An Acad Bras Cienc (2024) 96(1): e20230188 DOI 10.1590/0001-3765202420230188
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Redox profile of silver catfish challenged with *Aeromonas hydrophila* and treated with hexane extract of *Hesperozygis ringens* (Benth.) Epling through immersion bath

ISADORA A. ROSA, ADRIANE E. BIANCHINI, CAROLINE A. BRESSAN, FABÍOLA T. FERRARI, KARINE ARIOTTI, NATACHA C. MORI, GUERINO BANDEIRA JUNIOR, CARLOS G. PINHEIRO, MARIA AMÁLIA PAVANATO, JULIANA F. CARGNELUTTI, BERNARDO BALDISSEROTTO & BERTA MARIA HEINZMANN

Abstract: The growing increase in the fish farming sector has favored the establishment of bacterial outbreaks caused by *Aeromonas hydrophila* in several species. The hexane extract of *Hesperozygis ringens* (HEHR) (Lamiaceae) leaves increased the survival rate of silver catfish (*Rhamdia quelen*) experimentally infected by *A. hydrophila*. However, it is noteworthy that no reports have been found on the possible mechanisms of action of this extract in infected fish. This study aimed to evaluate the effect of the HEHR, administered through single immersion bath, on lipid peroxidation and antioxidant defenses in muscle and liver tissue of silver catfish challenged with *A. hydrophila*. The results showed that the oxidative status of silver catfish was altered, although oxidative stress was not triggered during the experiment. HEHR at 30 mg/L (HEHR30) was not characterized as a pro-oxidant agent in the presence of infection, unlike florfenicol and HEHR at 15 mg/L treatments in some cases. In short, HEHR30 provided an important increase in hepatic catalase activity, characterizing one of the possible mechanisms involved in the greater survival of fish experimentally infected by *A. hydrophila*. Additionally, HEHR30 did not induce lipid peroxidation, nor reduced antioxidant defenses of silver catfish infected or not by *A. hydrophila*.

Key words: action mechanism, antioxidant defenses, catalase, bacterial infection, fish, *Rhamdia quelen*.

INTRODUCTION

Food from aquaculture is considered one of the main sources of protein consumed worldwide and the expansion of this sector contributes to the economic and financial growth of producing countries (FAO 2020). However, with the intensification of this sector, fish are commonly subjected to stressful conditions, such as increased stocking density in the ponds, poor water quality, inadequate transport, and handling conditions (Segner et al. 2012, Souza et al. 2019). As a result, the reproduction, growth, and immunity of animals are harmed, facilitating the installation of infections by opportunistic pathogens (Souza et al. 2019).

Thus, several authors converge in the sense that bacterial diseases are one of the main obstacles that hinder the improvement and sustainability of aquaculture (Pilarski et al. 2011, Algammal et al. 2020). Bacterial outbreaks are associated with notable economic losses due to the increase in mortality, decrease in the growth

rate, and expenses with drugs for the prophylaxis and treatment of bacteriosis (Romero et al. 2012, Tavares-Dias & Martins 2017). Currently, only two antimicrobial drugs have been approved in Brazil (florfenicol and oxytetracycline) for the treatment of bacterial diseases in fish intended for human consumption (SINDAN 2022). In the United States, in addition to the aforementioned drugs, sulfamerazine and sulfadimethoxine/ormethoprim can also be used for this purpose (FDA 2022).

Aeromonas hydrophila, a facultative anaerobic Gram-negative rod with multifactorial virulence, is one of the most threatening pathogens that have caused great annual losses among fish populations (Pemberton et al. 1997, Pandey et al. 2010, Algammal et al. 2020). The clinical condition established by this bacterium in fish is usually characterized by cutaneous hemorrhage in the body and fins, ulcerations with loss of epithelium, ascites, tissue swelling, and hemorrhagic septicemia (Barcellos et al. 2008, Algammal et al. 2020). The gills and skin are characterized as the main entry routes, with subsequent adhesion and proliferation of this microorganism, reaching the muscle (Chu & Lu 2008). According to Algammal et al. (2020), the liver is one of the organs most affected by *A. hydrophila*. Several species are affected by this pathogen, including *Labeo rohita* (rohu carp) (Chandran et al. 2002), *Clarias batrachus* (catfish) (Llobrera & Gacutan 1987), *Oreochromis niloticus* (tilapia) (Yambot 1998), *Anguilla anguilla* (eels) (Esteve et al. 1993), and *Carassius auratus* (goldfish) (Harikrishnan et al. 2010).

Rhamdia quelen (Heptapteridae, Siluriformes) is a neotropical teleost, known as silver catfish, which has zootechnical and commercial characteristics that make it attractive for large-scale production, such as satisfactory performance at low temperatures, rapid growth, and easy adaptation to different environments and artificial diets (Gomes et al. 2000, Figueiredo et al. 2014, Signor et al. 2020). This species has suffered notable damage in its cultivation due to outbreaks of *A. hydrophila* (Barcellos et al. 2008).

Infections caused by bacteria of *Aeromonas* genus usually cause oxidative damage. However, several factors can influence the oxidative status of the affected animal (Bandeira Junior & Baldisserotto 2020). Oxidative stress (OS) can be established when fish are subjected to stressful conditions, such as poor water quality, high stocking density, transport, and management. It can also result from bacterial infections, either by excessive generation or impaired removal of free radicals and/or reactive oxygen species (ROS) (Monserrat et al. 2007, Baldissera et al. 2018, Souza et al. 2019).

The deleterious effects of pro-oxidant substances can be minimized with the use of plant extracts (Citarasu 2010). These are increasingly used in aquaculture, since they can function as antimicrobials, immunostimulants, growth promoters, sedatives, and anesthetics, among others (Citarasu 2010, Silva et al. 2013, Sutili et al. 2015). Therapeutic approaches from plant sources are characterized as promising alternatives with less environmental impact, which allows the reduction of harmful side effects generated by using synthetic substances, such as residual effects (Citarasu 2010).

Thus, the leaves of *Hesperozygis ringens* (Benth.) Epling (Lamiaceae), popularly known as "espanta-pulga", have several pharmacological properties applicable to the fish farming sector. The essential oil (EO) of its leaves is larvicidal (Silva et al. 2014), anesthetic (Silva et al. 2013), antibacterial (Sutili et al. 2015, Bandeira Junior et al. 2017), and antiparasitic (Bandeira Junior et al. 2017). Studies conducted by our research group showed that the hexane extract of *H. ringens* (HEHR) leaves also presented potential

use in fish farming, as it provided an increase in the survival rate of silver catfish (*R. quelen*) experimentally infected by *A. hydrophila*, at a higher rate than the florfenicol (Rosa et al. 2019). These results have encouraged us to search for mechanisms of action that explain the greater survival of fish challenged with *A. hydrophila* when treated with HEHR (Rosa et al. 2019). For this reason, this study aimed to evaluate the effect of HEHR, administered through single immersion bath, on the level of lipid peroxidation and the antioxidant defenses in muscle and liver tissue of silver catfish experimentally infected by *A. hydrophila*.

MATERIALS AND METHODS

Plant material and extraction procedure

Permission for the collection of plant material, which was carried out in the district of Santo Antão, Santa Maria, RS, Brazil, was granted by the Biodiversity Authorization and Information System (SISBIO, number 74776-3). *H. ringens* was identified by forest engineer Carlos Garrido Pinheiro and a voucher specimen was deposited in the Herbarium of the Forest Science Department at the Universidade Federal de Santa Maria (UFSM) (HDCF 6720). In addition, this study with this plant species was registered in the National System For The Management Of Genetic Heritage And Associated Traditional Knowledge (SisGen, number A89F417). The processing of the leaves and the obtention of the HEHR were performed as described by Rosa et al. (2019).

Fish and culture conditions

One hundred and twenty silver catfish juveniles (8.24 ± 0.28 g; 11.25 ± 0.25 cm) were obtained from a local fish farm (Arroio Grande, Santa Maria, Southern Brazil) and acclimatized at the Fish Physiology Laboratory at UFSM for three days in 250 L tanks (50 fish/tank), continuously aerated by a water recirculation system fitted with activated charcoal/stone filters. Water quality parameters were monitored daily using a YSI Model Y5512 oximeter for dissolved oxygen, a DMPH-2 pH meter for pH and a commercial kit (Labcon Test) to determine the total ammonia level. The temperature was also monitored and remained stable and adequate for the fish species throughout the acclimation and experimental period (temperature 20.98 ± 0.29 °C, dissolved oxygen 7.22 ± 0.11 mg/L, pH 7.45 ± 0.06 and non-ionized ammonia 0.023 ± 0.01 mg/L). Animals were fed once daily to satiety with commercial feed (42% crude protein; Supra-Alisul Alimentos®, Cruz Alta, Brazil).

The methodology used in this experiment was approved by the Ethical and Animal Welfare Committee of UFSM, Santa Maria, RS, Brazil (protocol number 074/2014) and met the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA, Brazil).

Bacterial strain

A clinical isolate of *A. hydrophila* was used, which was obtained from naturally infected silver catfish (*R. quelen*) juveniles. This strain was isolated and identified through biochemical and molecular tests by Bandeira Junior et al. (2018). The sequence is deposited in the GenBank under number MF 372510.

Challenge with *A. hydrophila* and experimental design

After acclimation, the fish received 15 µL of saline solution (n = 60) or 15 µL of suspension of *A. hydrophila* MF 372510 (1.95 x 10^8 colony forming units, CFU/mL; 0,3 OD 600 nm) (n = 60) and were distributed in plastic boxes (15 L) (n = 5 fish per box, in triplicate), according to the methodology described by Rosa et al. (2019). After 5 hours, the treatments were added to

the water, and fish were submitted to a single immersion bath. Healthy and infected animals were submitted to the following treatments: control, HEHR 15 mg/L, HEHR 30 mg/L, and FLOR 4 mg/L (florfenicol 40%, Maxflor®, Virbac), totaling eight experimental groups. Therefore, the aforementioned compounds were administered to the fish through immersion bath carried out only on the first experimental day. The control consisted of the presence of only water. The concentrations used in this study are in accordance with those used by Rosa et al. (2019), who detected *in vitro* antibacterial action for HEHR. In addition, these concentrations were determined from long exposure tests on silver catfish, which did not cause an anesthetic effect or behavioral side effects (RODRIGUES P, Unpublished data). Before being added to the bath water, the HEHR was diluted in 96% ethanol (1:10). According to Baldissera et al. (2017), the ethanol concentration used in the present study did not interfere with the oxidative parameters of silver catfish, and, for this reason, the effect of ethanol on the redox profile was not evaluated. Dead fish were removed daily, and part of the water was renewed from the second experimental day without replacing the extract/antimicrobial. The experimental period was 7 days.

Sample collection and tissue homogenization

After four and seven days post-infection (DPI), 2 fish from each tank (6 fish per treatment) in both collections, totaling 96 animals, were anesthetized with eugenol (50 mg/L) and euthanized by spinal cord sectioning to collect the liver and muscle. Both tissues were subsequently stored at –80 °C until analysis of OS parameters. Tissues were individually homogenized in ice-cold 100 mM sodium phosphate buffer, pH 7.4, and 1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged at 1000 g for 10 min at 4 °C. These supernatants were used for all analyses, except for the determination of lipid peroxidation (LPO). Protein content was evaluated by the Lowry et al. (1951) method using bovine serum albumin as standard.

Determination of oxidative stress indicators

Lipid peroxidation

Lipid hydroperoxides (LOOH) were determined to detect the primary products of lipid peroxidation using the oxidation of Fe^{2+} by LOOH in an acidic medium with xylenol orange dye, which forms a complex with Fe^{3+}. The liver and muscle tissues were homogenized with ice-cold 100% methanol and centrifuged at 2800 g for 10 min at 4 °C. Aliquots of the obtained supernatant were added to a working solution containing the following reagents, which were added in the subsequent order: 0.25 mM iron sulfate, 0.05 N H_2SO_4, 0.1 mM xylenol orange and distilled water. The samples were incubated for 1 h at room temperature (RT). The absorbances were read in cuvettes using a spectrophotometer at 580 nm. Thereafter, 8 µL of hydrogen peroxide (H_2O_2) was added. Then, the samples were incubated again for 40 min at RT and the absorbance was recorded at 580 nm. LOOH levels were expressed as nmol/g of tissue and calculated as ($A_{580\ nm}$ sample/$A_{580\ nm}$ 8 nmol H_2O_2) x 8 nmol H_2O_2 x 1000 / V1 x 6, where V1 is the volume of sample aliquot used in the assay and the factor "6" presumes a 1:5 (w/v) methanolic homogenate (Hermes-Lima et al. 1995).

Antioxidant defenses

Catalase (CAT) activity was evaluated by the decomposition of H_2O_2 following the decrease in absorbance at 240 nm and was reported as ρmol/min/mg of protein (Aebi 1984). Glutathione S-transferase (GST) activity was assayed based on the conjugation reaction with reduced glutathione (GSH), using

1-chloro-2,4-dinitrobenzene (CDNB) as substrate (Habig et al. 1974). Sample aliquots were added to the assay mixture containing 100 mM phosphate buffer (pH 6.5), GSH, and CDNB at a final concentration of 1 mM each. GST activity was calculated from the changes in absorbance in 1 min at 340 nm and was expressed as ρmol/min/mg of protein. The total antioxidant capacity (TAC) assay was carried out as described by Cao & Prior (1998). Briefly, sample aliquots were added to a solution containing 20 mM phosphate buffer, pH 7.4, 5 mM 2,2'azobis (2-amidinopropane) dihydrochloride, and 0.15 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). The absorbance was recorded for 1 min at 734 nm. TAC assay was calibrated against ascorbic acid standard and was expressed as µmol/mg of protein.

Statistical analysis

Statistical analyses were performed using the software Statistica® 7.0. The graphs were obtained using the software Graphpad Prism®. Levene's test was used to verify whether the data were parametric. A two-way analysis of variance followed by the Tukey test was performed to assess the differences. Results were expressed as mean ± standard error (SEM) and the minimum significance level was set at 95% ($p < 0.05$).

RESULTS

At the time of muscle and liver tissue collection, no clinical signs of aeromonosis (or other diseases) were observed in healthy animals. However, among the fish infected by *A. hydrophila*, especially in those that were not treated, characteristic lesions were observed such as lesions in the caudal peduncle and barbels, and ulcerative and hemorrhagic lesions throughout the body. These findings are consistent with those described by Barcellos et al. (2008) in silver catfish infected by this bacterium. The survival rate of silver catfish experimentally infected with *A. hydrophila* and treated with FLOR, HEHR15, or HEHR30 under the same conditions as in the present study was described by Rosa et al. (2019).

Oxidative status in the muscle tissue on the 4th day post-infection

In this study, lipid hydroperoxide (LOOH) levels in the muscle tissue were reduced at 4 DPI by *A. hydrophila* compared to the healthy control group, which received only saline injection ($p = 0.012354$) (Figure 1a). Although the healthy animals treated with HEHR30 did not differ statistically from the healthy control group, they showed higher values than healthy animals treated with FLOR ($p = 0.000188$) and HEHR15 ($p = 0.000732$). Regarding the groups of infected animals, there was no statistical difference between treatments. However, LOOH levels in the group infected and treated with HEHR30 were significantly reduced ($p = 0.00013$) when compared to the levels in its group *per se*. Catalase (CAT) activity in control groups was not affected at 4 DPI by *A. hydrophila* (Figure 1b). On the other hand, the treatment with FLOR in healthy animals demonstrated significantly higher CAT activity ($p = 0.029824$) compared to the group treated with HEHR30. Although there was no statistical difference between the infected fish, the group treated with FLOR showed a significant reduction ($p = 0.039924$) in CAT activity when compared to its group *per se*. As with CAT activity, glutathione S-transferase (GST) activity was unchanged at 4 DPI, since controls did not differ from each other (Figure 1c). The healthy fish groups did not differ statistically from each other, and the same was verified among the infected animals. However, the group infected and treated with HEHR15

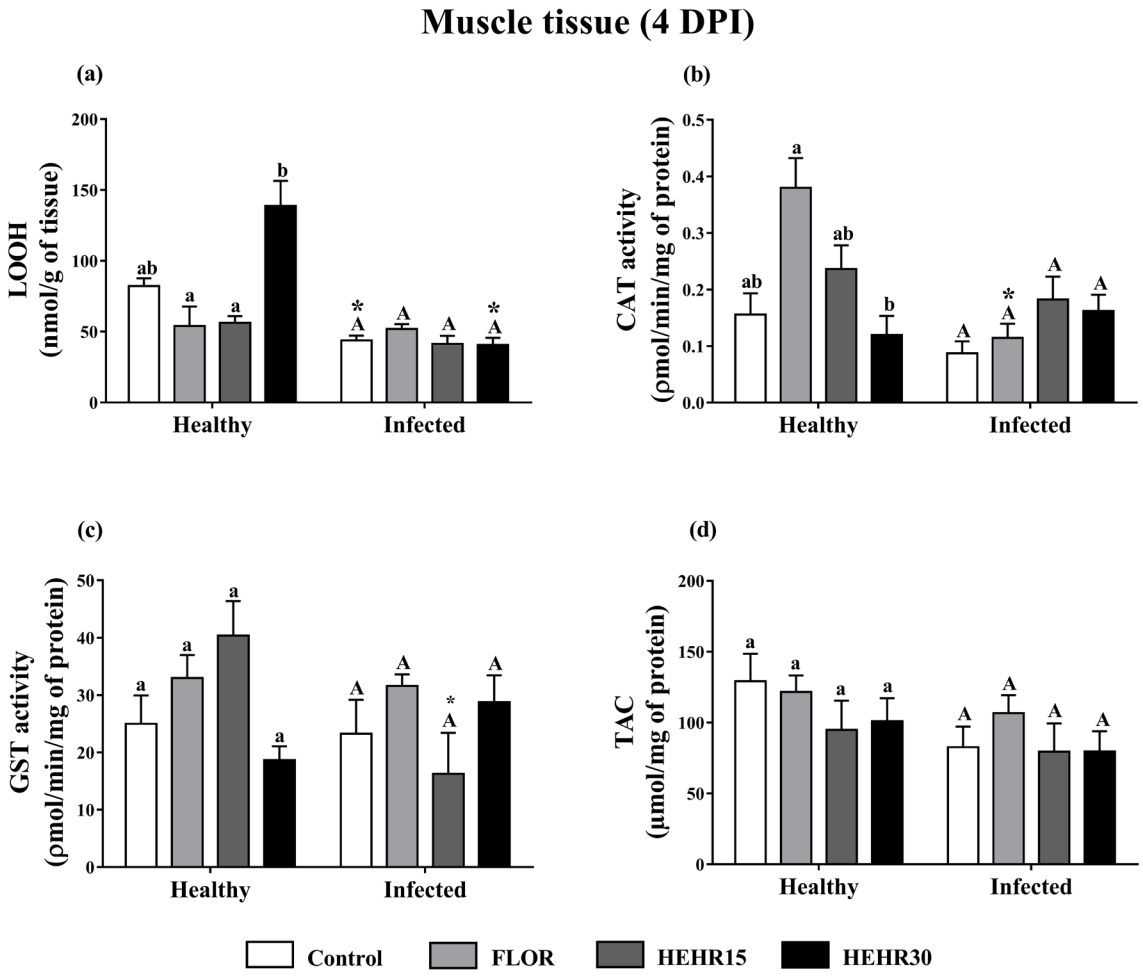

Figure 1. (a) Lipid hydroperoxide (LOOH) levels, (b) catalase (CAT) activity, (c) glutathione S-transferase (GST) activity, and (d) total antioxidant capacity (TAC) of muscle tissue collected on the fourth experimental day with silver catfish (*Rhamdia quelen*) healthy or experimentally infected by *Aeromonas hydrophila* (4 DPI) treated with florfenicol (FLOR), hexane extract of *Hesperozygis ringens* 15 mg/L (HEHR15), HEHR30, and control. Different lowercase letters indicate a significant difference between healthy fish. Different capital letters indicate a significant difference between infected fish. (*) indicate significant differences from healthy fish in the same treatment. All the values are expressed as mean ± SEM. Two-way ANOVA and Tukey test ($p < 0.05$).

showed a significant reduction ($p = 0.031124$) compared to its group *per se*. Total antioxidant capacity (TAC) was unaffected by treatments and at 4 DPI by *A. hydrophila* (Figure 1d).

Oxidative status in the muscle tissue on the 7th day post-infection

On the seventh day post-infection (7 DPI) by *A. hydrophila*, LOOH levels in the silver catfish muscle tissue did not show any statistical difference between healthy and infected controls, as well as between groups referring to healthy fish (Figure 2a). The groups of infected animals treated with HEHR15 showed a significant increase in relation to the control ($p = 0.001277$) and in relation to its group *per se* ($p = 0.000663$), with no statistical difference between the other treatments. Regarding CAT activity, there was no statistical difference between healthy and infected controls, nor between treatments referring to healthy fish (Figure 2b). However, the group infected and treated with

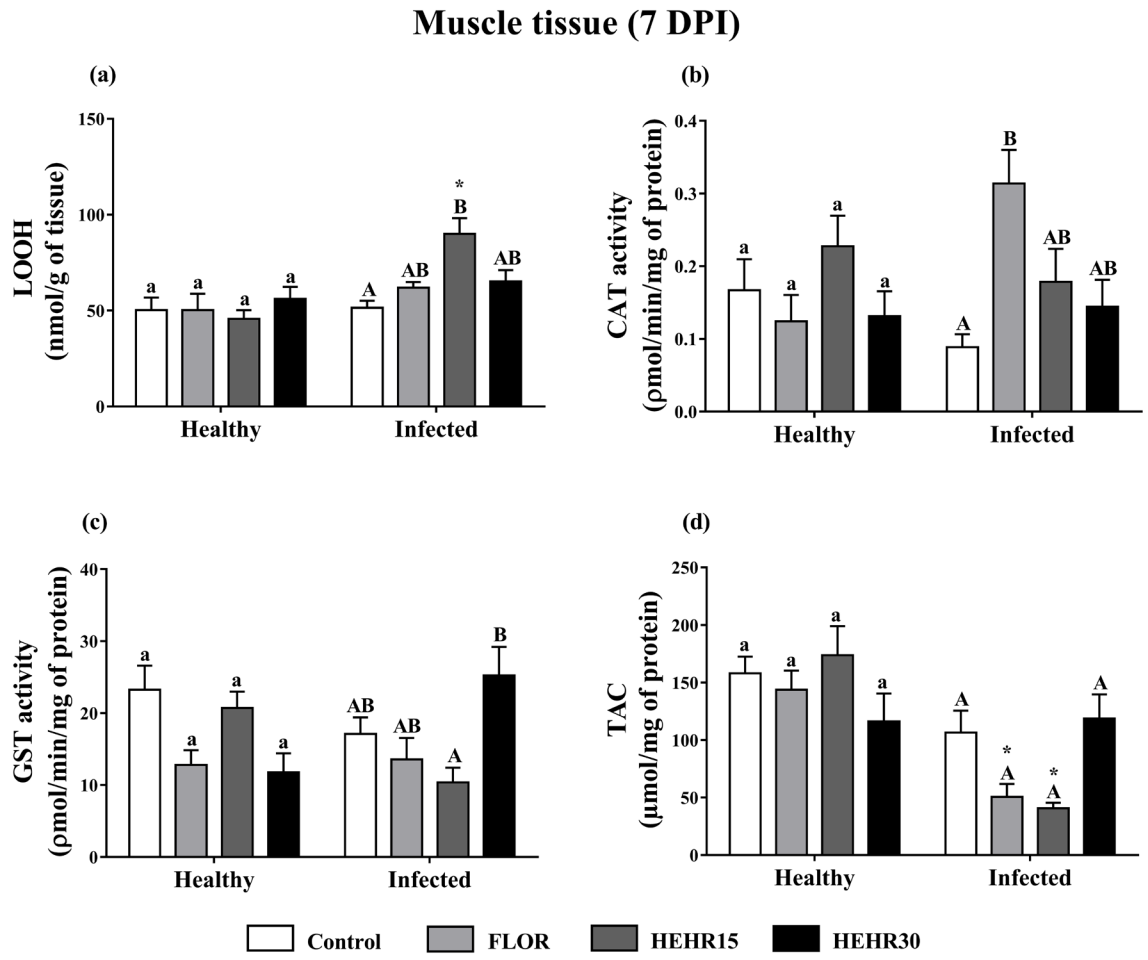

Figure 2. (a) Lipid hydroperoxide (LOOH) levels, (b) catalase (CAT) activity, (c) glutathione S-transferase (GST) activity and (d) total antioxidant capacity (TAC) of muscle tissue collected on the seventh experimental day with silver catfish (*Rhamdia quelen*) healthy or experimentally infected by *Aeromonas hydrophila* (7 DPI) treated with florfenicol (FLOR), hexane extract of *Hesperozygis ringens* 15 mg/L (HEHR15), HEHR30, and control. Different lowercase letters indicate a significant difference between healthy fish. Different capital letters indicate a significant difference between infected fish. (*) indicate significant difference from healthy fish in the same treatment. All the values are expressed as mean ± SEM. Two-way ANOVA and Tukey test ($p < 0.05$).

FLOR showed significantly higher activity ($p = 0.019253$) when compared to the infected control. Similar to what was verified for CAT activity, GST activity showed no significant difference between healthy and infected controls, as well as between groups referring to healthy animals (Figure 2c). On the other hand, between infected animals, the group treated with HEHR30 showed a significant increase ($p = 0.028187$) in GST activity compared to the group treated with HEHR15. The TAC showed no statistical difference between healthy and infected controls, neither between treatments referring to healthy fish, neither between treatments referring to infected fish (Figure 2d). However, the groups infected and treated with FLOR ($p = 0.030499$) and HEHR15 ($p = 0.000232$) showed a significant reduction in TAC compared to their healthy groups *per se*.

Oxidative status in the liver tissue on the 4th day post-infection

In the liver tissue, at 4 DPI by A. hydrophila, LOOH levels were unchanged as the infected control group did not differ statistically from the healthy control group (Figure 3a). The same was verified between the groups referring to healthy fish, which did not differ from each other. Among the infected fish, the group treated with HEHR15 showed a significant reduction ($p = 0.047340$) in LOOH levels compared to the FLOR group; however, there was no statistical difference compared to the control and HEHR30. The A. hydrophila infection was not able to modify CAT activity, as healthy and infected controls did not show any statistical difference (Figure 3b). Moreover, there was no statistical difference between treatments in healthy fish. However, among the infected fish, those treated with HEHR30 presented the highest CAT activity ($p = 0.000167$, 0.000168, and 0.000170). HEHR30 also showed an increased CAT activity in

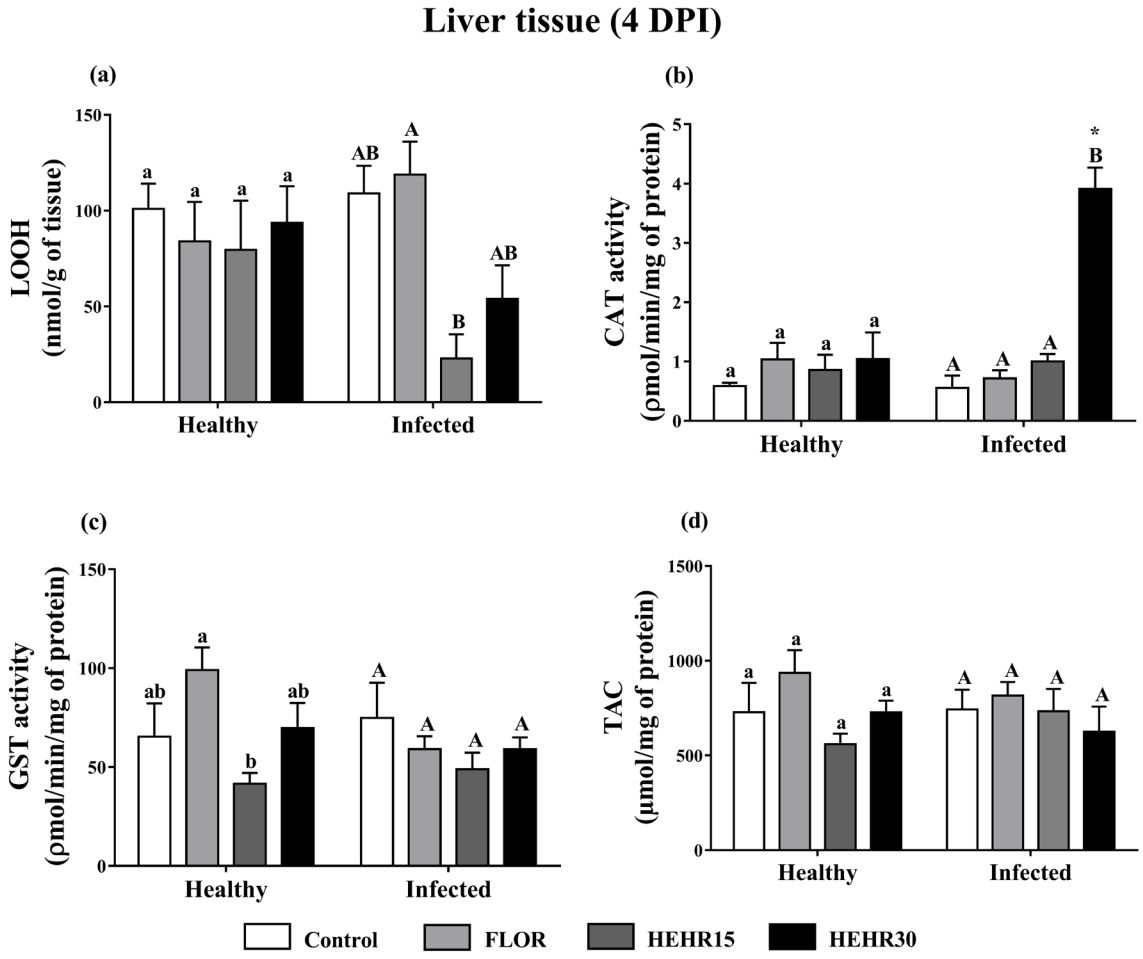

Figure 3. (a) Lipid hydroperoxide (LOOH) levels, (b) catalase (CAT) activity, (c) glutathione S-transferase (GST) activity and (d) total antioxidant capacity (TAC) of liver tissue collected on the fourth experimental day with silver catfish (*Rhamdia quelen*) healthy or experimentally infected by *Aeromonas hydrophila* (4 DPI) treated with florfenicol (FLOR), hexane extract of *Hesperozygis ringens* 15 mg/L (HEHR15), HEHR30, and control. Different lowercase letters indicate a significant difference between healthy fish. Different capital letters indicate a significant difference between infected fish. (*) indicate significant differences from healthy fish in the same treatment. All the values are expressed as mean ± SEM. Two-way ANOVA and Tukey test ($p < 0.05$).

infected fish, compared to its group per se (p = 0.000267). GST activity did not differ statistically between healthy and infected controls, nor between groups referring to infected fish (Figure 3c). However, between healthy animals, those treated with HEHR15 demonstrated significantly lower GST activity (p = 0.042231) compared to animals treated with FLOR. TAC was unaffected by treatments and at 4 DPI by A. hydrophila (Figure 3d).

Oxidative status in the liver tissue on the 7th day post-infection

Lipid hydroperoxide (LOOH) levels in the liver tissue of silver catfish at 7 DPI by *A. hydrophila* was significantly reduced (p = 0.031218) in the infected control group when compared to the healthy control group (Figure 4a). There was no statistical difference between healthy animals, neither between infected animals. The other oxidative parameters (CAT, GST, and TAC) in liver

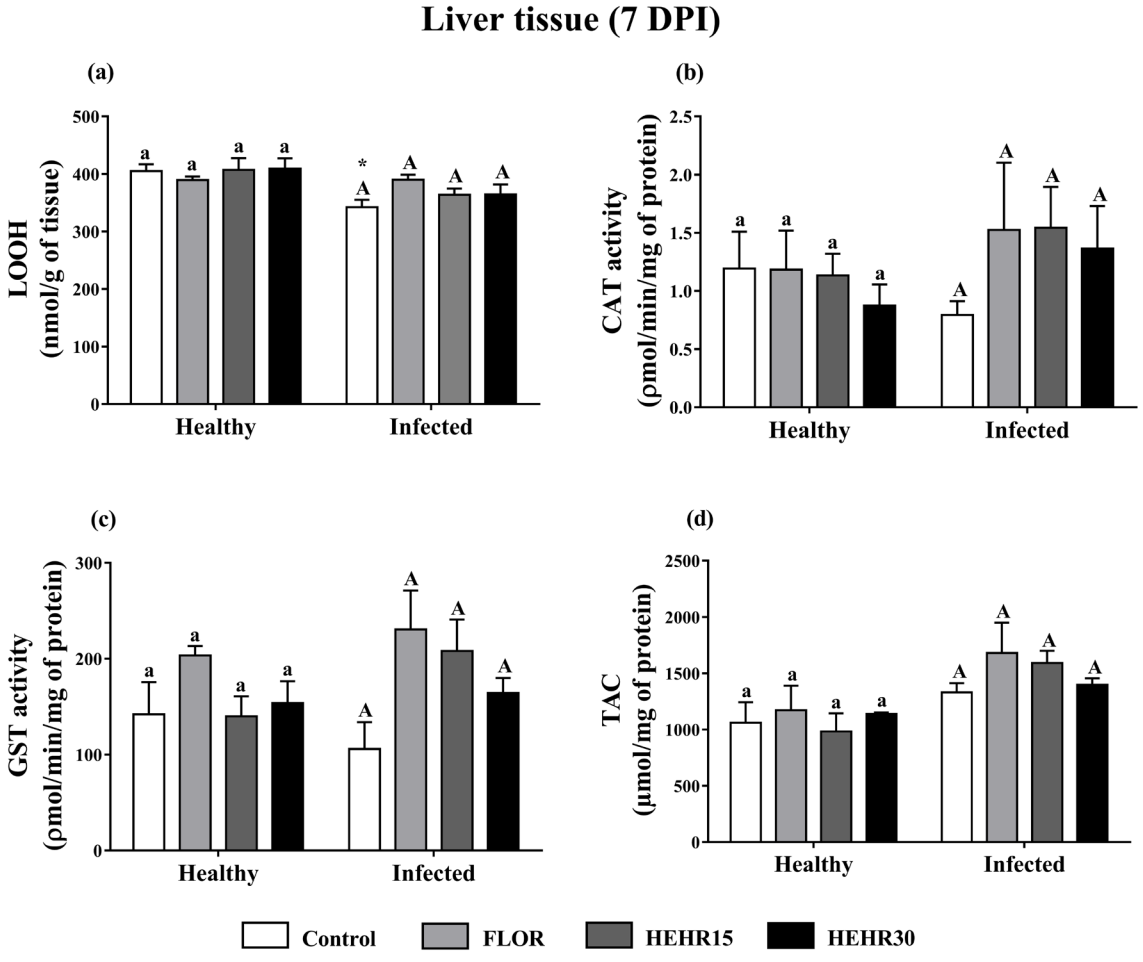

Figure 4. (a) Lipid hydroperoxide (LOOH) levels, (b) catalase (CAT) activity, (c) glutathione S-transferase (GST) activity and (d) total antioxidant capacity (TAC) of liver tissue collected on the seventh experimental day with silver catfish (*Rhamdia quelen*) healthy or experimentally infected by *Aeromonas hydrophila* (7 DPI) treated with florfenicol (FLOR), hexane extract of *Hesperozygis ringens* 15 mg/L (HEHR15), HEHR30, and control. Different lowercase letters indicate a significant difference between healthy fish. Different capital letters indicate a significant difference between infected fish. (*) indicate significant differences from healthy fish in the same treatment. All the values are expressed as mean ± SEM. Two-way ANOVA and Tukey test (p < 0.05).

tissue were not modified at 7 DPI by *A. hydrophila* (Figure 4b, c, and d, respectively).

DISCUSSION

In this study, there was a reduction in LOOH levels in the muscle tissue of juvenile silver catfish 4 DPI by *A. hydrophila*. Studies conducted by Harikrishnan et al. (2010) described a reduction in the respiratory burst (RBA) and nitric oxide (NO) levels of blood leukocytes at 3, 6, and 12 h post-infection (HPI) by *A. hydrophila* in *Carassius auratus* (goldfish). Although previous research configure assays with non-enzymatic markers of oxidative status and distinct fish species, the cited studies detected reduced levels of pro-oxidant agents after infection by *A. hydrophila*.

Regarding the levels of LOOH in the muscle tissue 7 DPI by *A. hydrophila* in silver catfish, in this study, there was no statistical difference between the healthy and infected controls. It is known that the infected control group showed a tendency to increase LOOH levels since infected controls at 4 DPI had a reduced level of this biomarker. In experiments conducted with *Carassius auratus gibelio* (gibel carp) (Yang et al. 2015a) and *Ictalurus punctatus* (channel catfish) (Yang et al. 2015b), the challenge with *A. hydrophila* did not provide statistical variation in plasmatic MDA levels 7 DPI, corroborating the present study, despite different determinations for the assessment of oxidative damage and distinct fish species.

Fish infected and treated with HEHR15 demonstrated an increase in LOOH levels in muscle tissue 7 DPI when compared to the infected control group. This result may be linked to the fact that fish are producing more ROS to eliminate microorganisms (Bandeira Junior & Baldisserotto 2020) since this treatment in healthy fish did not favor the increase of LPO levels. At the same time, this treatment may be acting as a pro-oxidant agent and promoting, along with the infection, the increase in LOOH levels. Lipid peroxidation consists of a chain reaction of unsaturated or polyunsaturated fatty acids (UFA or PUFA) in membranes, where reactive peroxidation products are formed, such as LOOH, which can change their permeability, fluidity, and integrity (Monserrat et al. 2007, França et al. 2013, Lushchak 2014).

In the present study, LOOH levels in the liver tissue of the infected control remained unchanged at 4 DPI by *A. hydrophila*. Similarly, Abasubong et al. (2018) found that 4 DPI by *A. hydrophila* in *Megalobrama amblycephala* (blunt snout bream) hepatic MDA levels remained unchanged. However, in the current study, a reduction in hepatic LOOH levels was observed in infected control fish 7 DPI. Tang et al. (2018) also found a reduction in LPO levels, but in hepatic MDA content, 24 HPI, whose levels were reestablished (statistically unchanged) after 48 and 72 h.

Catalase activity remained unchanged in the infected control, compared to the healthy control group. Thus, its activity did not show an increase or a reduction in muscle and liver tissue, at 4 and 7 DPI by *A. hydrophila*. Yang et al. (2015b) described that infection by this bacterium in channel catfish did not alter CAT activity 7 DPI, corroborating the present study. CAT is responsible for promoting the degradation of H_2O_2, protecting from the resulting cell damage (Rio et al. 2007). It is worth noting that CAT activity in fish does not have a defined pattern after infection by *A. hydrophila*, which may be decreased, increased, or unchanged even considering the organ and collection time after infection (Bandeira Junior & Baldisserotto 2020).

Hepatic CAT activity in silver catfish infected and treated with HEHR30 on the 4th day was significantly higher compared to the other

treatments. However, for 7 DPI, the activity did not differ from the other treatments. According to Rosa et al. (2019), HEHR30 added to fish culture water through a single therapeutic bath allowed an increase in the survival rate of juvenile silver catfish experimentally infected with *A. hydrophila*. The increase in hepatic CAT activity may be directly related to a defense mechanism that the extract at a concentration of 30 mg/L can provide in favor of the elimination of this pathogen, as well as to attenuate scientifically proven virulence factors of this bacterial species (Barcellos et al. 2008, Bandeira Junior et al. 2018). Rosa et al. (2019) described that at 4 DPI by *A. hydrophila* about 40% of infected and untreated animals had died, which characterizes a crucial post-infection period either for disease evolution or for possible recovery. This report highlights the present finding regarding CAT activity on the 4th day for fish that received HEHR30. Recently, it was found that the dry extract of *Curcuma longa* (Motore™) provides an increase in CAT activity in the liver tissue of silver catfish experimentally infected by *A. hydrophila* (Franco et al. 2021) when it is added to the silver catfish feed at a concentration of 750 mg/kg.

The combination of bioactive terpenoids in plant extractives can result in actions on different pharmacological targets, including increased solubility of one or more constituents (Wagner 2011, Elangovan & Mudgil 2023), inhibition and/or prevention of biofilm formation (Bandeira Junior et al. 2018, Pernando et al. 2022), blockage of the bacteria's efflux pumping system (Wagner 2011), interference with membrane permeability, transport processes and protein synthesis (Pernando et al. 2022, Sousa et al. 2023) and disruption of the bacterial cytoplasmic membrane (Sousa et al. 2023). In this way, HEHR, which has bioactive terpenoids such as pulegone, the major constituent of its volatile fraction (Rosa et al. 2019, Shahdadi et al. 2023), can favor the survival of silver catfish affected by *A. hydrophila* through different mechanisms of action, as mentioned above, in conjunction with the increase in hepatic CAT activity, in addition to others not yet elucidated. On the other hand, synthetic compounds such as florfenicol usually have a dominant and, in this case, well-known mechanism of action, which is through the inhibition of bacterial protein synthesis (Schwarz et al. 2004). However, the increase in the levels of reactive species in the gills of healthy silver catfish and those infected by *A. hydrophila* could be observed for florfenicol via bath (Bandeira Junior et al. 2021). These harmful effects corroborate the present study, which verified that florfenicol favored the reduction of muscle TAC 7 DPI.

Similar to CAT, GST activity was not directly influenced by *A. hydrophila* infection, remaining unchanged in the control groups of both tissues analyzed at 4 and 7 DPI. According to Baldissera et al. (2018), silver catfish infected by *A. caviae* demonstrated a 40% decreased hepatic GST activity at 4 DPI when compared to the uninfected control group. Fish infected and treated with HEHR15 demonstrated significantly decreased GST activity in muscle tissue at 4 DPI in relation to its group *per se*, possibly demonstrating to be acting as a stressor or pro-oxidant in the presence of the infection. Although this activity at 7 DPI did not differ significantly from its group *per se*, fish infected and treated with HEHR30 had a higher GST activity when compared to fish infected and treated with HEHR15 suggesting that it is a concentration-dependent effect of this extract. GST catalyzes conjugation reactions between GSH and oxidized molecules, in addition to being involved in the detoxification of LPO products (Lushchak & Bagnyukova 2006). According to Bandeira Junior & Baldisserotto (2020), the activity or gene expression of fish antioxidant enzymes, such as GST, can decrease

after infection by bacteria of the genus *Aeromonas*, but it can also be increased or even remain unchanged.

Total antioxidant capacity (TAC) remained unchanged at 4 and 7 DPI by *A. hydrophila*, both in muscle and liver tissue. The same was verified by Yang et al. (2015a) and Liu et al. (2013) in plasma and liver tissue, respectively, of gibel carp 7 DPI by this pathogen. Zhao et al. (2019) found that hepatic TAC remained unchanged at 28 DPI by *A. hydrophila* and reduced after 56 days in grass carp. In the current study, fish infected and treated with FLOR and HEHR15 had significantly lower TAC levels in muscle tissue at 7 DPI compared to their groups *per se*. Given this result, the respective treatments seem to be acting as pro-oxidants. Such effect was not verified for HEHR30 treatment.

Infection by *A. hydrophila* in the current study did not show to cause a condition of oxidative stress, characterized by the imbalance between pro-oxidant and antioxidant compounds in favor of the former (Sies 2015), since LPO markers did not increase, nor did reduce antioxidant defenses (CAT, GST, and TAC) in infected control groups. Nevertheless, there was a tendency towards a reduction in the activity of the enzymes studied, although there was no detected significant difference. Additionally, some species of bacteria of the *Aeromonas* genus have their own antioxidant systems, which enable them to overcome the harmful effects of ROS on their cells and thus confer greater resistance to the host's immune defenses (Bandeira Junior & Baldisserotto 2020).

The concentration of bacterial suspension used, equivalent to that described by Rosa et al. (2019), was lower when compared to other studies (Baldissera et al. 2017, Da Rosa et al. 2019). The concentration used in our study provided mortality of approximately 53% of silver catfish during the aeromonosis induction protocol. As described by Chen et al. (2020), the oxidative damage and reduction of antioxidant defenses are dependent on the concentration of the suspension of *A. hydrophila* to which the fish are subjected. These authors found that the greater the bacterial concentration, the greater the reduction in defense enzymes, such as SOD, GSH, and lysozyme, as well as the greater the level of LPO over time. For this reason, the concentration of the bacterial suspension used in the challenge with *A. hydrophila* seems to be a key point for the absence of oxidative stress.

In short, it is common to verify a variation in oxidative stress parameters within the same experimental infection by *A. hydrophila*. Several authors initially describe unchanged levels of antioxidant defenses and/or LPO biomarkers, and, over the days, elevated and reduced levels are observed or vice versa (Zhao et al. 2019, Chen et al. 2020). Changes in LOOH levels in muscle and liver tissue were demonstrated on different days of collection in the current study, while antioxidant defenses were more affected in the presence of a secondary stressor. In the present study, the main stressor for animals was characterized by bacterial infection. The addition of a compound to the immersion bath, FLOR and/or HEHR15, in some cases, favored the reduction of the activity of some antioxidant defenses. For this reason, these compounds can be considered as additional and/or secondary stressors to the bacterial infection, since this reduction was not verified by their effect *per se*. According to Lushchak (2014), one of the main problems regarding the activity of antioxidant enzymes is the complexity of their response. Moreover, depending on the intensity of the oxidative stress, their activity can increase, decrease, or even not change.

To the best of our knowledge, this is the first study of the effect of HEHR on the redox profile of healthy or *A. hydrophila*-infected fish. The volatile

fraction of HEHR has as its main constituent the monoterpenoid pulegone (content of 47%), with the other constituents identified as menthone (5.8%), menthol (3.86%), isopulegone (1.84%), spathulenol (4.35%), and caryophyllene (2.19%), as described by our research group (Rosa et al. 2019). Regarding these terpenoids, menthone, pulegone, and caryophyllene exhibited low or almost null antioxidant activity in *in vitro* assays (Torres-Martínez et al. 2017). On the other hand, Hoseini et al. (2020) verified that menthol can increase the activity of antioxidant enzymes and reduce the oxidative stress of rainbow trout (*Oncorhynchus mykiss*). Other studies found that isopulegone (Silva et al. 2012) and spathulenol (Nascimento et al. 2018) have a strong *in vitro* antioxidant potential. As this is a follow-up study of our research group, in the present study, the same batch of HEHR used by Rosa et al. (2019) was utilized for the assays, and therefore, it has the same phytochemical composition. Based on this, the phytoconstituents present in the HEHR30 may be acting in a synergistic, additive, or potentiation way, given their antioxidant properties described in the literature, contributing to the increase in the activity of hepatic CAT, as well as helping to maintain LOOH levels and antioxidant defenses.

CONCLUSIONS

In conclusion, our results showed that the presence of a secondary stressor for fish, in addition to the infection by *A. hydrophila*, provided a pro-oxidant effect on the antioxidant defenses, verified in some cases for FLOR and HEHR15 treatments. The HEHR30 treatment did not show a deleterious effect in the absence of infection. In addition, it was not characterized as a pro-oxidant agent in the presence of infection, neither inducing lipid peroxidation nor reducing antioxidant defenses, which demonstrates that it has a beneficial effect on silver catfish when administered through immersion bath. One of the possible mechanisms of action of HEHR30 that contribute to the increase in the survival rate of silver catfish challenged by *A. hydrophila* verified in previous studies is via the increase in hepatic CAT activity, demonstrated in the present study. For this reason, further investigations should be conducted to elucidate the effects of HEHR30 on other fish species, the mechanisms of action involved, safety, and efficacy for future applications in aquaculture.

Acknowledgments

We thank MSc Patricia Rodrigues for her assistance during the collection of fish tissues. The authors recognize and are grateful for the financial support in part to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) – Financial Code 001: Postdoctoral fellowship to C. G. Pinheiro, Ph.D. fellowships to I. A. Rosa, A. E. Bianchini, C. A. Bressan and G. Bandeira Junior and MSc fellowship to K. Ariotti and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). B. Baldisserotto is the recipient of a research fellowship from CNPq (grant number 301,225/2017-6). F. T. Ferrari received a technological initiation fellowship from PIBITI-CNPq.

REFERENCES

ABASUBONG KP, LIU W, ZHANG D, YUAN X, XIA S, XU C & LI X. 2018. Fishmeal replacement by rice protein concentrate with xylooligosaccharides supplement benefits the growth performance, antioxidant capability and immune responses against *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*). Fish Shellfish Immunol 78: 177-186. https://doi.org/10.1016/j.fsi.2018.04.044.

AEBI H. 1984. Catalase *in vitro*. Meth Enzymol 105: 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3.

ALGAMMAL AM, MOHAMED MF, TAWFIEK BA, HOZZEIN WN, EL KAZZAZ WM & MABROK M. 2020. Molecular typing, antibiogram and PCR-RFLP based detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. Pathogens 9(3): 238. https://doi.org/10.3390/pathogens9030238.

BALDISSERA MD, SOUZA CF, BANDEIRA JUNIOR G, VARGAS AC, BOLIGON AA, CAMPOS MMA, STEFANI LM & BALDISSEROTTO B.

2017. *Melaleuca alternifolia* essential oil enhances the non-specific immune system and prevents oxidative damage in *Rhamdia quelen* experimentally infected by *Aeromonas hydrophila*: Effects on cholinergic and purinergic systems in liver tissue. Fish Shellfish Immunol 61: 1-8. https://doi.org/10.1016/j.fsi.2016.12.016.

BALDISSERA MD, SOUZA CF, PARMEGGIANI B, LEIPNITZ G, VERDI CM, SANTOS RCV, STEFANI LM & BALDISSEROTTO B. 2018. The disturbance of antioxidant/oxidant balance in fish experimentally infected by *Aeromonas caviae*: Relationship with disease pathophysiology. Microb Pathog 122: 53-57. https://doi.org/10.1016/j.micpath.2018.06.011.

BANDEIRA JUNIOR G ET AL. 2017. Potential uses of *Ocimum gratissimum* and *Hesperozygis ringens* essentials oils in aquaculture. Ind Crops Prod 97: 484-491. https://doi.org/10.1016/j.indcrop.2016.12.040.

BANDEIRA JUNIOR G & BALDISSEROTTO B. 2020. Fish infections associated with the genus *Aeromonas*: a review of the effects on oxidative status. J Appl Microbiol 131(3): 1083-1101. https://doi.org/10.1111/jam.14986.

BANDEIRA JUNIOR G, SOUZA CF, DA SILVA HNP, BIANCHINI AE, RODRIGUES P, DA COSTA ST, HEINZMANN BM, CARGNELUTTI JF & BALDISSEROTTO B. 2021. Combined effect of florfenicol with linalool via bath in combating *Aeromonas hydrophila* infection in silver catfish (*Rhamdia quelen*). Aquaculture 545: 737247. https://doi.org/10.1016/j.aquaculture.2021.737247.

BANDEIRA JUNIOR G, SUTILI FJ, GRESSLER LT, ELY VL, SILVEIRA BP, TASCA C, REGHELIN M, MATTER LB, VARGAS APC & BALDISSEROTTO B. 2018. Antibacterial potential of phytochemicals alone or in combination with antimicrobials against fish pathogenic bacteria. J Appl Microbiol 125(3): 655-665. https://doi.org/10.1111/jam.13906.

BARCELLOS LJG, KREUTZ LC, RODRIGUES LB, SANTOS LR, MOTTA AC, RITTER F, BEDIN AC & SILVA LB. 2008. *Aeromonas hydrophila* in *Rhamdia quelen*: macroscopic and microscopic aspect of the lesions and antibiotic resistance profiles. Bol Inst Pesca 34(3): 355-363. http://refhub.elsevier.com/S0044-8486(21)00910-8/rf0060.

CAO G & PRIOR RL. 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 44: 1309-1315. https://doi.org/10.1093/CLINCHEM/44.6.1309.

CHANDRAN MR, ARUNA BV, LOGAMBAL SM & DINAKARAN MICHAEL R. 2002. Immunisation of Indian major carps against *Aeromonas hydrophila* by intraperitoneal injection. Fish Shellfish Immunol 13(1): 1-9. https://doi.org/10.1006/fsim.2001.0374.

CHEN J, LIU N, ZHANG H, ZHAO Y & CAO X. 2020. The effects of *Aeromonas hydrophila* infection on oxidative stress, nonspecific immunity, autophagy, and apoptosis in the common carp. Dev Comp Immunol 105: 103587. https://doi.org/10.1016/j.dci.2019.103587.

CHU W-H & LU C-P. 2008. *In vivo* fish models for visualizing *Aeromonas hydrophila* invasion pathway using GFP as a biomarker. Aquaculture 277(3-4): 152-155. https://doi.org/10.1016/j.aquaculture.2008.03.009.

CITARASU T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquac Int 18: 403-414. https://doi.org/10.1007/s10499-009-9253-7.

DA ROSA VM ET AL. 2019. Dietary addition of rutin impairs inflammatory responses and protects muscle of silver catfish (*Rhamdia quelen*) from apoptosis and oxidative stress in *Aeromonas hydrophila*-induced infection. Comp Biochem Physiol C Toxicol Pharmacol 226: 108611. https://doi.org/10.1016/j.cbpc.2019.108611.

ELANGOVAN S & MUDGIL P. 2023. Antibacterial properties of *Eucalyptus globulus* essential against MRSA: a systematic review. Antibiotics 12(3): 474. https://doi.org/10.3390/antibiotics12030474.

ESTEVE C, BIOSCA EG & AMARO C. 1993. Virulence of *Aeromonas hydrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in fresh water. Dis Aquat Org 16: 15-20. https://doi.org/10.3354/dao016015.

FAO – FOOD AND AGRICULTURE ORGANIZATION. 2020. The state of world fisheries and aquaculture. Sustainability in action. Food and Agriculture Organization, Rome, Italy. https://www.fao.org/documents/card/en/c/ca9229en/. Accessed 19 August 2020.

FDA – FOOD AND DRUG ADMINISTRATION. 2022. Approved Aquaculture Drugs. Food and Drug Administration. https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs. Accessed 29 June 2022.

FIGUEIREDO AB, TANCREDO KR, HASHIMOTO GSO, ROUMBEDAKIS K, MARCHIORI NC & MARTINS ML. 2014. Haematological and parasitological assessment of silver catfish *Rhamdia quelen* farmed in Southern Brazil. Rev Bras Parasitol Vet 23(2): 157-163. https://doi.org/10.1590/S1984-29612014028.

FRANÇA BK, ALVES MRM, SOUTO FMS, TIZIANE L, BOAVENTURA RF, GUIMARÃES A & ALVES JUNIOR A. 2013. Peroxidação lipídica e obesidade: métodos para aferição do estresse oxidativo em obesos. GE Jornal Português de Gastrenterologia. 20(5): 199-206. https://doi.org/10.1016/j.jpg.2013.04.002.

FRANCO FW, BANDEIRA JUNIOR G, DOLCI GS, BARBOSA LB, OLIVEIRA CES, SOUZA CF, BURGER ME, BALDISSEROTTO B & HEINZMANN BM.

2021. The influence of dietary Motore™ supplement on antioxidant status to *Aeromonas hydrophila* infection in *Rhamdia quelen*. Microb Pathog 154(4): 104871. https://doi.org/10.1016/j.micpath.2021.104871.

GOMES LC, GOLOMBIESKI JI, GOMES ARC & BALDISSEROTTO B. 2000. Biologia do jundiá *Rhamdia quelen* (Teleostei, Pimelodidae). Cienc Rural 30(1): 179-185. https://doi.org/10.1590/S0103-84782000000100029.

HABIG WH, PABST MJ & JAKOBY WB. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

HARIKRISHNAN R, KIM M, KIM J, HAN Y, JANG I, BALASUNDARAM C & HEO M. 2010. Immune response and expression analysis of cathepsin K in goldfish during *Aeromonas hydrophila* infection. Fish Shellfish Immunol 28(4): 511-516. https://doi.org/10.1016/j.fsi.2009.12.005.

HERMES-LIMA M, WILLMORE WG & STOREY KB. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radic Biol Med 19(3): 271-280. https://doi.org/10.1016/0891-5849(95)00020-X.

HOSEINI SM, MIRGHAED AT, PARAY BA, HOSEINIFAR SH & DOAN HV. 2020. Effects of dietary menthol on growth performance and antioxidant, immunological and biochemical responses of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 524: 735260. https://doi.org/10.1016/j.aquaculture.2020.735260.

LIU B, XU L, GE X, XIE J, XU P, ZHOU Q, PAN L & ZHANG Y. 2013. Effects of mannan oligosaccharide on the physiological responses, HSP70 gene expression and disease resistance of Allogynogenetic crucian carp (*Carassius auratus gibelio*) under *Aeromonas hydrophila* infection. Fish Shellfish Immunol 34: 1395-1403. https://doi.org/10.1016/j.fsi.2013.02.028.

LLOBRERA AT & GACUTAN RQ. 1987. *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. Aquaculture 67(3-4): 273-278. https://doi.org/10.1016/0044-8486(87)90211-0.

LOWRY OH, ROSEBROUGH NJ, FARR AL & RANDALL RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193(1): 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6.

LUSHCHAK VI. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 224: 164-175. https://doi.org/10.1016/j.cbi.2014.10.016.

LUSHCHAK VI & BAGNYUKOVA TV. 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comp Biochem Physiol B 144: 283-289. https://doi.org/10.1016/j.cbpb.2006.02.014.

MONSERRAT JM, MARTINEZ PE, GERACITANO LA, AMADO LL, MARTINS CMG, PINHO GLL, CHAVES IS, FERREIRA-CRAVO M, VENTURA-LIMA J & BIANCHINI A. 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comp Biochem Physiol C Toxicol Pharmacol 146: 221-234. https://doi.org/10.1016/j.cbpc.2006.08.012.

NASCIMENTO KF ET AL. 2018. Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw. and spathulenol. J Ethnopharmacol 210: 351-358. https://doi.org/10.1016/j.jep.2017.08.030.

PANDEY A, NAIK M & DUBEY SK. 2010. Hemolysin, protease, and EPS producing pathogenic *Aeromonas hydrophila* strain An4 shows antibacterial activity against marine bacterial fish pathogens. J Mar Biol 2010: 1-9. https://doi.org/10.1155/2010/563205.

PEMBERTON JM, KIDD SP & SCHMIDT R. 1997. Secreted enzymes of *Aeromonas*. FEMS Microbiol Lett 152: 1-10. https://doi.org/10.1111/j.1574-6968.1997.tb10401.x.

PERNANDO NSA, HAMZAH H, WIRNAWATI & SIREGAR KAAK. 2022. Antibacterial and antibiofilm activity *Staphylococcus aureus* from plants containing essential oils: a mini-review. Lett Appl NanoBioSci 12(1): 5. https://doi.org/10.33263/LIANBS121.005.

PILARSKI F, ISHKAWA MM, SEBASTIÃO FA, PADUA SB & SAKABE R. 2011. Columnariose: etiologia, sinais clínicos e envio de amostras para análise laboratorial, Embrapa Agropecuária Oeste-Documentos (INFOTECA-E) 109: 1-32. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/901115. Accessed 18 July 2022.

RIO RVM, ANDEREGG M & GRAF J. 2007. Characterization of a catalase gene from *Aeromonas veronii*, the digestive-tract symbiont of the medicinal leech. Microbiology 153(6): 1897-1906. https://doi.org/10.1099/mic.0.2006/003020-0.

ROMERO J, FEIJOÓ CG & NAVARRETTE P. 2012. Antibiotics in aquaculture – use, abuse and alternatives. In: HEALTH AND ENVIRONMENT IN AQUACULTURE, CARVALHO E (Ed), p. 159-198, Rijeka, Croatia: InTech. https://www.intechopen.com/chapters/35141.

ROSA IA, RODRIGUES P, BIANCHINI AE, SILVEIRA BP, FERRARI FT, BANDEIRA JUNIOR G, VARGAS APC, BALDISSEROTTO B & HEINZMANN BM. 2019. Extracts of *Hesperozygis ringens* (Benth.) Epling: *in vitro* and *in vivo* antibacterial activity against fish pathogenic bacteria. J Appl Microbiol 126(5): 1353-1361. https://doi.org/10.1111/jam.14219.

SCHWARZ S, KEHRENBERG C, DOUBLET B & CLOECKAERT A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 28(5): 519-542. https://doi.org/10.1016/j.femsre.2004.04.001.

SEGNER H, SUNDH H, BUCHMANN K, DOUXFILS J, SUNDELL KS, MATHIEU C, RUANE N, JUTFELT F, TOFTEN H & VAUGHAN L. 2012. Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. Fish Physiol Biochem 38: 85-105. https://doi.org/10.1007/s10695-011-9517-9.

SHAHDADI F, FARYABI M, KHAN H, SARDOEI AS, FAZELI-NASAB B, GOH BH, GOH KW & TAN CS. 2023. *Mentha longifolia* essential oil and pulegone in edible coatings of alginate and chitosan: effects on pathogenic bacteria in lactic cheese. Molecules 28: 4554. https://doi.org/10.3390/molecules28114554.

SIES H. 2015. Oxidative stress: a concept in redox biology and medicine Redox Biol 4: 180-183. https://doi.org/10.1016/j.redox.2015.01.002.

SIGNOR AA, NETO CCB, FIGUEIREDO ES, SIGNOR FRP, WATANABE AL, FERREIRA HKA & REIDEL A. 2020. Manejo alimentar de juvenis de jundiá (*Rhamdia quelen*) cultivado em tanques-rede: tipos e rações, taxas de arraçoamento e estratégia alimentar. Braz J Dev 6(7): 48531-48546. https://doi.org/10.34117/bjdv6n7-483.

SILVA DT ET AL. 2014. Larvicidal activity of Brazilian plant essential oils against *Coenagrionidae larvae*. J Econ Entomol 107(4): 1713-1720. https://doi.org/10.1603/EC13361.

SILVA LL, SILVA DT, GARLET GI, CUNHA MA, MALLMANN CA, BALDISSEROTTO B, LONGHI SJ, PEREIRA AMS & HEINZMANN BM. 2013. Anesthetic activity of Brazilian native plants in silver catfish (*Rhamdia quelen*). Neotrop Ichthyol 11(2): 443-451. https://doi.org/10.1590/S1679-62252013000200014.

SILVA OA, OLIVEIRA FRAM, LIMA TC, SOUSA DP, SOUZA AA & FREITAS RM. 2012. Evaluation of the antioxidante effects *in vitro* of the isopulegone. Free Radic Antioxid 2(4): 50-55. https://doi.org/10.5530/ax.2012.4.9.

SINDAN – SINDICATO NACIONAL DA INDÚSTRIA DE PRODUTOS PARA SAÚDE ANIMAL. 2022. Compêndio de Produtos Veterinários. https://sistemas.sindan.org.br/cpvs/pesquisar.aspx. Accessed 29 June 2022.

SOUSA DP, DAMASCENO ROS, AMORATI R, ELSHABRAWY HÁ, CASTRO RD, BEZERRA DP, NUNES VR, GOMES RC & LIMA TC. 2023. Essential oils: chemistry and pharmacological activities. Biomolecules 13(7): 1144. https://doi.org/10.3390/biom13071144.

SOUZA CF, BALDISSERA MD, BALDISSEROTTO B, HEINZMANN BM, MARTOS-SITCHA JA & MANCERA JM. 2019. Essential oils as stress-reducing agents for fish aquaculture: a review. Front Physiol 10: 785. https://doi.org/10.3389/fphys.2019.00785.

SUTILI FJ, SILVA LL, GRESSLER LT, GRESSLER LT, BATTISTI EK, HEINZMANN BM, VARGAS APC & BALDISSEROTTO B. 2015. Plant essential oils against *Aeromonas hydrophila*: *in vitro* activity and their use in experimentally infected fish. J Appl Microbiol 119(1): 47-54. https://doi.org/10.1111/jam.12812.

TANG Y, HAN L, CHEN X, XIE M, KONG W & WU Z. 2018. Dietary supplementation of probiotic *Bacillus subtilis* affects antioxidant defenses and immune response in grass carp under *Aeromonas hydrophila* challenge. Probiotics Antimicrob Proteins 11: 545-558. https://doi.org/10.1007/s12602-018-9409-8.

TAVARES-DIAS M & MARTINS ML. 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms, J Parasit Dis 41(4): 913-918. https://doi.org/10.1007/s12639-017-0938-y.

TORRES-MARTÍNEZ R, GARCÍA-RODRÍGUEZ YM, RÍOS-CHÁVEZ P, SAAVEDRA-MOLINA A, LÓPEZ-MEZA JE, OCHOA-ZARZOSA A & GARCIGLIA RS. 2017. Antioxidant activity of the essential oil and its major terpenes of *Satureja macrostema* (Moc. and Sessé ex Benth.) Briq Pharmacogn Mag 13(4): S875-S880. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5822514/.

WAGNER H. 2011. Synergy research: approaching a new generation of phytopharmaceuticals. Fitoterapia 82: 34-37. https://doi.org/10.1016/j.fitote.2010.11.016.

YAMBOT AV. 1998. Isolation of *Aeromonas hydrophila* from *Oreochromis niloticus* during fish disease outbreaks in the Philippines. Asian Fish Sci 10: 347-354. https://www.asianfisheriessociety.org/publication/downloadfile.php?id=269&file=Y0dSbUx6QTVOVE0zTVRJd01ERXpOVFU0TURNeU5qZ3VjR1Jt.

YANG B, WANG C, HU H, TU Y, HAN D, ZHU X, JIN J, YANG Y & XIE S. 2015a. Repeated handling compromises the immune suppression and improves the disease resistance in overwintering channel catfish (*Ictalurus punctatus*). Fish Shellfish Immunol 47: 418-428. https://doi.org/10.1016/j.fsi.2015.09.010.

YANG B, WANG C, TU Y, HU H, HAN D, ZHU X, JIN J, YANG Y & XIE S. 2015b. Effects of repeated handling and air exposure on the immune response and the disease resistance of gibel carp (*Carassius auratus gibelio*) over winter. Fish Shellfish Immunol 47: 933-941. https://doi.org/10.1016/j.fsi.2015.10.013.

ZHAO H, LUO Y, WU Z, ZHOU Y, GUO D, WANG H & CHEN X. 2019. Hepatic lipid metabolism and oxidative stress responses of grass carp (*Ctenopharyngodon idella*) fed diets of two different lipid levels against *Aeromonas hydrophila* infection. Aquaculture 509: 149-158. https://doi.org/10.1016/j.aquaculture.2019.05.029.

How to cite

ROSA IA ET AL. 2024. Redox profile of silver catfish challenged with *Aeromonas hydrophila* and treated with hexane extract of *Hesperozygis ringens* (Benth.) Epling through immersion bath. An Acad Bras Cienc 96: e20230188. DOI 10.1590/0001-3765202420230188.

Manuscript received on February 23, 2023;
accepted for publication on December 19, 2023

ISADORA A. ROSA[1]
https://orcid.org/0000-0003-3612-9837

ADRIANE E. BIANCHINI[1]
https://orcid.org/0000-0002-9141-0085

CAROLINE A. BRESSAN[1]
https://orcid.org/0000-0003-4986-6350

FABÍOLA T. FERRARI[2]
https://orcid.org/0009-0005-2815-5930

KARINE ARIOTTI[1]
https://orcid.org/0000-0003-0385-3845

NATACHA C. MORI[1,3]
https://orcid.org/0000-0003-0863-2857

GUERINO BANDEIRA JUNIOR[1]
https://orcid.org/0000-0002-7697-398X

CARLOS G. PINHEIRO[4]
https://orcid.org/0000-0002-1634-8015

MARIA AMÁLIA PAVANATO[1,5]
https://orcid.org/0000-0002-1348-3828

JULIANA F. CARGNELUTTI[6]
https://orcid.org/0000-0002-3160-3643

BERNARDO BALDISSEROTTO[1,5]
https://orcid.org/0000-0002-8770-0100

BERTA MARIA HEINZMANN[1,2,7]
https://orcid.org/0000-0002-6509-949X

[1]Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[2]Universidade Federal de Santa Maria, Curso de Farmácia, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[3]Universidade de Cruz Alta (UNICRUZ), Curso de Farmácia, Rodovia Municipal Jacob Della Mea, s/n, km 5,6, 98020-290 Cruz Alta, RS, Brazil

[4]Programa de Pós-Graduação em Engenharia Florestal, Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[5]Universidade Federal de Santa Maria, Departamento de Fisiologia e Farmacologia, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[6]Universidade Federal de Santa Maria, Departamento de Medicina Veterinária Preventiva, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[7]Universidade Federal de Santa Maria, Departamento de Farmácia Industrial, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

Correspondence to: **Berta Maria Heinzmann**
E-mail: berta.heinzmann@gmail.com

Author contributions

Isadora Aguirre Rosa: Conceptualization, Methodology, Validation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Visualization. Adriane Erbice Bianchini: Conceptualization, Methodology, Validation, Formal analysis. Caroline Azzolin Bressan: Conceptualization, Methodology, Formal Analysis, Writing – Review & Editing. Fabíola Tonelli Ferrari, Karine Ariotti, Natacha Cossettin Mori, Guerino Bandeira Junior and Carlos Garrido Pinheiro: Methodology. Maria Amália Pavanato and Juliana Felipetto Cargnelutti: Resources. Bernardo Baldisserotto: Formal analysis, Resources, Writing – Review & Editing, Supervision, Funding Acquisition. Berta Maria Heinzmann: Conceptualization, Methodology, Formal Analysis, Resources, Writing – Review & Editing, Supervision, Funding Acquisition.