



***Sapindus saponaria* (SAPINDACEAE): CHEMICAL COMPOSITION AND TOXIC EFFECT ON *Artemia salina* (ARTEMIIDAE) AND *Aedes aegypti* (CULICIDAE)**

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ABSTRACT

Purpose: Phytochemical analysis of *Sapindus saponaria* leaves extracts, evaluating their effect on *Artemia salina* and *Aedes aegypti* larvae.

Theoretical framework: Dengue is a disease transmitted by *Aedes aegypti* mosquitoes and considered a serious public health problem. Its control is done through the management of adult forms of *A. aegypti*, which occurs through the application of synthetic insecticides. However, its constant use has selected populations of insects resistant to chemical products and for this reason alternative solutions are sought, such as the use of phytoinsecticides.

Method/design/approach: Leaves were collected in areas of native vegetation, from which the ethanolic, aqueous and acetonitrile extracts were obtained, in addition to the ethanolic extract fractions, submitted to phytochemical analysis, with *A. aegypti* and *A. salina* larvae used to evaluate the toxic effect of extracts and fractions.

Results and conclusion: The ethanol extract showed the greatest diversity of secondary metabolites, the best CL90 and 100% larval mortality, negatively affecting larval time and larval and pupal mortality, in addition to decreasing the number of formed alates. The inhibitory effect is probably linked to the presence of cardiotonic saponins and heterosides, known for their deleterious action in organisms.

Research implications: The research indicates the potential use of *Sapindus saponaria* leaves in the control of the dengue vector.

Originality/value: Viability of plant strata for vector control, reducing the application of synthetic insecticides and improving environmental quality.

Keywords: Vector Control, Larvicide Activity, Dengue.

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***Sapindus saponaria* (SAPINDACEAE): COMPOSIÇÃO QUÍMICA E EFEITO TÓXICO EM *Artemia salina* (ARTEMIIDAE) E *Aedes aegypti* (CULICIDAE)**

RESUMO

Objetivo: Análise fitoquímica de extratos de folhas de *Sapindus saponaria*, avaliando seu efeito em larvas de *Artemia salina* e *Aedes aegypti*.

Referencial teórico: A dengue é uma doença transmitida pelo mosquito *Aedes aegypti* e considerada um grave problema de saúde pública. Seu controle é feito por meio do manejo das formas adultas de *A. aegypti*, o que ocorre por meio de aplicação de inseticidas sintéticos. Entretanto seu uso constante tem selecionado populações de insetos resistentes aos produtos químicos e por este motivo buscam-se soluções alternativas, tais como o uso de fitoinseticidas.

Método/design/abordagem: Folhas foram coletadas em áreas de vegetação nativa, das quais foram obtidos os extratos etanólico, aquoso e acetonitrila, além das frações do extrato etanólico, submetidos a análise fitoquímica, com larvas de *A. aegypti* e *A. salina* utilizadas para avaliar o efeito tóxico de extratos e frações.

Resultados e conclusão: O extrato etanólico apresentou a maior diversidade de metabólitos secundários, melhor CL90 e 100% de mortalidade das larvas, afetando negativamente o tempo larval e mortalidade larval e pupal, além de diminuir o número de alados formados. O efeito inibitório provavelmente está ligada a presença de saponinas e heterosídeos cardiotônicos, conhecidos por sua ação deletéria em organismos.

Implicações da pesquisa: A pesquisa indica o potencial de uso das folhas de *Sapindus saponaria* no controle do vetor da dengue.

Originalidade/valor: Viabilidade dos extratos vegetais para o controle do vetor, reduzindo a aplicação de inseticidas sintéticos e melhorando a qualidade ambiental.

Palavras-chave: Controle de Vetores, Atividade Larvicida, Dengue.

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1 INTRODUCTION

Dengue is a disease caused by viruses of the Flaviviridae family, *Flavivirus genus*, which includes four immunological types (DENV-1, 2, 3 and 4) (Brazil, 2002), transmitted by the *Aedes aegypti* L. mosquitoes (Diptera: Culicidae), widely distributed in all Brazilian regions (Catão and Guimarães, 2011). The disease is characterized as infectious, acute, febrile and with a wide spectrum of clinical manifestations, which can be serious and lethal (Brazil, 2002), which has been worrying medical health authorities due to constant epidemics, being a serious public health problem. (Mendonça, Souza and Dutra, 2009).

In this sense, dengue is considered one of the main arboviruses in the world. According to Mendonça et al. (2009), the spread of mosquitoes and viruses has led to the global resurgence of epidemic dengue and the emergence of hemorrhagic fever (a more serious form of the disease) in the last 25 years, with around 50 million infections occurring per year, 500 thousand cases of hemorrhagic fever, leading to thousands of deaths annually.

The problem is worsened by deficiencies in environmental sanitation in underdeveloped or developing countries, a common situation in several Brazilian regions (Prythoon, El-Deir, Baía, Aragão Júnior & Santana, 2022), despite basic sanitation being a right guaranteed by the Constitution Brazilian Federal (Cabral, Cintra, Araújo and Soares, 2022). In this way, the



objective was to carry out the phytochemical analysis of extracts and fractions of *Sapindus leaves saponaria* and evaluate its toxic effect on *Artemia salina* and *Aedes aegypti* larvae .

2 THEORETICAL FRAMEWORK

One of the ways to combat dengue is by controlling the adult forms of the *A. aegypti* mosquito , which occurs through the application of synthetic insecticides, such as organochlorines, organophosphates and pyrethroids (Horta, Castro, Rosa, Daniel and Melo, 2011). However, the constant use of these products has selected populations of insects that are more resistant to chemical products, making vector management more complex (Horta et al., 2011), which has led to intensified applications of insecticides (Guirado and Bicudo, 2009) , causing damage to human health and harming populations of insects beneficial to the environment, among other environmental problems (Torres et al., 2014).

Given the increase in vector resistance to the chemicals used, in addition to the problems caused by their intensive use, alternative solutions are being sought, such as the use of phytoinsecticides (Spletozer , Santos, Sanches and Garlet , 2021). For this reason, several plant species are objects of research for the population control of insect vectors of diseases, in a search for low-cost and effective active substances (Vieira, Fernandes and Andrei, 2017), such as the research carried out by Sumitha and Thoppil (2016), evaluating the use of *Ocimum essential* oil *gratissimum* L. in the control of *Aedes albopictus* larvae Skuse or Pauliquevis et al. (2021) using extracts from *Piper umbellatum* L. to control *Aedes aegypti* .

Brazilian ecosystems are known for their great biodiversity and different species used in folk medicine, for example. The Cerrado, a savanna with large areas in the Central-West region, is considered one of the most diverse savannas in the world, with flora that is still little explored (Simon et al., 2009). This biome, as it is located in an area subjected to seasonal water stress, has developed a flora with diverse adaptations to the environment (Scariot , Souza-Silva and Felfili , 2005), which provides the presence of different secondary metabolites, often a form of adaptation to the environment.

Some families are known for their characteristics of scientific interest, such as Sapindaceae , which has compounds with proven insecticidal activity (Athayde, Taketa , Gosmann and Schenkel , 2017; Vieira et al., 2017). Among the species studied is *Sapindus saponaria* L., known as soldier's soap, a tree species that reaches up to 12 meters in height, found in semi-deciduous forests in the North and Central-West regions of Brazil and commonly used as a medicinal and insecticidal plant by traditional peoples (Pott and Pott , 1994). According to Barreto (2005), the extract from its fruit peels causes morpho-histological changes in *A. aegypti* larvae and according to Athayde et al. (2017), this activity is related to the presence of saponins and steroidal or triterpene heterosides , which have already recognized hemolytic and insecticidal activity.

However, it is important not only to investigate the insecticidal action of plant extracts, but also to evaluate their toxicological potential, making their use safe in different environmental conditions. One methodology used in this process is testing using larvae of *Artemia salina* L., a small arthropod (up to 10 mm) from the crustacean group, found in continental brackish water bodies (Dumitrascu , 2011). The species is a useful tool for determining the lethal concentration (CL₅₀) of plant extracts, fractions and bioactive compounds (Sarmiento et al., 2014), in addition to being an agile and safe testing method (Queiroz, Wolfender , Hostettmann and Vieira, 2014).



3 METHODOLOGY

3.1 Collection Area

Sapindus leaves *saponaria* were collected from 10 adult specimens in a Legal Reserve area in the rural area of the municipality of Campo Grande, Mato Grosso do Sul, with a branch used to assemble the exsiccata, deposited in the Herbarium of Universidade Anhanguera-Uniderp. The collected material was transported to the Environmental Systems and Biodiversity Laboratory and the leaves were fragmented using pruning shears and dried at room temperature (27 ± 2 °C). After drying, they were crushed in an industrial mill (Tecnal Willye / TE-650, 1,725 rpm, 20 mesh) and the resulting powder stored in a hermetically closed amber glass bottle and kept under refrigeration (± 5 °C).

3.2 Preparation of Extracts

The dried leaf powder (800 g) was subjected to extraction using different solvents (ethanol, water and acetonitrile). Initially, extraction took place in an ultrasound device (Unique[®], 1450) for 60 minutes, followed by 48 hours of extraction by static maceration with each solvent separately. After maceration, the material was filtered and extracted for seven days, until the plant drug was exhausted. The solvent from each extraction step was eliminated using a rotary evaporator (Tecnal, Model MA120) to obtain the ethanolic extract (Ext_{EtOH}), aqueous extract (Ext_{H₂O}) and acetonitrile extract (Ext_{MeCN}), calculating the yield.

3.3 Chemical Fractionation

Fractionation (semi-purification of the extract by different polarities) (Cechinel Filho and Yunes, 2001) was carried out only for the ethanolic extract, the most active in preliminary bioassays with *A. aegypti* (Cavalcanti et al., 2001). A part of the extract (20.04 g) was suspended in methanol/water (80%) and subjected to liquid-liquid partition, with the solvents: hexane (hexane fraction = F_{Hex}), dichloromethane (dichloromethane fraction = F_{CH₂Cl₂}), ethyl acetate (ethyl acetate fraction = F_{AcOEt}), and n-butanol (butanolic fraction = F_{n-BuOH}), with the partition residue characterized as the hydromethanolic fraction (F_{H₂O/MeOH}).

3.4 Classical Phytochemical Analysis

The extracts (Ext_{EtOH}, Ext_{H₂O} and Ext_{MeCN}) and fractions (F_{Hex}, F_{AcOEt}, F_{CH₂Cl₂}, F_{n-BuOH} and F_{H₂O/MeOH}) were dissolved in ethanol (20%) and subjected to phytochemical analysis, through a series of characterization reactions: phenolic compounds (precipitation reaction with ferric chloride), naphthoquinone (acid/base reaction), flavonoids (cyanidin and sulfuric acid reaction), tannins (reaction with iron salts and precipitation of proteins), coumarins (KOH/ultraviolet light), triterpenes and steroids (Liebermann-Burchard reaction), cardiotonic heterosides (Baljet test and Kedde), saponins (Lieberman-Buchard reaction) and reducing sugars (Benedict reaction) (Matos, 2009).

The analyzes were carried out in triplicate and the results were compared with the control sample, observing changes in color and precipitation, with procedures based on Fontoura et al. (2015). Changes in color were classified as partial (10%), low (25%), moderate (50%), medium (75%), high intensity (100%) or negative (0%) (Fontoura et al., 2015). Tests with precipitate formation (phenolic compounds and tannins) were carried out in graduated tubes and the results were classified as partial intensity (<0.2 cm), low intensity (0.21-0.50 cm), moderate intensity (0.51-0.70 cm) and high intensity (0.71-1.0 cm) (Fontoura et al., 2015).



3.5 Visible Spectrophotometric Analysis and High Performance Liquid Chromatography (HPLC)

Confirmation of the majority constituents was carried out using the UV-visible spectrum scanning technique (FEMTO[®], 800XI), with an aliquot of 10 mg mL⁻¹ of samples dissolved in methanol (HPLC/Merck). Absorption spectra were determined in the wavelength range 190 to 750 nm, with the absorption bands compared to the literature (Silverstein, Webster, Kiemle, & Bryce, 2019).

The characterization of the most active extract and fraction was carried out using High Performance Liquid Chromatography (HPLC), using a liquid chromatograph from Shimadzu SCL - 10AVP[®] (ranges of 220, 254 and 340 nm) equipped with an LC 10AD pump and detector by ultraviolet spectrum scanning DAD SPD M10A. The chromatographic column used was the RP-18 (20 mm x 4.6 ID) from Merck[®] and pre-column RP-18 (250 mm x 4.6 ID, 5 µm). The mobile phases were composed of the solvents acetonitrile and acidified milliQ water pH 3.5 (glacial acetic acid). For the detection of polar compounds, the following programming was considered: acetonitrile = 10% (0 min), 90% (80 min) and 10% (90 min). For nonpolar compounds, H₂O = 90% (0 min), 10% (80 min) and 90% (90 min), with a sample flow of 1.0 mL/minute, considering a temperature of 40 °C and spectra analysis carried out based on literature (Marston, 2007).

3.6 Quantification of Phenolic and Flavonoid Compounds

Total phenols were quantified using the Folin-Ciocalteu Method, using gallic acid (10 to 350 mg/mL) as standard ($y = 0.781x - 0.0031$; $R^2 = 0.9959$) (Sousa et al., 2007). Flavonoids were evaluated using the Aluminum Chloride Method, using quercetin as standard ($y = 0.0088x - 0.0015$; $R^2 = 0.988$), to construct the calibration curve (Peixoto Sobrinho et al., 2008). The data were subjected to analysis of variance and, when significant, a comparison of means was carried out using the Tukey test, at 5% probability, with results processed with the aid of statistical software.

3.7 Determination of Antioxidant Activity Using the Free Radical Scavenging Method (DPPH)

The potential antioxidant activity was determined based on the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extracts and fractions were diluted to concentrations of 250, 200, 150, 100, 50 and 25 µg/mL, to which 2 mL of a DPPH solution in methanol (24 mg/100 mL of methanol) was added. After 30 minutes, the absorbance was determined using a UV-VIS spectrophotometer (wavelength 515 nm). The DPPH solution in methanol was used as a negative control and BHT (butylhydroxytoluene) as a positive control, with the same concentration used in the samples (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos and Byrne, 2006). The percentage of antioxidant activity and DPPH remaining in the reaction medium (%AA) was calculated according to Sousa et al. (2007). The determination of EC₅₀ (efficient concentration), that is, the concentration of the sample or standard that causes 50% inhibition of the initial concentration of DPPH, was obtained by linear regression. The results were subjected to analysis of variance and, when significant, a comparison of means was carried out using the Tukey test, at 5% probability, with results processed with the aid of statistical software.



3.8 Toxicological Test Against *Artemia Salina*

The toxicity assessment was developed according to the methodology of McLaughlin , Chang and Smith (1993), expressed in Average Lethal Concentration (CL_{50}), in which the extracts were diluted in DMSO (1%) and saline solution, at concentrations of 500, 250, 125, 62.5 and 31.2 $\mu\text{g}/\text{mL}$. At each concentration, 10 larvae were used, and the tests were carried out in quadruplicate, with dechlorinated water as a positive control (pH 6.5-7.0) and a negative control, rotenone solution, using test tubes containing a final volume of 5 milliliters. In all tests, the larvae were left in contact with the solutions for 36 hours and then the number of dead and alive larvae was determined, with data expressed as percentage of mortality and Toxicity Rate to *Artemia salina* (TAS).

3.9 Ineticidal Activity

Larvae in the 3rd instar of *A. aegypti* were used, cultivated in permanent colonies, at room temperature ($25 \pm 2^\circ\text{C}$) and fed with crushed feline food, whose nutritional value met the growth and maintenance needs (30% protein, 11% of lipids, 10% moisture and ash, 4% fiber, 0.8% phosphorus and 0.2-0.8% calcium) (Silva, Silva, Oliveira and Elias, 1998). Adult individuals (winged) were fed with a sugar solution (8%) and three weekly blood meals (using poultry) for 1 hour and 30 minutes to maintain female fertility and egg viability. The eggs hatched in a plastic tray containing one and a half liters of dechlorinated water (pH between 6.5 and 7.0).

The biological cycle bioassay used solutions of ethanolic, aqueous and acetonitrile extracts and 3rd instar larvae of *A. aegypti* (Silva et al., 1998). A standard solution was prepared with the extracts, pre-solubilized in DMSO and dissolved in water, in sufficient quantity to obtain a concentration of 1 mg mL^{-1} . From this solution, a series of dilutions were prepared to obtain solutions with concentrations of 500, 250, 125 and 62.5 mg/mL . 25 larvae were added to 25 mL of each solution, with bioassays carried out in quadruplicates, in addition to the control in dechlorinated water pupal mortality, with readings taken every 24 hours of exposure and a total follow-up time of 38 days. The experimental design was completely randomized, with four replications per treatment and 25 larvae per experimental unit. Data on the duration of the phases (larval and pupal) were grouped into hours and the one-way ANOVA analysis of variance test and Tukey's post test ($p < 0.05$) were applied to compare the means.

For the lethality assessment test, expressed in Average Lethal Concentration (CL_{50}), the larvae were kept in solution for 48 hours, using the proportion of one larva/ mL to 25 mL, with eight replicates, in containers covered with mesh. nylon. Dechlorinated water (pH 6.5-7.0) was used as a positive control and rotenone solution was used as a negative control at already established mortality concentrations, between 0.2 and 20.99 g/L for 10, 50 and 90% of the exposed population.

After a period of 48 hours, the number of deaths was observed through stimulation with the aid of a Pasteur pipette. The lethal concentrations (10, 50 and 90%) of the population exposed to each assay were determined using the Probit model (McLaughlin et al., 1993), using the Leora[®] software. The mortality of larvae and pupae was expressed as a percentage of mortality for each treatment, with only treatments that presented corrected mortality established between 5 and 20% being accepted as adequate (Abbott, 1925).



4 RESULTS AND DISCUSSION

4.1 Phytochemical Analysis

Analysis of the extracts (Figure 1) demonstrated that the extracting solvent that provided a greater number of classes of secondary metabolites (9 classes) was ethanol, a solvent with a polarity lower than water (7 classes) and higher than acetonitrile (7 classes). (Figure 1), which also presented the highest yield (28.8 g), higher than Ext_{H₂O} (23.8 g) and Ext_{MeCN} (10.6 g).

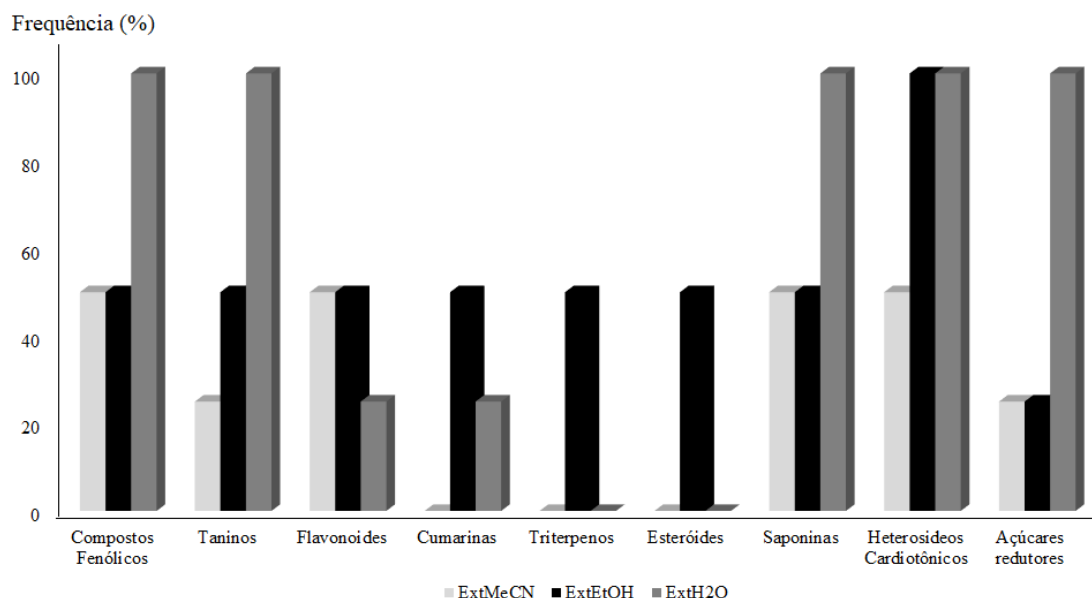


Figure 1. Classes of secondary metabolites and their frequencies in extracts Ext_{MeCN}, Ext_{EtOH} and Ext_{H₂O}, leaves of *Sapindus saponaria*.

Source : the authors.

The results indicate that the phytoconstituents have affinity with higher solvents. polarity (water > ethanol) (solutes solvate strongly through hydrogen bonds), relative to acetonitrile , lower polarity and aprotic solvent (solvates solutes through their positive and negative dipoles) (Solomons , 2012).

Regarding the frequency of metabolites (Figure 1), water proved to be a solvent that made it possible to obtain a higher frequency of phenolic compounds, tannins, saponins, cardiotonic heterosides and reducing sugars (100%), which may justify the higher yield of the Ext_{H₂O} in relation to Ext_{MeCN} . _ Ext_{EtOH} , which has the largest number of classes, presented a higher frequency of only cardiotonic heterosides (100%) , with moderate intensity for the other classes (except for reducing sugars, low intensity).

Regarding fractions, the highlight is F_{H₂O/MeOH} with 7 classes and highest frequency (100%) for phenolic compounds, saponins, cardiotonic heterosides and reducing sugars and, F_{AcOEt} , with 8 classes, highlighting phenolic compounds (100 %) (Figure 2). The other fractions presented a number of classes that varied between three and six. The results demonstrate that semi-purification with solvents of different polarities was able to separate components present in the leaves of *S. saponaria* .

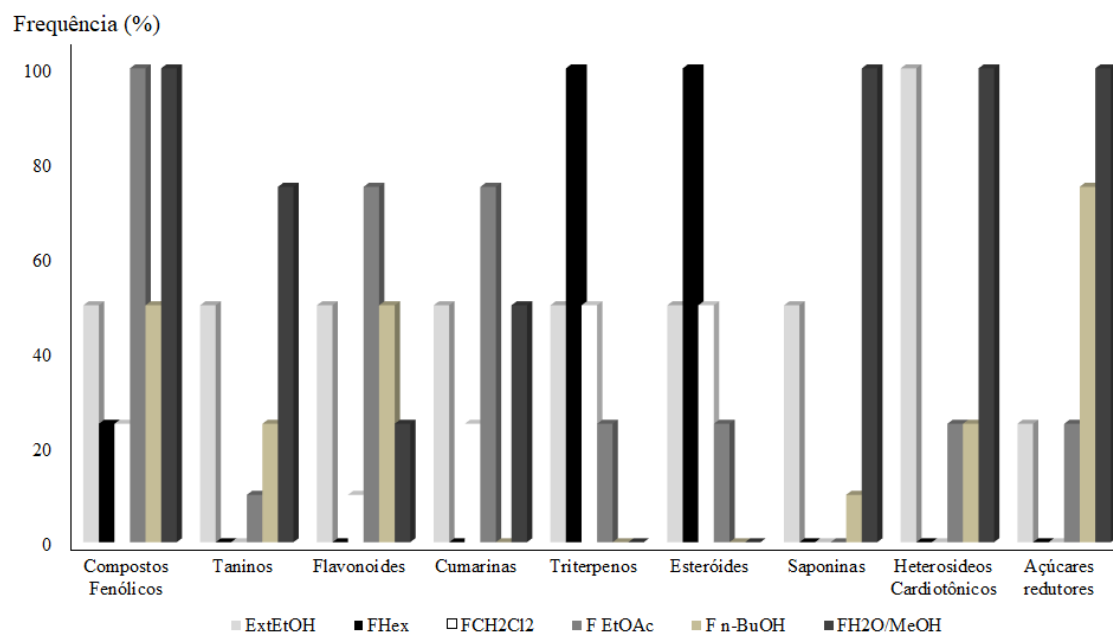


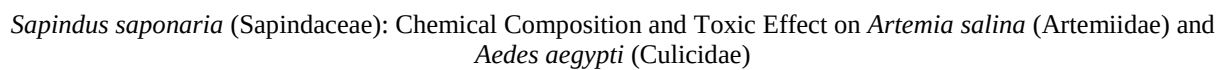
Figure 2. Classes of secondary metabolites and their frequencies in the hexane (F_{Hex}), dichloromethane (F_{CH₂Cl₂}), ethyl acetate (F_{AcOEt}), butanolic (F_{n-BuOH}) and hydromethanolic (F_{H₂O/MeOH}) fractions, *Sapindus saponaria* .

Source: the authors.

In work with fruits of the same species, Grisi, Ranal, Gualtieri and Santana (2012) identified high levels of acetylated triterpene saponins and glycosylated sesquiterpenes, while Murgu et al. (2008) and Medina, Gómez, Valencia, Jamarillo and Maya (2013), phenolic compounds, flavonoids, saponins, glycosylated sesquiterpenes and reducing sugars, demonstrating that the presence of certain secondary metabolites occur frequently in leaves and fruits. Amaral, Murgu , Rodrigues-Fo, Souza and Moura (2008) also mentioned saponins, flavonoids, tannins, triterpenes and sesquiterpenoids in the peels, indicating the presence of important metabolites in various plant structures, which justifies their use as an insecticide, among other forms. of use (Pott and Pott , 1994).

Similar results were cited by Valdés et al. (2015), investigating fruits, seeds and stems, showing the presence of saponins, tannins, flavonoids and reducing sugars, with the highest concentration of saponins found in the pericarp of the fruit. For leaves, Grisi et al. (2015) confirmed the presence of terpenes and a monoterpene acid , demonstrating the secondary metabolite production potential of *S. saponaria* . This information indicates that the species has compounds with medicinal action, justifying its popular use in combating fever , cough and rheumatism, among other diseases (Pott and Pott , 1994) .

The retention times of the molecules that emit electrical signals, recorded in the form of peaks for qualification (Figure 3), confirmed the presence of phenolic and flavonoid compounds, through bands in the region of 240 to 360 nm, characteristic of these metabolites .



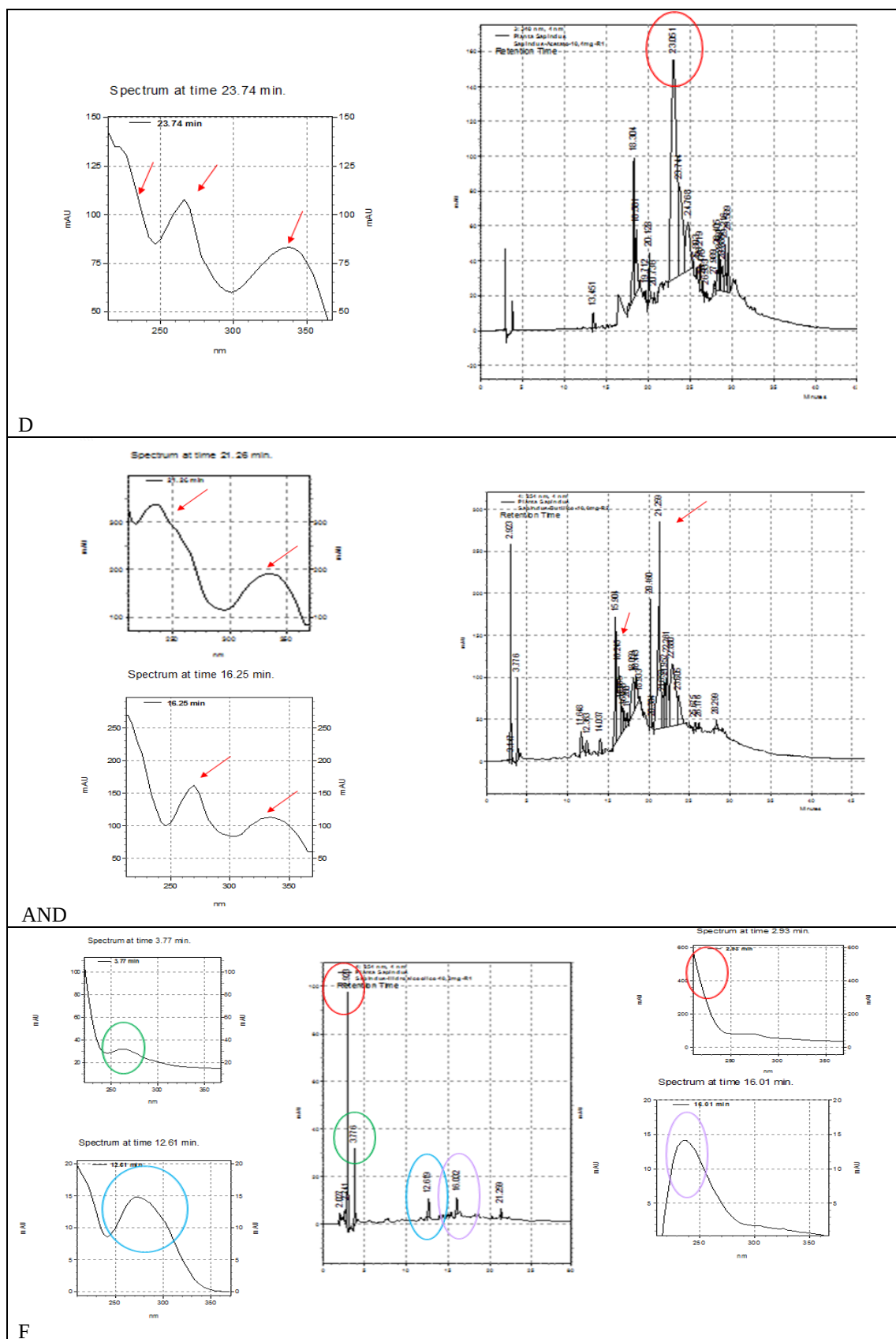


Figure 3. UV visible spectrum , HPLC chromatographic profile and maximum absorption, (A) ethanolic extract (241, 260 and 370 nm) and fractions: (B) hexane (241, 260 and 370 nm) , (C) dichloromethane (331, 220 and 250 nm) , (D) ethyl acetate (240, 260 and 340 nm) , (E) butanolic (240, 260 and 340 nm) and (F) hydromethanolic (240 nm) , *Sapindus leaves saponaria* .

Source: the authors.



The quantification of phenolic compounds and antioxidant potential of the extracts demonstrates that Ext_{H₂O} stands out, with the highest concentration of total phenols and flavonoids, which also resulted in greater antioxidant activity (capacity to scavenge free radicals) (Table 1), which would be expected.

Table 1. Quantification of total phenols, flavonoids and antioxidant activity of extracts and fractions obtained from *Sapindus saponaria* leaves

Extract/Fraction	Total phenols (mg/g)	Flavonoids (mg/g)	Antioxidant activity IC ₅₀ (µ/ mL)
Acetonitrile	87.3±0.3 and	95.1±1.1b	48.7±1.4 and
Ethanol	182.9±0.6c	95.1±2.4 b	85.2±1.2c
Aqueous	322.4±0.1 a	119.8±0.9 a	115.6±1.9 a
Hexanica	145.9±4.3d	39.2±1.9d	31.8±3.0 f
Dichloromethane	150.2±3.9d	51.6±1.6c	58.5±0.9 and
Ethyl acetate	260.1±1.2b	131.4±1.2 a	98.9±1.2b
Butanolic	177.3±3.2c	93.8±1.5b	71.4±1.2d
Hydromethanolic	259.0±1.4b	78.7±3.6b	95.1±0.4 bc
Witness	-	-	-

Mean±SD . Means followed by the same lowercase letter in the column are not statistically different from each other (Tukey test , 5% probability) .

Source: the authors.

F_{AcOEt} and F_{H₂O/MeOH} fractions also stood out, with the second best antioxidant activity, resulting from the second best concentration of total phenols and flavonoids (Table 1). The effect of plant drugs with antioxidant potential and their relationship with the presence of phenols and flavonoids is the subject of studies by different research groups, with the aim of justifying the therapeutic effect of certain medicinal plants, such as *Sapindus saponaria* (Athayde et al., 2017).

Despite its medicinal use, no data were found on the quantification of phenolic compounds and flavonoids or antioxidant activity for *S. saponaria* . However, in studies with different extracts of leaves and fruits with another species of the same genus (*Sapindus mukorossi* Gaertn .), Singh and Kumari (2015) pointed out that there is a positive correlation between antioxidant activity and polyphenolic compounds (total phenolic and flavonoid content). The authors stated that the reducers present in the extracts are the main contributors to the antioxidant potential and that these extracts could be used for pharmaceutical purposes .

Singh and Kumari (2015) also demonstrated that the maximum levels obtained for total phenols, methanolic extract of fruits (469.0±0.6 mg/g) and flavonoids, maximum content obtained in the methanolic extract of leaves (540.1±0 .9 mg/g), are higher than those found for *Sapindus saponaria* , which may be related to the species itself or environmental conditions at the collection sites, which may interfere with the presence and quantity of the compounds produced.

4.2 Toxicity to *Artemia Salina*

The tests indicated high toxicity (Table 2), as the reference standard indicates that the substance or extract that causes 50% mortality at concentrations below 0.1 mg/ mL is toxic (McLaughlin et al., 1993). For Lacerda et al. (2011), the defined lethality standards consider dosages lower than 0.08 mg/ mL as toxic values . Therefore, Ext_{MeCN} and Ext_{EtOH} are the most toxic extracts , with dosages varying between 0.02 and 0.1 mg/ mL for the lowest and highest dosages (Table 2).



Table 2. Extracts obtained from *Sapindus* leaves *saponaria* , mean dosage, confidence interval and lethal concentration against *Artemia salina*

Extracts	n	Average dosage 95% (mg/L)	Lethal concentration (CL)
Aqueous	32	0.032 (0.029-0.036)	CL ₁₀
	32	0.099 (0.097-0.101)	CL ₅₀
	32	0.309 (0.304-0.311)	CL ₉₀
Acetonitrile	32	0.028 (0.025-0.028)	CL ₁₀
	32	0.069 (0.059-0.064)	CL ₅₀
	32	0.169 (0.163-0.174)	CL ₉₀
Ethanol	32	0.027 (0.025-0.027)	CL ₁₀
	32	0.064 (0.060-0.066)	CL ₅₀
	32	0.154 (0.150-0.156)	CL ₉₀

Source : the authors.

The two extracts demonstrated high toxicity, which can lead to protein denaturation, enzyme inhibition and membrane disintegration in organisms, leading to their death. This situation may occur due to the interaction between the phytoconstituents present in the extracts, in a synergy process, which can enhance one of the classes found, such as saponins and cardiotonic heterosides , known for their harmful actions on cells of living organisms (Yunes and Calixto, 2001).

In a complementary way, when associating the chemical profile of the extracts with the tests with *A. salina* , it is clear that Ext_{EtOH} and Ext_{MeCN} They have in common the presence of triterpenes , which does not occur in Ext_{H2O} (Figure 1). It is important to highlight that saponins in species of the genus *Sapindus* (Grisi et al. , 2012) indicate the insecticidal potential (toxicity) of the species, with its hemolytic activity (triterpene saponins) associated with the ability to interact with the components of the cell membrane of erythrocytes, mainly with cholesterol molecules, inducing deformation in the membrane with the consequent extravasation of intracellular contents (Karabaliev and Kochev , 2003).

The fact that Ext_{H2O} has lower toxicity may be related to the strong antioxidant capacity of the extract, with the highest values of phenolic compounds and flavonoids (Table 1). The greater presence of these compounds may have reduced the action of saponins and their synergistic processes, reducing the mortality rate of the organisms. On the other hand, the presence of phenolic compounds and flavonoids justifies the pharmaceutical potential and medicinal use of the species (Pott and Pott , 1994), since in traditional medicine it is common to use water as an extractor, in the form of leaf tea, for example (Oliveira et al., 2023).

On the other hand, although the leaves have metabolites with medicinal action, the high intensity of saponins and heterosides cardiotonics in Ext_{H2O} is a risk to human health when consumed constantly. Saponins have hemolytic action and extracts with high intensity are not recommended for regular human consumption, as in aqueous media they are aphrogenic and have high solubility, and can cause liver and kidney damage due to their interaction with enzyme receptors (Athayde et al., 2017). Regarding heterosides , there is a range of toxic heterosides that occur in vascular plants, the most abundant being heterosides cardiotoxic , highly toxic even in low concentrations, with harmful action on the heart muscle, which can lead to death when used inappropriately (Aguiar and Veiga Júnior, 2021).

Regarding TAS (Table 3), it was observed that Ext_{EtOH} stood out, with the highest rate of toxicity and mortality, a factor probably associated with the presence of saponins, as they have a strong hemolytic capacity, associated with aglycone , a triterpenic or steroid nucleus with high affinity for cholesterol in cell membranes, leading to their rupture (Karabaliev and Kochev , 2003). Santiago et al. (2005), evaluating saponins obtained from different plant species, also describe that this group has larvicidal activity, leading to the death of *Aedes aegypti* .



Another factor that may have caused the greater deleterious action of Ext_{EtOH} is the presence of coumarins at a moderately moderate intensity, also identified in Ext_{H₂O}, although at a low intensity (Figure 1). Coumarin is used as an insecticide, in addition to other biological activities, with use in medicine (Scio, 2004).

Table 3 . Toxicity and mortality rate of *Artemia salina* larvae submitted to extract and fractions obtained from *Sapindus saponaria* leaves

Extract and Fractions	Toxicity Rate	Mortality (%)
Ethanolic extract	100.0 to	100.0 to
Hexane fraction	82.4b	17.6b
Dichloromethane fraction	83.6b	16.4b
Ethyl acetate fraction	85.1b	14.9b
Butanolic fraction	83.6b	16.4b
Fraction hydromethanolic	84.5b	15.5b
Negative control	82.5b	17.5b
Positive control	9.2c	2.1c

Means followed by the same lowercase letter in the column do not differ statistically from each other (Tukey test , 5% probability).

Source : the authors.

Saponins can exert broad biological activity, with insecticidal and larvicidal effects, determining the cytotoxic action of *S. saponaria* (Valdés et al. , 2015) against *A. salina* . Its absence in the fractions (Figure 2) may explain the lower toxicity of these solutions. On the other hand, its intensity in Ext_{EtOH} is moderately moderate (equal to Ext_{MeCN}), while in Ext_{EtOH} it has high intensity. Therefore, it can be assumed that not only saponins acted on the larvae, but other metabolites, such as cardiotonic heterosides, found in high intensity in Ext_{EtOH} (Figure 2).

Heterosides are well known for their deleterious actions on cells of living organisms (Yunes and Calixto , 2001), with hemolytic and insecticidal activity already recognized (Rates, Bridi , Braga and Simões, 2017), which may explain their effects on larvae. Saponins and glycosides, alone or in a synergistic process, are probably responsible for the mortality observed in *A. salina* larvae .

4.3 Insecticide Activity Facing of *Aedes Aegypti* Larvae

The results obtained demonstrated that the highest concentrations (500 mg L⁻¹ - Ext_{MeCN} and Ext_{EtOH} ; 500 and 250 mg L⁻¹ - Ext_{H₂O}) were the most effective in increasing larval time and larval and pupal mortality , in addition to decreasing the number of alates formed, for all extracts (Exception for larval time, Ext_{EtOH}). Ext_{EtOH} had no effect on larval duration, but achieved the highest larval mortality (73.0%) and 100% pupal mortality , preventing the formation of alates, the best result among the extracts (Table 4).

Table 4 . Effect of extracts (Ext_{MeCN} , Ext_{EtOH} and Ext_{H₂O}) obtained from *Sapindus leaves saponaria* , concentrations of 500, 250, 125 and 62.5 mg/L in larval duration, larval and pupal mortality and formation of alates, *Aedes aegypti* larvae

Concentration mg/L	Larval duration (h)	Larval mortality (%)	Pupal mortality (%)	Wings formed (%)
Ext _{MeCN} 500	248.7 a	61.0b	41.0 to	14.4 to
250	253.0 to	50.0c	44.2 a	29.0b
125	260.0 to	46.0c	37.6 a	31.0b
62.5	210.0b	51.0c	45.0 to	31.0b
Positive control	146.0c	0.0d	15.6b	85.2c
Negative control	-	100 to	-	-
Ext _{EtOH} 500	154.4 a	73.0b	100.0 to	0.0 to



250	142.3 a	37.0c	36.5b	40.0b
125	145.5 a	40.0c	33.3b	34.8b
62.5	153.4 a	36.0c	29.3b	33.0b
Positive control	146.0 to	0.0d	15.6c	85.2c
Negative control	-	100 to	-	-
Ext H ₂ O 5 00	222.0 to	58.7b	55.5 to	22.0 to
250	224.0 to	55.0b	58.3 a	16.8 to
125	201.0b	25.0c	41.3b	36.0b
62.5	169.0c	32.0d	24.0c	38.0b
Positive control	146.0d	0.0d	15.6d	85.2c
Negative control	-	100 to	-	-

Means followed by the same lowercase letter in the column do not differ statistically from each other (Tukey test, 5% probability).

Source: the authors.

Ext EtOH was the most effective extract in controlling the target species, with adverse effects on the insect's biology, with its action probably associated with higher frequencies of saponins and heterosides, in addition to the presence of coumarins, with well-known deleterious biological activity (Yunes and Calixto, 2001; Valdés et al., 2015; Athayde et al., 2017; Kuster and Rocha, 2017; Rates et al., 2017). These metabolites, alone or in synergistic processes, are probably responsible for the mortality observed in *A. aegypti* larvae, as occurred with *A. salina*.

5 CONCLUSIONS

Sapindus leaves *saponaria*, in different extracts, showed a toxic effect on *Artemia salina* larvae, although the fractions evaluated did not show good results. On the other hand, Ext EtOH was the extract with the best larvicidal effect, causing the death of most larvae and pupae, preventing the hatching of adults, despite having no effect on the larval duration of *Aedes aegypti*, demonstrating potential for population control of this vector. The activity of the leaves, in the form of extracts, can be related to the presence of some secondary metabolites, such as saponins and cardiotonic heterosides, known for their harmful action on organisms, alone or through synergistic effects.

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REFERENCES

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2), 265-267.
- Aguiar, A. T. C. & Veiga Júnior, V. F. (2021). O jardim venenoso: a química por trás das intoxicações domésticas por plantas ornamentais. *Química Nova*, 44(8), 1093-1100. <https://doi.org/10.21577/0100-4042.20170746>



- Amaral, L. S., Murgu, M.; Rodrigues-Fo, E., Souza, A. Q. & Moura, S. M. I. (2008). A saponin tolerant and glycoside producer xylariaceous fungus isolated from fruits of *Sapindus saponaria*. *World Journal of Microbiology and Biotechnology*, 24(8), 1341-1348. <https://doi.org/10.1007/s11274-007-9607-0>
- Athayde, M. L., Taketa, A. T. C., Gosmann, G. & Schenkel, E. P. (2017). Saponinas. In Simões, C. M. O., Schenkel, E. P., Mello, J. C. P., Mentz, L. A. & Petrovick, P. R. (Orgs.). *Farmacognosia: do produto natural ao medicamento* (pp. 285-303). Porto Alegre: Artmed.
- Barreto, C. F. (2005). *Aedes aegypti* - Resistência aos inseticidas químicos e as novas alternativas de controle. *Revista Eletrônica Faculdade Montes Belos*, 1(2), 62-73.
- Brasil. (2002). Ministério da Saúde. Fundação Nacional de Saúde. *Dengue: aspectos epidemiológicos, diagnóstico e tratamento*. Brasília: Ministério da Saúde, Fundação Nacional de Saúde. Brasília: Fundação Nacional de Saúde (Série A. Normas e Manuais Técnicos, n.º 176).
- Cabral, A. C. A., Cintra, Y. C., Araújo, M. G. & Soares, V. S. (2022). Mapeamento do setor de saneamento no Brasil sob a perspectiva da Teoria Ator-Rede. *Revista de Gestão Social e Ambiental*, 16(2), e02966. <https://doi.org/10.24857/rgsa.v16n2-020>
- Catão, R. C. & Guimarães, R. B. (2011). Mapeamento da reemergência do dengue no Brasil – 1981/82-2008. *Hygeia*, 7(13), 173-185.
- Cavalcanti, S. B., Teles, H. L., Silva, D. H. S., Furlan, M., Young, M. C. M. & Bolzani, V. (2001). New tetra-acetylated oligosaccharide diterpene from *Cupania vernalis*. *Journal of the Brazilian Chemical*, 12, 413-416.
- Cechinel Filho, V. & Yunes, R. A. (2001). Estratégias para a obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceitos sobre modificação estrutural para a otimização da atividade. *Química Nova*, 21, 99-105.
- Dumitrascu, M. (2011). *Artemia salina*. *Balneo and PRM Research Journal*, 2(4), 119-122. <https://doi.org/10.12680/balneo.2011.1022>
- Fontoura, F. M., Matias, R., Ludwig, J., Oliveira, A. K. M., Bono, J. A. M., Martins, P. D. F. R. B. & Guedes, N. M. R. (2015). Seasonal effects and antifungal activity from bark chemical constituents of *Sterculia apetala* (Malvaceae) at Pantanal of Miranda, Mato Grosso do Sul, Brazil. *Acta Amazonica*, 45(3), 283-292. <http://dx.doi.org/10.1590/1809-4392201500011>
- Grisi, P. U., Forim, M. R., Costa, E. S., Anese, S., Franco, M. F., Eberlin, M. N. & Gualtieri, S. C. J. (2015). Phytotoxicity and identification of secondary metabolites of *Sapindus saponaria* L. leaf extract. *Journal of Plant Growth Regulation*, 34(2), 339-349. <https://doi.org/10.1007/s00344-014-9469-2>
- Grisi, P. U., Ranal, M. A., Gualtieri, S. C. J. & Santana, D. G. (2012). Allelopathic potential of *Sapindus saponaria* L. leaves in the control of weeds. *Acta Scientiarum Agronomy*, 34(1), 1-9. <https://doi.org/10.4025/actasciagron.v34i1.11598>
- Guirado, M. M. & Bicudo, H. M. C. B. (2009). Alguns aspectos do controle populacional e da resistência a inseticidas do controle populacional e da resistência a inseticidas em *Aedes aegypti* (Diptera, Culicidae). *Bepa Epidemiológica Paulista*, 6(64), 5-14.



- Horta, M. A. P., Castro, F. I., Rosa, C. S., Daniel, M. C. & Melo, A. L. (2011). Resistance of *Aedes aegypti* (L.) (Diptera: Culicidae) to temephos in Brazil: a revision and new data for Minas Gerais State. *BioAssay*, 6(7), 1-6. <https://doi.org/10.14295/BA.v6.0.103>
- Karabaliev, M. & Kochev, V. (2003). Interaction of solid supported thin lipid films with saponin. *Sensors and Actuators B*, 88(1), 101-105. [https://doi.org/10.1016/S0925-4005\(02\)00311-8](https://doi.org/10.1016/S0925-4005(02)00311-8)
- Kuster, R. M. & Rocha, L. M. (2017). Cumarinas, cromonas e xantonas. In Simões, C. M. O., Schenkel, E. P., Mello, J. C. P., Mentz, L. A. & Petrovick, P. R. (Orgs.). *Farmacognosia: do produto natural ao medicamento* (pp. 537-556). Porto Alegre: Artmed.
- Lacerda, A. M., Modolo, A. K., Matias, R. C., Pistori, H., Yano, M., Roel, A. R. & Porto, K. R. A. (2011). Screening de plantas com potencial fitotóxico. *Revista Brasileira de Farmacognosia*, 92(4), 352-355.
- Marston, A. (2007). Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry*, 68(22/24), 2785-2797. <https://doi.org/10.1016/j.phytochem.2007.08.004>
- Matos, F. J. A. (2009). *Introdução a fitoquímica experimental* (3a ed.). Fortaleza: Edições UFC.
- McLaughlin, J. L., Chang, C. J. & Smith, D. L. (1993). Simple bench-top bioassays (Brine shrimp and potato discs) for the discovery of plant antitumor compounds. In Kinghorn, A. D. & Balandrini, M. F. (Orgs.). *Human medicinal agents from plants* (pp. 112-137). New York: American Chemical Society.
- Medina, L. O., Gómez, Á. Á., Valencia, V. A., Jaramillo, Á. C. & Maya, W. C. (2013). Actividad espermicida y citotóxica del extracto de *Sapindus saponaria* L. (jaboncillo). *Revista Cubana de Plantas Medicinales*, 18(2), 187-200.
- Mendonça, F. A., Souza, A. V. & Dutra, D. A. (2009). Saúde pública, urbanização e dengue no Brasil. *Sociedade & Natureza*, 21(3), 257-269.
- Murgu, M., Santos, L. F. A., Soouza, G. D. D., Daolio, C., Schneider, B., Ferreira, A. G. & Rodrigues-Filho, E. (2008). Hydroxylation of a hederagenin derived saponin by a Xylareaceous fungus found in fruits of *Sapindus saponaria*. *Journal of the Brazilian Chemical Society*, 19(5), 831-835. <https://doi.org/10.1590/S0103-50532008000500004>
- Oliveira, A. K. M., Matias, R., Dourado, D. M., Fernandes, R. M., Abreu, C. A.A. & Silva, B. C. F. L. (2023). Ethnobotanical survey of medicine species used in Taboco Village, Maracaju mountains, Brazil, and healing activity of the specie with higher value of use (*Maytenus ilicifolia*). *Boletim Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 22 (4): 537-559 (2023). <https://doi.org/10.37360/blacpma.23.22.4.40>
- Pauliquevis, C. F., Oliveira, A. K. M., Silva, P. G., Zanella, D. F. P., Roel, A. R., Porto, K. R. & Matias, R. (2021). Efeito larvicida do extrato etanólico de *Piper umbellatum* sobre o mosquito *Aedes aegypti*. *South American Journal of Basic Education Technical and Technological*, 8(1), 84-101.
- Peixoto Sobrinho, T. J. S., Silva, C. H. T. P., Nascimento, J. E., Monteiro, J. M., Albuquerque, U. P. & Amorim, E. L. C. (2008). Validação de metodologia espectrofotométrica para



quantificação dos flavonóides de *Bauhinia cheilantha* (Bongard) Steudel. *Revista Brasileira de Ciências Farmacêuticas*, 44(4), 683-689. <https://doi.org/10.1590/S1516-93322008000400015>

Pott, A. & Pott, V. J. (1994). *Plantas do Pantanal*. Brasília: Embrapa.

Prysthon, K. B. T., El-Deir, S. G., Baía, F. B. T. P., Aragão Júnior, W. R. & Santana, R. F. (2023). Socioeconomic biases: Analysis of popular perception about sanitary sewage services in Pernambuco. *Revista de Gestão Social e Ambiental*, 17(1), e03158. <https://doi.org/10.24857/rgsa.v17n1-015>

Queiroz, E. F., Wolfender, J. L., Hostettmann, K. & Vieira, P. C. (2014). *Princípios ativos de plantas superiores* (2a ed.). São Carlos: EDUFSCAR.

Rates, S. M. K., Bridi, R., Braga, F. C. & Simões, C. M. O. (2017). Heterosídeos cardioativos. In Simões, C. M. O., Schenkel, E. P., Mello, J. C. P., Mentz, L. A. & Petrovick, P. R. (Orgs.). *Farmacognosia: do produto natural ao medicamento* (pp. 271-284). Porto Alegre: Artmed.

Santiago, G. M. P., Viana, F. A., Pessoa, O. D. L., Santos, R. P., Pouliquen, Y. B. M., Arriaga, A. M. C., Andrade-Neto, M. & Braz-Filho, R. (2005). Avaliação da atividade larvicida de saponinas triterpênicas isoladas de *Pentaclethra macroloba* (Willd.) Kuntze (Fabaceae) e *Cordia piauhiensis* Fresen (Boraginaceae) sobre *Aedes aegypti*. *Revista Brasileira de Farmacognosia*, 15(3), 187-190. <https://doi.org/10.1590/S0102-695X2005000300003>

Sarmiento, P. A., Rocha, A. T., Barbosa, A. P. F.; Araújo-Júnior, J. X.; Lúcio, I. M. L. & Bastos, M. L. A. (2014). Evaluation of the extract of *Zeyheria tuberculosa* with a view to products for wound healing. *Revista Latino-Americana de Enfermagem*, 22(1), 165-172. <https://doi.org/10.1590/0104-1169.3143.2385>

Scariot, A., Souza-Silva, J. C. & Felfili, J. M. (Orgs.). (2005). *Cerrado: ecologia, biodiversidade e conservação*. Brasília: Ministério do Meio Ambiente.

Scio, E. (2004). Cumarinas encontradas no gênero *Kielmeyera* - Família Clusiaceae. *Revista Brasileira de Farmácia*, 85(1), 27-31.

Silva, H. H. G., Silva, I. G., Oliveira, C. L. N. S. & Elias, C. N. (1998). Adaptação do *Aedes aegypti* (Linnaeus, 1762) em criadouros artificiais com água poluída. *Entomologia y Vectores*, 6, 383-391.

Silverstein, R. M., Webster, F. X., Kiemle, D. & Bryce, D. (2019). *Identificação espectrométrica de compostos orgânicos* (8a ed.) Barueri: LTC.

Simon, M. F., Grether, R., Queiroz, L. P., Skeme, C., Pennington, R. T. & Hughes, C. E. (2009). Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences*, 106(48), 34-45. <https://doi.org/10.1073/pnas.0903410106>

Singh, R. & Kumari, N. (2015). Comparative determination of phytochemicals and antioxidant activity from leaf and fruit of *Sapindus mukorossi* Gaertn. – A valuable medicinal tree. *Industrial Crops and Products*, 73, 1-8. <https://doi.org/10.1016/j.indcrop.2015.04.012>

Solomons, T. W. G. (2012). *Química orgânica* (10a ed.). São Paulo: Editora LTC.



Sousa, C. M. M., Silva, H. R., Vieira Jr., G. M., Ayres, M. C. C., Costa, C. L. S., Araújo, D. S., Cavalcante, L. C. D., Barros, E. D. S., Araújo, P. B. M., Brandão, M. S. & Chaves, M. H. (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova*, 30(2), 351-355. <https://doi.org/10.1590/S0100-40422007000200021>

Spletozer, A. G., Santos, C. R., Sanches, L. A. & Garlet, J. (2021). Plantas com potencial inseticida: enfoque em espécies amazônicas. *Ciência Florestal*, 31(2), 974-997. <https://doi.org/10.5902/1980509832244>

Sumitha, K. V. & Thoppil, J. E. (2016). Larvicidal efficacy and chemical constituents of *Ocimum gratissimum* L. (Lamiaceae) essential oil against *Aedes albopictus* Skuse (Diptera: Culicidae). *Parasitology Research*, 115(2), 673-680. <https://doi.org/10.1007/s00436-015-4786-3>

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6/7), 669-675. <https://doi.org/10.1016/j.jfca.2006.01.003>

Torres, A. F., Lasmar, O., Carvalho, G. A., Santa-Cecília, L. V. C., Zanetti, R. & Oliveira, D. (2014). Atividade inseticida de extratos de plantas no controle de formiga cortadeira, em cafeeiro. *Coffee Science*, 8(3), 371-378.

Valdés, M. L., Santos, T. B., Olivet, S. E., Paredes, P. L. E., Hernández, B. Y., González, O. A. & González, S. G. (2015). Determinación de saponinas y otros metabolitos secundarios en extractos acuosos de *Sapindus saponaria* L. (Jaboncillo). *Revista Cubana de Plantas Medicinales*, 2(1), 106-116.

Vieira, P. C., Fernandes, J. B. & Andrei, C. C. (2017). Plantas inseticidas. In Simões, C. M. O., Schenkel, E. P., Mello, J. C. P., Mentz, L. A. & Petrovick, P. R. (Orgs.), *Farmacognosia: do produto natural ao medicamento* (pp. 903-918). Porto Alegre: Artmed.

Yunes, R. A. & Calixto, J. B. (2001). *Plantas medicinais sob a ótica da química medicinal moderna: métodos de estudo, fitoterápicos e fitofármacos, biotecnologia, patente*. Chapecó: Argos.