Research, Society and Development, v. 9, n. 9, e634997853, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i9.7853

Sobrevivência de larvas de Zebrafish (*Danio rerio*) expostas ao extrato hidroalcoólico de *Baccharis dracunculifolia*

Survival of zebrafish larvae (*Danio rerio*) exposed to hydroalcoholic extract of *Baccharis dracunculifolia*

Supervivencia de larvas de Zebrafish (*Danio rerio*) expuestas al extracto hidroalcohólico del *Baccharis dracunculifolia*

Received: 24/08/2020 | Reviewed: 30/08/2020 | Accept: 31/08/2020 | Published: 01/09/2020

Tatiane Rogelio Ramos*
ORCID: https://orcid.org/0000-0003-0752-2373
Universidade Estadual de Maringá, Brasil
E-mail: taatirramos@hotmail.com
Kennyson Alves de Souza
ORCID: https://orcid.org/0000-0002-5347-4264
Universidade Federal do Recôncavo da Bahia, Brasil
E-mail: kennysonalves@hotmail.com
Rodrigo Augusto Cortez Passetti
ORCID: https://orcid.org/0000-0002-3332-2562
Universidade Estadual de Maringá, Brasil
E-mail: racpassetti@gmail.com
Jaísa Casetta
ORCID: https://orcid.org/0000-0001-9994-077X
Universidade Estadual de Maringá, Brasil
E-mail: jaisacasettta@hotmail.com
Ana Carolina Pelaes Vital
ORCID: https://orcid.org/0000-0002-6033-6898
Universidade Estadual de Maringá, Brasil
E-mail: ana_carolv@hotmail.com
Ricardo Pereira Ribeiro
ORCID: https://orcid.org/0000-0001-7752-3692
Universidade Estadual de Maringá, Brasil
E-mail: rpribeiro@uem.br

Benício Alves de Abreu Filho
ORCID: https://orcid.org/0000-0002-3271-808X
Universidade Estadual de Maringá, Brasil
E-mail: baafilho@uem.br
Ivanor Nunes do Prado
ORCID: https://orcid.org/0000-0003-1058-7020
Universidade Estadual de Maringá, Brasil
E-mail: inprado@uem.br

Resumo

O objetivo deste trabalho foi testar o efeito tóxico de diferentes concentrações de extrato hidroalcoólico da planta *Baccharis dracunculifolia*, utilizando larvas de zebrafish (*Danio rerio*) de 8 dias pós-fertilização. No primeiro ensaio, 50,0, 25,0, 12,5, 6,25, 3,125, 1,563 e 0,781 mg/mL de extrato foram diluídos em H_2O e foram realizadas observações em 0, 1 e 2 horas das larvas expostas ao extrato. No segundo ensaio, as diluições foram 2,0; 1,0; 0,40 e 0,20 mg/mL do extrato em H_2O e exposição das larvas no extrato em 0, 4, 8 e 12 horas. A partir do desenvolvimento dos dois testes pode-se verificar que quando as larvas foram expostas a extratos de *B. dracunculifolia* com diluições superiores a 2,0 mg/mL, obteve-se 100% de mortalidade assim que a larva foi exposta. A exposição de larvas de zebrafish (*Danio rerio*) aos oito dias pós-fertilização nos diferentes níveis de diluição do extrato hidroalcoólico de *B. dracunculifolia* apresentou efeitos letais. Porém, quando foram testadas diluições a partir de 0,40 mg/mL, obteve-se um baixo percentual de mortalidade.

Palavras-chave: Compostos fenólicos; Flavonoides; Modelo animal; Mortalidade; Toxicologia.

Abstract

The objective of this work was to test the toxic effect of different concentrations of hydroalcoholic extract of the *Baccharis dracunculifolia* plant, using Zebrafish (*Danio rerio*) larvae of 8 days' post fertilization. In the first assay, 50.0, 25.0, 12.5, 6.25, 3.125, 1.563 and 0.781 mg mL^{-1} of extract were diluted in H_2O and observations at 0, 1 and 2 hours of the larvae exposed to the extract were made. In the second assay, the dilutions were 2.0, 1.0, 0.40 and 0.20 mg mL^{-1} of extract in H_2O and exposure of the larvae on the extract at 0, 4, 8 and 12 hours. From the development of the two tests it can be seen that when the larvae were exposed to extracts of *B. dracunculifolia* with dilutions higher than 2.0 mg mL^{-1}, 100%

mortality was obtained as soon as the larvae was exposed. The exposure of larvae of Zebrafish (*Danio rerio*) to eight days after fertilization at the different dilution levels of the hydroalcoholic extract of *B. dracunculifolia* showed lethal effects. However, when dilutions were tested from 0.40 mg mL^{-1}, a low percentage of mortality was obtained.

Keywords: Animal model; Flavonoids; Mortality; Phenolic compounds; Toxicology.

Resumen

El objetivo de este trabajo fue probar el efecto tóxico de diferentes concentraciones de extracto hidroalcohólico de la planta *Baccharis dracunculifolia*, utilizando larvas de zebrafish (*Danio rerio*) 8 días después de la fertilización. En el primer ensayo, se diluyeron 50.0, 25.0, 12.5, 6.25, 3.125, 1.563 y 0.781 mg / mL de extracto en H_2O y se realizaron observaciones a las 0, 1 y 2 horas de las larvas expuestas al extracto. En la segunda prueba, las diluciones fueron 2,0; 1,0; 0,40 y 0,20 mg/mL del extracto en H_2O y exposición de las larvas en el extracto a las 0, 4, 8 y 12 horas. Del desarrollo de las dos pruebas se puede observar que cuando las larvas fueron expuestas a extractos de *B. dracunculifolia* con diluciones mayores a 2.0 mg/mL, se obtuvo una mortalidad del 100% tan pronto como la larva estuvo expuesta. La exposición de larvas de zebrafish (*Danio rerio*) a los ocho días de la fertilización a diferentes niveles de dilución del extracto hidroalcohólico de *B. dracunculifolia* tuvo efectos letales. Sin embargo, cuando se probaron diluciones a partir de 0,40 mg/mL, se obtuvo un bajo porcentaje de mortalidad.

Palabras clave: Compuestos fenólicos; Flavonoides; Modelo animal; Mortalidad; Toxicología.

1. Introduction

The species *Baccharis dracunculifolia* (Asteraceae), popularly known as Alecrim-do-Campo or Vassourinha, is a Brazilian bush, with perennial growth and can be found throughout South America, mainly in temperate and tropical areas (Abad & Bermejo, 2007). This plant has been widely used in folk medicine, in the prevention of dysfunctions such as anemia, inflammation, diabetes, liver disorders and prostate (Verdi et al., 2005). This is because some studies have shown that the species *Baccharis dracunculifolia* presents compounds, such as: flavonoids (isosakuranetin, aromadendrin-4'-methyl ether) terpenes (baccharin) and phenolic acids (artepillin C, caffeic acid, p-coumaric acid, ferulic acid) (Loots et al., 2006; Mendez, 2005; Silva Filho et al., 2004). It is described as the main plant

source of green propolis, so called because of its coloration (Yong K Park et al., 2004). Studies have demonstrated some of the biological properties of green propolis, including cytotoxicity (Matsuno et al., 1997), anti herpes (Vynograd et al., 2000), antitumor (Kimoto et al., 1998), antioxidant (Basnet et al., 1997), antimicrobial (Park et al., 1998) e anti-HIV activities (Ito et al., 2001). Some authors state that the biological activities of Brazilian green propolis are due to the high levels of prenylated p-coumaric acids, mainly Artepillin C (3,5-diprenyl-p-coumaric acid), which is also present in *B. dracunculifolia* (Bankova, 2005; Messerli et al., 2009; Nguyen et al., 2016).

The Zebrafish (*Danio rerio*) popularly known in Brazil as the freshwater Paulistinha belonging to the family Cyprinidae, can be found naturally in south and southeast Asia, around the Ganges and Brahmaputra rivers. They have been widely used in research evaluating antioxidant agents (Kirkwood et al., 2012) determination of reactive oxygen species and other biomarkers of processes related to oxidative stress (Caro et al., 2016). These studies mainly report the oxidation process related to the presence of chemicals in the aquatic environment (Bartoskova et al., 2014; Blahová et al., 2013; Chen et al., 2016). In addition, studies have demonstrated that Zebrafish embryos and larvae are useful for toxicology analysis from survival and developmental tests (Zhao et al., 2013).

This study was realized to evaluate in which concentration the hydroalcoholic extract of *B. dracunculifolia* does not present lethal effect to Zebrafish larvae (*Danio rerio*) with 8 days' post fertilization.

2. Methodology

2.1. Ethical considerations

The toxicity analysis using Zebrafish larvae was approved by the Animal Ethics Committee of the (CEUA Nº 6119170518).

2.2. Plant collection and extraction of bioactive compounds

The *Baccharis dracunculifolia* was collected in the city of Maringá, Paraná, Brazil (23°27'23.9"S 51°58'33.6"W), in August 2017 and taken to the Herbarium of the Universidade Estadual de Maringá (HUEM), to be recognized. Then, the plant was taken to the Animal Food and Nutrition Laboratory (Laboratório de Alimentos e Nutrição Animal -

LANA) of the Department of Animal Science – Universidade Estadual de Maringá, without root, was dried, in a forced circulation oven at 40 °C for 72 hours until complete drying, and grinded in mill knife with 1 mm thick sieve. After processing, the dried and milled plant was stored in a refrigerator at 4 °C.

The extraction of the bioactive compounds from the plant was carried out using a hydroalcoholic extract, where 1 gram of sample was added to the beaker with an analytical balance (BEL Engineering, M214Ai) and then 80 mL of ethanol (99.9% of purity) and 20 mL of distilled H_2O, homogenized on magnetic stirrer (TE-0851) for 20 seconds and resting for 10 minutes. This stirring and rest process was repeated four times, then the extract was filtered and placed in a water bath at 45 °C for 24 hours for total evaporation of the alcohol, resulting in an aqueous extract with a dilution of 50.0 mg mL^{-1} of extract in H_2O.

2.3. Reproduction and breeding of larvae of Zebrafish (Danio rerio)

The breeding of the larvae was carried out at the Laboratório de Zebrafish do Núcleo de Pesquisa PeixeGen – Management, genetic improvement and molecular genetics in freshwater fish culture, located at the Department de Zootecnia of the Universidade Estadual de Maringá. The fish were kept in controlled environment, with lighting cycle 14 -10 hours of dark-light and temperature between 26 and 28 ºC, with adequate water quality parameters as recommended for species. Six adult Zebrafish couples were housed in 30 liter aquariums and kept separately until mating to obtain the larvae.

2.4. Survival analysis

The present study was carried out in two tests. The first test being carried out using 240 larvae with 8 days post fertilization (dpf). Seven treatments with different dilutions of the hydroalcoholic extract of *B. dracunculifolia*, being 50.0, 25.0, 12.5, 6.25, 3.125, 1.563 and 0.781 mg mL^{-1} of extract in H_2O and as control treatment was used dechlorinated water. Three times of exposition observations were made to the extract (0, 1 and 2 hours). In 96-well micro plates, 200 µL of extract per well was pipetted and then the larva was added, with each well having a larva. Immediately after placing the larva in contact with the extract, observations were made of the presence of swimming movements in the micro plates. Then, with the help of a Pasteur pipette, the larva was placed on a slide and with the aid of a light microscope (Motic BA310E) with a 10-fold magnification objective and Moticam 5.0 MP

coupled camera, it was observed if there were body changes, blood circulation and heart beats. When it was verified the absence of these movements the larva was considered dead. Thirty larvae were used per treatments and the result obtained was expressed as % mortality per hour of exposure.

In the second test, 180 larvae of eight dpf were used and four treatments were tested with dilutions of hydroalcoholic extracts of *B. dracunculifolia* (2.0, 1.0, 0.40 and 0.20 mg mL^{-1} of extract in H_2O) in four observation times (0, 4, 8 and 12 hours). The assay was performed following the same procedures as the first assay. A total of 36 larvae were used per treatment and the result obtained was expressed as % of mortality over the exposure time. Toxicity tests on Zebrafish larvae were carried out in accordance with the OECD Guideline N° 236: Acute Fish Toxicity Test (FET) with adaptation.

3. Results

In the first trial (Table 1), the observation of swimming movements in the larvae of eight dpf was carried out in seven different dilutions of the hydroalcoholic extract of *B. dracunculifolia*, where it can be observed that the larvae exposed to the extracts in dilutions of 50.0, 25.0, 12.5 and 6.25 mg mL^{-1} of extract in H_2O did not show swimming movements immediately after exposure. In the same way, the presence of blood circulation and heart beats were not observed in microscopy.

Thus, 100% mortality was considered at zero observation hour. When analyzing the treatments with dilutions of 3.125, 1.563 and 0.781 mg mL^{-1} of extract in H_2O at zero hour of exposure, 0% mortality was obtained. However, for treatment 3,125 mg mL^{-1} of extract in H_2O in 1 hour of exposure, 100% mortality was obtained, whereas for treatments 1,563 and 0.781 mg mL^{-1} of extract in H_2O, 50 and 20% of mortality in 1 hour of exposure and 60 and 50 % mortality in 2 hours of exposure were obtained, respectively. On the other hand, when performing the observations in the control treatment, 0% mortality was observed in the three exposure times, indicating that the mortalities obtained in the study is related to exposure to *B. dracunculifolia* extract.

In the second trial (Table 2), the larvae exposed to the extract with dilutions 2.0, 1.0, 0.40 and 0.20 mg mL^{-1} of extract in H_2O did not presented mortality at zero observation time, however, it was observed an increase in mortality as the exposure time increased, and with 4 hours of exposure to the extract 100 % mortality was obtained for the treatment 2.0 mg mL^{-1} of extract in H_2O.

Table 1. Lethal effect of *B. dracunculifolia* extract in seven different dilutions (50.0, 25.0, 12.5, 6.25, 3.125, 1.563 and 0.781 mg mL^{-1} of extract in H_2O) at three observation times (0, 1 and 2 hours).

Dilution, mg mL^{-1} of extract in H_2O	Mortality, %		
	0 hour	1 hour	2 hours
CON	0.00	0.00	0.00
50.0	100.00	100.00	100.00
25.0	100.00	100.00	100.00
12.5	100.00	100.00	100.00
6.25	100.00	100.00	100.00
3.125	0.00	100.00	100.00
1.563	0.00	50.00	60.00
0.781	0.00	20.00	50.00

Source: Authors.

Table 2. Lethal effect of *B. dracunculifolia* extract in seven different dilutions (2.0, 1.0, 0.40 and 0.20 mg mL^{-1} of extract in H_2O) at four observation times (0, 4, 8 e 12 hours).

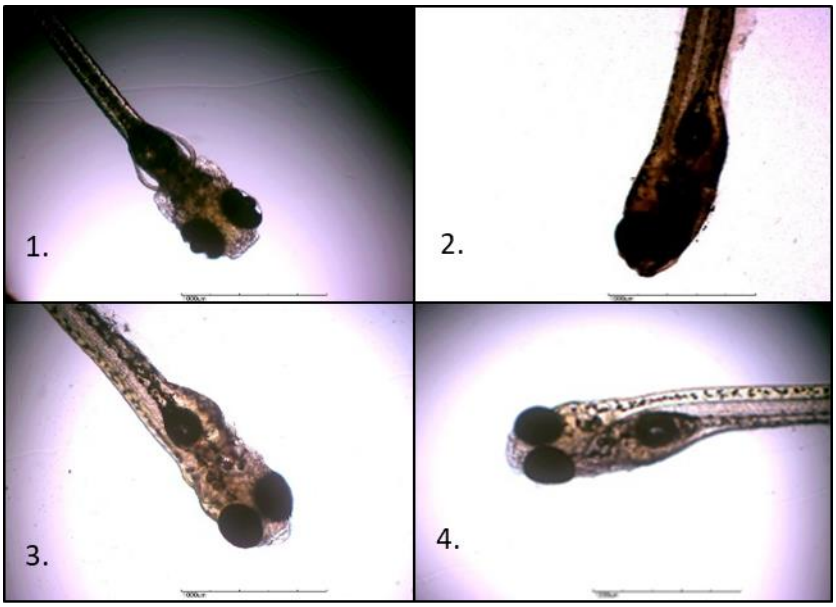
Dilution, mg mL^{-1} of extract in H_2O	Mortality, %			
	0 hour	4 hours	8 hours	12 hours
CON	0.00	0.00	0.00	0.00
2.0	0.00	100.00	100.00	100.00
1.0	0.00	19.44	75.00	100.00
0.40	0.00	8.33	36.11	41.67
0.20	0.00	0.00	11.11	25.00

Source: Authors.

The treatment 1.0 mg mL^{-1} of extract in H_2O had 100 % mortality in 12 hours of exposure (Table 2). Treatment 0.40 mg mL^{-1} of extract in H_2O presented 41.47% mortality in 12 hours of exposure. On the other hand, treatment 0.20 mg mL^{-1} of extract in H_2O did not present mortality in up to 4 hours of exposure, while in 8 and 12 hours of exposure, 11 and 25% of mortality were obtained, respectively. When analyzing the control treatment, no

mortality was observed, indicating that mortalities are directly related to the extract of *B. dracunculifolia* (Figure 1).

Figure 1. Photos of larvae of zebrafish (*Danio rerio*) exposed to hydroalcoholic extracts of *Baccharis dracunculifolia* at Zero hour.1. Treatment control; 2. Treatment 50.0 mg mL^{-1} of extract in H_2O; 3. Treatment 2.0 mg mL^{-1} of extract in H_2O; 4. Treatment 0.20 mg mL^{-1} of extract in H_2O.



Source: Authors.

4. Discussions

From the results obtained, it was observed that the *Braccharis dracunculifolia* plant presents a high lethal effect when it was used with dilution extracts of higher than 2.0 mg mL^{-1} of extract in H_2O, corroborating with studies that indicated that some species of the genus Baccharis showed a high toxicity (Jarvis et al., 1996; Varaschin & Alessi, 2003). Furthermore, studies investigating the possible genotoxic and mutagenic effects of *B. dracunculifolia* in mice demonstrated that the plant's aqueous extract increased damage to DNA, blood and liver tissues and the frequency of the micronucleus in the bone marrow (Rodrigues et al., 2009). Studies carried out by Fukuda et al. (2006) analyzing the cytotoxic effect of compounds isolated from *Baccharis dracunculifolia* demonstrated that monoterpene phenols and sesquiterpene alcohols exhibited a strong cytotoxic effect. It is possible to suggest that the harmful effects found in the treatments with higher concentrations of *B.*

dracunculifolia (dilutions > 2.0 mg mL^{-1} of extract in H_2O) are related to higher concentrations of flavonoids and phenolic acids present in the extracts.

5. Final considerations

The exposure of Zebrafish larvae (*Danio rerio*) to eight days post-fertilization at the different dilution levels of the aqueous extract of *B. dracunculifolia* proposed in the present study, showed toxic effects for dilutions of higher than 2.0 mg mL^{-1} of extract in H_2O with 100 % of mortality as soon as the animals were exposed. However, when dilutions were tested from 0.40 mg mL^{-1} of extract in H_2O, a low mortality rate was obtained. The *B. dracunculifolia* as demonstrated, has numerous bioactive compounds, which can be used as a medicament in alternative medicine. However, due to its toxicity the use requires care, with the need to develop future studies in order to elucidate the action mechanisms of the isolated bioactive compounds present in this plant.

Reference

Abad, M. J., & Bermejo, P. (2007). Baccharis (Compositae): a review update. *Arkivoc*, *7*(7), 76–96.

Bankova, V. (2005). Chemical diversity of propolis and the problem of standardization. *Journal of Ethnopharmacology*, *100*(1), 114–117.

Bartoskova, M., Dobsikova, R., Stancova, V., Pana, O., Zivna, D., Plhalova, L., Blahova, J., & Marsalek, P. (2014). Norfloxacin—toxicity for zebrafish (Danio rerio) focused on oxidative stress parameters. *BioMed Research International*, *2014*, 1–6. https://doi.org/http://dx.doi.org/10.1155/2014/560235.

Basnet, P., Matsuno, T., & Neidlein, R. (1997). Potent free radical scavenging activity of propol isolated from Brazilian propolis. *Zeitschrift Für Naturforschung*, *52*(11–12), 828–833.

Blahová, J., Plhalová, L., Hostovský, M., Divišová, L., Dobšíková, R., Mikulíková, I., Štěpánová, S., & Svobodová, Z. (2013). Oxidative stress responses in zebrafish Danio rerio after subchronic exposure to atrazine. *Food and Chemical Toxicology*, *61*, 82–85.

https://doi.org/https://dx.doi.org/10.1016/j.fct.2013.02.041.

Caro, M., Iturria, I., Martinez-Santos, M., Pardo, M. A., Rainieri, S., Tueros, I., & Navarro, V. (2016). Zebrafish dives into food research: effectiveness assessment of bioactive compounds. *Food & Function*, *7*(6), 2615–2623. https://doi.org/http://dx.doi.org/10.1039/C6FO00046K.

Chen, M., Yin, J., Liang, Y., Yuan, S., Wang, F., Song, M., & Wang, H. (2016). Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. *Aquatic Toxicology*, *174*, 54–60. https://doi.org/https://doi.org/10.1016/j.aquatox.2016.02.015.

Fukuda, M., Ohkoshi, E., Makino, M., & Fujimoto, Y. (2006). Studies on the constituents of the leaves of Baccharis dracunculifolia (Asteraceae) and their cytotoxic activity. *Chemical and Pharmaceutical Bulletin*, *54*(10), 1465–1468. https://doi.org/http://dx.doi.org/10.1248/cpb.54.1465.

Ito, J., Chang, F.-R., Wang, H.-K., Park, Y. K., Ikegaki, M., Kilgore, N., & Lee, K.-H. (2001). Anti-AIDS agents. 48. Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from Brazilian propolis. *Journal of Natural Products*, *64*(10), 1278–1281. https://doi.org/http://dx.doi.org/10.1021/np010211x.

Jarvis, B. B., Wang, S., Cox, C., Rao, M. M., Philip, V., Varaschin, M. S., & Barros, C. S. (1996). Brazilian Baccharis toxins: livestock poisoning and the isolation of macrocyclic trichothecene glucosides. *Natural Toxins*, *4*(2), 58–71. https://doi.org/https://doi.org/10.1002/19960402NT2.

Kimoto, T., Arai, S., Kohguchi, M., Aga, M., Nomura, Y., Micallef, M. J., Kurimoto, M., & Mito, K. (1998). Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. *Cancer Detection and Prevention*, *22*(6), 506–515. https://doi.org/http://dx.doi.og/10.1046/j.1525-1500.1998.00020.x.

Kirkwood, J. S., Lebold, K. M., Miranda, C. L., Wright, C. L., Miller, G. W., Tanguay, R. L., Barton, C. L., Traber, M. G., & Stevens, J. F. (2012). Vitamin C deficiency activates the purine nucleotide cycle in zebrafish. *Journal of Biological Chemistry*, *287*(6), 3833–3841. https://doi.org/http://dx.doi.org/10.1074/jbc.M111.316018.

Loots, D. T., van der Westhuizen, F. H., & Jerling, J. (2006). Polyphenol composition and antioxidant activity of Kei-apple (Dovyalis caffra) juice. *Journal of Agricultural and Food Chemistry*, *54*(4), 1271–1276. https://doi.org/http://dx.doi.org/0.1021/jf052697j.

Matsuno, T., Matsumoto, Y., Saito, M., & Morikawa, J. (1997). Isolation and characterization of cytotoxic diterpenoid isomers from propolis. *Zeitschrift Für Naturforschung C*, *52*(9–10), 702–704. https://doi.org/https://doi.org/10.1515/znc-1997-9-1020.

Mendez, J. (2005). Dihydrocinnamic acids in Pteridium aquilinum. *Food Chemistry*, *93*(2), 251–252. https://doi.org/https://doi.org/10.1016/j.foodchem.2004.09.019.

Messerli, S. M., Ahn, M., Kunimasa, K., Yanagihara, M., Tatefuji, T., Hashimoto, K., Mautner, V., Uto, Y., Hori, H., & Kumazawa, S. (2009). Artepillin C (ARC) in Brazilian green propolis selectively blocks oncogenic PAK1 signaling and suppresses the growth of NF tumors in mice. *Phytotherapy Research*, *23*(3), 423–427. https://doi.org/https://doi.org/10.1002/ptr.2658.

Nguyen, B. C. Q., Taira, N., Maruta, H., & Tawata, S. (2016). Artepillin C and other herbal PAK1-blockers: Effects on hair cell proliferation and related PAK1-dependent biological function in cell culture. *Phytotherapy Research*, *30*(1), 120–127. https://doi.org/http://dx.doi.org/10.1002/ptr.5510.

Park, Y K, Koo, M. H., Abreu, J. A., Ikegaki, M., Cury, J. A., & Rosalen, P. L. (1998). Antimicrobial activity of propolis on oral microorganisms. *Currents Microbiological*, *36*(1), 24–28. http://www.ncbi.nlm.nih.gov/pubmed/9405742

Park, Yong K, Paredes-Guzman, J. F., Aguiar, C. L., Alencar, S. M., & Fujiwara, F. Y. (2004). Chemical constituents in Baccharis dracunculifolia as the main botanical origin of southeastern Brazilian propolis. *Journal of Agricultural and Food Chemistry*, *52*(5), 1100–1103. https://doi.org/http://dx.doi.org/10.1021/jf021060m.

Rodrigues, C. R. F., Dias, J. H., Semedo, J. G., Silva, J., Ferraz, A. B. F., & Picada, J. N. (2009). Mutagenic and genotoxic effects of Baccharis dracunculifolia (DC). *Journal of*

Ethnopharmacology, *124*(2), 321–324. https://doi.org/https://doi.org/10.1016/j.jep.2009.04.022.

Silva Filho, A. A., Bueno, P. C. P., Gregório, L. E., Silva, M. L. A., Albuquerque, S., & Bastos, J. K. (2004). In-vitro trypanocidal activity evaluation of crude extract and isolated compounds from Baccharis dracunculifolia DC (Asteraceae). *Journal of Pharmacy and Pharmacology*, *56*(9), 1195–1199. https://doi.org/http://dx.doi.org/10.1211/0022357044067.

Varaschin, M. S., & Alessi, A. C. (2003). Poisoning of mice by Baccharis coridifolia: an experimental model. *Veterinary and Human Toxicology*, *45*(1), 42–44.

Verdi, L. G., Brighente, I. M. C., & Pizzolatti, M. G. (2005). Gênero Baccharis (Asteraceae): aspectos químicos, econômicos e biológicos. *Química Nova*, *28*(1), 85–94.

Vynograd, N., Vynograd, I., & Sosnowski, Z. (2000). A comparative multi-centre study of the efficacy of propolis, acyclovir and placebo in the treatment of genital herpes (HSV). *Phytomedicine*, *7*(1), 1–6. https://doi.org/https://doi.org/10.1016/S0944-7113(00)80014-8.

Zhao, X., Wang, S., Wu, Y., You, H., & Lv, L. (2013). Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. *Aquatic Toxicology*, *136*, 49–59. https://doi.org/https://doi.org/10.1016/j.aquatox.2013.03.019.

Percentage of contribution of each author in the manuscript

Tatiane Rogelio Ramos – 25%

Kenysson Alves de Souza – 10%

Rodrigo Augusto Cortez Passetti – 10%

Jaísa Casetta – 10%

Ana Carolina Pelaes Vital – 10%

Ricardo Ribeiro Pereira – 10%

Benício Alves de Abreu Filho – 10%

Ivanor Nunes do Prado – 15%