

Evaluation of angiogenic and embryotoxic activity of the extract of *Anadenanthera peregrina* (Angico-do-Cerrado)

Tamírís Augusto Marinho^{1,5}, Patrícia Silva Nunes^{2,5}, Ruy de Souza Lino Junior², Paulo Roberto de Melo Reis⁴, Mônica Rodrigues Ferreira Machado³, Nelson Jorge Silva Jr^{1,4}, Antonio Carlos Pereira de Menezes Filho⁶ & Matheus Vinicius Abadia Ventura⁷

¹ Universidade Federal de Goiás, UFG, Instituto de Patologia Tropical e Saúde Pública (IPTSP), Programa de Pós-Graduação em Biotecnologia e Biodiversidade (PGBB), Goiânia, Goiás State, Brasil.

² Universidade Federal de Goiás, UFG, Instituto de Patologia Tropical e Saúde Pública (IPTSP), Programa de Pós-Graduação em Medicina Tropical, Goiânia, Goiás State, Brasil.

³ Universidade Federal de Jataí, UFJ, Departamento de Ciências Biológicas, Laboratório de Pesquisa e Reprodução de Zebrafish (LABFISH), Jataí, Goiás State, Brasil.

⁴ Pontifícia Universidade Católica de Goiás, (PUC – Goiás), Laboratório de Estudos Experimentais e Biotecnológicos, Goiânia, Goiás State, Brasil.

⁵ Instituto Federal de Educação, Ciência e Tecnologia de Goiás, (IFG), Núcleo de Estudos e Pesquisas em Promoção da Saúde (NUPPS), Goiânia, Goiás State, Brasil.

⁶ Instituto Federal Goiano (IFGoiano), Laboratórios de Química Tecnológica e de Hidráulica e Irrigação, Rio Verde, Goiás State, Brasil.

⁷ Faculdade UniBRAS Rio Verde (UniBRAS), Rio Verde, Goiás State, Brasil

Correspondence: Antonio Carlos Pereira de Menezes Filho, Laboratórios de Química Tecnológica e de Hidráulica e Irrigação, Instituto Federal Goiano, Rio Verde, Goiás State, Brasil. E-mail: astronomoamadorgoias@gmail.com

Received: June 05, 2022

Accepted: June 30, 2022

Published: September 01, 2022

Abstract

The genus *Anadenanthera* has been reported in the literature with antioxidant, anti-inflammatory, antimicrobial effect and healing action in wound treatment. The study aimed to evaluate, *in vivo*, the angiogenic and embryotoxic activities of *A. peregrina* extract. Angiogenesis in chicken embryo egg chorioallantoic membrane and zebrafish embryotoxicity was performed. *A. peregrina* extract at concentrations 62 mg mL⁻¹ and 124 mg mL⁻¹ were angiogenic. For embryotoxicity, the mortality rate increased with increasing concentration and increased dose and time dependent embryotoxicity was observed. The lethal concentration (LC₅₀) ranged from 0.331mg mL⁻¹ over the 24 hpf period to 0.007 mg mL⁻¹ at 168 hpf ($\Delta\% = -97.9$), decreasing with increasing exposure. The heart rate decreased progressively and significantly with increasing concentration at all tested exposure times. In conclusion, it was evidenced that the extract of *Anadenanthera peregrina* has angiogenic activity. Nonetheless, embryotoxic effects were observed at high concentrations.

Keywords: Angiogenesis inducing agents, Plants, Drugs, Toxicity, Zebrafish

Resumo

O gênero *Anadenanthera* tem sido relatado na literatura com ação antioxidante, anti-inflamatória, antimicrobiana e cicatrizante no tratamento de feridas. O estudo teve como objetivo avaliar, *in vivo*, as atividades angiogênica e embriotóxica do extrato de *A. peregrina*. Foi realizada angiogênese em membrana corioalantóide de ovo de embrião de galinha e embriotoxicidade de peixe-zebra. O extrato de *A. peregrina* nas concentrações de 62 mg mL⁻¹ e 124 mg mL⁻¹ foi angiogênico. Para embriotoxicidade, a taxa de mortalidade aumentou com o aumento da concentração e observou-se aumento da dose e embriotoxicidade dependente do tempo. A concentração letal (CL₅₀) variou de 0,331mg mL⁻¹ ao longo do período de 24 hpf a 0,007 mg mL⁻¹ a 168 hpf ($\Delta\% = -97,9$), diminuindo com o aumento da exposição. A frequência cardíaca diminuiu progressiva e significativamente com o aumento da concentração em todos os tempos de exposição testados. Em conclusão, foi constatado que o extrato de *Anadenanthera peregrina* possui atividade angiogênica. No entanto, efeitos embriotóxicos foram

observados em altas concentrações.

Palavras-chave: Agentes indutores de angiogênese, Plantas, Drogas, Toxicidade, Peixe-zebra

Resumen

El género *Anadenanthera* ha sido reportado en la literatura con acción antioxidante, antiinflamatoria, antimicrobiana y cicatrizante en el tratamiento de heridas. El estudio tuvo como objetivo evaluar, *in vivo*, las actividades angiogénicas y embriotóxicas del extracto de *A. peregrina*. La angiogénesis se realizó en la membrana corioalantoidea del huevo de embrión de pollo y la embriotoxicidad del pez cebra. El extracto de *A. peregrina* en concentraciones de 62 mg mL⁻¹ y 124 mg mL⁻¹ fue angiogénico. Para la embriotoxicidad, la tasa de mortalidad aumentó con el aumento de la concentración y se observó una mayor dosis y embriotoxicidad dependiente del tiempo. La concentración letal (CL₅₀) varió de 0,331 mg mL⁻¹ durante el período de 24 hpf a 0,007 mg mL⁻¹ a las 168 hpf ($\Delta\% = -97,9$), disminuyendo con el aumento de la exposición. La frecuencia cardíaca disminuyó progresiva y significativamente con el aumento de la concentración en todos los tiempos de exposición probados. En conclusión, se encontró que el extracto de *Anadenanthera peregrina* tiene actividad angiogénica. Sin embargo, se observaron efectos embriotóxicos a altas concentraciones.

Palabras clave: Agentes indutores de angiogénesis, Plantas, Fármacos, Toxicidad, Pez cebra

1. Introduction

The use of traditional and complementary medicine (TCM) for therapeutic purposes represents an old practice in health care (WHO, 2020). In Brazil, despite the growth of the pharmaceutical industry, TCM is part of health care due to the high cost of medicines, difficult access to the public health system and availability of plant species (Dutra et al. 2016).

In the Cerrado, the second largest Brazilian biome, is found the *Anadenanthera peregrina* (Angico-do-Cerrado), belonging to the Fabaceae family. Within this genus yet another specie *Anadenanthera colubrina*. Multiple uses of species *Anadenanthera* have been reported in the literature (Ishara; Maimoni-Rodella, 2010; Cunha et al. 2020). The use of bark and seeds have healing action in wound treatment (Pessoa et al. 2012; Pessoa et al. 2015), antioxidant effect, anti-inflammatory, antimicrobial and use in respiratory diseases (Weber et al. 2001; Gama et al. 2018).

The hydroethanolic 50% (v/v) extract of *A. peregrina* bark, present high content of total phenolic compounds (583 mg of GAE g⁻¹ extract) and antioxidant activity of moderate intensity with an average IC₅₀ value of 13 µg mL⁻¹ compared with 2 mg mL⁻¹ for Trolox. The bark of *A. peregrina* is a potential source of polar extracts, enabling the extraction of tannins that represent approximately 17% of the bark (173.3 mg CE g⁻¹ bark) and 59% of the hydroalcoholic extract (in Catechin equivalents) (Mota et al. 2017).

In this context, highlight for the use of medicinal plants in the treatment of wounds favoring healing through angiogenic activity, including in infected wounds (Araújo et al. 2016). Toxicity studies of plant species for safe use are critical and zebrafish is shown to be a correlative *in vivo* model due to the high degree of genomic homology with humans, embryo and larvae transparency allowing real-time evaluation (Jayasinghee; Jayawardena, 2019).

Previous studies using zebrafish have shown that this method enables the evaluation of various toxic aspects of the tested substances, including decreased heart rate, yolk sac edema, pericardial edema, spinal cord alteration, hatch rate inhibition, lethal concentration (LC₅₀) and mortality rate (Thiagarajan et al. 2019).

However, even though *A. peregrina* is a popular medicinal plant, there are no reports in the literature of its possible angiogenic and embryotoxicity effects. Thus, the present study aimed to evaluate, *in vivo*, the angiogenic activities and embryotoxicity of *A. peregrina* concentrated liquid extract.

2. Materials and Methods

Collection and identification of plant material

The bark of *Anadenanthera peregrina* stem was collected from three specimens located in the Botanical Garden of Goiânia, Goiás State, Brasil, (16°43'22"S and 49°22'54"W). The species was identified and authenticated by Dr^a. Lorena Lana Camelo Antunes, at the Laboratory of Plant Morphology and Taxonomy of the Federal University of Goiás, Goiânia, Goiás, Brasil, and a sample was deposited in the herbarium of the same university

(Plant Voucher Registration Number: 61.014).

Production of Anadenanthera peregrina bark extract

To obtain the extract, the barks were ground in a knife mill with Tamis 20 mesh (TE-625; Tecnal Ltd., São Paulo, Brasil); then, 1.000 g of the ground barks sample was percolated (Revitec Ltd., São Paulo, Brasil) with 5.000 mL of hydroethanolic solution (50:50 v/v) for 24 h in a metal percolator with a Tamis 200 mesh lined with a layer of paper towel and cotton to filter the barks particles. Next, it was extracted exhaustively (0.2 mL min^{-1}) at room temperature (percolation phase). Subsequently, the extract was evaporated at $40 \text{ }^\circ\text{C}$ in a rotary evaporator (TE211; Tecnal Ltd., São Paulo, Brasil) under reduced pressure (vacuum pump - TE0581; Tecnal Ltd., São Paulo, Brasil). The extract obtained (2500 mL) was stored in a closed refrigerated container ($-2 \text{ }^\circ\text{C}$ to $+8 \text{ }^\circ\text{C}$) until further analysis. Posteriorly, after the rotavaporated hydroalcoholic extract was produced and using the Moisture Meter with an infrared heat source (ID 200; Scientific Mars), at $150 \text{ }^\circ\text{C}$, the extract concentration was determined as 124 mg mL^{-1} , based on the content of solids in triplicate.

Evaluation of angiogenic activity

Chicken embryonated eggs (*Gallus domesticus*) were incubated in an automatic oven at $37 \text{ }^\circ\text{C}$ and 60-70% relative humidity for sixteen days. On the fifth day of incubation, a circular hole opening in the eggshell was performed in a laminar flow chamber using a microretify (Dremel, Multi Pro Grinder, São Paulo, Brasil). Immediately thereafter, a drop of 0.9% (w/v) NaCl was added over the vascularized chorioallantoic membrane (CAM). The opening was sealed with tape and the incubation was carried on.

At the end of the thirteenth day of incubation, filter paper discs containing concentrated *A. peregrina* liquid extract were added directly to the CAM at three different concentrations (EX1: 25% of initial concentration = 31 mg/mL ; EX2: 50% of initial concentration = 62 mg mL^{-1} ; EX3: 100% of the initial concentration = 124 mg mL^{-1}). In addition, negative control: sterile distilled water (DW) (Samtec Biotechnology, São Paulo, Brasil), induction control: Regederm® (Pele Nova Biotecnologia, São Paulo, Brasil) and inhibition control: Injectable Dexamethasone 4 mg mL^{-1} (Aché Pharmaceutical Laboratories S.A., São Paulo, Brazil) was tested under sterile conditions. The eggs returned to incubation by the sixteenth day.

On the sixteenth day of incubation the CAMs were removed, fixed with formaldehyde solution (3.7%) for 5 minutes and cuted with blunt curved scissors and kept in Petri dishes in the presence of 10% formaldehyde solution to obtain photo registration (640x480 pixels; RGB 24 bits) for analysis and quantification of a newly formed vascular. They were then fixed in a 10% formaldehyde solution, embedded in a paraffin block, and then made $5 \mu\text{m}$ thick histological sections on a Spencer microtome (Ao 820, Spencer Buffalo, New York, United States) and stained with hematoxylin-eosin (HE). Ten membranes were analyzed for each test group and controls for the following parameters: number of blood vessels, cellular infiltrate, blood vessel size and cellular pyknosis. The results were classified by the intensity of each parameter: absent (0), discrete (1), moderate (2) and intense (3). The length, caliber, number of junctions and number of blood vessel complexes formed in the CAM were measured using the AngioQuant software version 6.5 (Niemistö et al., 2005).

Embryotoxicity assessment

Acute embryotoxicity tests on zebrafish embryos followed OECD 236 recommendations. Experimentation was performed with embryos from adult fish placed under ideal conditions, 60 males and 20 females in aquariums with water recirculation system ($28.5 \pm 2 \text{ }^\circ\text{C}$, 80% humidity) and the photoperiod was adjusted to a 14 h light/10 hours dark cycle, fed four times a day with commercial floccular feed (FlakesFood®) and *Artemia salina*. For experiments, embryos from reproduction were collected, transferred to Petri dishes containing E3 solution and classified aided by stereomicroscope (Olympus CX 31, Olympus, Tokyo, Japan) as good, intermediate, bad and non-fertilized, using as standard: cell coloration, disposition and proliferation, in addition to egg fertilization and malformation.

After selection, the embryos classified exclusively as good were placed in polypropylene plates, white in color and transparent bottom with 96-wells. Twelve different serial dilutions of *A. peregrina* concentrated liquid extract was tested at the initial concentration of 124 mg mL^{-1} and negative control (E3 solution) in 5 replicates. The solutions were changed daily and kept at room temperature. Embryonic developmental stages were evaluated for: decreased heart rate, yolk sac edema, pericardial edema, spinal cord alteration, hatching rate

inhibition, lethal concentration (LC₅₀) and mortality rate through photos and/or videos were obtained in the periods of 0, 48, 72, 96, 120, 144 and 168 hours after fertilization (hpf) using the light microscope (LEICA DM750, Leica Microsystems, Wetzlar, Germany) coupled to the ICC50 HD digital camera and the LAS[®]EZ3.0.0 software (Nagel, 2002).

Statistical analysis

Data on angiogenic activity and embryotoxicity were analyzed using SPSS version 24.0 and GraphPadPrism version 7.0. Initially, the normality variables of the study were verified by the *Shapiro-Wilk* test (Razali; Wah, 2011). The parameters measured in angiogenic activity were presented for each group as median and interquartile range (QII). To compare the values found, the *Kruskal-Wallis* nonparametric test for independent samples was performed, followed by post hoc analysis by Dunn's test for multiple comparisons in case of statistical significance (Kruskal & Wallis, 1952; Dunn, 1964). The parameters evaluated for embryotoxicity were presented as average and standard error of average (SEM). The comparison of the values found in relation to the control group was performed by the Mann-Whitney test for independent samples, *p*-values *p* < 0.05 were considered statistically significant. To determine the LC₅₀ the Probit method was used.

Ethical aspects

This study was approved by the Research Ethics Committee of Pontifícia Universidade Católica de Goiás, PUC-Goiás (Consubstantiated Report n° 8235150816/2018).

3. Results

Evaluation of angiogenic activity

Table 1 and Figure 1 summarize the descriptive and comparative intergroup analysis. *Kruskal-Wallis* test for independent samples showed a statistically significant difference among groups for all parameters evaluated (*p*-value < 0.001) (Table 1). The intergroup multiple comparison analysis showed that for the parameter length, size, number of complexes and vessel junctions, EX2 and EX3 were angiogenic when compared to Dexamethasone inhibition control and Regederm[®] induction control.

Table 1. Descriptive and comparative data of angiogenesis parameters (length, size, complexes and number of blood vessel junctions) intergroups.

Parameters *	Dexamethason e	Water	Regederm [®]	EX1	EX2	EX3	H (g.l.)	p-value
Length	2.553,95 (1.223,55)	6.225,75 (1.277,00)	13.384,72 (1.325,85)	6.162,55 (803.35)	12.612,75 (777.48)	11.840,90 (1.303,23)	72.80 [†] (5)	< 0.001
Size	15.382,50 (3.189,75)	33.329,50 (12.193,75)	60.176,50 (12.813,25)	38.184,00 (11.730,25)	54.663,00 (7.965,25)	58.059,89 (4.610,75)	46.62 [†] (5)	< 0.001
Complexes	21.00 (10.25)	39.00 (5.50)	49.50 (6.50)	33.00 (10.25)	46.50 (12.00)	44.00 (10.50)	50.60 [†] (5)	< 0.001
Junctions	32.00 (8.75)	123.50 (7.00)	274.00 (25.75)	125.50 (34.50)	243.00 (73.00)	242.00 (55.25)	49.68 [†] (5)	< 0.001

Note: Abbreviations: g.l. = degrees of freedom; * Data presented as median (IQR); † *Kruskal-Wallis* test for independent samples. Source: Authors, 2020.

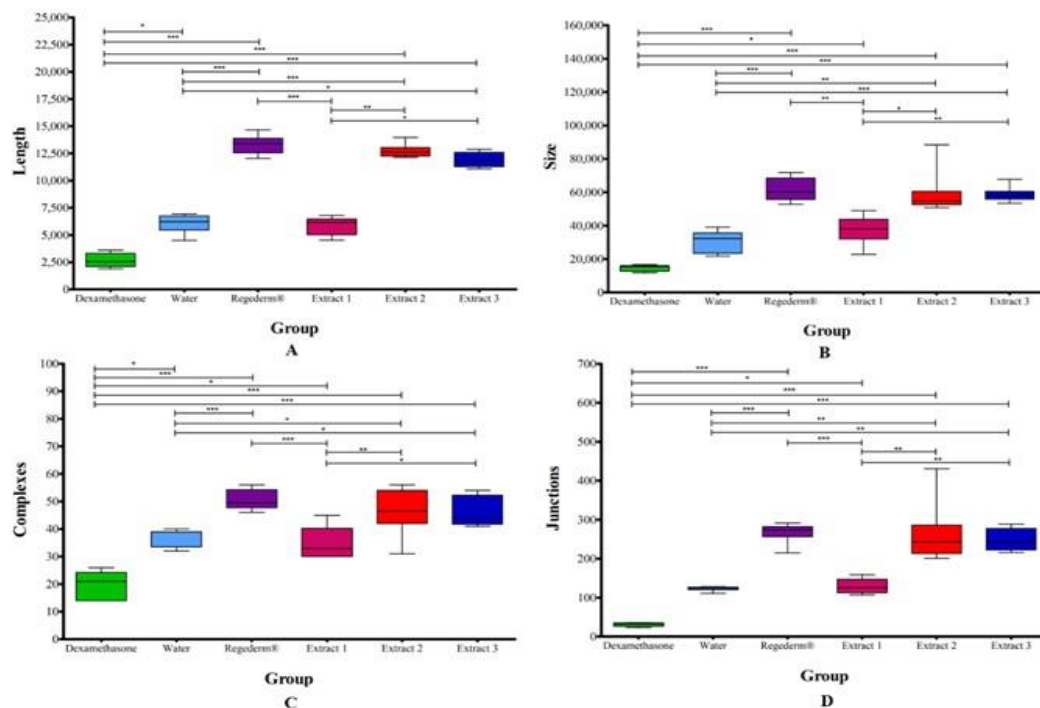


Figure 1. Multiple comparisons of angiogenesis parameters intergroups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **A** - length. **B** - size. **C** - complexes. **D** - number of blood vessel junctions. Source: Authors, 2020.

Figure 2 shows the chorioallantoic membranes (A) and photomicrographs (B) treated with 3 μL of the studied products for 72 hours. In membrane (i) Dexamethasone, there were reduced blood cells and vessels; In (ii) DW, there was a slight presence of inflammatory cells and vessels, a pattern similar to that found in (iv) EX1; In (iii) Regederm[®] there was a moderate presence of inflammatory cells and vessels, in addition to increased connective tissue in the parenchyma, a pattern similar to that observed in (v) EX2; In (vi) EX3, there was an important presence of inflammatory cells and vessels, as well as increased connective tissue in the parenchyma.

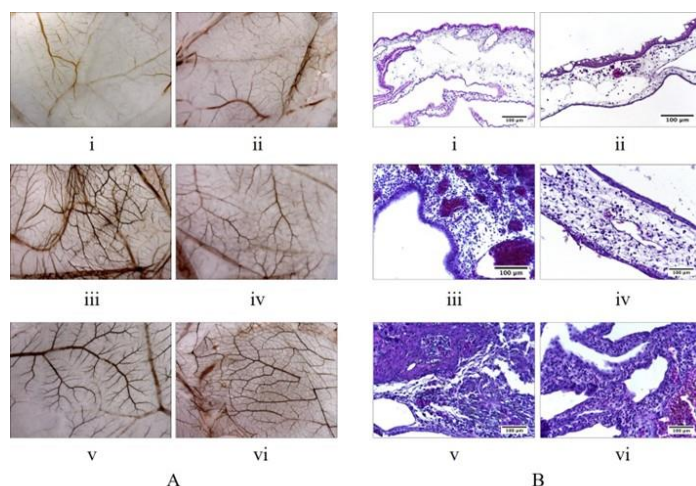


Figure 2. Chorioallantoic membranes and photomicrographs intergroups within 72 hours. **A** - Chorioallantoic membranes. **B** - Photomicrographs. **i** - Dexamethasone. **ii** - DW. **iii** - Regederm[®]. **iv** - EX1. **v** - EX2. **vi** - EX3. Source: Authors, 2020.

Embryotoxicity assessment

Figure 3 shows the embryos zebrafish mortality rate, LC_{50} , heart rate, and hatching rate of *A. peregrina* concentrated liquid extract. Regarding mortality, an increase in tax with increased concentration was observed; from the concentration 1.954 mg mL^{-1} to 62 mg mL^{-1} this rate was 100% at all times analyzed. In addition,

exposure to the extract in zebrafish embryos caused increased dose and time-dependent embryonic toxicity (Figure 3A).

The LC₅₀ of *A. peregrina* concentrated liquid extract ranged from 0.331 mg mL⁻¹ over the 24 hpf period to 0.007 mg mL⁻¹ in 168 hpf ($\Delta\% = -97.9$). LC₅₀ decreased with increased exposure (p -value = 0.001) (Figure 3B).

Heart rate decreased progressively and significantly with increasing concentration at all exposure times: 48 hpf (p -value < 0.001), 72 hpf (p -value = 0.002) and 96 hpf (p -value = 0.001). Significant heart rate inhibitions were observed in embryos treated with extract *A. peregrina* at concentrations greater than or equal to 0.1210 mg mL⁻¹ at 96 hpf. At 48 hpf and 72 hpf, significant inhibition of heart rate was found at concentrations equal to or greater than 0.241 mg mL⁻¹ (Figure 3C).

Regarding the hatching rate, compared to the control, *A. peregrina* concentrated liquid extract showed low hatching rate or no hatching. From concentration 0.0605 mg mL⁻¹ the hatching rate was zero for all periods (Figure 3D). † Data presented has mean or \pm mean and standard error mean. * p -value < 0.05 (Mann-Whitney test) when compared to control.

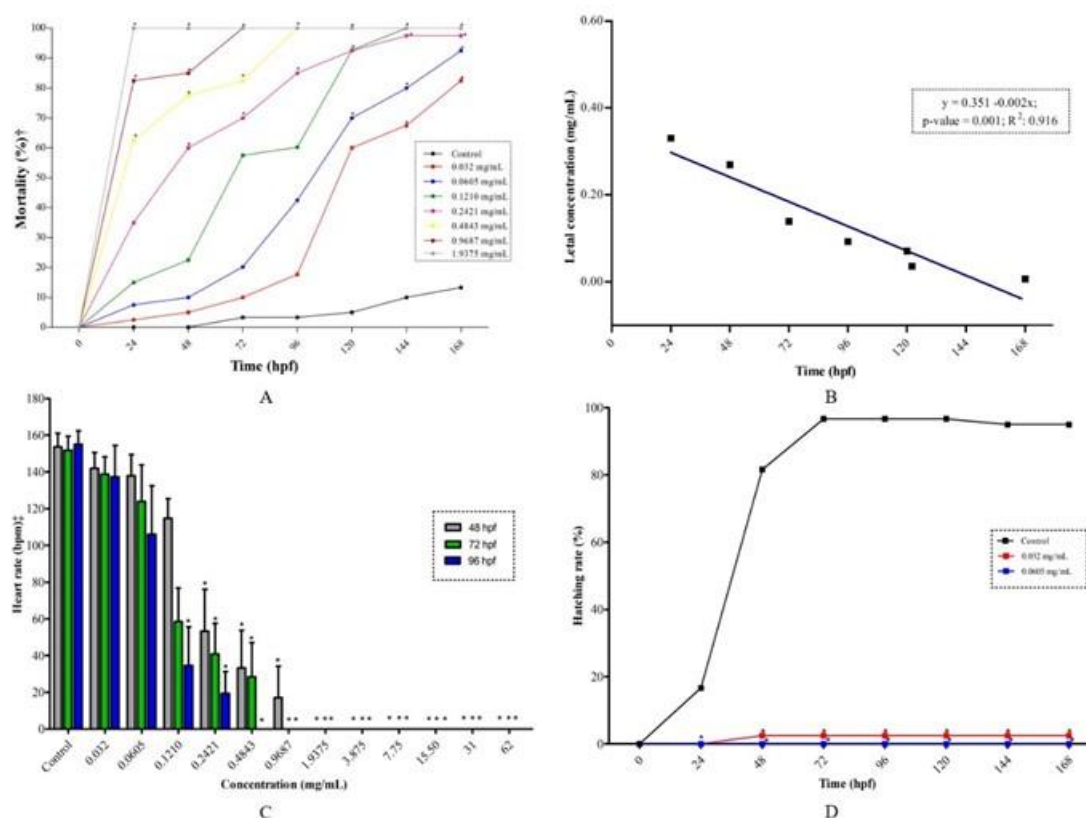


Figure 3. The mortality rate, LC₅₀, heart rate and hatching rate in zebrafish embryos exposed to *A. peregrina* concentrated liquid extract. **A** - Mortality rate. **B** - LC₅₀. **C** - Heart rate. **D** - Hatching rate. Source: Authors, 2020.

Malformations

Concentrated liquid extract of *A. peregrina* induced a set of malformations in zebrafish embryos, such as spinal alteration, pericardial edema, and yolk sac edema. However, there was no significant difference in pericardial edema rate ($H = 20,086$; p -value = 0.065), as well as in the yolk sac edema rate ($H = 9,445$; p -value = 0.665), and spinal alteration ($H = 12,000$; p -value = 0.446) when compared to the control group (Figure 4).

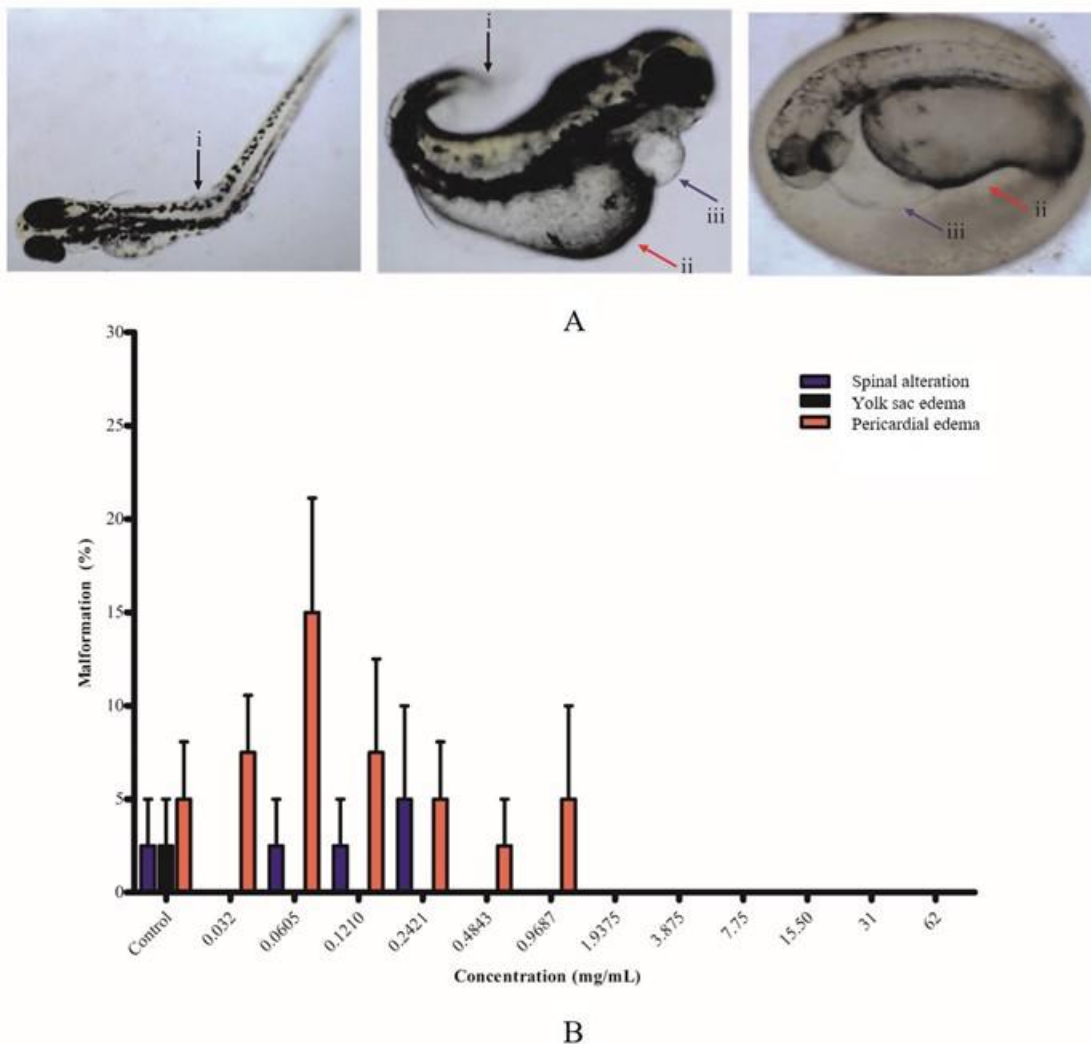


Figure 4. Malformations found in zebrafish embryos exposed to *A. peregrina*. **A** = Examples of the malformations identified. i - spinal alteration. ii - yolk sac edema. iii - pericardial edema. **B** = Malformation rate. Source: Authors, 2020.

4. Discussion

The genus *Anadenanthera* is described in the literature as a popular medicinal plant. In the present study *A. peregrina* presented angiogenic results for the concentrated liquid extract in the highest concentrations. Considering other studies with the genus *Anadenanthera*, an antimicrobial effect was observed against *Staphylococcus aureus* and *Escherichia coli* (Araújo et al., 2016), potentiated action of neomycin, Amikacin against *Staphylococcus aureus* (Barreto et al. 2016), as well as synergism when combined with Fluconazole (Nunes et al., 2015). Also, in other researches, antifungal potential (Lima et al., 2014), biofilm inhibition (Trentin et al., 2013), and relevance in pain management have been reported (Santos et al., 2013). The association of angiogenic effect and antimicrobial potential becomes relevant for use in infected wounds.

In a study that evaluated the effect of *Anadenanthera colubrina* extract on rat skin wounds, the morphology and morphometric analysis improved the healing process of lesions on the fourth, seventh and fourteenth postoperative days. On the fourth day, large lumens were found, as well as thickening in the fibrin-leukocyte layer of the vessels, and on days seven and fourteen the blood vessels were more dilated (Pessoa et al., 2012; Pessoa et al., 2015). This same study identified a large amount of proanthocyanidins in the hydroalcoholic extract, as well as reducing sugars, flavonoids, leucoanthocyanidin, saponins, triterpenes and steroids. In other study, tannis, phenols, flavones/flavonol/xanthones and alkaloids were identified (Farias et al., 2010). These results obtained in the literature are consistent and justify the angiogenic effects evidenced in the present study.

The angiogenic action verified in the concentrated liquid extract of *A. peregrina* is in agreement with other researches that evaluated healing plant extracts in rats, such as Sanativo Elixir® product based on a blend hydroalcoholic extracts, including in its composition 20% of the *Anadenanthera colubrina* extract, which has

shown a positive effect on wound healing and low toxicity (Lima et al., 2006). As in other plant species, the aqueous extract of barbatimão (*Stryphnodendron adstringens*) induced angiogenic activity in chicken embryonic egg chorioallantoic membrane (Chaves et al., 2016), copaiba oil demonstrated an increase in the number and caliber of blood vessels on the seventh day of postoperative skin wounds (Estevão et al., 2009), and a study on the angiogenic activity of the ethanolic extract of *Calendula officinalis* that showed benefit in healing (Estevão et al., 2009). Regarding the embryotoxicity evaluated in zebrafish embryos in the present study, there is a dose and time dependent mortality rate; in contrast, the observed malformations were not statistically significant when compared to the control group. For *Curcuma longa* extract, also having E3 medium as a control, toxicity effects and dose-dependent mortality were also observed, as well as malformations such as spinal alteration and yolk sac edema (Alafiatayo et al., 2019).

Still regarding malformations, *Sutherlandia frutescens* a medicinal plant used as immunostimulant was tested at concentrations of 5 $\mu\text{g mL}^{-1}$ to 50 $\mu\text{g mL}^{-1}$ for developmental evaluation in zebrafish embryos and showed embryotoxicity effects such as pericardial edema and edema yolk sac, being the aqueous extract less toxic than the ethanolic. Such malformations were also observed in the concentrated liquid extract of *A. peregrina*. The concentrated liquid extract used here was rotaevaporated hydroalcoholic, to exclude possible alcoholic interference in the results (Chen et al., 2018).

The reduction in hatching rate and non-hatching observed for *A. peregrina* concentrated liquid extract may be due to impregnation of substances present in the extract that prevented the rupture of the chorion in the expected time. Similarly, to that observed in ethnomedicinal plants *Andrographis paniculata*, *Canela zeylanicum*, *Curcuma xanthorrhiza*, *Eugenia polyantha* and *Orthosiphon stamineus* used from fever to metabolic disease. When tested for embryotoxicity in zebrafish, they showed after 48 hpf, particularly *Cinnamon zeylanicum* and *Eugenia polyantha*, increased mortality rate, malformations, abnormal heartbeat and delayed hatching rates, suggesting that the chorion protected embryos by decreasing diffusion of substances present in extracts, which may delay embryotoxic effects until hatching (Ismail et al., 2017).

The LC_{50} observed for the concentrated liquid extract of *A. peregrina*, as the concentration of the extract increased the LC_{50} decreased, and the LC_{50} values were dependent on the exposure time. For *Momordica charantia* seed extract, popularly known as “cabaço amargo”, LC_{50} values 50 $\mu\text{g mL}^{-1}$ were observed in zebrafish embryos and multiple malformations at sub-lethal concentrations (Khan et al., 2019). This same medicinal plant still had a growing mortality rate and a decreased hatching rate as the extract concentration increased and no hatching at the highest concentration was observed (1.000 $\mu\text{g mL}^{-1}$) (Thiagarajan et al., 2019).

In relation to the cardiocirculatory system, in the zebrafish embryos the system is closed and heart is the first organ to be formed during embryonic development, as in other vertebrates, being the physiology highly representative in humans (Dahme et al., 2009). A study of the cardiovascular system using ethanolic extract from *Cynodon dactylon* and *Sida acuta* showed early heart rate alteration with 3 hpf and LC_{50} of 32.6 $\mu\text{g mL}^{-1}$ and 20.9 $\mu\text{g mL}^{-1}$, respectively (Kannan; Vincent, 2012). In the present study, there was a significant decrease in heart rate in zebrafish embryos at increasing concentrations of *A. peregrina* concentrated liquid extract and as the concentration of the LC_{50} extract decreased statistically.

4. Conclusions

In conclusion, it was evidenced that the concentrated liquid extract of *A. peregrina* has angiogenic activity as described for popular use in wound healing. However, medicinal plants with potential therapeutic effect can still possess certain toxic effects on embryos and development of larvae especially at higher dosage. Usually, medicinal plants or compounds derived from natural sources are often used before they are investigated for toxicity and side effects. Thus, it is suggested that further pharmacological studies should be performed and may elucidate questions regarding safe dose, duration of treatment and phytochemical screening should be conducted to identify the specific components which exhibit respective toxic effects.

5. Acknowledgments

The Authors are grateful to the Universidade Federal de Goiás, Pontifícia Universidade Católica de Goiás, Universidade Federal de Jataí, Instituto Federal de Goiás, Brasil, for providing necessary infrastructural facility.

6. References

- Alafiatayo, A. A., Lai, K. S., Syahida, A., Mahmood, M. & Shaharuddin, N. A. (2019). Phytochemical Evaluation, Embryotoxicity, and Teratogenic Effects of Curcuma longa Extract on Zebrafish (*Danio rerio*). *Evidence-Based Complementary Alternative Medicine*, 2019, 1-10.
- Araújo, E. R. D., Oliveira, D. C., Soares, T. C., Langassner, S. M. Z., Tavares, J. C. M. & Cavalcanti e Silva, D. G. K. (2015). Avaliação do potencial antimicrobiano de extrato hidroalcoólico e aquoso da espécie *Anadenanthera colubrina* frente à bactérias gram negativa e gram positiva. *Biota Amazônia*, 5, 66-71.
- Araújo, L. A., Araújo, R. G. M., Gomes, F. O., Lemes, S. R., Almeida, L. M., Maia, L. J. Q., Gonçalves, P. J., Mrué, F., Silva-Jr, N. J. & Melo-Reis, P. R. (2016). Physicochemical/photophysical characterization and angiogenic properties of *Curcuma longa* essential oil. *Anais da Academia Brasileira de Ciências*, 88, 1889-1897.
- Barreto, H. M., Coelho, K. M., Ferreira, J. H., Dos Santos, B. H., De Abreu, A. P., Coutinho, H. D., Da Silva, R. A., De Sousa, T. O., Citó, A. M. & Lopes, J. A. (2016). Enhancement of the antibiotic activity of aminoglycosides by extracts from *Anadenanthera colubrina* (Vell.) Brenan var. *cebil* against multi-drug resistant bacteria. *Natural Products Research*, 30, 1289-1292.
- Chaves, D. A., Lemes, S. R., Araujo, L. A., Sousa, M. A. M., Freitas, G. B., Lino-Junior, R. S., Mrué, F. & Melo-Reis, P. R. (2016). Avaliação da atividade angiogênica da solução aquosa do barbatimão (*Stryphnodendron adstringens*). *Revista Brasileira de Plantas Mediciniais*, 18, 524-530.
- Chen, L., Xu, M., Gong, Z., Zonyane, S., Xu, S. & Makunga N. P. (2019). Comparative cardio and developmental toxicity induced by the popular medicinal extract of *Sutherlandia frutescens* (L.) R.Br. detected using a zebrafish Tuebingen embryo model. *BMC Complementary and Alternative Medicine*, 18, 1-11.
- Cunha, M. C. L., Lima, T. L., Ferreira, T. C. & Santos, Y. M. (2020). Seed, seedling, tirodendro morphology and germination of *Anadenanthera macrocarpa* (Benth.) Brenan (Fabaceae, Mimosoideae). *Hoehnea*, 47, e592020.
- Dahme, T., Katus, H. A. & Rottbauer, W. (2009). Fishing for the genetic basis of cardiovascular disease. *Diseases Models and Mechanisms*, 2, 18-22.
- Dutra, R. C., Campos, M. M., Santos, A. R. & Calixto, J. B. (2016). Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. *Pharmacological Research*, 112, 4-29.
- Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics*, 6, 241-252.
- Estevão, L. R. M., Medeiros, J. P., Scognomillo-Zsabó, M. V. R., Baratella Evêncio, L., Guimarães, E. C., Câmara, C. A. G. & Evêncio-Neto, J. (2009). Neoangiogênese de retalhos cutâneos em ratos tratados com óleo de copaíba. *Pesquisa Agropecuária Brasileira*, 44, 406-412.
- Farias, D. F., Cavalheiro, M. G., Viana, M. P., Queiroz, V. A., Rocha-Bezerra, L. C., Vasconcelos, I. M., Morais, S. M. & Carvalho, A. F. (2010). Water extracts of Brazilian leguminous seeds as rich sources of larvicidal compounds against *Aedes aegypti* L. *Anais da Academia Brasileira de Ciências*, 82, 585-594.
- Gama, A. D. S., De Paula, M., Da Silva, R. R. V., Ferreira Júnior, W. S. & Medeiros P. M, D. (2018). Exotic species as models to understand biocultural adaptation: Challenges to mainstream views of human-nature relations. *PLoS ONE*, 13, 1-18.
- Ishara, K. L. & Maimoni-Rodella, R. C. S. (2010). Community structure and comparative analysis of the woody component of a Cerrado remnant in Southeastern Brazil. *Hoehnea*, 37, 199-210.
- Ismail, H. F., Hashim, Z., Soon, W. T., Rahman, N. S. A., Zainudin, A. N. & Majid, F. A. A. (2017). Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo. *Journal of Traditional and Complementary Medicine*, 7, 452-465.
- Jayasinghe, C. D. & Jayawardena, U. A. (2019). Toxicity Assessment of Herbal Medicine Using Zebrafish Embryos: A Systematic Review. *Evidence-Based Complementary Alternative Medicine*, 2019, 1-17.
- Kannan, R. R. & Vincent, S. G. P. (2012). *Cynodon dactylon* and *Sida acuta* extracts impact on the function of the cardiovascular system in zebrafish embryos. *Journal of Biomedical Research*, 26, 90-97.
- Khan, M. F., Abutaha, N., Nasr, F. A., Alqahtani, A. S., Noman, O. M. & Wadaan, M. A. M. (2019). Bitter gourd (*Momordica charantia*) possess developmental toxicity as revealed by screening the seeds and fruit extracts in zebrafish embryos. *BMC Complementary Alternative Medicine*, 19, 1-13.

- Kruskal W. H. & Wallis A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47, 583-621.
- Lima, R. D. E. F., Alves, E. P., Rosalen, P. L., Ruiz, A. L., Teixeira Duarte, M. C., Góes, V. F., De Medeiros, A. C., Pereira, J. V., Godoy, G. P. & Melo de Brito Costa, E. M. (2014). Antimicrobial and Antiproliferative Potential of *Anadenanthera colubrina* (Vell.) Brenan. *Evidence-Based Complementary Alternative Medicine*, 2014: 1-7.
- Lima, C. R., Costa-Silva, J. H., Lyra, M. M. A., Araújo, A. V., Arruda, V. M., Dimech, G. S., Baratella-Evêncio, L., Fraga, M. C. C. A., Lafayette, S. S. L. & Wanderley, A. G. (2006). Atividade cicatrizante e estudo toxicológico pré-clínico do fitoterápico Sanativo®. *Acta Farmacéutica Bonaerense*, 25, 544-549.
- Mota, G. S., Sartori, C. J., Miranda, I., Quilhó, T., Mori, F. A. & Pereira H. (2017). Bark anatomy, chemical composition and ethanol-water extract composition of *Anadenanthera peregrina* and *Anadenanthera colubrina*. *PLoS One*, 12, 1-14.
- Nagel, R. (2002). DarT: the embryo test with the zebrafish *Danio rerio* - a general model in ecotoxicology and toxicology. *ALTEX*, 19, 38-48.
- Niemistö, A., Dunmire, V., Yli-Harja, O., Zhang, W. & Shmulevich, I. (2005). Robust Quantification of In Vitro Angiogenesis Through Image Analysis. *IEEE Transactions on Medical Imaging*, 24, 549-553.
- Nunes, L. E., Viana, A. P. P. & Rocha, W. R. V. (2015). *In vitro* evaluation of antifungal activity and interactive effect of *Anadenanthera colubrina* (Benth). *African Journal of Microbiology Research*, 9, 2006-2012.
- Parente, L. M. L., Andrade, M. A., Brito, L. A. B., Moura, V. M. B. D., Miguel, M. P., Lino-Júnior, R. S., Tresvenzo, L. F. M., Paula, J. R. & Paulo N. M. (2011). Angiogenic activity of *Calendula officinalis* flowers L. in rats. *Acta Cirúrgica Brasileira* 26: 19-24
- Pessoa, W.S., Estevão, L.R., Simões, R.S., Barros, M.E., Mendonça, F.D.E.S., Baratella-Evêncio, L. & Evêncio-Neto, J. 2012. Effects of angico extract (*Anadenanthera colubrina* var. *cebil*) in cutaneous wound healing in rats. *Acta Cirúrgica Brasileira*, 27, 655-670.
- Pessoa, W. S., Estevão, L. R., Simões, R. S., Mendonça, F. D. E. S., Evêncio-Luz, L., Baratella-Evêncio, L., Florencio-Silva, R., Sá, F.B. & Evêncio-Neto J. 2015. Fibrogenesis and epithelial coating of skin wounds in rats treated with angicoextract (*Anadenanthera colubrina* var. *cebil*). *Acta Cirúrgica Brasileira*, 30, 353-358.
- Razali, N. M. & Wah, Y. B. (2011). Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *Journal of Statistical Modeling and Analytics*, 2, 21-33.
- Santos, J. S., Marinho, R. R., Ekundi-Valentim, E., Rodrigues, L., Yamamoto, M. H., Teixeira, S. A., Muscara, M. N., Costa, S.K. & Thomazzi, S. M. (2013). Beneficial effects of *Anadenanthera colubrina* (Vell.) Brenan extract on the inflammatory and nociceptive responses in rodent models. *Journal of Ethnopharmacology*, 148, 218-222.
- Thiagarajan, S. K., Rama Krishnan, K., Ei, T., Husna Shafie, N., Arapoc, D. J. & Bahari, H. (2019). Evaluation of the Effect of Aqueous *Momordica charantia* Linn. Extract on Zebrafish Embryo Model through Acute Toxicity Assay Assessment. *Evidence-Based Complementary Alternative Medicine*, 2019, 1-9.
- Trentin, D. S., Silva, D. B., Amaral, M. W., Zimmer, K. R., Silva, M. V., Lopes, N. P., Giordani, R. B. & Macedo, A. J. (2013). Tannins possessing bacteriostatic effect impair *Pseudomonas aeruginosa* adhesion and biofilm formation. *PLoS ONE*, 8, 1-13.
- Weber, C. R., Soares, C. M. L., Lopes, A. B. D., Silva, T. S., Nascimento, M. S. & Ximenes, E. C. P. A. (2001). *Anadenanthera colubrina*: a therapeutic potential study. *Revista Brasileira de Farmácia*, 92: 235-244.
- World Health Organization - WHO (2019). WHO global report on traditional and complementary medicine. ISBN 978-92-4-151543-6. [serie en Internet]. [cited 25 Jun 2020], 46(26).

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).