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Stryphnodendron adstringens bark extracts per solvents different: chemical quantification and antioxidant activity *in vitro* and *in silico*

Extratos de casca de *Stryphnodendron adstringens* por diferentes solventes: quantificação química e atividade antioxidante *in vitro* e *in silico*

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Plants are considered important sources of molecules with biological potential. The objective of this investigation was to identify tannic compounds, determine antioxidant activity, parameters of PH and density, as well as the contents of soluble solids and total flavonoids, in addition to the phytochemical profile by spectrophotometry in the visible ultraviolet and molecular docking simulation between the major compounds present in plant extracts of *Stryphnodendron adstringens* (Mart.) Coville. The extracts were formulated using a homogeneous powder obtained by grinding the bark of *S. adstringens*, they were formulated at 10% (w/v) by the extractive technique of dynamic maceration. The hydroethanolic, ethanolic and methanolic extract had the highest flavonoid contents. The antioxidant activity was greater than 50% for the acetonic and aqueous extracts. The visible ultraviolet spectroscopy showed the majority presence of flavonoids for all evaluated extracts. Molecular docking simulations showed the high antioxidant potential of the major phytochemicals studied, supporting the results obtained in the *in vitro* assays. Therefore, considering the inherence of the other conditions observed during the extract production process, it is inferred that the choice of certain solvents can qualitatively and quantitatively influence the obtaining of certain groups of plant metabolites with biological potential in *S. adstringens.*
Keywords: biological activity, biomolecules, flavonoids.

As plantas são consideradas fontes importantes de moléculas com potencial biológico. O objetivo desta investigação foi identificar compostos taninos, determinar a atividade antioxidante, parâmetros de pH e densidade, bem como os teores de sólidos solúveis e flavonoides totais, além do perfil fitoquímico por espectrofotometria no ultravioleta visível e simulação de acoplamento molecular entre os principais compostos presentes em extratos de plantas de *Stryphnodendron adstringens* (Mart.) Coville. Os extratos foram formulados usando um pó homogêneo obtido pela moagem da casca de *S. adstringens*, eles foram formulados a 10% (p/v) pela técnica extrativa de maceração dinâmica. Os extratos hidroetanólico, etanólico e metanólico apresentaram os maiores teores de flavonoides. A atividade antioxidante foi superior a 50% para os extratos acetônico e aquoso. A espectroscopia ultravioleta visível mostrou a presença majoritária de flavonoides para todos os extratos avaliados. As simulações de acoplamento molecular mostraram o alto potencial antioxidante dos principais fitoquímicos estudados, corroborando os resultados obtidos nos ensaios in vitro. Portanto, considerando a inerência das outras condições observadas durante o processo de produção do extrato, infere-se que a escolha de certos solventes pode influenciar qualitativa e quantitativamente a obtenção de certos grupos de metabólitos vegetais com potencial biológico em *S. adstringens*.
Palavras-chave: atividade biológica, biomoléculas, flavonoides.

1. INTRODUCTION

The plant *Stryphnodendron adstringens* (Mart.) Coville is a component of the rich family Fabaceae, is native to the Cerrado, and is endemic to the brazilian neotropical region. Adult individuals of this species reach an average of five meters in height, have a coarse trunk of approximately 30 cm in diameter, and compound or bipinnate leaves [1]. Many dies point to the potential of this plant as a source of biomolecules, especially the phenolic substances that are found abundantly in the bark, stem, and leaves of this vegetable. *S. adstringens* has been used for decades,

in traditional populations (rural communities and quilombolas) and natives as a form of treatment for genitourinary fungal infections and as an anti-inflammatory and healing agent against lesions and skin wounds and stomach ulcers [2].

The application of this vegetable by conventional populations is established through empirical use and extensive knowledge that forms the basis of ethnobotany [3, 4]. The World Health Organization stipulates that 80% of the population of underdeveloped and developing countries use some type of plant resource for the treatment of illnesses [5]. Plants are considered potential sources of molecules with biological capacity [6, 7]. These molecules come from secondary or special plant metabolism. Special metabolites do not have intrinsic value for the plant, but their evolutionary adaptive values must be considered as they act as insecticides, herbicides, and antimicrobials, helping to maintain plant integrity [8].

Plant metabolism and its primary and secondary products show high structural and functional diversity. Secondary routes produce three groups of special metabolites: phenolic compounds, terpenes, and nitrogenous substances. Of these, phenolic substances exhibit the greatest functional diversity and are found abundantly in higher plants. These substances are the most diverse, ranging from simple monocyclic phenolics to long-chain, high-molecular-weight heterocyclic compounds [9].

The most representative phenolic substances derived from plant metabolism are flavonoids (FIGURE 1) which have high anti-inflammatory, antimicrobial, and antioxidant capacity. The most representative group of these molecules are the anthocyanins, which have significant ecological value for the coevolution between plants and animals, in addition, these molecules have a high capacity to sequester reactive forms of oxygen, acting as powerful antioxidants [10]. Tannins are also important phenolic molecules because they have a high antimicrobial capacity thanks to the high potential that these molecules must bind in a non-specific way to certain microbial polymers (polypeptides/enzymes and polysaccharides) through hydrogen bonds. They also have a high adaptive value for plants because at high concentrations they reduce the digestibility of parts that are attractive to herbivores (precipitation of glycoproteins and enzymes) [11].

Therefore, in the same way that special biomolecules act actively in physiology, maintenance of integrity, and plant interactions, they can be dynamically used in animal systems to obtain a certain benefit [12]. Analytical assays, in vitro, in vivo, and in silico tests that seek to investigate the biological potential of molecules originating from secondary routes are essential for advancing the development of new therapeutic drugs.

Obtaining these molecules can be performed using different techniques and extractive methods. There are countless variables that influence the obtaining of the metabolites of interest, these variables can act quantitatively, that is, on the total number of molecules to be obtained and on which molecules can be obtained, in a qualitative way [13]. Granulometry, temperature, pressure, and solvent used in the extractive method are examples of variables and must be considered [14].

Due to the need to identify new sources with biological capacity, the objective of this research was to evaluate ethanolic, hydroethanolic, methanolic, acetone, and aqueous extracts obtained from the bark of *S. adstringens* in terms of hydrogen ion potential, density, total soluble solids content and quantitative analysis of flavonoids, as well as establishing a phytochemical parameter by visible ultraviolet spectroscopy to identify major functional groups. Was determined antioxidant activity by the radical oxidant the DPPH, antioxidant activity, study in this enzyme CPY450 was selected as a target for molecular docking studies as it accounts to produce reactive oxygen species (ROS) during metabolism and some molecules described to *S. adstringens* in literature with potential antioxidant were determined as hits.

2. MATERIAL AND METHODS

2.1 Obtaining plant material

Bark, leaf, and branch samples were collected in the rural area of Santa Rita do Sapucaí (22°17'8.082” S, 45°48'18.174” W) Minas Gerais, Brazil. The samples were used to identify the plant species based on morphological criteria. After identification, a specimen was prepared and

deposited in the Univas Herbarium of the Faculty of Philosophy, Sciences, and Letters Eugênio Pacelli of the University of Vale of Sapucaí, under the code Univas-007.

2.2 Formulation of phytoextracts

The peels were processed in a knife mill (Solab, model SL – 30) to obtain a fine and uniform powder. Hydrated ethyl alcohol 70%, absolute ethyl alcohol, absolute methyl alcohol, distilled water, and acetone were used as solvents for preparing the extractive solutions. The extractive solutions were formulated at 10% (w/v), and the extraction was performed using the extractive technique of dynamic maceration using a shaker table (Criemaq®, model C-200) at room temperature (26°C) for 48 hours at 40 rpm. After extraction, the extractive solutions were filtered and the extracts were stored in an amber bottle, protected from light and under refrigeration.

2.3 Determination of total soluble solids content

Total soluble solids were determined using the desiccation technique in an oven (7Lab®, SSA – 30L) at 55°C for 48 hours, following the method adopted by Reis et al. (2021) [15]. Aliquots of extracts were added to beakers with mass previously noted (mi), then taken to an oven for drying and after that, they had their mass measured (mf). The determination was made according to the formula: Sst = mf – mi. Where: Sst = total soluble solids content (mg/mL); mi = initial mass of the beaker (g); mf = final mass of the beaker (g).

2.4 Determination of hydrogen potential (pH)

The hydrogen ion potential was measured by solubilizing 1 g of soluble solids in 100 mL of distilled water in a volumetric flask using a properly calibrated pH meter (Kasvi model k-390014PA) [16].

2.5 Density determination

Density determination was performed according to the Brazilian Pharmacopoeia [17]. For this, samples of the dry extract were added to test tubes with annotated mass, then the test tubes were sealed and submitted to the technique of manual conformation of solids. Density was determined according to the following formula: D = mf – mi / V. Where: D = density (g/cm³); mi= mass of the test tube (g); mf = mass of the test tube + solids (g); V = volume of solids in the test tube (cm³).

2.6 Identification of tannic compounds

The identification of tannic compounds was carried out using the method of complexation reaction with ferric chloride (FeCL3) adopted by Lima et al. (2006) [18] with adaptations. In test tubes, 2 mL of extracts at a concentration of 100 mg/mL, 5 mL of absolute ethyl alcohol, and 3 mL of 2% ferric chloride aqueous solution were added. The evaluation is done visually, the blue color indicates hydrolyzable tannins, and the brown color indicates condensed tannins.

2.7 Quantification of total flavonoids

To determine the total flavonoid content, the method of complexation with aluminum chloride (AlcL3) at 10% was used, according to the method adopted by Formagio et al. (2018) [19], with modifications. A methanolic quercetin solution was used as a reference standard. The curve was constructed at concentrations of 1.5 µg/mL, 3.0 µg/mL, 6.0 µg/mL, and 12.0 µg/mL by combining 4 mL of methanolic quercetin solution with 4 mL of ethanolic quercetin solution aluminum in test tubes. The evaluation of the test group followed the same method used in the curve, however with the replacement of the reference standard by aliquots of standardized extracts at a concentration of 100 mg/mL and in triplicate. The test tubes remained at rest for 40 minutes in the dark. Readings

were performed in triplicate by spectrophotometry (BEL® model UV-M51 spectrophotometer) at 425 nm. The absorbance values obtained were used to solve the standard curve equation using the least squares method. The results were expressed in equivalent milligrams of quercetin per gram of dry extract (mg EQ/g).

2.8 Antioxidant activity

The extracts' antioxidant potential was evaluated by scavenging the free radical of DPPH (2,2-Diphenyl-1-Picrylhydrazyl) using a 0.06 mM ethanolic solution [20]. Test tubes received 100 µL of extracts, then the tubes received 3.9 mL of DPPH ethanolic solution and remained for 30 minutes in the dark. For the control group, the same method used for the test was adopted, but with the replacement of the extract by the solvent used in its production. The reading was performed by spectrophotometry at a wavelength of 517 nm. All extracts were evaluated in triplicate. To obtain the percentage of DPPH radical inhibition, the absorbance values obtained were entered in the following formula: %I = (ABS Control – ABS Sample) / ABS Control x 100. Where: %I = Percentage of DPPH radical capture; ABS Control = absorbance reading of the control solution; ABS Sample = recorded reading for the test sample.

2.9 Chemical profiling by visible ultraviolet (UV-Vis) spectroscopy

In this assay, the extracts were diluted in test tubes, obtaining ethanolic solutions at a concentration of mg/mL. The determination was made using a UV-Vis spectrophotometer (BEL® model UV-M51 spectrophotometer) between 190 nm and 400 nm with readings every 5 nm. An Excel spreadsheet was used to express the results and create linear regression graphs following the method adopted by Marcucci and collaborators [21].

2.10 Phenolic molecules of *S. adstringens* with biological potential

Aiming additional the result of antioxidant potential was realized in the literature review about the principal molecules derived from *S. adstringens* related to antioxidant capacity. Substances identified in crude extract of bark in *S. adstringens*: gallic acid, gallocatechin, epigallocatechin, catechin, and epigallocatechin gallate [22, 23].

2.11 Molecular Docking Simulations

In this study, the enzyme CPY450 was selected as a target for molecular docking studies due to its role in producing reactive oxygen species (ROS) during metabolism. The three-dimensional geometry model of the CYP450 enzyme was obtained from the Protein Data Bank (PDB), based on the crystallographic information of its active state (PDB ID: 1OG5, resolution 1.75 Å). The model was preprocessed using the AutoDockTools 1.5.6 software, which involved adding missing hydrogens, removing co-crystallized ligands and water molecules, and checking for potential structural errors, especially those related to the recognition site to be explored. Some molecules described for *S. adstringens* in the literature with potential antioxidant properties were identified as ligands. The phytochemicals were modeled and subjected to parameterization and geometric optimization by molecular mechanics, using the AM1 force field implemented in the MOPAC software. The total energies of the energetically optimized conformers were calculated using the ab initio HF/6-31G* method, and the electrostatic potential distribution was determined through the calculation of CHarges from ELectrostatic Potentials using a grid-based method (ChelpG) with the ORCA software [24].

Redocking studies were conducted prior to the simulations, using the AutoDock 1.5.6 software. To prepare the active state geometry, the co-crystallized ligand was removed. The compounds were then docked to the active enzyme with the following GridBox adjustments: X, Y, and Z coordinates were set to cover the specific active site of the macromolecule (X = -20.257, Y = 86.991, Z = 38.581). Two runs, each with fifty resulting poses, were performed to generate a set of 100 possible

conformers for analysis. All other AutoDock software parameters were kept as default in this study. The resulting docking poses were ranked according to their Score values and analyzed using the Biovia Discovery Studio Visualizer software [25]. All possible interactions and the steric complementarity obtained for the phytochemicals were analyzed considering a maximum distance of 3.0 Å for hydrogen interactions. Interactions involving electronic π systems, such as π-cation, π-stacking, and ion-dipole interactions, were also considered, according to pre-set criteria in Biovia Discovery Studio Visualizer.

3. RESULTS AND DISCUSSION

3.1 Semiquantitative and qualitative chemical characterization and antioxidant activity

The results for soluble solids and total flavonoids, as well as for density, pH, antioxidant activity, and tannic compounds are shown in Table 1. The levels of soluble solids and total flavonoids were higher in methanolic, ethanolic, and hydroethanolic extracts, respectively. The antioxidant activity was greater than 50% in the acetonic and methanolic extracts. The presence of tannic compounds was identified in all evaluated extracts, in these same extracts the pH behaved similarly.

Table 1. Results for total soluble solids content (mg/mL), density (g/cm3), pH (range), total flavonoids (mg EQ/g), tannic compounds (+/c or h) and antioxidant activity (%) of Stryphnodendron adstringens bark extracts.

Extracts	Tests					
	Soluble solids	Density	pH	Total flavonoids	Compounds tannic	Antioxidant activity
Aqueous	22.07 ± 0.02	0.90 ± 0.009	4,4	632.17 ± 0.01	+/h	32.12
Acetonic	27.59 ± 0.03	0.52 ± 0.02	4,5	884.34 ± 0.02	+/c	56.29
Ethanolic	30.46 ±0.02	0.67 ± 0.01	4,8	1051.30 ± 0.02	+/c	33.42
Hydroethanolic	28.19 ± 0.01	0.73 ± 0.01	4,1	1239.13 ± 0.02	+/c	28.75
Methanolic	30.98 ± 0.02	0.64 ± 0.01	4,1	1160.86 ± 0.03	+/c	60.01

+/c = positive reaction for the majority presence of condensed tannins; +/h = positive reaction for the majority presence of hydrolysable tannins.

Countless studies have tried to investigate the biological potential of *S. adstringens*. In addition to the antioxidant activity, the biological potential of this vegetable is also investigated in terms of its antitumor, cytotoxic, and antimicrobial properties. Eventually, all these biological potentialities are related to the ability of the metabolites from this plant to interact and act synergistically or antagonistically in certain processes and cellular aspects [26, 27]. By capturing and stabilizing free radicals (such as reactive oxygen species), phenolic molecules, such as flavonoids, can act by reducing degenerative processes that are harmful to cells, since these reactive forms can degrade important molecules for cell maintenance and control, such as the DNA. Flavonoids are important phenolics whose biological potential is very well described [28]. The aqueous extract showed a positive reaction for the presence of hydrolyzable tannic compounds, the other extracts showed a positive reaction for condensed tannic compounds. These results corroborate results obtained in other studies where the presence of tannins in extracts from *S. adstringens* is also detected. With just a few quantitative variations, the flavonoid contents found for *S. adstringens* extracts are like those found in other studies, which point to average values of 800 mg EQ/g for this species [29].

Therefore, as observed in other studies, it is considered that the solvent plays an essential role in obtaining phytochemical groups [30]. The bioprospecting of compounds with antioxidant potential was versatile since different percentages were found that varied between 28.75% (hydroethanolic) and 60.1% (methanolic). The acetonic (56.29%) and methanolic extracts were more effective in obtaining substances with antioxidant capacity, however, new studies are needed,

considering that in this research the highest antioxidant percentages are not necessarily related to the highest levels of flavonoids. Therefore, flavonoids which harbor classes of powerful antioxidants (such as anthocyanins) [31, 27] are apparently not quantitatively related to the antioxidant activity for *S. adstringens* [27, 32].

3.2 Chemical profiling by visible ultraviolet (UV-Vis) spectroscopy

Figure 1 shows the UV-Vis spectrum of the five extracts evaluated in this work, highlighting the absorbance peaks between 250 nm and 300 nm.

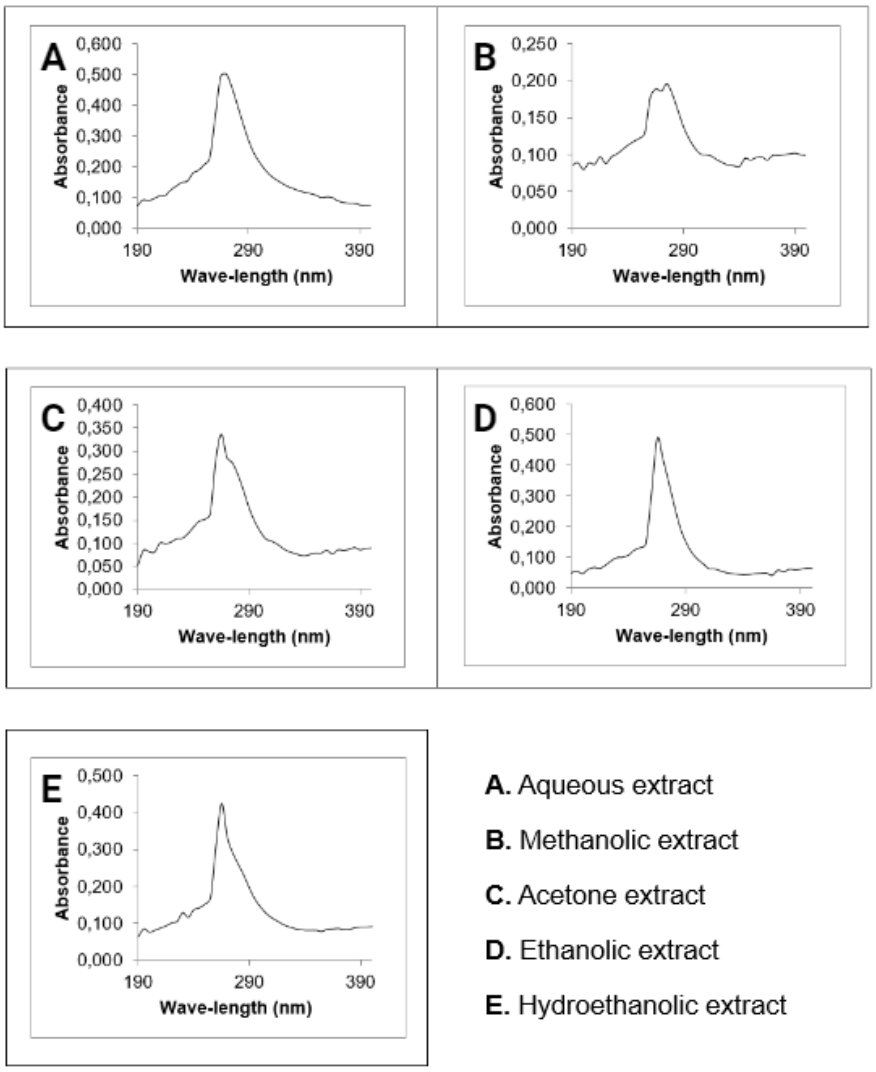

Figure 1. Phytochemical profile of the extracts.

The acetone, ethanolic, and hydroalcoholic extracts showed the highest peak of absorbance at wavelength of 265 nm, the aqueous extract showed the highest peak at 270 nm and the methanolic extract showed the highest peak of absorbance at a wavelength of 275 nm. As mentioned, the highest absorbance peaks were identified between the wavelengths of 250 nm and 300 nm, in literature peaks at 280 nm in UV-Vis Spectro are assigned the unit of gallic acid, flavan-3-ol, and proanthocyanidins [33], the picks found in this study shows the majority presence of these compounds or related compounds among the other secondary compounds that can be found for the bark of *S. adstringens.*

3.3 Molecular docking simulation

The best score values obtained for each pose, hydrogen, and hydrophobic interactions are shown in Table 2. The binding affinity is a tool used to evaluate the fitness of the ligand into the target as it results from the docking simulation.

Table 2. Interactions of CP450 target (PDB ID: 1OG5) amino acid residues with ligands at activity site.

Ligand	Score Binding affinity, ΔG (Kcal/mol)	Amino acids involved and distance (Å)	
		Hydrogen Interactions	Hydrophobic Interactions
1	-	**VAL113** (2.18), **GLY98** (2.03)	**PHE114** (4.24) **ARG97** (5.22) **ILE99** (4.82), **ALA103** (4.56), **LEU366** (5.25), **PRO367** (4.93)
2a	-10.58	**GLY98** (2.27), **PHE476** (2.10)	**PHE114** (4.56), **PHE476** (4.54), **ALA103** (4.51), **PRO367** (4.14), **PHE100** (5.33) **LEU366** (5.34), **PRO367** (4.11), **ALA103** (4.64), **LEU366** (4.58), **PRO367** (4.81)
2b	-10.72	**GLY98** (2.25), **PHE476** (2,02)	**PHE114** (4.47), **PHE476** (4.69), **ALA103** (4.48), **PRO367** (4.08), **PHE100** (5.33), **LEU366** (5.23), **PRO367** (4.23), **ALA103** (4.61), **LEU366** (4.65), **PRO367** (4.86)
3a	-10.78	**VAL113** (2.46), **PHE476** (2.13)	**PHE114** (4.45), **PHE476** (4.70), **ALA103** (4.48), **PRO367** (4.19), **PHE100** (5.33), **LEU366** (5.21), **PRO367** (4.22), **ILE99** (5.45), **ALA103** (4.37), **LEU366** (4.92), **PRO367** (4.84)
3b	-10.84	**PHE100** (2.16), **GLY98** (2.31), **VAL113** (2.42), **PHE476** (2.00)	**PHE114** (4,45), **PHE476** (4.68), **ALA103** (4.49), **PRO367** (4.12), **PHE100** (5.31), **LEU366** (5.23), **PRO367** (4.21), **ALA103** (4.45), **LEU366** (4.84), **PRO367** (4.84)
4	-10.75	**PHE100** (1.86), GLN214 (1.83), **GLY98** (2.15), **ASN217** (2.29), GLN214 (2.24), **PRO367** (3.25)	**ALA103** (4.29), **ARG97** (4.37), **ILE99** (4.44), **PHE114** (4.51), LEU208 (5.33), **ALA103** (3.32), **PRO367** (4.82)
5	-15.75	**ALA103** (1.83), **THR364** (2.02), **ASN217** (2.13), **ASN217** (2.38), **PHE476** (2.13), **GLY98** (2.21), **GLY98** (2.09)	**PHE114** (3.83), **PHE476** (4.20), **LEU366** (5.44), **PRO367** (4.10), ALA103 (4.22), **ILE99** (5.32), **ALA103** (4.52), **LEU366** (5.08)
6	-14.88	**ALA103** (2.03), **ASN217** (2.08), **ASN217** (2.41), **THR364** (2.85), **PHE476** (1.84), **VAL113** (2.35), **GLY98** (2.81)	**PRO367** (3.70), **PHE114** (3.76), **PHE476** (4.56), **ARG97** (3.62), **ILE99** (3.25), **PHE114** (3.95), **LEU366** (5.12), **ALA103** (4.19), **ALA103** (4.51), **LEU366** (4.92)
7	-14.23	**PHE100** (2.33), **ASN217** (1.96), **ASN217** (2.08), **GLY98** (1.93), **GLY98** (1.92), **VAL113** (2.59), **THR364** (2.72), **SER365** (1.92), GLN214 (3.09), **ALA103** (4.84)	LEU208 (3.82), **PHE114** (4.43), **ALA103** (3.74), **LEU102** (4.64), **PHE100** (5.32), **LEU366** (5.02), **PRO367** (4.33), **ALA103** (4.62), **LEU366** (4.70), **PRO367** (4.86), **LEU102** (5.08)
8	-10.40	**PHE476** (2.11), **THR364** (1.67), **THR364** (2.58), **GLY98** (1.80)	**PHE476** (4.23), **ALA103** (4.24), **PRO367** (5.12), **ILE99** (5.43), **ALA103** (3.64), **PRO367** (4.72), **PRO367** (4.29)
9	-10.16	**PHE100** (1.98), **ALA103** (1.76), **ASN217** (2.16), **ASN217** (2.34), **GLY98** (2.21)	**PHE100** (5.47), **ALA103** (4.16), **PRO367** (3.87), **LEU366** (3.99), **ALA103** (4.11)

According to Costa et al. (2018) [34], the active site of interaction is coated by ARG97, GLY98, ILE99, PHE100, LEU102, ALA103, VAL113, PHE114, ASN217, THR364, SER365, LEU366, PRO367 and PHE476 residues. As presented in Table 2, a high similarity of interactions was observed between the studied pharmacological target CP450 and the phytochemicals majorly present in *Stryphnodendron adstringens* extracts. These results for the tested molecules together with good binding energy values indicate a good antioxidant capacity of these molecules. All the phytochemicals studied showed great performance in the molecular docking simulation, however we selected and present in Figures 2A, 2B and 2C the three best results observed, i.e., the compounds that showed more potential to perform hydrogen interactions with the target studied.

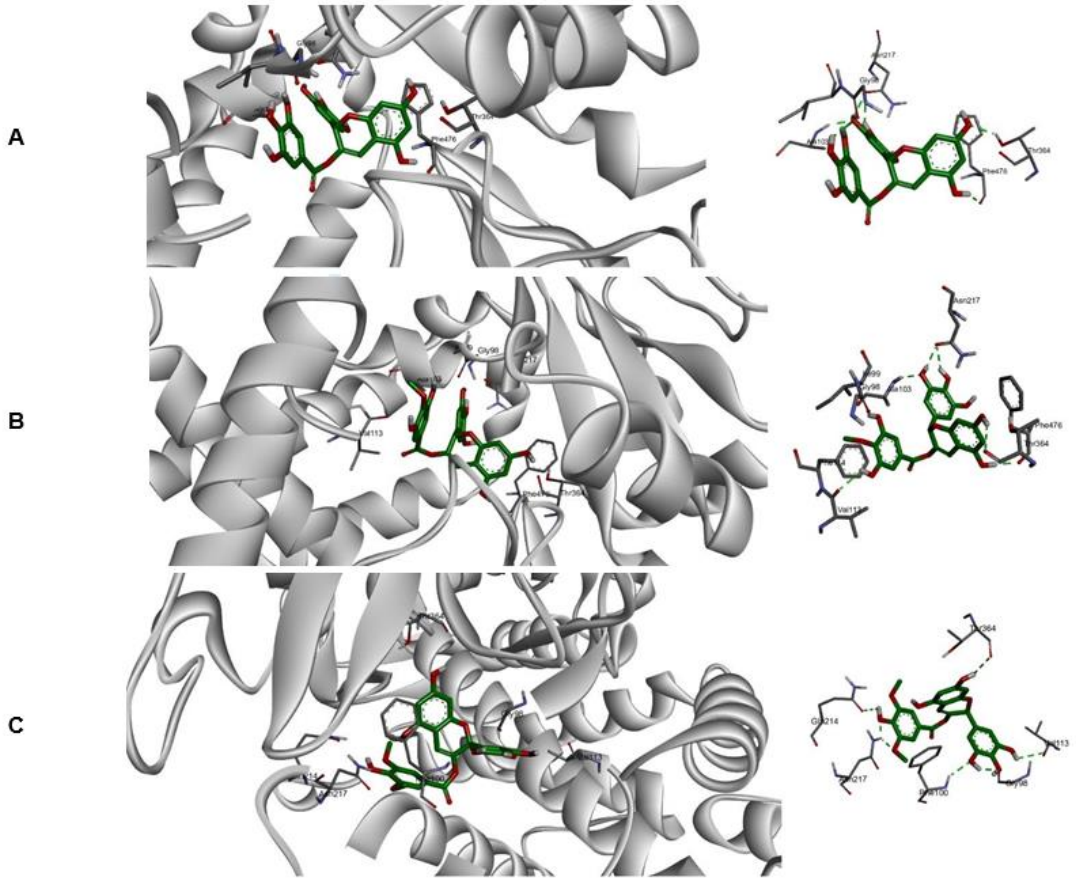

Figure 2. Best poses obtained in molecular docking simulations with compounds 5 (A), 6 (B) e 7 (C). The Left image represents the active site and highlights the residues with which the compounds were able to establish interactions. On the right are hydrogen interactions, represented by dotted lines in green. Oxygen is represented in red, nitrogen in blue, carbons in gray, and polar hydrogens in white.

Phytochemicals 5, 6 and 7, besides presenting the lowest binding affinity values, show interaction potential with residues GLY98, PHE 100, ALA103, VAL113, ASN217, THR364, SER365 and PHE476, all residues present in the active site. Compound 5 exhibits a total of seven hydrogen interactions, two interactions between phenolic hydrogens R1 and R2 of the active compound and residue GLY98. Similarly, two phenolic hydrogens of the C ring interact simultaneously with ASN217. On this same ring, there is additionally an interaction between the one phenolic oxygen and the hydrogen of ALA103. On ring A, the two phenolic hydrogens interact with THR364 and PHE476. Phytochemical 6 also showed a total of seven hydrogen interactions. Two of these interactions occur between the two phenolic hydrogens of ring A with PHE476 and THR364. On ring C three interactions are observed, two of them with one of the phenolic

hydrogens and residues ALA103 and ASN217 and one more interaction with ASN217. The hydrogens of the two R2 substituents interact with VAL113 and GLY98.

Finally compound 7, which showed the highest number of interactions, totalizing ten. On ring A only one interaction was observed between one of the phenolic hydrogens and THR364. The hydrogens of the R1 substituents interact with GLY214 and one of the R2 substituents has two simultaneous interactions with ASN217. On ring C four interactions occur, i.e., all phenolic hydrogens on this ring are amenable to interaction. Residue GLY98 interacts simultaneously with two of the three phenolic hydrogens and additionally residues VAL113 and PHE100 also showed interaction potential. Considering the better result demonstrated by compound 7, an overlapping structural comparison model was generated, which is presented in Figure 3.

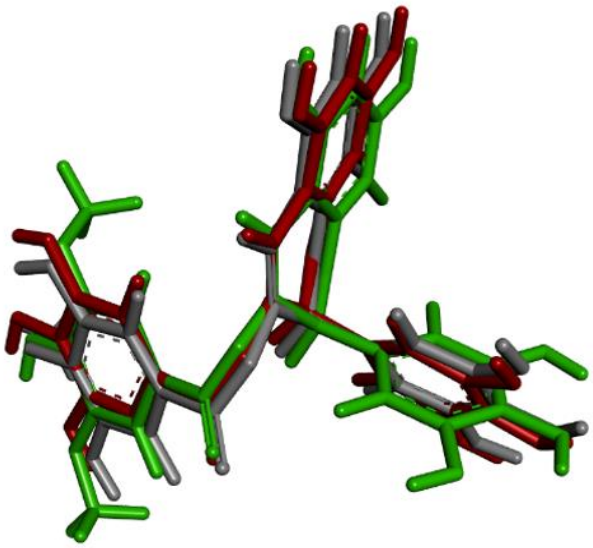

Figure 3. Superposition of the optimized three-dimensional structures of compounds 5 (red), 6 (gray) and 7 (green).

It is possible to observe that the C ring does not align with the other two C rings of compounds 5 and 6. The position of the C-ring of compound 7 seems to be essential for a higher number of hydrogen interactions to be realized, since all phenolic hydrogens showed potential interaction with the residues found in the active site.

4. CONCLUSION

The results obtained suggest that different solvents used in the extractive method influence the quantitative bioprospection of flavonoids and, therefore, on the biological capacities of the *S. adstringens* extracts such as the antioxidant potential, which was proven by the excellent performance of main phytochemicals evaluated by molecular docking against the CP450 target.

5. ACKNOWLEDGMENTS

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