

## Chemical-biological analysis of the barks of *Anacardium occidentale* Linn and *Psidium guajava* Linn

Rafael Binow Schmidt<sup>1</sup>, Jeferson de Oliveira Salvi<sup>1</sup>, Weverson Alves Ferreira<sup>1</sup>

<sup>1</sup>Department of Pharmacy, Lutheran University Center of Ji-Paraná (CEULJI/ULBRA), Brazil.

Corresponding Author: Rafael Binow Schmidt

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**Abstract:** The use of medicinal plants is a very common practice. *Anacardium occidentale* L. and *Psidium guajava* L. are widely used because of their antidiarrheal properties. This study aimed to perform phytochemical analyses and to evaluate cytotoxicity and antibacterial potential of the barks of *A. occidentale* L. and *P. guajava* L. The exsiccates were prepared and deposited at the Antônio Dalla Martha Herbarium, located at CEULJI/ULBRA. The ethanolic extracts were carried out by maceration and the aqueous extracts by decoction. The phytochemical prospection was performed through colorimetric and precipitant tests, *Artemia salina* was used to evaluate the cytotoxicity, and the antibacterial analyses were carried out by disc diffusion. In both plants was confirmed presence of secondary metabolites, the ethanolic extracts showed active against *S. aureus* and *E. coli.*, and the aqueous extracts demonstrated antibacterial activity only against *S. aureus*. The preparations of *A. occidentale* and *P. guajava* did not demonstrate toxicity against *A. salina*. The bioassays executed demonstrated great therapeutical potentials. Investigative studies about medicinal plants are extremely important, since some constituents may present drug interactions or interfere in laboratory tests. Therefore, further studies about the chemical constituents of the species in question are suggested, this way is possible to infer the occurrence of possible potential interactions.

**Keywords:** Anacardiaceae, Antibacterial analysis, Medicinal plants, Myrtaceae, Phytochemical prospection

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### I. INTRODUCTION

Since the origins of mankind, plant preparations were produced and used by healers in rituals, with the purpose of healing the evils that afflicted the body and soul [1]. The Emperor of China, Shan Nung (2838-2698 BCE), had great importance in the history of medicinal plants for being the first to catalog a wide variety of herbs with medicinal and toxic effects [2]. In Brazil, the practice of the use of medicinal plants has indigenous, Portuguese and African origin, which allowed a vast culture of information about the preparation and use of herbs [3].

In 2006, Brazil inserted through the Portaria No. 971 the use of medicinal plants and phytotherapics in the National Policy of Integrative and Complementary Practices (NPICP) of the Sistema Único de Saúde (SUS) [4]. In this context, aiming to guarantee access and rational use of medicinal plants and phytotherapics was created the Decree No. 5813, of June 22, 2006, which beyond incorporation of more therapeutic options, brought back again a millenarian practice that covers both scientific and popular knowledge [5].

The use of medicinal plants by rural and distant communities from the great centers is very common due to the precariousness of access to the SUS, the high costs of industrialized medicines, the difficulties of transportation and the option for natural treatments [6, 7]. Among the several plants used with medicinal purposes, stands out *Anacardium occidentale* L. and *Psidium guajava* L., widely used due to their antidiarrheal properties [8, 9].

The *Anacardium occidentale* L., belonging to the family Anacardiaceae, popularly known as cashew tree, is originated from the northeastern region of Brazil, has a tortuous crown and soft accessory fruits, colored with yellow and/or red, and its fruit, the cashew nut is similar to a human kidney. The bark of the cashew tree is used as an astringent and tonic (anacardine), its branches produce a yellow resin able to replace the gum arabic, and its sap can be used to produce paints. It is widely used in the traditional medicine for presenting therapeutic effects such as: hypoglycemic, pain relief, arthritis, intestinal colic, bronchitis and asthma, anti-inflammatory activity and inhibition of the enzyme acetylcholinesterase [9, 10, 11, 12].

The *Psidium guajava* L, from the Myrtaceae family, popularly known as guava tree, is native throughout America, except Canada and Mexico, being considered a weed in the United States. The tree presents tortuous trunks, its fruits are green and/or yellowish berries, with sweet-acidulated juicy pulp. Widely

used in the traditional medicine, it has therapeutic activities such as: antidiarrheal, antispasmodic, topical antibacterial, hypoglycemic and platelet aggregator [8, 13].

Because they are frequently used in association in the popular medicine, scientific investigation of the therapeutic potential of these plants is extremely relevant. Therefore, the present study aimed to perform phytochemical analyses, and to evaluate the cytotoxicity and the antibacterial potential of the barks of *A. occidentale* L. and *P. guajava* L.

## II. METHODOLOGY

### 2.1 Collection of plant material and preparation of exsiccate

The fertile samples of the plants were collected in June 2018, in the city of Ji-Paraná (RO). The following geographical coordinates identify the sites of collection of *A. occidentale* and *P. guajava*, respectively, 10.85°93.6" S 61.96°61.3" W and 10.85°93.5" S 61.96°59.7" W.

The exsiccates were prepared in accordance with the methodology of Simões et al. (2010) [14], and subsequently deposited at the Antônio Dalla Martha Herbarium, located at the University Center of Ji-Paraná, CEULJI/ULBRA. The plants were properly identified according to Simões et al. (2017) [16], and registered under the codes JPCU 319 (*P. guajava* L.) and JPCU 320 (*A. occidentale* L.).

### 2.2 Plant material preparation and extraction

The barks used in the present study, were dried at 45±1°C in a circulating air oven, and subsequently ground in an electric mill [14, 17].

The maceration to obtain the ethanolic extract (EE) was performed through the blending of 300 mL of ethanol Chemco (99.5%) and 200g of plant material of each species. These preparations were kept in amber bottles at room temperature, for seven days, and after being filtered, a concentrate was made with the crude extracts with the aid of the rotary evaporator Quimis (Q344B1), under a pressure of -400 PSI at the temperature of 40°C [18].

The barks of *A. occidentale* L. and *P. guajava* L. were separately submitted to the decoction process, by adding 20 grams of them in 240 mL of boiling water to obtain the aqueous extract (AE) [14, 19].

To calculate the yield, the weight of each concentrate extract obtained was divided by the weight of the dried material (200g) and was multiplied by 100 [20].

### 2.3 Phytochemical prospection

To be evaluated, the AE and EE were separately prepared in the concentrations of 83 mg/mL and 500 mg/mL, respectively. All the samples were performed in triplicate.

**Table 1:** Based in colorimetric, precipitant and foam tests.

Secondary metabolites	Tests and reagents	Positive results
Alkaloids	Dragendorff and Mayer reagents; organic and inorganic acids.	Precipitate formation.
Coumarins	Observation under ultravioleta light; diethyl ether.	Bright green or blue fluorescence
Flavonoids	Cyanidin or Shinoda test; Hydrochloric acid and magnesium.	Orange or red coloration.
Phenols	Ferric chloride	Blue or bluish green coloration.
Saponins	Hemolytic reaction	Formation of persistent foam.
Tannins	Reaction of 10% ferric chloride solution	Blue and/or green coloration.
Triterpenes	Salkowski reaction; Chloroform and sulfuric acid.	Red and/or steady yellow coloration.

Source: Adapted from Who (1980) [21], Simões *et al.* (2010) [14], Radi (2007) [22] and Teixeira (2012) [23].

The phytochemical prospection to identify secondary metabolites was carried out through colorimetric, precipitant and foam tests, according to Table 1.

### 2.4 Bioassay with *Artemia salina*

In a flat bottom flask, a solution of sea salt (35g/L - pH 8.5) was prepared, and the cysts of *A. salina* were added. The eggs were kept under aeration, at the temperature of 25°C (± 2) and 100 watts light, for 48 hours to complete the hatching [24].

The samples of the AE were prepared in the concentration of 83 mg/mL and the EE at 10 mg/mL. After that, a serial dilution was made (1:1, 1:2, 1:5, 1:10 and 1:20). Then, approximately 10 *A. salina* nauplii were transferred to each test tube containing the prepared dilutions. As negative control, the saline solution (35 g/L) was used, and as positive control was used 0.1% potassium dichromate [25].

Passed 24 hours, after the addition of the nauplii, the live specimens were counted and the percentage of mortality (LD<sub>50</sub>) was calculated [26]. The tests were performed in triplicates.

### 2.5 Antibacterial activity analysis

*A. occidentale* L. and *P. guajava* L. ethanolic extracts tested were prepared from the following samples: *A. occidentale* L. (3 mg/mL); *P. guajava* L. (2 mg/mL) and from the association of *A. occidentale* L. and *P. guajava* L. (2 mg/mL each). Three samples, prepared by barks' decoction, were also evaluated: *A. occidentale* L. (83 mg/mL); *P. guajava* L. (83 mg/mL); *A. occidentale* L. and *P. guajava* L. (83 mg/mL each). The disc diffusion technique was used according to the methodology standardized by the Clinical and Laboratory Standard Institute (CLSI, 2011) using the Mac Farland scale [18, 28].

The culture medium used was Mueller-Hinton agar [29], and the bacterial strains chosen were: *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), obtained from the bacteria storage of CEULJI/ULBRA's Microbiology Laboratory.

The negative control was done with distilled water, and the positive control with Clindamycin (2µg) for *S. aureus* and Ceftriaxone (10µg) for *E. coli*. The plates were incubated in an oven (37°C/24h), then the inhibition halos were evaluated. The samples, whose inhibition halo was higher than 6mm, in diameter, were considered active [30]. All analyses were performed in triplicate.

## III. RESULTS AND DISCUSSION

### 3.1 Phytochemical prospection

The yield of the ethanolic extracts obtained were 6.32% (12.64g) and 5.6% (11.2 g) for *A. occidentale* L. and *P. guajava* L., respectively.

The secondary metabolites found in the studied plant species are expressed in Table 1.

**Table 2:** Secondary metabolites evaluated in the *Anacardium occidentale* L. and *Psidium guajava* L. preparations.

Secondary metabolites	<i>A. occidentale</i> L. EE	<i>P. guajava</i> L. EE	<i>A. occidentale</i> L. AE	<i>P. guajava</i> L. AE
Alkaloids	--	--	--	--
Coumarins	--	--	--	--
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Triterpenes	+	+	+	+

**Label:** (AE) Aqueous Extract; (EE) Ethanolic Extract; (+) Present; (--) Absent.

The results, of the phytochemical prospection of this work, are consistent with data found in the literature. Bouzada et al. (2009) [31], when performing the phytochemical analysis of the ethanolic extract of the leaves of *A. occidentale* L., confirmed the presence of triterpenes, tannin, flavonoids, alkaloids and anthraquinones. Santos and Rodrigues (2011) [32], confirmed the presence of tannins, phenols and alkaloids in the ethanolic extract of the barks of *A. occidentale* L. Related to *P. guajava* L., Silva et al. (2013) [33], found flavonoids, tannins and saponins in its leaves' ethanolic extract.

Many environmental factors may influence in the presence of secondary metabolites in plants [34]. It should be mentioned here, that the samples collected for this study originate from trees that grew in the midst of dense vegetation and were in the flowering period.

The presence of flavonoids in plants is a mechanism of chemical defense against microorganisms, besides influence in the attraction of pollinators [14, 35, 36]. The flavonoids bind to glycoproteins present in viruses, and in some cell receptors, being able to trigger a significant antiviral activity already at the stage of virus' penetration into the cell [37]. Acacetin and amentoflavone are some examples of flavonoids with antiviral activity, while quercetin and rutin have antibacterial and anti-inflammatory activities [38].

The antibacterial activity of saponins and triterpenes, is related to their amphiphilic behavior and their ability to make complexes with proteins, steroids and membrane phospholipids, altering the cell permeability and damaging the functioning, by osmotic imbalance [39]. In relation to the tannins, the antibacterial activity occurs by three different mechanisms: inhibiting enzymes, through a tannin-protein complex, that prevents the

bacterial protein synthesis; modifying the metabolism in the microorganisms' cell membranes; and binding to metal ions, decreasing their availability for microbial metabolism [40, 41].

The presence of phenolic compounds was also evidenced in the studied preparations. It is known that some phenolic compounds are able to inhibit cyclooxygenase (COX) and lipoxygenase pathways, enzymes that are involved in inflammatory responses [38].

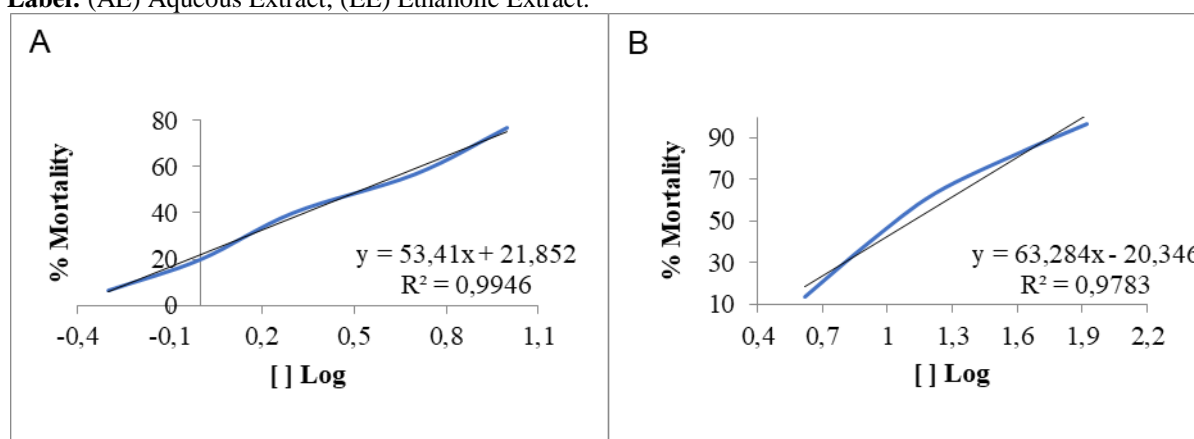
### 3.2 Bioassay with *Artemia salina*

The median lethal dose (LD<sub>50</sub>) was obtained by the linear regression method based on the correlation of the concentrations' log as a function of the percentages of the recorded mortalities. To the straight line equation obtained ( $y = a.x + b$ ), it is attributed one half of the possible deaths to the value of y (ordinates) and, to the result x (abscissas), it was applied the antilogarithm to determine the concentration in µg/mL [26].

**Table 3:** Median lethal dose obtained from different preparations of the barks of *Anacardium occidentale* L. and *Psidium guajava* L. evaluated against *Artemia salina*.

	Ways of samples preparation			
	AE DL <sub>50</sub>	R <sup>2</sup>	EE DL <sub>50</sub>	R <sup>2</sup>
<i>A. occidentale</i> L.	1425 µg/mL	0,966	4980 µg/mL	0,990
<i>P. guajava</i> L.	3437 µg/mL	0,994	2500 µg/mL	0,981
<i>A. occidentale</i> L. + <i>P. guajava</i> L.	1305 µg/mL	0,978	3360 µg/mL	0,994

**Label:** (AE) Aqueous Extract; (EE) Ethanolic Extract.



**Figure 1:** Linear mortality of *A. salina* depending on the logarithm of concentration of the ethanolic extracts (A) and aqueous extract (B) prepared by association of *A. occidentale* L. and *P. guajava* L.

When evaluated according to the parameters described by Meyer et al. (1982) [24], the biological activity of the aqueous extracts was classified as non-toxic to *A. salina*. In other words, all samples had LD<sub>50</sub>>1000 µg/mL. The biological activity of the ethanolic extracts was also classified as non-toxic to *A. salina*, since all the samples had a LD<sub>50</sub>> 500 µg/mL [42].

Motti et al. (2015) [43], when evaluating the toxicity of a formulation, obtained from cashew nuts (*A. occidentale* L.), against *A. salina*, obtained DL<sub>50</sub> of 3147 µg/mL. Durães et al. (2015) [44], obtained DL<sub>50</sub> of 1133 µg/mL, when investigating the leaves' toxicity of *P. myrsinites* DC against *A. salina*. These results corroborate to the present study.

### 3.3 Antibacterial activity analysis

The LD<sub>50</sub>, obtained from the bioassay with *A. salina*, was used as a parameter, to perform the analysis of the EE's antibacterial activity of the barks of *A. occidentale* L. and *P. guajava* L. The obtained results are expressed in Table 2, and demonstrate that there was antibacterial activity in different concentrations. In the positive control, the inhibition halo was of 28 mm for *S. aureus* and 25 mm for *E. coli*, and in the negative control, no halo formation was observed.

The separated EE, as well as their association, are shown to be active against *S. aureus* starting from 0.5 mg/mL, once they presented inhibition halo greater than 0.6 mm. But for *E. coli*, only the EE association presented to be active at this concentration, however, when tested separately, both were active in a concentration of 1.5 mg/mL.

**Table 4:** Antibacterial activity of the barks of *Anacardium occidentale* L. and *Psidium guajava* L. evaluated against strains of *Staphylococcus aureus* and *Escherichia coli* in different dilutions.

Tested concentrations and inhibition halos						
	3,0 mg/mL	2,0 mg/mL	1,5 mg/mL	1,0 mg/mL	0,5 mg/mL	0,25 mg/mL
<b><i>A. occidentale</i> L. EE</b>						
<i>Staphylococcus aureus</i>	16 mm	14 mm	13 mm	11 mm	09 mm	04 mm
<i>Escherichia coli</i>	13 mm	11 mm	08 mm	04 mm	--	--
<b><i>P. guajava</i> L. EE</b>						
<i>Staphylococcus aureus</i>	**	13 mm	10 mm	09 mm	06 mm	--
<i>Escherichia coli</i>	**	11 mm	09 mm	05 mm	--	--
<b><i>A. occidentale</i> L and <i>P. guajava</i> L. EE</b>						
<i>Staphylococcus aureus</i>	**	16 mm	13 mm	11 mm	08 mm	05 mm
<i>Escherichia coli</i>	**	13 mm	11 mm	09 mm	06 mm	--

**Label:** (EE) Ethanolic Extract, (\*\*) Concentration not tested, (--) There was no inhibition halo.

In 2006, Konan [45] reported in a study that the hydroethanolic extract of the leaves of *A. occidentale* L. showed antibacterial activity for *S. aureus* strains in the concentration of 0.320 mg/mL, these results are close to those found in the present study, which was 0.500 mg/mL. In another study, the hydroethanolic extract of *P. guajava* L. 0.100 mg/mL demonstrated activity against *S. aureus*, but not against *E. coli* [46]. According to the classification by Holetz et al. (2002) [47], the extracts of the present study showed weak antibacterial activity.

The AE of the barks were also evaluated, and they showed up antibacterial activity against *S. aureus*, a gram-positive bacterium. It was checked, inhibition halos of 12, 11 and 13 mm, respectively, for *A. occidentale* L., *P. guajava* L. and their association. On the other hand, the AE were inactive against *E. coli*, a gram-negative bacterium. It is known that the gram-positive bacteria's sensitivity is more common to verify, since the plants secondary metabolites are more active against this type of bacteria [48,49], which confirms the results obtained in this study.

The antibacterial activity of the studied plants is related to the presence of the following secondary metabolites: phenols, flavonoids, saponins, tannins and triterpenes, that were identified in the aqueous and ethanolic extracts. In this way, the antibacterial activity could have occurred by many different mechanisms of action, such as: the amphiphilic behavior of saponins and triterpenes in the cell membranes, that causes cellular apoptosis by osmotic imbalance [18, 39]; phenols and flavonoids, that can cause nuclear fragmentation and chromatin condensation, due to the block of the tyrosine kinase enzyme, unleashing the process of cellular apoptosis [36, 37, 38]; and tannins blocking the protein synthesis and decreasing the availability of essential ions to the bacterial metabolism [40].

When carrying out a search in the literature about the antibacterial activities of the studied plants, different results can be observed according to each study. This difference can be explained due to the number of phytochemical constituents that may vary according to climatic changes, soil differences, plants development stage, collection and preparation ways, part of the studied plant, among others [50].

#### IV. CONCLUSION

The preparations of the studied plants, *Anacardium occidentale* L. and *Psidium guajava* L., presented great phytochemical potential, which was proved by the bioassays performed. About the toxicity test made with *A. salina* L., the different preparations analyzed showed to be non-toxic.

The bacteriological studies, allowed to conclude that the samples obtained by aqueous extracts were active against *S. aureus*, but inactivate against *E. coli*. On the other hand, the ethanolic extracts, demonstrated activity against both bacteria studied, being observed better results over *S. aureus*.

Investigative studies about the different substances present in medicinal plants are extremely important, once some constituents can present drug interactions or can interfere in laboratory tests. Thus, new studies about the chemical constituents of the species in question are suggested, this way it is possible to infer the occurrence of possible potential interactions.

#### REFERENCES

- [1] Firmo, WCAF, Menezes VJM, Passos CEC, Dias CN, Alves LPL, Dias ICL, Neto MS, and Olea RSGO. Contexto histórico, uso popular e concepção científica sobre plantas medicinais. *Cad. Pesq.*, 18(1), 2011, 90-95.
- [2] Vale NB. A farmacobotânica, ainda tem lugar na moderna anestesiologia?. *Rev. Bras. Anestesiol.*, 52(3), 2002, 368-380.
- [3] Nogueira MJC. Fitoterapia popular e enfermagem comunitária. [tese]. São Paulo (SP): Escola de Enfermagem da USP; 1983.

- [4] BRASIL. Ministério da Saúde. Política Nacional de Práticas Integrativas e Complementares. Portaria n.º 971. Diário Oficial da União, n.º 84, seção I, p. 20- 24, Brasília, 04 maio 2006.
- [5] BRASIL. Ministério da Saúde. Secretaria de Assistência à Saúde. Departamento de Atenção Básica. Política Nacional de Práticas Integrativas e Complementares no SUS: PNPIC-SUS. Brasília: Ministério da Saúde, 2006.
- [6] Fonseca MCM. Epamig pesquisa, produção de Plantas Medicinais para Aplicação no SUS. (*Espaço para o produtor. Viçosa. 2012*).
- [7] Silva SMM, Silva CAG, Bazzo YMF, Magalhães PO, and Silveir D. *Eugenia dysenterica Mart. ExDC.(cagaita): planta brasileira com potencial terapêutico. Revista Infarma Ciências Farmacêuticas, 27(1), 2015, 49-95.*
- [8] Gutierrez RMP, Mitchell S, and Solis RV. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology, 117(1), 2008, 1–27.*
- [9] Gois MAF, Lucas FCA, Costa JCM, Moura PHB, and Lobato GJM. Etnobotânica de espécies vegetais medicinais no tratamento de transtornos do sistema gastrointestinal. *Rev. Bras. Pl. Med., 18(2), 2016, 547-557.*
- [10] Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, and Modesto-Filho J. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. *Rev. Bras. Farmacognosia, 15(1), 2005, 392-413.*
- [11] Falcão HS, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, and Batista LM. Review of the plants with anti-inflammatory activity studied in Brazil. *Revista Brasileira de Farmacognosia, 15(1), 2005, 381-391.*
- [12] Barbosa-Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde-Filho PF, Silva MS, Cunha EVL, and Almeida JRGS, Quintans-Júnior LJ. Natural products inhibitors of the enzyme acetyl cholinesterase. *Rev. Bras. Farmacognosia, 16(1), 2006, 258-285.*
- [13] Alonso J R. Tratado de fitomedicina – bases clínicas e farmacológicas. (*Editora Isis, Buenos Aires, Argentina, 1998*).
- [14] Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, and Petrovick PR (Orgs). Farmacognosia: da planta ao medicamento. (*6.ed. Porto Alegre: Editora da UFRGS: Florianópolis: Editora da UFSC, 2010. 1104p*).
- [15] Lorenzi H. Árvores brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil, (*Instituto Plantarum, 5 ed., 2008*).
- [16] Simões CMO, Schenkel EP, Mello JCP, Mentz LA, and Petrovick PR (Orgs). Farmacognosia: da planta ao medicamento. (*Porto Alegre: Editora Artmed, e-PUB, 2017*).
- [17] Oliveira F, Akisue G, and Akisue MK. Farmacognosia. (*São Paulo: Editora Atheneu, p. 15, 1998*).
- [18] Dias DLPP, Novais VP, Schmidt RB, Carmago EES, and Silva FC. Análise da atividade antibacteriana dos bulbos de *Eleutherine plicata herb.* (Iridaceae). *BJSR, 23(2), 2018, 07-11.*
- [19] Dickel OE, Aguiar RB, Geracitano L, Monserrat JM, and Barros DM. Efeitos comportamentais e neurotóxicos do extrato aquoso de *Brugmansia suaveolens* em ratos. *Rev. Bras. Farm., 91(4), 2010, 189-99.*
- [20] Santos AS, Alves SM, Baker D, and Rocha Neto O. Descrição de sistema e métodos de extração de óleos essenciais e determinação de umidade de biomassa em laboratório. (*Belém: Embrapa Amazônia Oriental, 2004, 6p*).
- [21] Who, World Health Organization. Marini-Betollo GB. Preliminary chemicals screening of medicinal plants in Field conditions. (*Roma:DPM, 1980*).
- [22] Radi PA, and Terrones MGH. Metabólitos secundários de plantas medicinais. *Rev. Bras. Farm.20(2), 2007,18-22.*
- [23] Teixeira LN, Stringheta PC, Oliveira FA. Comparação de métodos para quantificação de antocianinas. *Revista Ceres, 55(4), 2008, 297-304.*
- [24] Meyer BM, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, and Mclaughlin JL. Brineshrimp: A convenient general bioassay for active plant constituents. *Journal of Medical Plant Research, 45(1), 1982, 31-34.*
- [25] Hocayen PAS, Campos LA, Pochapski MT, and Malfatti CRM. Avaliação da Toxicidade do extrato bruto metanólico de *Baccharis dracunculifolia* por meio do bioensaio com *Artemia salina*. *INSULA Revista de Botânica Florianópolis, 41(1), 2012, 23-31.*
- [26] Rajeh MAB, Kwan YP, Zakaria Z, Latha LY, Jothy SL, and Sasidharan S. A cutetotoxicity impacts of *Euphorbia hirta* L extract on behavior, organs body weight index and histopathology of organs of the mice and *Artemia salina*. *Pharmacognosy Research, 4(3), 2012,170-177.*

- [27] CLSI. Publication M100-S21. Suggested Grouping of US-FDA Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Non-fastidious Organisms by Clinical Laboratories. Hardy Diagnostics, 2011, 1-3.
- [28] Lennette EH, Balows A, Hausler WJ, and Shadomy HJ. Manual of Clinical Microbiology. American Society for Microbiology, Washington, D.C., 1985.
- [29] Anvisa. Descrição dos Meios de Cultura Empregados nos Exames Microbiológicos. 2004. Disponível em <[http://www.anvisa.gov.br/servicos/medicamentos/manuais/microbiologia/mod\\_4\\_2004.pdf](http://www.anvisa.gov.br/servicos/medicamentos/manuais/microbiologia/mod_4_2004.pdf)> acesso em 18/06/2018.
- [30] Bona E, Silva PFG, Fruet TK, Jorge TCM, and Moura A. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (CIM) de extratos vegetais aquosos e etanólicos. *Arq do Inst Biol*, 81(3), 2014, 218-25.
- [31] Bouzada MLM, Fabri RL, Duarte GG, and Scio E. Busca de novas drogas antimicrobianas a partir de vegetais. *Revista Principia*, 11(1), 2007, 1-8.
- [32] Santos FO, and Rodrigues OG. Atividades biológicas de *Anacardium occidentale* (Linn). Dissertação, UFCG, Patos, PB, 2011.
- [33] Silva ICA, Aleixo AA, Aleixo AM, Figueiredo AP, Lemuchi MO, and Lima LARS. Análise fitoquímica e atividade antioxidante do Extrato hidroetanólico das folhas de *Psidium guajava* L. (Goiabeira). *Biochemistry and Biotechnology Reports*, 2(2), 2013, 76-78.
- [34] Neto LG, and Lopes NP. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quim. Nova*, 30(2), 2007, 374-381.
- [35] Zuanazzi JAS, and Montanha JA. Flavonóides. In: Simões CMO, Schenkel EP, Gormann G, Mello JCP, Mentz LA, Petrovick PR. Farmacognosia: da planta ao medicamento. (Porto Alegre/Florianópolis: Editora da UFRGS, 5.ed. p.577-614, 2002).
- [36] Machado H, Nagem TJ, Peters VM, Fonseca CS, and Oliveira TT. Flavonóides e seu potencial terapêutico. *Boletim do Centro de Biologia da Reprodução*, 27(1/2), 2008, 33-39.
- [37] Carvalho OV, Oliveira FS, Saraiva GL, Botelho CV, Ferreira HCC, Santos MR, Silva Júnior A, and Almeida MR. Potencial antiviral da quercetina sobre o parvovírus canino. *Bras. Med. Vet. Zootec*, 65(2), 2013, 353-358.
- [38] Santos DS, and Rodrigues MMF. Atividades farmacológicas dos flavonóides: um estudo de revisão. *UNIFAP*, 7(3), 2017, 29-35.
- [39] Carminate B, Carvalho AC, Pacheco TF, Natalli VD, and Silva MB. Investigação antibacteriana *in vitro* de extratos etanólicos das folhas e cascas de *Cedrela fissilis* Vell. *Ciência e Natura*, 36(1), 2014, 335-340.
- [40] Pereira AV, Azevêdo TKB, Santos HSS, Santana GM, Trevisan LFA, Azevedo SS, Pereira MV, and Paula AFR. Taninos da casca do Cajueiro: atividade antimicrobiana. *Agropecuária Técnica*. 2015;36(1):121-7.
- [41] Saidu A, Akanya H, Dauda B, and Ogbadoyi E. Antibacterial and comparative hypoglycemic effect of *Anacardium occidentale* leaves. *International Research Journal of Biochemistry and Bioinformatics*. 2(1), 2012, 6-10.
- [42] Dolabela MF. Triagem *in vitro* para atividade antitumoral e anti *Trypanosoma cruzi* de extratos vegetais, produtos naturais e substâncias sintéticas. (Minas Gerais, 1997, 130).
- [43] Motti PR, Porto KRA, and Roel AR. Toxicidade da formulação obtida a partir do líquido da castanha do caju *Anacardium occidentale* L. em *Artemia salina* Leach, *Multitemas, Campo Grande*, 47(1), 2015, 9-19.
- [44] Durões ERB, Faria LR, Clementino CO, Paula JAM, and Naves PLF. Toxicidade em *artemia salina* L. das folhas de *psidium myrsinites* dc. (myrtaceae) e composição química do óleo essencial, *Anais de Congresso UEG*, 2(1), 2015, 1-6.
- [45] Konan NA, and Bacchi EM. Antiulcerogenic effect and acute toxicity of a hydroethanolic extract from the cashew (*Anacardium occidentale* L.) leaves. *Journal of ethnopharmacology*, 112(2), 2007, 237-42.
- [46] Gonçalves FA, Neto MA, Bezerra JN, Macrae A, Souza OV, Fonteneles-Filho AA, and Vieira RH. Antibacterial activity of guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob Shrimp. *Rev. Inst. Med. Trop.* 50(1), 2008, 11-15.
- [47] Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, and Dias-Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Memórias do Instituto Oswaldo Cruz*, 97(7), 2002, 1027-1031.
- [48] Carvalhal ML, and Alterthum F. Morfologia e estrutura da célula bacteriana. In: TRABULSI; L.R.; ALTERTHUM, F. Microbiologia. (4 Ed. São Paulo, Atheneu, 2004. 718).
- [49] Eller CB, Burgess SSO, and Oliveira RS. Environmental controls in the water use patterns of a tropical cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *Tree Physiol* 35(1), 2015, 387-399.

- [50] Lopes DCXP, Freitas ZMF, and Santos EP, Tomassini TCB. Atividades antimicrobiana e fototóxica de extratos de frutos e raízes de *Physalis angulata* L., *Rev. Bras. Farmacogn*, 16(2), 2006, 206-210.

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