AVALIAÇÃO DO POTENCIAL ANTIOXIDANTE, HEMOLÍTICO E ANTIMICROBIANO DE EXTRATOS E FRAÇÕES DE *Monteverdia evonymoides* (Reissek) Biral

EVALUATION OF THE ANTIOXIDANT, HEMOLYTIC AND ANTIMICROBIAL POTENTIAL OF EXTRACTS AND FRACTIONS OF *Monteverdia evonymoides* (Reissek) Biral

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RESUMO:
Monteverdia evonymoides (Reissek) Biral é uma espécie que pertence ao gênero *Monteverdia,* popularmente conhecida como coração de bugre, amplamente distribuída nos biomas brasileiros. O objetivo deste trabalho foi investigar o potencial antioxidante da planta, avaliar sua segurança quanto ao uso e verificar sua atividade antimicrobiana. O material vegetal, composto por folhas e galhos secos e estabilizados, foi moído e submetido à extração em Soxhlet modificado. O potencial antioxidante foi avaliado por meio de três testes diferentes: ABTS, FRAP e DPPH. A citotoxicidade foi verificada através do teste hemolítico utilizando células sanguíneas de carneiro. A atividade antimicrobiana foi testada utilizando cepas de bactérias e fungos bem conhecidos, como *S. aureus* (ATCC® 25913), *E. coli* (ATCC® 25922), *P. aeruginosa* (ATCC® 27853) e *C. albicans* (ATCC® 14053). Os resultados mostraram que tanto o extrato quanto as frações de *M. evonymoides* apresentaram uma capacidade antioxidante significativa. No entanto, não foram observados efeitos citotóxicos sobre os glóbulos vermelhos de carneiro, indicando que a planta é segura para uso em termos de toxicidade celular. Por outro lado, a atividade antimicrobiana não foi detectada nas condições testadas, sugerindo que a planta pode não ser eficaz contra as cepas microbianas utilizadas neste estudo. Os resultados obtidos indicam que *Monteverdia evonymoides* é uma espécie promissora devido ao seu potencial antioxidante significativo e à ausência de citotoxicidade. Esses achados fazem da espécie um alvo seguro e interessante para futuros estudos e aplicações terapêuticas, especialmente na área de antioxidantes naturais. No entanto, mais pesquisas são necessárias para explorar outras possíveis atividades biológicas e confirmar a segurança do uso prolongado da planta
Palavras chaves: *Monteverdia evonymoides*. Metabólitos secundários, Toxicidade.

ABSTRACT
Monteverdia evonymoides (Reissek) Biral is a species belonging to the genus *Monteverdia*, commonly known as coração de bugre, widely distributed across Brazilian biomes. The objective of this study was to investigate the plant's antioxidant potential, evaluate its safety for use, and verify its antimicrobial activity. The plant material, consisting of dried and

stabilized leaves and branches, was ground and subjected to extraction using a modified Soxhlet apparatus. The antioxidant potential was assessed using three different tests: ABTS, FRAP, and DPPH. Cytotoxicity was evaluated through a hemolytic test using sheep blood cells. The antimicrobial activity was tested using well-known bacterial and fungal strains, such as *S. aureus* (ATCC® 25913), *E. coli* (ATCC® 25922), *P. aeruginosa* (ATCC® 27853), and *C. albicans* (ATCC® 14053). The results showed that both the extract and fractions of *M. evonymoides* exhibited significant antioxidant capacity. However, no cytotoxic effects were observed on sheep red blood cells, indicating that the plant is safe for use in terms of cellular toxicity. On the other hand, antimicrobial activity was not detected under the conditions tested, suggesting that the plant may not be effective against the microbial strains used in this study. The results indicate that Monteverdia evonymoides is a promising species due to its significant antioxidant potential and lack of cytotoxicity. These findings make the species a safe and interesting target for future studies and therapeutic applications, especially in the area of natural antioxidants. However, further research is necessary to explore other possible biological activities and to confirm the safety of prolonged use of the plant in humans**Keywords**: Monteverdia evonymoides. Secondary metabolites, Toxicity.
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1. INTRODUCTION

Polyphenols (flavonoids and tannins) are widely distributed in the plant kingdom and are associated with therapeutic effects (NEGRI; POSSAMAI; NAKASHIMA, 2009). The antioxidant action of phenolic compounds is related to the oxidation-reduction power, thus acting as reducing agents, hydrogen donors, enzyme inhibitors and can also eliminate free radicals (GAWLIK-DZIKI et al., 2013). In view of the above, phytochemical and toxicological studies involving sensitive, practical and reliable methods become indispensable tools in this search, which aims to identify plants with potential antioxidant capacity, biological activity and that are safe in terms of toxicity (BEDNARCZUK et al., 2011).

The genus *Monteverdia* has 49 species distributed in the Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa and Pantanal (FLORA E FUNGA DO BRASIL, 2022). Species belonging to the genus are widely used in folk medicine, such as: *Monteverdia ilicifolia* (Mart. ex Reissek) Biral and *Monteverdia aquifolia* (Mart.) Biral (MARIOT; BARBIERI, 2008; FLORA E FUNGA DO BRASIL, 2022) traditionally used by Indians, in the preparation of medicinal teas and infusions, against gastric disorders (VELOSO et al.,2017). Reports in the literature describe the occurrence of numerous biological activities attributed to secondary metabolites, such as flavonoids, triterpenes and tannins in other species of the genus *Monteverdia* (MARTINS et al., 2021), including analgesic and antiulcerogenic activities (VELOSO et al. , 2017), larvicidal effect against *Aedes aegypti* (DOS ANJOS, et al., 2023), allelopathic effect (DIAS et al., 2005), anti-inflammatory effect (MOTA et al., 2008,

MARTINS et al., 2012) and may be related to the presence of antioxidant compounds (DOS ANJOS, 2024).

Monteverdia evonymoides (Reissek) Biral is a species that belongs to the genus *Monteverdia*, popularly known as buggy heart (FLORA E FUNGA DO BRASIL, 2022). Its investigation is scarce in the literature. Considering the biological potential described in the genus *Monteverdia*, the present study aimed to verify the antioxidant potential, cytotoxicity, and antimicrobial activity of extracts and fractions of *M. evonymoides.*

2. MATERIAL AND METHODS

The collection of leaves and stems of the species was carried out in the city of Curitiba, Paraná, Brazil (25°26`55``S, 49°14`22``W). The identification was carried out by Forestry engineer Inti Souza. An exsicata was produced and deposited in the Herbarium Escola de Florestas Curitiba-EFC under number EFC 19467. The study was registered with SISGEN (the body responsible for registering and authorizing the use of genetic heritage) under number A13E001.

The plant material (leaves and stem) was dried at room temperature in the shade, then crushed in a knife and hammer mill, and subjected to extraction in a modified Soxhlet (CARVALHO et al., 2009). The crude ethanolic extract was prepared with 80° GL ethanol (1:10; w/v) under continuous reflux for a period of 60 consecutive hours at 80°C. The fractions were prepared using PA grade organic solvents in increasing order of polarity (hexane, chloroform, ethyl acetate) in a modified Soxhlet apparatus using the liquid-liquid partition technique (CARVALHO et al., 2009). The extracts obtained from the total extraction were called Crude Leaves and Stem Extract (EBF)/(EBC), Hexane Leaves and Stem Fraction (FHF)/(FHC), Chloroform Leaves and Stem Fraction (FCF)/(FCC), Fraction Ethyl Acetate Leaves and Stem (FAEF)/(FAEC), Remaining Fraction of Leaves and Stem (FRF)/(FRC).

The dosage of total phenolic compounds was determined according to the methodology originally described by Singleton and Rossi (1965) with adaptations (HORNUNG et al. 2020). A diluted sample (20 µL) was mixed with 240 µL of ultrapure water and 15 µL of freshly diluted Folin-Ciocalteu reagent solution in a 96-well microplate. Then, 15 µL of sodium carbonate solution (20% w/v) was added and the samples were kept in the dark for 60 min at room temperature (25 ± 2 °C). Absorbance was measured at 725 nm on an Epoch microplate spectrophotometer (Synergy-BIOTEK, USA). Gallic acid was used as

the standard. Results were expressed as μmol of gallic acid equivalents (GAE) per 100 gr of samples (dry weight basis).

The method described by Zhishen; Mengcheng; Jianming, (1999), for the determination of total flavonoids, with minor modifications. The diluted sample (20 μL) was mixed with 90 μL of sodium nitrite in a microplate. The solution was mixed and allowed to react for 5 min. Then, 10 μL of 10% aluminum chloride solution was added to each well, and the solution allowed to react for another 5 min. Then, 90 μL of a 1 mol L−1 NaOH solution was added to the wells, and the absorbance was read at 510 nm. The measurement was compared to a catechin calibration curve, and results were expressed as milligrams of catechin equivalents (CE) per 100 gr of sample.

The ABTS•+ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical reduction method was originally described by Re et al. (1999) and adapted for microplate by Hornung et al. (2020). Stock solutions of 7 mmol L−1 ABTS solution and 2.45 mmol L−1 potassium persulfate solution were prepared and allowed to react for 16 h. at room temperature (25 ± 2 °C) in the dark. The working solution was prepared by mixing 4.0 to 4.5 mL of ABTS radical cation solution with 250 mL of distilled water to obtain an absorbance of 0.70 at 750 nm. The diluted samples (10 μL) were added to 300 μL of diluted ABTS solution and after 30 min incubation in the dark, the absorbance was read at 750 nm. Results were compared to a standard Trolox curve and expressed as μmol of Trolox equivalents (TE) per 100 gr of sample (based on dry weight).

To evaluate the antioxidant potential by DPPH•, the technique described by Brand-Williams was used; Cuvelier; Berset (1995), with modifications (HORNUNG et al., 2020). 190μL of DPPH solution (0.12 mmol/L) and 10 μL of sample (1000 μg/mL) or standard solution (Trolox – 0.00008 to 0.0016 mmol/L) were added to the microplate wells. After 30 minutes of rest in the dark, the absorbance was measured at 517nm. Trolox was used as standard ($y= -1.9879x + 2.0643$; $R^2=0.9917$).

The iron reducing antioxidant capacity method (FRAP) is based on directly measuring the antioxidant power of a given sample, including biological fluids and aqueous solutions. The test was carried out following the methodology proposed by Pulido, Bravo, Saura-Calixto (2000), being adapted for 96-well microplates (HORNUNG et al., 2020). The FRAP solution was composed of 100 mL of 300 mM acetate buffer pH 3.63 (1.87 g of anhydrous sodium acetate, 16 mL of glacial acetic acid and distilled water to make up 1 L), 10 mL of 20 mM ferric chloride and 10 mL of 10 mM TPTZ solution in 40 mM HCL. In the assay, 300 μL of FRAP solution and 10 μL of sample (1000 μg/mL) or standard solution

(Trolox – 0.00008 to 0.0016 mmol/L) were added to the wells and, after resting in the dark for 30 minutes, absorbance was measured at 570 nm. The standard was used Trolox (y= 1.0173x – 0.089; R^2=0.9918).

To verify the differences between the means in the quantification of total phenols, flavonoids and antioxidant assays, the ANOVA test was used, followed by Tukey $p<0.05$, using the statistical program Sisvar version 5.6 (SCOTT-KNOTT et al., 2001).

Hemolytic activity was conducted following the methodology described by (ASLAM et al, 2011) with modifications. The red blood cell (RBC) solution was obtained from defibrinated sheep blood cells (Laborclin®, lot 210805031). A 3 mL aliquot of homogenized blood was weighed and centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and 5 mL of ice-cold PBS solution (NaCl 58.44 g/mol, KCl 74.5513 g/mol, Na2HPO4 141.96 g/mol, KH2PO4 136.086 g/mol and distilled water) at 4°C with pH 7.4 was added, followed by centrifugation. This step was repeated three times until a clear supernatant was obtained. The final volume of red blood cells was diluted in PBS at 4°C (5g/100mL). 20 µL of the sample or controls and 180 µL of the red blood cell solution (2.5%) were added to the microtubes. Samples and controls were previously solubilized in PBS (containing 10% methanol) and diluted in PBS at concentrations of 1000, 750, 500, 250, 100 and 75 µg/mL. Rutin was the antihemolytic control, saponin was the hemolytic control. The blank consisted of 20 µL of the sample and 180 µL of PBS. After incubation with the lid open in an oven at 37°C for 30 minutes, the microtubes were placed in an ice bath for 5 minutes and then centrifuged at 3000 rpm for 5 minutes. The supernatant (100 µL) was diluted in 900 µL of PBS in a new microtube, and 200 µL of this solution was transferred to a 96-well microplate. Absorbance readings were performed at 576 nm using a microplate reader (Multiskan TM Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan), and results were expressed as percentage hemolysis.

Antimicrobial activity was performed against bacterial strains of *Staphylococcus aureus* (ATCC® 25913), *Escherichia coli* (ATCC® 25922), *Pseudomonas aeruginosa* (ATCC® 27853) and antifungal activity against strain of *Candida albicans* (ATCC® 14053). The broth microdilution technique was used, as described by Veiga et al. (2019), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012) (COCKERILL et al. 2012). The assay was conducted in sterile 96-well U-bottom microplates. Stock solutions of the extracts were prepared at a concentration of 20 mg/mL in methanol. Inocula of *S. aureus, E. Coli* and *P. aeruginosa* were prepared in 0.85% saline and adjusted to a standard turbidity of 0.5 McFarland (1.0×10^8 CFU/mL). This suspension was then diluted

1:20 (5 × 10^5 CFU/mL). Serial dilutions of the extracts (1000; 500; 250; 125; 62.5; 31.25; 15.62; and 7.8 µg/mL) were prepared in Mueller Hinton broth in a 96-well microplate (100µL). 10 µL of the bacterial suspension was added to each well. Bacterial viability (positive control) was prepared with 100 µL of MHB and 10 µL of bacterial inocula. In the negative control, the inhibitory activity of the DMSO diluent was used, in which 100 µL of 1% DMSO solution was added to 100 µL of MHB and 10 µL of bacterial inocula. The microplates were incubated in a bacteriological oven (35°C) for a period of 20 hours. Subsequently, 20 µL of 0.5% aqueous TTC solution (Triphenyl Tetrazolium Chloride – Merck, Darmstadt, Germany) was added and the microplates were reincubated for a period of 3 hours (35 °C). The *C. Albicans* inoculum was prepared in RPMI 1640 medium (Roswell Park Memorial Institute - Gibco/Invitrogem, New York, USA), following the same bacterial concentrations (CLSI, 2012), 100 µL of this preparation was inoculated into the holes of the microplates, which were incubated (35 °C) for a period of 48 hours. Then, 20 µL of 0.5% TTC was added and the microplates were incubated again for 3 hours (35 °C). Visual readings of the wells were carried out. Visualization of red color indicates absence of antimicrobial activity. To determine the MIC, the concentration of the extract that caused the inhibition of bacterial growth was considered, as observed by the absence of turbidity to the naked eye and expressed in micrograms per milliliter (µg/mL). The antimicrobial activity of the extracts was classified based on the MIC as good (< 100 µg/mL), moderate (100 to 500 µg/mL), weak (500 to 1000 µg/mL) and inactive (> 1000 µg/mL) (PESSINI et. al., 2003).

3. RESULTS AND DISCUSSION

Total Phenolics and Total Flavonoids from extracts and fractions of *M. Evonymoides* are shown in TABLE 1. The quantification of both was calculated using linear regression equations and expressed in mg equivalent of gallic acid (mg.EAG.100mg^{-1}) and mg equivalent of catechin (mg.EC. 100mg^{-1}) respectively. The total composition of phenolic compounds and flavonoids is related to the sample's ability to donate electrons or capture free radicals, therefore, it can be said that the greater the amount of phenolics and flavonoids in the sample, the greater its antioxidant capacity (SILVA et al., 2016).

TABLE 1 - CONTENT OF TOTAL PHENOLIC COMPOUNDS, FLAVONOIDS AND ANTIOXIDANT CAPACITY OF EXTRACTS AND FRACTIONS OF LEAVES AND STEM OF *M. evonymoides* FROM THE EAG, CE AND TE CURVE

Samples	TPC (mg.EAG. 100mg^{-1})	TFC (mg.CE. 100mg^{-1})	ABTS (µmol.TE. 100mg^{-1})	DPPH• (µmol.TE. 100mg^{-1})	FRAP (µmol.TE. 100mg^{-1})
EBF	653,40±13,09^{a4}	65,29±0,52^{a4}	**4065,08±9,08^{a5}**	**1886,18±6,57^{a5}**	**6250,41±61,90^{a6}**
FHF	301,84±7.,25^{a1}	60,21±0,74$^{a1\ a2}$	1573,19±7,00^{a1}	1043,40±0,68^{a1}	1264,01±65,31^{a1}
FCF	400,32±5,57^{a2}	60,58±0,64 $^{a1\ a2\ a3}$	2515,13±14,38^{a2}	1146,05±11,31^{a2}	2682,54±43,08^{a3}
FAEF	601,20±11,44^{a4}	61,62±0,16 $^{a2\ a3}$	2492,22±8,96^{a2}	1119,99±15,91^{a2}	**5983,52±36,64^{a6}**
FRF	387,48±8,66^{a2}	58,79±0,21 ^{a1}	**3384,85±36,19^{a4}**	1216,04±17,43^{a4}	3099,22±32,64^{a4}
EBC	**935,58±9,74^{a5}**	**67,98±0,75^{a5}**	**3220,65±50,32$^{a3\ a4}$**	1521,26±11,21^{a4}	**8033,00±24,00^{a7}**
FHC	506,72±11,27^{a3}	60,67±0,67 $^{a1\ a2\ a3}$	2285,31±45,47^{a2}	1193,97±3,38^{a3}	1813,39±30,63^{a2}
FCC	**933,87±11,28^{a5}**	62,46±0,62 ^{a3}	**3181,64±37,33^{a3a4}**	**2433,55±31,84^{a6}**	4041,23±66,74^{a5}
FAEC	**1553,16±8,28^{a6}**	**68,00±1,22^{a5}**	**6372,03±57,13^{a6}**	**2859,08±24,63^{a7}**	**6097,87±34,63^{a6}**
FRC	645,25±27,84^{a4}	61,51±0,53 $^{a2\ a3}$	3112,32±51,58^{a3}	1496,34±6,46^{a4}	1495,66±36,35^{a1a2}

NOTE: Results for phenolic compound content, flavonoid content and antioxidant capacity expressed as: TPC: Phenolic compound content (mg.EAG.100mg^{-1}); TFC: Flavonoid content (mg.EC. 100mg^{-1}); ABTS (µmol.TE.100mg^{-1}); DPPH• (µmol.TE. 100mg^{-1}); FRAP (µmol.TE. 100mg^{-1}). EBF: Crude Leaf Extract; FHF: Hexane Leaf Fraction; FCF: Chloroform Leaf Fraction; FAEF: Ethyl Acetate Leaf Fraction; FRF: Residual Leaf Fraction; EBC: Crude Stem Extract; FHC: Hexane Stem Fraction; FCC: Chloroform Stem Fraction; FAEC: Ethyl Acetate Stem Fraction; FRC: Stem Residual Fraction; GAE: gallic acid equivalent; EC: catechin equivalent; TE: Trolox equivalent. Results expressed as arithmetic mean and ± Standard deviation. The same letters and numbers in the same column do not differ statistically from each other at a 5% level of significance using Tukey's post-hoc ANOVA test.

It is important to highlight that more than one methodology is necessary to indicate the antioxidant capacity of a compound (MERINO, et al, 2015; OLIVEIRA, 2015; SILVA et al., 2018). The evaluation of the antioxidant capacity of M evonymoides extracts and fractions was determined using the ABTS·+, DPPH• and FRAP tests. These are methodologies based on the transfer of electrons or hydrogen atoms (SIRIVIBULKOVIT; NOUANTHAVONG; SAMEENOI, 2018). The results are presented in TABLE 01.

Phenol concentrations above 50 mg.EAG are considered high. g^{-1} and medium-high 50-30 mg.EAG. g^{-1}, medium-low 30-10 mg.EAG. g^{-1} and low <10 mg.EAG. g^{-1} (CHEW et al., 2011). According to the classification proposed by CHEW et al., (2011) and carrying out the appropriate unit conversions, it can be seen that among all the samples tested, FAEC presented the highest concentration of total phenolics (1553.16 ± 8.28 mg EAG.100mg^{-1}), followed by EBC and FCC (935.58 ± 9.74; 933.87 ± 11.28 mg EAG.100mg^{-1}), respectively. In the quantification of flavonoids, FAEC also presented the highest yield (68.00 ± 1.22 mg.EC.100mg^{-1}), followed by EBC (67.98 ± 0.75 mg.EC.100mg^{-1}). The results observed for phenolic compounds and flavonoids showed low capacity, when compared to gallic acid and catechin standards respectively. When comparing with other species of the genus, it is observed that the concentration of flavonoids and total phenolics was lower than the results observed by CANSIAN et al., (2015) for *M aquifolium, M ilicifolia, M dasyclada*, respectively being 24 ± 0.96, 50 ± 1.42 and 22 ± 1.03 mg EAG. g^{-1}, respectively (CANSIAN et al., 2015).

On the other hand, it was superior when compared to the study with ethanolic extract of M. boaria leaves, which presented 0.021 mg.EAG/gr and 0.024 mg.EQ/gr respectively (SOTO-MALDONADO et al., 2022).

The ABTS·+ (2,2'-Azino-bis (3-Ethylbenzothiazolin) 6-Sulphonic Acid) and DPPH• (2,2-Diphenyl-1-Picrylhydrazyl) capture assays are widely used to evaluate the antioxidant potential of natural products. These are methods based on the scavenging of free radicals by a proton donor substance, their quantification occurs indirectly (LINS NETO et al., 2016). They require relatively standardized equipment and provide rapid and reproducible results. Furthermore, the ABTS·+ assay is particularly interesting in plant extracts because the absorption wavelength at 734 nm eliminates color interference (DUDONNÉ et al., 2009). The FRAP (Ferric Reducing Anti-oxidant Power;) in turn, measures the antioxidant capacity directly, in this test it is possible to determine how much iron was reduced by the sample (HALVORSEN et al., 2002). Therefore, the three tests are used in the screening of synthetic chemical compounds and natural products, becoming important as a preliminary test for determining the antioxidant potential of an extract and/or fraction, or even pure substance.

The results of the antioxidant capacity according to the applied methodology can be expressed in equivalent quantity of the standard tested in the sample, for example, µmol.TE. g^{-1}, in terms of IC50 (which expresses the necessary amount of sample to reduce the initial concentration of the donor/reducing agent by 50%) and also be expressed as a percentage of inhibition (NEGRI; POSSAMAI; NAKASHIMA, 2009; CANSIAN et al., 2015). When the result is expressed in an equivalent amount of the standard tested in the sample, the higher the result found, the greater the antioxidant potential observed. When the result is expressed in IC50, the lower the result found, means that the sample has a greater antioxidant capacity. When the result is expressed as a percentage of inhibition, the closer to 100% the greater the antioxidant capacity of the sample.

In the ABTS+· capture assay, FAEC (6372.03 ± 57.13 µmol.TE. 100mg^{-1}) presented the best result among the fractions, followed by EBF (4065.08 ± 9.08 µmol.TE. 100mg $^{-1}$). Other species of the genus Monteverdia were also evaluated for their antioxidant potential using the ABTS·+ method, among which M dasyclada stands out. and *M. cassineformis* (SCHWANZ, 2012), *M. ilicifolia* (VELLOSA et al., 2006), *M. guyanensis* (LIMA; VARGAS; POHLIT, 2010), *Maytenus pedunculari*, *Maytenus procumbens, Maytenus senegalensis, Maytenus undata* (AHMED; MCGAW; ELOFF, 2013), *Maytenus royleanus* (SHABBIR; KHAN; SAEED, 2013).

In the DPPH· assay, the most significant result was observed in FAEC (2859.08 ± 24.63 µmol.TE.100mg^{-1}), demonstrating significant antioxidant potential, followed by FCC and EBF (2433.55 ± 31.84; 1886, 18 ± 6.57 µmol.TE.100mg^{-1}) and corroborate the result found in the quantification of total phenolic compounds and flavonoids.

PESSUTO, et al (2009) in their studies quantified total phenolics in *M. ilicifolia* leaves, comparing alcoholic extract, aqueous fraction and ethyl acetate fraction, correlating the results of total phenols with the antioxidant capacity, for this purpose, the authors calculated the IC50 using the DPPH· method and observed that the ethyl acetate fraction presented a higher antioxidant capacity than other extracts (PESSUTO et al., 2009). In studies conducted by NEGRI; MAY I; NAKASHIMA, (2009), with the same species, the authors dried the samples at different temperatures and subsequently quantified the IC50 using the DPPH· method. The results observed were 4.02 µg/mL at 40 ºC and 7.07 µg/mL at 80 ºC, indicating that temperature can influence the antioxidant capacity of the sample (NEGRI; POSSAMAI; NAKASHIMA, 2009).

In the FRAP assay, EBC showed promising antioxidant capacity (8033.00 ± 24.00 µmol.TE. 100mg^{-1}). The EBF, FAEC and FAEF showed statistically similar results (6250.41 ± 61.90; 6097.87 ± 34.63; 5983.52 ± 36.64 µmol.TE. 100mg^{-1}). The FRAP method was carried out with *Maytenus pyria*, which presented 190 ± 0.87 µM trolox/g in the extract (SOOBRATTEE et al., 2008). This result corroborates the result found in the present study, which showed that crude extracts of leaves and stems had a greater capacity to reduce FRAP.

TABLE 2 demonstrates that all samples presented results similar to the non-hemolytic standard (Rutin). Highlights include EBF, FHF and FAEF, which presented hemolytic activity lower than the Rutin standard at all concentrations tested. In relation to the methanol and PBS controls, all samples showed a lower % of hemolysis, demonstrating the cell protection capacity. Rutin is a flavonoid with recognized antioxidant capacity, due to its ability to scavenge free radicals, it can protect the erythrocyte, thus preventing hemolysis (ABE et al., 2014). Furthermore, hemolytic and non-hemolytic capacity is related to the presence, quantity and synergisms with other compounds present in the planar (AHMED et al., 2019).

TABLE 2 - EVALUATION OF THE HEMOLYTIC PROPERTY OF EXTRACTS AND FRACTIONS OF LEAVES AND STUMS OF *M. evonymoides*

	concentration µg/mL						
Samples	**75**	**100**	**250**	**500**	**750**	**1000**	**Controls**
EBF	4,15 ± 0,11 ^{a1}	0,80 ± 0,05 ^{a1}	1,74 ± 0,25 ^{a1}	1,44 ± 0,27 ^{a1}	3,69 ± 0,83 ^{a1}	1,92 ± 0,11 ^{a1}	
FHF	2,82 ± 0,27 ^{a1}	1,63 ± 0,25 ^{a1}	1,01 ± 0,05 ^{a1}	1,27 ± 0,11 ^{a1}	2,45 ± 0,46 ^{a1}	2,02 ± 0,11 ^{a1}	
FCF	4,01 ± 0,44 ^{a1}	3,44 ± 0,12 ^{a1}	5,18 ± 0,68 ^{a1}	1,53 ± 0,27 ^{a1}	3,41 ± 0,37 ^{a1}	3,03 ± 0,16 ^{a1}	
FAEF	1,70 ± 0,21 ^{a1}	0,37 ± 0,05 ^{a1}	0,85 ± 0,11 ^{a1}	0,85 ± 0,11 ^{a1}	1,38 ± 0,11 ^{a1}	2,13 ± 0,14 ^{a1}	
FRF	7,49 ± 1,17 ^{a1}	5,11 ± 0,49 ^{a1}	6,88 ± 0,53 ^{a1}	6,53 ± 0,33 ^{a1}	17,56 ± 1,50 $^{a1\ a2\ a3}$	10,32 ± 0,59 ^{a1}	
EBC	6,07 ± 0,16 ^{a1}	5,70 ± 0,60 ^{a1}	2,79 ± 0,40 ^{a1}	7,72 ± 0,40 ^{a1}	5,22 ± 1,55 ^{a1}	5,91 ± 0,80 ^{a1}	
FHC	2,24 ± 0,16 ^{a1}	9,53 ± 1,13 ^{a1}	5,27 ± 0,16 ^{a1}	12,62 ± 1,39 $^{a1\ a2}$	10,91 ± 1,75 $^{a1\ a2}$	6,79 ± 0,88 ^{a1}	
FCC	11,82 ± 0,70 $^{a1\ a2}$	6,39 ± 0,32 ^{a1}	3,27 ± 0,24 ^{a1}	3,83 ± 0,64 ^{a1}	7,11 ± 0,56 ^{a1}	14,45 ± 2,18 $^{a1\ a2\ a3}$	
FAEC	3,99 ± 0,16 ^{a1}	8,09 ± 0,88 ^{a1}	7,03 ± 1,46 ^{a1}	11,90 ± 0,56 $^{a1\ a2}$	8,14 ± 1,21 ^{a1}	7,51 ± 0,32 ^{a1}	
FRC	5,99 ± 0,88 ^{a1}	3,91 ± 0,40 ^{a1}	4,55 ± 0,08 ^{a1}	2,71 ± 0,16 ^{a1}	7,83 ± 0,89 ^{a1}	3,51 ± 0,70 ^{a1}	
SAPONIN	112,58 ± 21,91 ^{a5}	146,38 ± 27,32 ^{a6}	157,61 ± 57,28 ^{a6}	216,22 ± 4,89 ^{a7}	149,68 ± 21,68 ^{a6}	242,52 ± 21,56 ^{a7}	
ROUTINE	5,56 ± 0,20 ^{a1}	10,42 ± 0,05 ^{a1}	3,41 ± 0,49 ^{a1}	5,00 ± 1,12 ^{a1}	6,35 ± 1,41 ^{a1}	3,67 ± 0,10 ^{a1}	
PBS							43,85 ± 7,82 ^{a4}
METHANOL							51,73 ± 7,13 ^{a4}

LEGEND: Crude Leaves Extract (EBF), Hexane Leaves Fraction (FHF), Chloroform Leaves Fraction (FCF), Ethyl Acetate Leaves Fraction (FAEF), Remaining Leaves Fraction (FRF), Crude Stem Extract (EBC), Hexane Stem Fraction (FHC), Stem Chloroform Fraction (FCC), Stem Ethyl Acetate Fraction (FAEC), Stem Remaining Fraction (FRC); Buffer Control (PBS). NOTE: Results expressed as arithmetic mean and ± Standard deviation. Statistical difference at 5% significance level using the Tukey test. Lowercase letters and equal numbers do not statistically differ from each other.

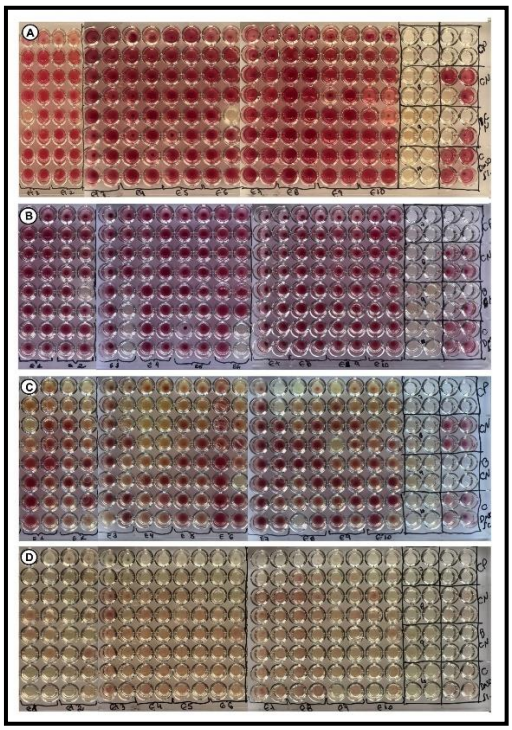
The hemolysis assay can be an important tool in the phytotoxic screening of plants and bioactive compounds, as it indicates toxicity and bioactivity (KUBLIK et al., 1996; GANDHI; CHERIAN, 2000; KALEGARI et al., 2011; COLACITE, 2015). RBC disruption is related to damage caused to the cell membrane of erythrocytes, which may involve modification of the structure of the lipid bilayer, the transport of specific ions or toxic effects that prevent control of cell volume (DE SOUZA LIMA; SOTO-BLANCO, 2010). When the sample is capable of causing red blood cell disruption in at least 50% of the erythrocytes, this concentration is considered

hemolytic in comparison to the positive control (DOS SANTOS JÚNIOR et al., 2010). Saponin (hemolytic control) has the ability to cause hemolysis, disrupting the erythrocyte membrane, even when very diluted (KARABALIEV; KOCHEV, 2003).

Other species of the genus *Monteverdia* have already been subjected to hemolysis testing, such as *M. ilicifolia*, which showed low toxicity (COLACITE, 2015). The negative result proves to be useful, as it justifies the ideal continuation of research with extracts and fractions to search for inputs with medicinal purposes. Furthermore, the antioxidant capacity observed in the present study reveals that the samples have a significant content of polyphenols and these, in turn, can contribute to cellular protection.

The antimicrobial activity of *M. evonymoides* fractions was tested against the microorganisms *S. aureus* (ATCC 25913), *E. coli* (ATCC 25922), *P. aeroginosa* (ATCC 27853) and *C. albicans* (ATCC 14053) respectively. The highest concentration tested was 1000 µg/mL. There was no inhibition at any of the concentrations tested (FIGURE 1).

FIGURE 1 – Antimicrobial and antifungal activity of extracts and fractions of *Monteverdia evonymoides*



CAPTION: A: Activity in front of *S. aureos*; B: Activity against *E. coli*; C: Activity against *P. aeruginosa*; D: Activity against *C. albicans*. e1: Crude leaf extract; e2: Hexane leaf fraction; e3: Leaf chloroform fraction; e4: Ethyl acetate leaf fraction; e5: Remaining fraction of leaves; e6: Crude stem extract; e7: Hexane stem fraction; e8: Stem chloroform fraction; e9: Stem ethyl acetate fraction; e10: Remaining stem fraction. CP: positive control; CN: negative control; B: white; 1% DMSO: negative control. Visual result of the antimicrobial and antifungal test.

However, studies related to antimicrobial activity were conducted with species of the genus. Rocha (2003) carried out a comparative study with crude methanolic extracts and fractions between two species, *M. ilicifolia* and *M. rigida*, against the strains *S. aureus, Proteus mirabilis, Micrococcus flavus, E. coli* using the disc diffusion technique, in which he evaluated the resistance and sensitivity. Only the methanolic crude extract of *M. ilicifolia* and *M. rigida* showed sensitivity to *S. aureus*. The authors attributed the activity to the triterpene class present in the samples. When evaluating antifungal activity against *C. albicans* and *C. krusei*, none of the samples were effective.

In the study conducted with *Maytenus distichophylla*, the authors carried out the test using the CIM methodology of hexane extract (EHE), chloroform extract (ECL), in addition to other fractions, against the strains *S. aureus, B. cereus, E. coli, S. typhimurium* and the yeast *C. albicans*. Among all the samples tested, only EHE was active against *B. cereus* and ECL was active against *S. aureus* only (FERREIRA, 2014). DA SILVA et al., (2018) conducted antimicrobial tests with hexane extract of *Maytenus guianensis* and reported activity against *S. aureus* and *S. pneumoniae* (0.37 µg/mL and 5 µg/mL, respectively). Both authors attribute the activity in the hexane fraction due to synergistic factors that may be present in the plant, in addition, triterpenes, widely found in the genus *Monteverdia* and present in the most nonpolar fraction (FERREIRA, 2014; DA SILVA et al., 2018).

Bruni et al (2006) followed the same methodology (MIC) with ethanolic extract of *Maytenus krukovii* bark against *Micrococcus luteus, S. aureus, Bacillus subtilis, Enterococcus foecalis, Klebsiella oxytoca, E. coli, P. aeruginosa, Proteles mirabilis* and observed negative results in all the strains tested, with the exception of weak antifungal activity against the phytopathogenic fungus *P. Ultimum* (BRUNI et al., 2006). Some factors may be attributed to the diversity of results found, such as: collection location, collection time, sample preparation, extraction method, phytochemical composition of the extracts, in addition to the synergism present in the phytochemical composition (ROCHA, 2003; RODRIGUES, 2011; SANTOS et al., 2011, FERREIRA, 2014; COLACITE, 2015; DA SILVA et al., 2018; VARGAS et al., 2020), a fact that may explain the negative result observed in the present study by the method listed.

4. CONCLUSION

Plants can present relative toxicity, and these in turn. they may be associated with varied phytochemical composition, related to intrinsic and extrinsic factors, such as

collection location, soil characteristics, harvest time, sample preparation, extraction method, in addition to the synergism present in the plant and multiplicity of species. *Monteverdia evonymoides* is a species native to Brazil, with different secondary metabolites and was shown to be safe in the present study.

The presence of flavonoids and phenolics was confirmed, the antioxidant potential was promising and did not cause hemolysis. Furthermore, data to prove the pharmacological effects are scarce in the literature. Antimicrobial activity is reported in the genus *Monteverdia*, the results were negative under pre-established conditions against the microorganisms chosen in the present study, which demonstrates that further studies are necessary to evaluate the antimicrobial potential of the species.

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