v. 2, n. 41, 2022

International Journal of Health Science

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *Cordia verbenacea* extracts

Lávia Karine M. Correia Monteiro
Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil
http://lattes.cnpq.br/7921827316446482

Simone Rocha de Oliveira
Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil
http://lattes.cnpq.br/2821258293062390

Simone Acrani
Department of Biochemistry, Pharmacology and Physiology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil
http://lattes.cnpq.br/6727855182136632

Karina F. Devienne
Department of Biochemistry, Pharmacology and Physiology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil
http://lattes.cnpq.br/2464488628312435

Abstract: The use of medicinal plants is an ancient practice for the treatment of diseases and, increasingly, they have been used by modern society, as it is an easily accessible therapy, has lower costs when compared to the acquisition of allopathic medicines and presents an idea of less aggression to the organism. *Cordia verbenacea* belongs to the Boraginaceae family, popularly known as erva baleeira, catinga de barão, balieira cambará, erva preta, maria milagrosa, maria preta. It is a native species of Brazil, widely distributed throughout the Brazilian territory. In ethnopharmacology, this plant is popularly used as an anti-inflammatory, analgesic and antiulcerogenic in the form of tea or infusions, while alcoholic extracts and decoctions for rheumatism, rheumatoid arthritis, gout, muscle pain, for topical or internal use. Considering that the extracting solvent can influence the chemical composition and, consequently, the biological activity, this study aimed to evaluate the cytotoxicity, antioxidant action and the presence of some phytochemical constituents in the aqueous and ethanolic extracts of *C. verbenacea*. The results of phytochemical characterization showed that the aqueous extract had higher phenolic and flavonols/flavones content compared to the ethanolic extract, which exhibited higher amounts of flavanones. Alkaloids were not identified in the evaluated preparations. Both extracts showed an antioxidant effect, and the ethanolic extract showed better ability to scavenger nitric oxide. The results showed that the ethanol extract, even with antioxidant capacity, induced a significant reduction in cell viability (concentrations ≥ 0.125 mg/mL), while in the aqueous preparation such effects were verified from 0.5 mg/mL. The greater toxicity exhibited by the ethanol extract may be related to the presence of more hydrophobic constituents than those found in the aqueous extract. In view of these results, it is extremely important to consider the method of preparation of extracts obtained from *Cordia verbenacea,* since it induces variations in the phytochemical composition and exerts an influence on biological and toxicological effects.

Keywords: *Cordia verbenacea*; phenolic content; antioxidant; cytotoxicity.

INTRODUCTION

The use of medicinal plants is an ancient practice, and they are increasingly being used by modern society, not only for their therapeutic properties, but also for being low cost. This is associated with the difficulties encountered in the acquisition of allopathic medicines and the deficiency of primary health care (SANTOS *et al.,* 2017).

The diversity and richness of bioactive compounds found in medicinal plants has motivated researchers to study plants for therapeutic purposes (SIMÕES *et al.,* 2017). Thus, Brazil, one of the greatest plant biodiversity, constitutes a relevant field for the development of such studies.

Among the plant species of interest for the development of phytotherapics is *Cordia verbenacea,* belonging to the Boraginaceae family (MS, 2014, 2017). This species is popularly known as cordia, erva baleeira, camarinha, catinga-de-barão, balieira cambará, maria preta, salicinia, catinga-preta, maria rezadeira, camarinha, camaramoneira-do-brejo. It is a species native to Brazil, widely distributed throughout the Brazilian territory, from Ceará to Rio Grande do Sul (TAYLOR, 2005; LORENZI; MATOS, 2008). In ethnopharmacology, in the form of tea or infusions, this species is used as anti-inflammatory, analgesic and anti-ulcer (VENTRELLA *et al.,* 2008; LORENZI; MATOS, 2008), alcoholic extracts and decoctions are used in the treatment of arthritis, rheumatism, gout, muscle pain,

for topical or internal use (PASSOS *et al.,* 2007). Some studies have reported its use in diseases such as prostatitis, neuralgia, bruises, in addition to being used as a tonic (LORENZI; MATOS, 2008; VENTRELLA *et al.,* 2008). The anti-inflammatory, anti-ulcer, antimicrobial and photoprotective effects of different preparations of *C. verbenacea* have been scientifically proven (SERTIÉ *et al.,* 2005; PASSOS *et al.,* 2007; PEREIRA *et al.*, 2021; MELO *et al.*, 2021) Currently, this medicinal plant is marketed for topical use, whose anti-inflammatory properties are related to the presence of α-humulene and trans-caryophyllene, extracted from the essential oil of *C. verbenacea*, although other studies indicate that artemetin has anti-inflammatory action, capable of inhibiting and/or reducing the synthesis and recruitment of inflammation mediators (SERTIÉ *et al.,* 1990; VAZ *et al.,* 2006; COUTINHO *et al.,* 2009). Phytochemical components such as flavonoids, terpenes and tannins have also been identified in preparations of *C. verbenacea* (GILBERT; FAVORETO, 2012; PINTO *et al.,* 2009).

Part of these substances are polyphenols, which are capable of modulating inflammatory processes and their bioactivity may be related to their antioxidant potential. Antioxidant substances have the ability to interact with reactive species from our metabolism and transform them into stable non-reactive species before they attack cells, causing lesions and triggering other diseases related to oxidative stress (SILVA *et al.,* 2018; ZANCO *et al.,* 2017). Oxidative stress results from the loss of balance between the production and degradation of reactive species, causing the accumulation of free radicals in the intracellular environment, culminating in the loss of cellular function. Currently, it is known that oxidative stress is related to inflammatory events (PIZZINO *et al.,* 2017). Thus, research about the antioxidant properties of natural products has been developed to minimize the deleterious effects of reactive oxygen and nitrogen species in the body.

The nitric oxide radical is an endogenous molecule of great importance in physiological processes, but in excess it contributes to the triggering of oxidative stress, leading to the loss of homeostasis. It is related to several pathological processes, including inflammatory processes and induced toxicity (ADEGBOLA *et al.*, 2017; FÖRSTERMANN *et al.*, 2017; MITTAL *et al.*, 2014; SILVA, DELGADO, 2020). Thus, the search for natural substances capable of scavenging free radicals can be of great value in the therapeutic field, helping to reduce the toxicity caused by reactive species, acting as a modulator of inflammatory processes (MAIA *et al.,* 2010).

Due to the interest in the study regarding the bioactivity of products of plant origin and the use of tea and ethanolic extract of *Cordia verbenacea* by the population in the treatment of different diseases, it is important to evaluate biological activities, including cytotoxic activity and the antioxidant potential of *Cordia*. Considering that the extracting solvent can influence the phytochemical composition and, consequently, the biological activity, this study aims to evaluate the cytotoxicity, antioxidant action and verify the presence of some phytochemical constituents in the aqueous and ethanolic extracts of *C. verbenacea*, aiming at the possibility of developing simple therapeutic alternatives to minimize the damage caused by free radicals in co-morbidities associated with oxidative stress.

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

The *C. verbenacea* (aerial parts) was acquired commercially and presented a quality control report, guaranteeing the

botanical identity and analysis of the micro and macroscopic aspects of the plant.

The aqueous extract was prepared according to the guidelines described in the Phytotherapic Form of the Brazilian Pharmacopoeia (ANVISA, 2011). The vegetable material was infused in water (10 g/L) for 10 minutes. Subsequently, the solution was filtered and lyophilized. For the preparation of the ethanolic extract, the vegetable material was subjected to the process of maceration in absolute ethanol (100 g/L), at room temperature, for one week. After this period, the mixture was filtered and subjected to solvent evaporation. This process was repeated at least three times.

PHYTOCHEMICAL CONSTITUENTS

Quantification of total phenols

The quantification of phenolic compounds in the extracts was performed using the modified Folin Ciocalteu method. (ROESLER *et al.,* 2007). The total phenols content was determined by interpolation of the absorbance of the samples against a calibration curve constructed with gallic acid standards. The analyzes were performed in triplicate and the results expressed in GAE (gallic acid equivalent).

Determination of flavonoids

The determination of flavonols and flavones present in the extracts was performed using a colorimetric method using aluminum chloride. The content of flavonols and flavones was determined by interpolation of the absorbance of the samples against a calibration curve constructed with different concentrations of quercetin. Analyzes were performed in triplicate and results expressed as QE (quercetin equivalent) (CHANG *et al.,* 2002). The determination of flavanones present in the extracts was performed according to the methodology described by Chang *et al.* (2002) which consists of the reaction of the ketone and aldehyde groups of the flavonoid with 2,4-dinitrophenylhydrazine, forming the product 2,4-dinitrophenylhydrazone. The flavanone content was determined by interpolation of the absorbance of the samples against a calibration curve constructed with different concentrations of naringin. Analyzes were performed in triplicate.

Qualitative test for alkaloid identification

The identification of alkaloids in the preparations of *C. verbenacea* was performed using the methodology described by Costa (2002), which uses the reagents of Mayer, Bertrand, Dragendorf and Wagner. A solution of pilocarpine, a natural alkaloid, was used as a positive reaction control.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH test (1,1-diphenyl-2-picrylhydrazyl)

The antioxidant activity of the extracts was determined by a spectrophotometric technique, which uses the free radical DPPH. The percentage of antioxidant activity (%AA) was determined through the equation: $\%AA = \{(A_0 - A)/A_0\} \times 100$; where A_0 is the absorbance of the DPPH without the test substance and A refers to the absorbance verified with the addition of the test sample. The experiments were carried out in triplicate and accompanied by a control antioxidant substance (gallic acid) (MENSOR *et al.,* 2001).

Nitric oxide scavenger

The nitric oxide scavenger was evaluated using the sodium nitroprusside (NPS) technique, followed by the Griess method (TSIKAS, 2007). The experiments were carried out in triplicate and accompanied by a control substance (gallic acid). The percentage of nitric oxide scavenging was calculated as described in item 2.3.1.

IN VITRO CYTOTOXIC ACTIVITY

McCoy cells (approximately 10^5 cells/mL) were inoculated into microplates (96 wells) and treated with different concentrations of aqueous and ethanolic extracts. After incubation for 24 hours, cell viability was evaluated by the Neutral Red test (BORENFREUD; PUERNER, 1985). The tests were accompanied by control growth (cells treated only with culture medium), performed in triplicate and repeated at least 3 times. The results were expressed as percentage of cell viability in relation to the control.

STATISTICAL ANALYSIS

The results of the experiments were presented as mean ± standard deviation. In order to verify significant differences between the extracts and concentrations evaluated, statistical analysis of variance was performed, followed by Tukey's test. The confidence interval was 95% (α=0.05) (LAPPONI, 2013).

RESULTS

PHYTOCHEMICAL SCREENING

The results showed that the aqueous extract presented a phenolic content higher than the ethanolic content, in all the concentrations evaluated, and these differences were significant (Table 1). The quantification of flavonols and flavones in the evaluated extracts was only possible at a concentration of 1 mg/mL, not being detected at lower concentrations. The aqueous extract showed significantly higher amounts of flavonols and flavone (0.030 ± 0.012 mg QE/extract concentration) compared to the ethanolic extract (0.009 ± 0.001 mg QE/extract concentration). A greater content of flavanones was observed in the ethanolic extract in relation to the aqueous extract, with a significant difference only in the highest concentration evaluated (Table 2). Through the technique used, alkaloids were not detected in the phytochemical preparations of *C. verbenacea*.

Total phenols content (mg GAE/extract concentration)		
Extract (mg/mL)	**Aqueous extract**	**Ethanolic extract**
1	0.138 ± 0.018	0.075 ± 0.009*
0.5	0.064 ± 0.015	0.030 ± 0.004*
0.25	0.034 ± 0.008	0.011 ± 0.000*

GAE: Gallic acid equivalent; *p < 0.05.

Table 1 - Content of total phenols present in aqueous and ethanolic extracts of *Cordia verbenacea.* Results expressed as mean ± standard deviation.

Flavanone content (mg NE/extract concentration)		
Extract (mg/mL)	**Aqueous extract**	**Ethanolic extract**
1	0.222 ± 0.010	0.923 ± 0.014*
0.5	0.105 ± 0.038	0.238 ± 0.022
0.25	0.071 ± 0.001	0.106 ± 0.031

NE: Naringin equivalent; *p < 0.05 in relation to the same concentration of aqueous extract.

Table 2 - Flavanone content present in aqueous and ethanolic extracts of *Cordia verbenacea.* Results expressed as mean ± standard deviation.

IN VITRO ANTIOXIDANT ACTIVITY

Antioxidant potential

The aqueous extract showed an antioxidant activity of approximately 70% at all concentrations evaluated, while the results verified for the ethanolic extract showed that its antioxidant capacity is directly proportional to the concentration of the extract (Table 3). The results of the activity in the scavenger of nitric oxide by the aqueous and ethanolic extract of *C. verbenacea* are shown in Table 3 and demonstrate that the ethanolic extract has a greater capacity to scavenger nitric oxide than the aqueous extract. At concentrations ≤ 0.5 mg/mL both have demonstrated the same antioxidant action.

Antioxidant activity				
	DPPH reduction (%)		Nitric oxide scavenging (%)	
Extract (mg/mL)	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
2	68.24 ± 2.11	87.91 ± 2.43*	55.98 ± 3.97	78.98 ± 2.55 #
1	70.48 ± 1.11	84.39 ± 0.23*	55.83 ± 3.63	70.30 ± 6.26 #
0.5	74.43 ± 2.10	58.00 ± 3.59*	57.16 ± 0.34	58.88 ± 7.18
0.25	75.81 ± 4.09	34.21 ± 6.57*	52.33 ± 0.25	53.24 ± 0.17

*, # $p < 0.05$ in relation to the same concentration of aqueous extract

Table 3 – Antioxidant activity of different concentrations of aqueous and ethanolic extract of *C. verbenacea*. Results expressed as mean ± standard deviation.

IN VITRO CYTOTOXIC ACTIVITY

The effects of different concentrations of aqueous and ethanolic extracts *C. verbenacea* on the viability of McCoy cells are shown in Table 4. The results showed that both extracts cause a dose-dependent reduction in viability, showing that the ethanolic extract has greater cytotoxicity when compared to the aqueous extract.

Cell viability (%)		
Extract (mg/mL)	Aqueous extract	Ethanolic extract
1	52.29 ± 1.50 #	43.85 ± 3.91* #
0.5	64.41 ± 7.22 #	49.71 ± 6.16* #
0.25	83.98 ± 0.02	79.16 ± 16.51* #
0.125	88.86 ± 15.14	75.54 ± 5.19 #
0.0625	89.58 ± 15.97	94.11 ± 1.22
0	94.99 ± 6.45	94.99 ± 6.45

* $p < 0.05$ in relation to the same concentration of the aqueous extract.

# $p < 0.05$ against negative control (zero concentration).

Table 4 - *In vitro* effect of aqueous and ethanolic extract of *Cordia verbenacea* on McCoy cell viability

DISCUSSION

Medicinal plants have been the subject of great scientific interest due to the search for new prototypes with medicinal properties. This therapeutic character usually comes from bioactive principles from the secondary metabolism of plants, which may have isolated, antagonistic or synergistic action (SCHAFRANSKI *et al.,* 2019).

One of the most used forms of medicinal plants by ethnopharmacology is the administration of infusions from leaves or aerial parts of the plant or through the decoction of bark, twigs and roots. On the other hand, much of the scientific research employs the process of maceration in organic solvents to extract active principles, differences that promote changes in the phytochemical content obtained and, consequently, cause variations in the pharmacological and toxicological effects (FIRMINO; MIRANDA, 2015). In addition, many studies were carried out with species cultivated in other countries, a fact that compromises the validation of the pharmacological effects of Brazilian plants, since the chemical composition of the plant is dependent on climatic, environmental, seasonal conditions, cultivation conditions (soil, water, minerals, fertilizers, among others), storage and the part of the plant used (JAYASEKERA *et al.,* 2011; SCOTTI *et al.*, 2007).

Research on the phytochemical characterization of *C. verbenacea* leaves indicated the presence of phenolic compounds,

flavonoids (flavonols, flavones, flavanones), xanthones, steroids, saponins, fatty acids, essential oil and alkaloids (ROLDÃO *et al.,* 2008; PEREIRA, 2013; ALVES *et al.*, 2015). Although authors have reported the existence of alkaloids in the preparations of *C. verbenacea,* such a phytochemical group in the aqueous and ethanolic extracts of the aerial parts of the species has not been identified. Alves *et al.* (2015) also did not identify this group of substances in the methanolic extract fraction of *Cordia* leaves. Due to these variations in the chemical composition of extracts, their phytochemical analysis is necessary and must be performed to validate the verified biological activities.

Many of the therapeutic properties of medicinal plants result from the presence of phenolic compounds, a heterogeneous chemical group characterized by having at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group (SIMÕES *et al.,* 2017). These substances can be extracted by methods of infusion, decoction and maceration in water or organic solvents (CASTRO-MUÑOZ *et al.,* 2016; VERRUCK *et al.,* 2018).

Studies report that extracts that use water as an extractant liquid are very effective methods for obtaining phenolic compounds (KO *et al.,* 2014; RODRIGUES, 2015). Santi *et al.* (2014) performed phytochemical analysis of different preparations of *C. verbenacea* leaves and found that the extract obtained by the infusion method had a higher content of total phenols compared to the ethanolic extract. These data corroborate the results of this study since a higher phenolic content was also observed in the aqueous extract.

Flavonols, flavones and flavanones were detected in the studied extracts. The aqueous extract had the highest content of flavonols, flavones and the ethanolic extract had the highest content of flavanones. Ameira *et al.*, 2009 identified the isoflavones 7,4'-dihydroxy-5'-carboxymethoxy-isoflavone and 7,4'-dihydroxy-5'-methyl-isoflavone in the fraction of the methanolic extract of leaves of *C. verbenaceae,* while in the hydroalcoholic extract of branches and leaves, the flavone artemetin (5-hydroxy-3,6,7,3',4'-pentamethoxyflavone) was identified (BAYEUX *et al.*, 2002; SERTIÉ *et al.,* 1990). Other phytochemical compounds such as rosmarinic acid, caffeic acid, gallic acid and chlorogenic acid were also found in *C. verbenacea* leaves (TICLI *et al.*, 2005; MATIAS *et al.*, 2013).

The gender *Cordia* is a promising source of secondary metabolites of different classes and the phytochemical constituents present in various phytotherapeutic preparations have been strongly correlated with therapeutic effects. Studies carried out in rodents verified that the hydroalcoholic and ethanolic extracts of *C. verbenacea* leaves were able to reduce inflammation, induce healing and promote the protection of the gastric mucosa. The methanolic extract was able to inhibit the formation of edema in mouse paws and these actions were attributed to the presence of compounds such as rosmarinic acid, flavonoid and the flavone artemetin (SERTIÉ *et al.*, 1988).

The antioxidant capacity of flavonoids and other phenolic compounds is widely known (POPESCU *et al.,* 2016). The results of this study showed that both aqueous and ethanolic extracts have antioxidant capacity, which may be due to the presence of these phytochemical constituents. The evaluation of the antioxidant activity of the infusion and the ethanolic extract of *C. verbenacea* leaves, carried out by Santi *et al.* (2014), showed that the ethanolic preparation has greater antioxidant capacity than the infusion, even with a lower phenolic content, however, it had a higher total flavonoid content than

that observed in the aqueous preparation. Our results also showed that the ethanolic extract, which has a lower phenolic amount and a higher flavanone content, presented a greater capacity in the scavenger of nitric oxide when compared to the aqueous extract.

Nitric oxide is a molecule present in several physiological mechanisms and essential bioregulatory processes, being important for the homeostasis of the organism. However, in high concentrations it can induce deleterious effects to the body (ADEGBOLA *et al.*, 2017; FÖRSTERMANN *et al.*, 2017; MITTAL *et al.*, 2014; SILVA, DELGADO, 2020).

The results of this work showed that the ethanolic extract, even with antioxidant capacity, induced a significant reduction in cell viability (concentrations $\geq$ 0.125 mg/mL), suggesting that other mechanisms are involved in cytotoxicity. The aqueous preparation showed lower toxicity compared to the ethanolic extract and this result may be related to the hydrophilic and lipophilic characteristics of the phytochemical constituents present in each extract. According to Woerdenbag *et al.* (1986), cytotoxicity increases with decreasing hydrophilicity, facilitating the penetration of molecules through the cytoplasmic membrane into the cell interior. Thus, considering that the process of infusion in water promotes obtaining more hydrophilic substances, the degree of water solubility and lipophilicity of the extracts may be directly related to the cytotoxicity exerted by extracts.

Other studies have also demonstrated the toxic effect of *C. verbenacea* preparations. Kviecinski *et al.* (2010) found that the supercritical extract of *C. verbenacea* induced cytotoxicity and apoptosis of Ehrlich carcinoma cells, showing an anticarcinogenic potential. Oliveira *et al.* (2010) showed that the ethanolic extract has high toxicity in bioassays performed with *Artemia salina*. Pereira *et al.* (2021) reported the strong cytotoxic effect of *C. verbenacea* essential oil on mammalian fibroblasts.

In view of the results this study, it is extremely important to consider the method of preparation of extracts obtained from *C. verbenacea*, since it induces variations in the phytochemical composition and exerts influence on biological and toxicological effects.

REFERENCES

ADEGBOLA, P.; ADERIBIGBE, I.; HAMMED, W.; OMOTAYO, T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. 2017. **American Journal of Cardiovascular Disease**, v.7, n.2, p.19-32, 2017.

ALVES, E.F. *et al.* Evaluation of antibacterial activity and modulatory effect of the hexane fraction from methanol extracto of *Cordia verbenacea* DC leaves. **Journal of Medicinal Plants Research**, Nigéria, v.9, n.12, p.395-99, 2015.

AMEIRA, O.A. *et al.* Estabelecimento de cultura de células em suspensão e identificação de flavonoides em *Cordia verbenacea* DC. **Revista Brasileira de Plantas Medicinais**, Campinas, v.11, n.1, p.7-11, 2009.

ANVISA, Brasil. **Formulário de Fitoterápicos Farmacopeia Brasileira**, p.1–126, 2011.

BAYEUX, M.; FERNANDES, A.; FOGLIO, M.; CARVALHO, J. Evaluation of the antiedematogenic activity of artemetin isolated from *Cordia curassavica* DC. **Brazilian Journal of Medical and Biological Research**, São Paulo, v.35, p.1229-32, 2002.

BORENFREUND, E.; PUERNER, J.A. Toxicity determined *in vitro* by morphological alterations and neutral red absorption. **Toxicol Letters**, Amsterdam, v.24, n.2-3, p.119-24, 1985.

CASTRO-MUÑOZ, R.; YÁÑEZ-FERNÁNDEZ, J.; FÍLA, V. Phenolic compounds recovered from agro-food by-products using membrane technologies: An overview. **Food Chemistry**, Amsterdam, v.213, p.753-62, 2016.

CHANG, C.C.; YANG, M.H.; WEN, H.M.; CHERN, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. **Journal of Food and Drug Analysis**, Taiwan, v.10, p.178-82, 2002.

COSTA, A.F. **Farmacognosia**. v.2. 4. ed. Lisboa: Fundação Calouste Gulbenkian, 2002. p.1120. 2002.

COUTINHO, M.A.S.; MUZITANO, A.S.; COSTA, S.S. Flavonoides: potenciais agentes terapêuticos para o processo inflamatório. **Revista Virtual de Química**, Niteroi, v.1, n.3, p.241-56, 2009.

FIRMINO, L.A.; MIRANDA, M.P.S. Polifenóis totais e flavonóides em amostras de chá verde (*Camellia sinensis* L.) de diferentes marcas comercializadas na cidade de Salvador-BA. **Revista Brasileira de Plantas Medicinais**, Campinas, v.17, n.3, p.436-43, 2015.

FÖRSTERMANN, U.; XIA, N.; LI, H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. **Circulation Research**, v.120, n.4, p.713–35, 2017.

GILBERT. B.; FAVORETO, R. *Cordia verbenacea* DC. Boraginaceae. **Revista Fitos**, Rio de Janeiro, v.7, n.1, p.17-25, 2012.

JAYASEKERA, S. *et al.* Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea. **Food Chemistry**, Amsterdam, v.125, p.536-41, 2011.

KO, M.; CHEEIGH, C.; CHUNG, M. Relationship analysis between flavonoids structure and subcritical water extraction (SWE). **Food Chemistry**, Amsterdam, v.143, p.147-55, 2014.

KVIECINSKI, M.R. Study of the antitumor potential of brazilian *Bidens pilosa* L., *Casearea sylvestris* S., *Cordia verbenacea* DC and *Croton celtidifolus* B. In: SIMPÓSIO BRASILEIRO DE PLANTAS MEDICINAIS, 21, 2010, João Pessoa. **Anais.** João Pessoa: Sociedade Brasileira de Plantas Medicinais, 2010.

LAPPONI, J.C. **Estatística usando Excel.** 4 ed. Rio de Janeiro: Elsevier, 2013. 496p.

LORENZI, H.; MATOS, F.J.A. **Plantas Medicinais no Brasil – nativas e exóticas.** Nova Odessa: Ed. Instituto Plantarum, 2008, 576p.

MAIA, R.M. *et al.* Avaliação do sequestro do óxido nítrico (NO) pelo extrato metanólico da alga *Bryothamnion triquetrum* (Gmelin) Howe. **Revista Brasileira de Farmacognosia**, São Paulo v.20, n.4, p.489-93, 2010.

MATIAS, E.F.F. *et al*. Biological Activities and Chemical Characterization of *Cordia verbenacea* DC. as Tool to Validate the Ethnobiological Usage. **Evidence-Based Complementary and Alternative Medicine**, New York, v.2013, 7p., 2013.

MELO, C.P.B. *et al*. Protective effect of oral treatment with *Cordia verbenacea* extract against UVB irradiation deleterious effects in the skin of hairless mouse. **Journal of Photochemistry and Photobiology B: Biology**, v.216, 112151. doi: 10.1016/j.jphotobiol.2021.112151.

MENSOR, L.L. *et al*. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. **Phytotherapy Research**, London, v.15, n.2 p.127-30, 2001.

MITTAL, M.; RIZWAN, M.S.; TRAN, K.; REDDY S.P.; MALIK, A.B. Reactive Oxygen Species in Inflammation and Tissue Injury. **Antioxidants & Redox Signaling**, v.20, n.7, p.1126-67, 2014.

MS (MINISTÉRIO DA SAÚDE). **Plantas de Interesse ao Sus**. 2014.

MS (MINISTÉRIO DA SAÚDE). **RENISUS: lista de plantas medicinais do SUS.** 2017.

OLIVEIRA, L.G.S. Avaliação da atividade antifúngica e toxicidade do extrato etanólico de *Cordia verbenacea.* In: SIMPÓSIO BRASILEIRO DE PLANTAS MEDICINAIS, 21, 2010, João Pessoa. **Anais.** João Pessoa: Sociedade Brasileira de Plantas Medicinais, 2010.

PASSOS, G.F. *et al*. Antiinflammatory and anti-allergic properties of the essential oil and active compounds from *Cordia verbenacea*. **Journal of Ethnopharmacology**, Lausanne, v.110, p.323-33, 2007.

PERREIRA, J.A.S. ***Cordia verbenacea* DC.: Perfil morfo-anatômico, histoquímico, farmacognóstico e avaliação da atividade anti-candida do extrato hidroetanólico e suas frações.** 2013. 52f. Dissertação (Mestrado em Ciências Farmacêuticas) - Faculdade de Ciências Farmacêuitcas de Araraquara, SP – UNESP.

PEREIRA, P.S. *et al*. Cytotoxicity of Essential Oil *Cordia verbenaceae* against *Leishmania brasiliensis* and *Trypanosoma cruzi*. **Molecules**. v.26, n.15, 4485, 2021.doi: 10.3390/molecules26154485.

PINTO, J.E.B.P.; CARDOSO, M.G.; ARRIGONI-BLANK, M.F. Estabelecimento de cultura de células em suspensão e identificação de flavonoides em *Cordia verbenacea* DC. **Revista Brasileira Plantas Medicinais**., v.11, n.11, p.7-11, 2009.

PIZZINO, G. *et al*. Oxidative Stress: Harms and Benefits for Human Health. **Oxidative Medicine and Cellular Longevity**, Austin, v.2, p.1-13, 2017.

POPESCU, A. *et al*. Antioxidant Comparative Activity and Total Phenolic Content of *Scirpus holoschoenus* L. (*Holoschoenus vulgaris* Link) Depending on Extraction Condition and the Solvent Used. **Revista de Chimie**, Bucharest, v.67, n.2, P.255-59, 2016.

RODRIGUES, V.C. **Compostos bioativos de chás: comparação das infusões a quente e a frio**. 2015. 37f. Dissertação (Mestrado em Tecnologia de Alimentos) - Universidade Tecnológica Federal do Paraná, Campo Mourão.

ROESLER, R. *et a*l. Atividade antioxidante de frutas do cerrado. **Food Science and Technology**, Campinas, v.27, n.1, p.53-60, 2007.

ROLDÃO, E.F. *et al*. Evaluation of the antiulcerogenic and analgesic activities of *Cordia verbenacea* DC. (Boraginaceae). **Journal of Ethnopharmacology**, Lausanne, v.119, n.1, p.94-98, 2008.

SANTI, M.M. *et al.* Determinação do perfil fitoquímico de extrato com atividade antioxidante da espécie medicinal *Cordia verbenacea* DC. por HPLC-DAD. **Revista Brasileira de Plantas Medicinais**, Campinas, v.16, n.2, p.256-61, 2014.

SANTOS, S.L.F. *et al*. Uso de plantas medicinais por idosos de uma instituição filantrópica. **Revista Brasileira de Pesquisa em Ciências da Saúde**, v.4, n.2, p.71-75, 2017.

SCHAFRANSKI, K. **Extração e caracterização de compostos fenólicos de folhas de amoreira preta (*Morus nigra* L.) e encapsulamento em esferas de alginato**. 2019. 100f. Dissertação (Mestrado em Biotecnologia) - Universidade Tecnológica Federal Do Paraná, Ponta Grossa.

SCOTTI. M.T. *et al*. Quantitative structure–activity relationship of sesquiterpene lactones with cytotoxic activity**. Bioorganic & Medicinal Chemistry**. v.15, n.8, p.2927-34, 2007.

SERTIÉ, J.A.A. *et al*. Pharmacological assay of *Cordia verbenacea*. Part 1. Anti-inflammatory activity and toxicity of the crude extract of the leaves. **Planta Medica**. Stuttgart, v.54, n.1, p.7–10, 1988.

SERTIÉ, J.A.A. *et al.* Antiinflammatory activity and sub-acute toxicity of artemetin, **Planta Medica**, Stuttgart, v.56, n.1, p.36-40, 1990.

SERTIÉ. J.A.A. *et al*. Pharmacological assay of *Cordia verbenacea* V: oral and topical anti-inflammatory activity, analgesic effect and fetus toxicity of a crude leaf extract. **Phytomedicine**, Stuttgart, v.12, n.5, p.338-44, 2005.

SILVA, E.C.A. et al. Antioxidantes polifenóis de plantas brasileiras utilizadas como inibidores do câncer. **International Journal Nutrology**, S24-S327, 2018.

SILVA, F.B., DELGADO, N.T.B. Aterosclerose e atividade do sistema antioxidante enzimático. **Revista Espaço Acadêmico**, v.9, n.2, p.35-47, 2020.

SIMÕES, C.M.O. *et al.* **Farmacognosia do produto natural ao medicamento**. Porto Alegre: Artmed, 2017, 486p.

TAYLOR, L. **The Healing Power of Rainforest Herbs.** New York: Square one Publishers, Inc, 2005, 535p.

TICLI, F.K. *et al.* Rosmarinic acid, a new snake venom phospholipase A2 inhibitor from *Cordia verbenacea* (Boraginaceae): antiserum action potentiation and molecular interaction. **Toxicon**, Oxford, v.46, n.3, p.318-27, 2005.

TSIKAS, D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L- arginine/ nitric oxide area of research. **Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences**, Amsterdam, v.851, n.1-2, p.51-70, 2007.

VAZ, A.A.A. *et a*l. Biomassa e composição química de genótipos melhorados de espécies medicinais cultivadas em quatro municípios paulista. **Pesquisa Agropecuária Brasileira**, v.41, n.5, p.869-72, 2006.

VENTRELLA, M.C., MARINHO, C.R. Morphology and histochemistry of glandular trichomes of *Cordia verbenacea* DC. (Boraginaceae) leaves. **Revista Brasileira de Botânica**, Brasília-DF v.31, p.457-67, 2008.

VERRUCK, S.; PRUDENCIO, E.S.; SILVEIRA, S.M. Compostos bioativos com capacidade antioxidante e antimicrobiana em frutas. **Revista do Congresso Sul Brasileiro de Engenharia de Alimentos**, Santa Catarina, v.4, n.1, p.111-24, 2018.

WOERDENBAG, H.J. *et al.* Evaluation of the *in vitro* cytotoxicity of some sesquiterpene lactones on a human lung carcinoma cell line using the fast green dye exclusion assay. **Planta Medica**, Stuttgart, v.2, p.112-14, 1986.

ZANCO, P.; SOUZA, E.F.; BONACORSI, C. Avaliação da atividade antimicrobiana e antioxidante de chá de folhas da amora-preta (*Morus nigra*). **Scientific Eletronic Archives**, v.10, n.1, p.150-57, 2017.