due to the absorption of metals and metalloids during mineral nutrition, which can affect its toxicological potential (Barbosa *et al.*, 2013).

CONCLUSION

Phytochemical analysis of *E. macrophyllus* extracts revealed the presence of phenolic compounds, including flavonoids and phenolic acids. The results of the DPPH and FRAP assays indicated significant antioxidant potential, while the assay with *A. salina* demonstrated low toxicity of the extracts. Extraction by SXT showed a higher extract yield and phenolic compound content. However, further investigations are needed to evaluate the quality of the material used in potential applications in the pharmaceutical industry.

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DISCLAIMER

None.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare no use of any AI and AI-assisted technologies in the article elaboration.

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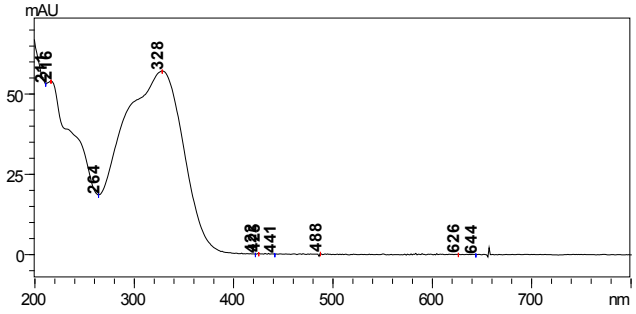
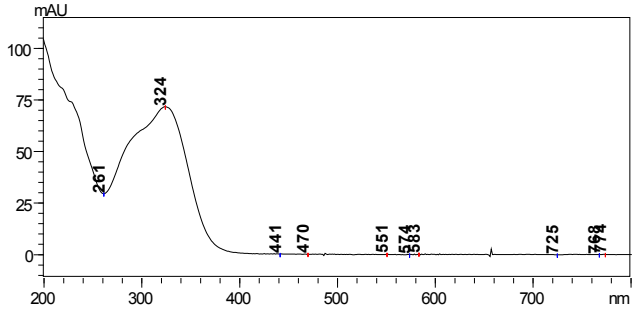
SUPPLEMENTARY MATERIAL

254 nm

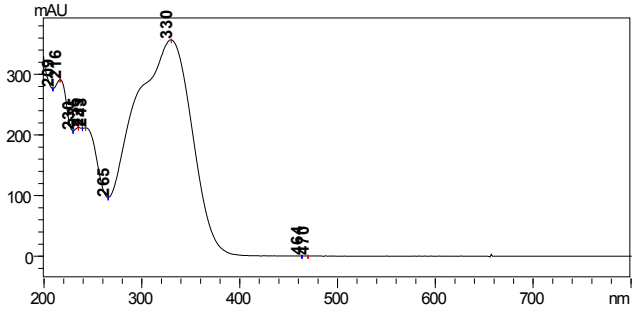
1. Min 7.617 – cinnamic acid derivative



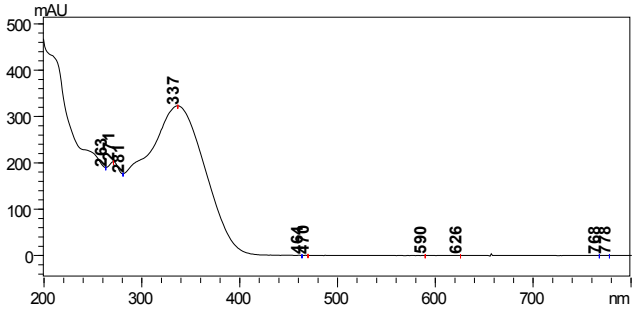
2. Min 8.389 – cinnamic acid derivative



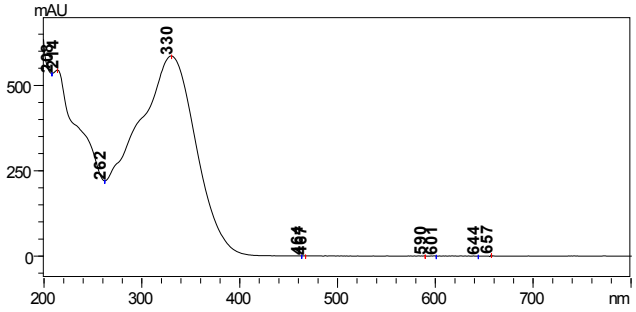
3. Min 8.893 - cinnamic acid derivative



4. Min 9.396 - cinnamic acid derivative

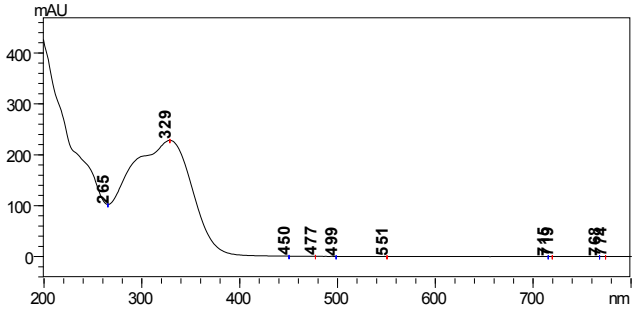


5. Min 10.034- cinnamic acid derivative

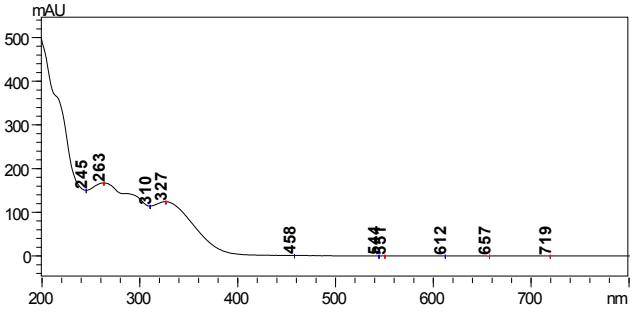


350nm

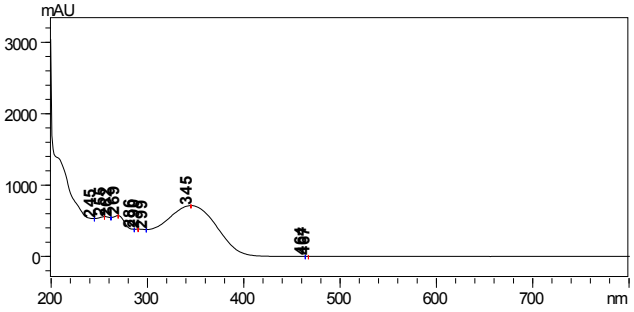
6. Min 8.960 – chlorogenic acid derivative



7. Min 9.060 – n.i.



8. Min 9.430 – flavone derivative



9. Min 9.966 - flavone derivative

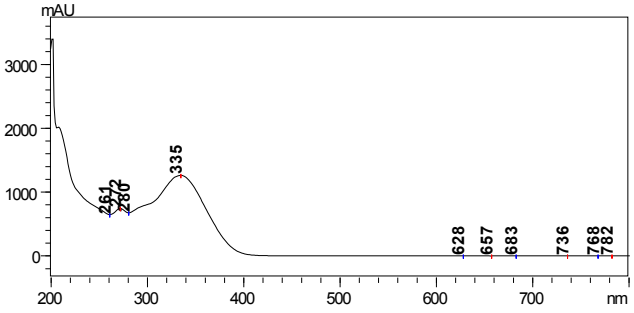


FIGURE 1S - Ultraviolet spectra of compounds detected in the extracts of the *Echinodorus macrophyllus*.