Magna Scientia UCEVA (2022) 2:2, 276-284
ISSN: 2805-6884 e-ISSN: 2805-6701
https://doi.org/10.54502/msuceva.v2n2a12

Research Article *Agricultural and Biological Sciences*

Phytochemical prospecting and cytotoxicity assays of potential bioproducts to control parasites in *Colossoma macropomum* Cuvier

Prospección fitoquímica y ensayos de citotoxicidad de bioproductos potenciales para el control de parásitos en *Colossoma macropomum* Cuvier

Érica Eloy Silva, Rodrigo Vieira Alves Amaral†, Weder Carlos de Souza Tomicha, Ricardo Henrique Bastos de Souza, Raul Dirceu Pazdiora and Creucí Maria Caetano

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Correspondence:

rodrigo.amaral@unir.br
Department of Fishing and Aquaculture Engineering (DEPA), Federal University of Rondônia, Presidente Médici campus (UNIR-PM), Brazil.

First draft submitted: 13-12-2021
Accepted for publication: 07-09-2022
Published on line: 01-12-2022

Key words:

Amazonian fish; *Artemia salina*; herbal medicines; nauplii; Tambaqui.

Palabras clave:

Artemia salina; hierbas medicinales; nauplios; peces amazónicos; Tambaqui.

Citation:

Silva EE, Amaral RVA, Tomicha WC, Bastos RH, Pazdiora RD, Caetano CM. Phytochemical prospecting and cytotoxicity assays of potential bioproducts to control parasites in *Colossoma macropomum* Cuvier. *Magna Scientia UCEVA* 2022;2:2 276-284. *https://doi.org/10.54502/msuceva.v2n2a12*

Abstract

With the aim of determining economic and environmental alternatives that support fish farming in the State of Rondônia, a phytochemical prospection of genetic resources of the Rondônia flora was carried out. Subsequently, cytotoxicity assays against *Artemia salina* L. nauplii, thus observing those herbal medicines supposedly with greater potential to control parasites in fish, with emphasis on Tambaqui (*C. macropomum*). With regard to cytotoxicity assays, at least four herbal medicines showed a high potential, reaching values of 100% mortality of microcrustaceans, for the tested concentrations. They were extracted from *Chenopodium ambrosioides* (herb of Santa Maria or mastruz), *Crotalaria* cf. micans (maraca), *Davilla* sp. (fire vine, sp. 1) and *Duranta* sp. (during). The bioprospecting of RFG is also positioned as a conservation strategy.

Resumen

Con el objetivo de determinar alternativas económicas y ambientales que apoyen la piscicultura en el Estado de Rondônia, se realizó una prospección fitoquímica de los recursos genéticos de la flora de Rondônia. Posteriormente, se realizaron ensayos de citotoxicidad contra *Artemia salina* L. nauplii, observándose así aquellas medicinas herbolarias supuestamente con mayor potencial para el control de parásitos en peces, con énfasis en Tambaqui (*C. macropomum*). En cuanto a los ensayos de citotoxicidad, al menos cuatro fitoterápicos mostraron un alto potencial, alcanzando valores del 100% de mortalidad de microcrustáceos, para las concentraciones ensayadas. Se extrajeron de *Chenopodium ambrosioides* (hierba de Santa María o Mastruz), *Crotalaria* cf. micans (maraca), *Davilla* sp. (vid de fuego, sp. 1) y *Duranta* sp. (durante). La bioprospección de RFG también se posiciona como una estrategia de conservación.

Introduction

Fishing is of great importance for the planet's food security, but it has been insufficient to meet world demand. Some fish stocks are at risk of depletion, particularly those of species of great economic value [1,2]. Thus, cultivation appears as an opportunity to meet these needs, becoming an important source of quality animal protein for human consumption.

However, any animal production enterprise is susceptible, regardless of the cultivation conditions, to the occurrence of problems related to health. Specifically, for the case of fish, there are many risks of infections, since pathogens are characterized by their opportunism, constituting a gateway to secondary infections with high mortalities and loss of the entire squad [3]. This picture is also present in the state of Rondônia, where despite leading the ranking in the production of Amazonian fish (especially Tambaqui) and the third place when analyzing the production of farmed fish, according to the Anuário PEIXE BR 2021, studies related to health in fish farming are incipient, mainly diseases and parasites [4], both on causal agents, predisposing agents and pathological aspects, as well as the development of protocols that establish the experimental infestation of the main parasites that affect fish species.

Considering that there are gaps related to ways of controlling infestations caused by *Perulaernea gamitanae* and *Neoechinorhynchus buttnerae* [5], economically important due to their impact on fish farming in Rondônia, especially Tambaqui [6,7], as well as the lack of knowledge about natural products of plant origin with antiparasitic potential, it is necessary to survey RFG (phytogenetic resources), phytochemical prospecting and evidence of associated cytotoxicity and efficacy.

Alternative products (herbal medicines, bioproducts) are a viable solution, as their use as an antiparasitic can: i) drastically reduce the use of chemotherapeutics and antimicrobials in fish farming, reducing production costs; ii) eradicate present parasites; iii) prevent the emergence of parasites and bacteria resistant to commonly used products, and also iv) minimize negative impacts on the environment [8,9].

Therefore, the aim of this research was to obtain extracts of species from the Rondônia flora with antiparasitic potential mainly for fish, which, once used, could reposition the state of Rondônia as a major producer of native fish at the national level, in the near future.

Methods

After each collection excursion (2021,2022) the materials were sent to the Animal Production Laboratory on the campus of the Federal University of Rondônia, located at Rua da Paz, 4376, Lino Alves Teixeira neighborhood, in the city of Presidente Médici, Brazil. In the laboratory, the plant samples were weighed, washed in running water and subjected to pre-drying at a temperature of 55°C for 96 hours in a ventilated oven. After this period, the samples were crushed in a blender, sieved and macerated in a solvent (ethanol or methanol) for 20 minutes, remaining in the solution for 48 hours.

At the end of this period, compounds were filtered three times on filter paper to remove the solid material. The filtrate was subjected to drying in a rotary evaporator, under reduced pressure at a temperature of 78°C for evaporation, until the total solvent elimination, remaining in the container only the crude extract (EB), which was dried in a desiccator, deposited in plastic bottles and weighed on an analytical balance. For phytochemical prospection, the butanolic, hexane and aqueous extracts were prepared, performing the following tests adapted from Matos [10]:

i) The phytochemical tests on butanolic, hexane and aqueous extracts for phenols and tannins procedure: three drops of ferric chloride solution were added to 3 ml of the extract, shaking well and observing any color variation or precipitate formation. A blank was prepared for comparison. Interpretation of results: variable color between blue and red, indicates the presence of phenols; dark precipitate with a blue hue indicates the presence of pyrogallic tannins; green precipitate indicates the presence of phlobaphene tannins.

ii) Phytochemical tests in aqueous extract for anthocyanins, anthocyanidins and flavonoids. Procedure: 3 ml of extract were added to three test tubes; acidified a tube to pH 3 with 3 drops of HCl, alkalized a tube to pH 8.5 with 3 drops of NaOH, and alkalized a tube to pH 11 with 8 drops of NaOH. Interpretation of results: possible changes observed in coloration were correlated with the presence of constituents or secondary metabolites, as shown in table 1.

Table 1 Prospection for anthocyanins, anthocyanidins and flavonoids, depending on color

Constituents	Coloration/average		
	pH 3.0	pH 8.5	pH 11
Anthocyanins and anthocyanidins	Red	Lilac	Blue-Purple
Flavones, flavononols, xanthones	-	-	Yellow
Chacons and auronas	-	-	Red Purple
Flavonols	-	-	Orange Red

iii) Phytochemical tests in aqueous extract for saponins. Procedure: 3 ml of extract, 5 ml of deionized water and 2 ml of chloroform were added into a test tube. Then, common filtration was performed, and it was stirred well for 3 minutes. Interpretation of results: Persistent foaming is indicative of the saponins presence.

iv) Phytochemical tests on hexane extract for alkaloids. Procedure: for each extract, three test tubes were used, containing 4 ml of the respective extract. In tube 1, three drops of Dragendorff's reagent (potassium iodine bismuthate) were added; in tube 2, three drops of Hager's reagent (saturated picric acid solution); in tube 3, three drops of Meyer's reagent. Interpretation of results: the formation of a flocculous precipitate in at least two tubes is indicative of alkaloids.

v) Phytochemical tests on butanolic extract for cyanidins. Procedure: 0.5 ml of concentrated HCl and 0.5 cm of magnesium tape (Mg) were added to a test tube containing 4 ml of extract solution. Interpretation of results: the appearance or intensification of an orange to red color in the solution, indicates the presence of flavonols, flavons, flavononols and/or xanthones.

To evaluate the cytotoxicity of each plant extract against *A. salina*, 1000ml of 35% saline solution was initially prepared for cyst incubation, which were exposed to artificial lighting (60W incandescent lamp) for 24 hours, until outbreak. To carry out the bioassays, a new 35% saline solution was prepared; later, 3% of Tween80 was added, for a better extract/water interaction. The extracts were weighed with fractions of 1, 10 and 100 $\mu g.ml^{-1}$. Each extract sample was added in triplicate in test tubes containing 10 ml of saline/Tween solution, each tube receiving ten specimens of *A. salina* nauplii. For comparison, control treatment was prepared under the same conditions; however, without the presence of extract. The tubes containing the samples were kept under artificial lighting for 24 hours at room temperature.

After this period, the number of live and dead nauplii exposed to each extract was counted, determining the percentage of mortality. Those organisms that showed movement when observed close to the light source for ten seconds, were considered alive. The LC50 (average lethal concentration for 50% of the test population) of each extract was determined, at a critical significance level of 95%. *A. salina* was used due to its traits, according to Nascimento et al. [11], reduced tolerance to environmental alterations and high specificity to external interferences, guaranteeing, therefore, the expression of clear results through small variations of environmental quality.

Results

Faced with the need to develop fish farming as an economically and environmentally sustainable activity, the adoption of natural sanitary measures certainly represents an important option for combating and preventing diseases and infestations. In this sense, the use of alternative techniques that can be easily implemented and executed by fish producers, deserves attention.

In plants, primary metabolism is conservative and universal. In turn, secondary metabolism products, common to taxonomic groups, or exclusive to a given species, offer advantages for the plant maintenance and development that synthesize them, including defense processes against pathogens and herbivores, attraction of pollinators, temperature tolerance extreme conditions, adaptation to water stress or nutrient and mineral deficiency in the soil. Such products correspond to herbal medicines, that is, those substances obtained exclusively from active vegetable raw materials, with safety and efficacy supported by scientific evidence and which, in addition, present constancy in their quality. They comprise three main groups: terpenes (such as carotenoids and saponins), phenolic compounds (here include flavonoids-anthocyanins, anthocyanidins, cyanidins, flavones, isoflavons, flavonols, flavononols, xanthones, etc., and condensed and hydrolysable tannins) and, finally, nitrogenous compounds (such as alkaloids, cyanogenic glycosides, glucosinolates and non-protein amino acids).

Herbal medicines with activity for the treatment of bacterial diseases and parasitic infestations of humans and animals have been described for centuries [12]. In the present study, taking as reference a survey of genetic resources of the Rondônia flora with potential antiparasitic properties, in which, 54 species were listed, 20 were selected for phytochemical prospecting and 25 for cytotoxicity tests. Table 2 shows the secondary metabolites identified, according to the phytochemical tests, in extracts of 20 species of the Rondônia flora collected in different municipalities of the State.

Table 2 Plant extracts of species (with their vernacular and scientific names, botanical family and Rondônia municipalities where they were collected) with the respective secondary metabolites present

Plant extracts	Identified secondary metabolites						
	Phenols	**Tannins**	**Flobabenic tannins**	**Cyanidins**	**Anthocyanins Anthocyanidins Flavonoids**	**Saponins**	**Alkaloids**
Cipó-de-fogo *Davilla* sp. Dilleniaceae (Ariquemes)	X*	X	--**	X	--	X	X
Alfazema-cabocla *Aloysia gratíssima* *V*erbenaceae (Cacoal)	X	--	X	--	X	X	X
Mentrasto *Hyptis* sp. Lamiaceae (Ariquemes)	X	--	X	--	X	--	X
Cranjiru *Arrabidaea chica* Bignoniaceae (Cacoal)	X	X	--	X	--	X	--
Cipó-de-fogo folhas *Davilla* sp. Dilleniaceae (Rolim de Moura)	X	X	--	--	--	X	--
Cipó-de-fogo fruto *Davilla* sp. Dilleniaceae (Rolim de Moura)	X	--	--	X	--	--	X
Cravo-de-urubu *Heliotropum* cf. *indicum* Boraginaceae (Ariquemes)	X	--	--	--	X	--	X
Maraca *Crotalaria* cf. *micans* Fabaceae (Tarilândia)	--	X	--	--	--	--	X
Erva-de-santa-maria *Chenopodium ambrosioides* Amaranthaceae (Cacoal)	X	X	--	--	--	--	--
Lantana *Lantana* sp. Verbenaceae (Ariquemes)	X	X	--	---	--	--	X
Cipó-de-são-joão *Pirostegia venusta* Bignoniaceae (Pimenta Bueno)	X	X	--	--	--	X	--
Neem *Azadirachta* cf. *indica* Meliaceae (Mirante da Serra)	X	X	--	--	--	X	--
Margaridinha *Tridax* cf. *procumbens*	X	X	--	--	--	--	--

Table 2 Continuation

Plant extracts	Identified secondary metabolites						
	Phenols	Tannins	Phlobabenic tannins	Cyanidins	Anthocyanins Anthocyanidins Flavonoids	Saponins	Alkaloids
Joá *Solanum aculeatissimum* Solanaceae (Alvorada d'Oeste)	X	X	--	--	X	--	--
Algodão-do- campo *Cochlospermum regium* Bixaceae (Pimenta Bueno)	X	X	--	--	--	--	--
Lobeira *Solanum lycocarpum* Solanaceae (Ariquemes)	X	--	--	--	X	--	X
Duranta *Duranta* sp. Verbenaceae (Ouro Preto d'Oeste)	X	X	--	--	--	--	--
Juá *Ziziphus* cf. *joazeiro* Rhamnaceae (Rolim de Moura)	X	--	--	--	--	--	--
Mamoninha- do-mato *Mabea* sp. Euphorbiaceae (Rolim de Moura)	X	X	--	--	--	X	--
Cipó-alho *Mansoa alliacea* Bignoniaceae (Ouro Preto d'Oeste)	X	--	X	--	--	X	--

*"X" indicates the presence of the tested metabolite in the extract; **"--" absence.

Once the extracts were obtained, they were applied in cytotoxicity bioassays against *A. salina*, with the aim of corroborating the effectiveness of each bioproduct and determining those with the greatest antiparasitic and/or antimicrobial potential. The lethality test is considered essential as a preliminary bioassay in the study of compounds with potential biological activity [11,13]. Initially, control tests (without the presence of bioproducts) were carried out in triplicate, considering the number of live *A. salina* nauplii. It is noteworthy that 10 live nauplii/test tube were packed. The average mortality rate of nauplii, in the control, was equal to 46.7, that is, less than 50%. Table 3 and figure 1 presents, for the extract of each evaluated species, the average mortality rates *A. salina* nauplii in concentrations (µg.ml^{-1}) 0.003; 0.03 and 0.3 at the end of 24 hours, in %.

Discussion

Among the species selected to obtain extracts for cytotoxicity assays, it is interesting to point out that, although some have been the subject of studies with herbal medicines, others have so far been little or not used, such as angel's finger, vine-garlic, fire vine, duranta, maraca and soursop leaves, among others. Bories et al. [14] and Moghadamtousi et at. [15], observed the antiparasitic activity of soursop seeds, and aqueous extract of the leaves, while projects carried out at the study area by Embrapa Western Amazon [16], have evidenced the goodness of working with the essential oil of vine-garlic. In the current study, ethanolic extracts were obtained from leaves of these two species.

Table 3 Cytotoxicity tests of 25 plant extracts (vernacular and scientific name) from the Rondônia flora against *Artemia salina* nauplii. Average mortality in %.

Vernacular name	Scientific name	Average mortality of nauplii %/Three replicates/concentration µg.ml^{-1}		
		0.3	0.03	0.003
Abóbora	*Cucurbita* sp.	90.0	73.3	76.7
Algodão-do-campo	*Cochlospermum regium* (Schrank) Pilg.	100.0	100.0	90.0
Alho	*Allium sativum* L.	100.0	26.7	40.0
Capim-limão	*Cymbopogon citratus* (DC.) Stapf	100.0	80.0	16.7
Cipó-alho	*Mansoa alliacea* (Lam.) A.H.Gentry	100.0	100.0	56.7
Cipó-de-fogo	*Davilla* sp. *1*	100.0	100.0	100.0
Cipó-de-fogo	*Davilla* sp. *2*	100.0	90.0	90.0
Cipó-de-são-joão	*Pyrostegia venusta* (Ker Gawl.) Miers	100.0	100,0	80,0
Dedo-de-anjo	*Euphorbia* sp.	100.0	90.0	56.7
Duranta	*Duranta* sp.	100.0	100.0	100.0
Erva-de-santa-maria	*Chenopodium ambrosioides* L.	100.0	100.0	100.0
Graviola	*Annona muricata* L.	100.0	100.0	30.0
Joá	*Solanum aculeatissimum* Jacq.	100.0	100.0	80.0
Hortelã-pimenta	*Mentha X piperita* L.	96.7	23.3	80.0
Lobeira	*Solanum lycocarpum* A. St.-Hil.	100.0	100.0	86.7
Mamão	*Carica papaya* L.	86.7	100.0	100.0
Mamoninha-do-campo	*Mabea* sp.	100.0	100.0	90.0
Mandioca	*Manihot esculenta* Crantz	100.0	76.7	43.3
Maraca	*Crotalaria* cf. *micans*	100.0	100.0	100.0
Maracujá-do-mato	*Passiflora foetida* L.	100.0	96.7	53.3
Margaridinha	*Tridax* cf. *procumbens*	100.0	100.0	43.3
Mentrasto	*Hyptis* sp. 1	100.00	96.7	100.0
Mentrasto	*Hyptis* sp. 2	100.0	96.7	90.0
Mussambé	*Cleome* sp.	100.0	80.0	86.7
Terramicina	*Alternanthera brasiliana* (L.) Kuntze	100.0	100.0	36.7

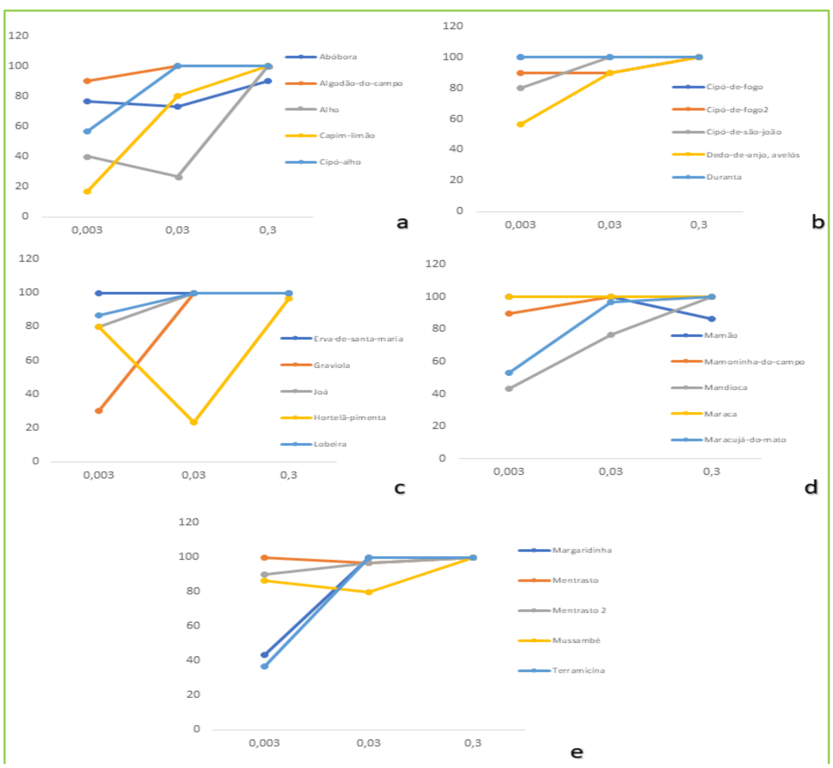


Figure 1 Behavior of 25 plant extracts used in cytotoxicity tests against *A. salina* nauplii, at three concentrations (a-e). It is considered in terms of average mortality (%)

It stands out, among the 25 phytotherapics, in relation to the cytotoxicity tests, that at least four, present a high potential, reaching values of 100% mortality in two microcrustaceans, for the tested concentrations. The same foram extracted from *Chenopodium ambrosioides* (erva-de-santa-maria or mastruz), *Crotalaria* cf. micans (maraca), *Davilla* sp. (cipó-de-fogo, sp. 1) and *Duranta* sp. (Duranta) (table 2; figure 1). The santa maria herb or mastruz (*Chenopodium ambrosioides*, Amaranthaceae) is a common species in all Brazilian states, cultivated or spontaneous. Its potential as an antiparasitic agent is widespread, due to the presence of ascaridol and saponins, tannins and flavonoids, in line with what was found by Jorge et al. [17] and Tapondjou et al. [18]; in the present study, phenols and tannins, they were the only ones detected. This result shows consistency, since flavonoids are included in the group of phenolic compounds. In cytotoxicity tests against *A. salina* nauplii, its extract was one of the most effective, reaching expressive numbers of 100% of average mortality, in all concentrations. Ketzis et al. [19] tested in vitro the oil of santa maria herb against an infestation developed by *Haemonchus contortus* in goats, where it proved to be 100.0% effective in the inhibition of the parasite eggs development.

In a study with sheep, Oliveira [20] showed that for the helminths belonging to the Trichostrongyloideae superfamily, a treatment with this same plant, showed an efficacy on the reduction of the number of eggs per gram of 52.97% on the seventh day and 47.5% on the fourteenth day after its application, when confronted with the control group. They also verified an efficacy of 73.82%, 57.4% and 93.94% on the genera Cooperia, *Trichostrongylus* and *Strongyloides*, respectively. *Crotalaria* cf. micans or maraca or guizo-de-cascavel, is a Fabaceae of wide occurrence, and spontaneously. In the present study, tannins and alkaloids were identified in the extract as secondary metabolites. Pyrrolizidine alkaloids, found mainly in the flowers of *Crotalaria micans*, are considered toxic to Africanized worker bees, genus Apis [11], as well as humans and other animals.

Already in the extract of *Davila* sp. (Dilleniaceae, sp. 1), popularly known as cipó-de-fogo or lixeirinha, a shrub collected in cerrado areas in Rondônia, the largest number of secondary metabolism products was detected, such as phenols, tannins, cyanidins, saponins and alkaloids. Among the 20 extracts evaluated, only *Aloysia gratissima* (Verbenaceae) or lavender-cabocla also showed a high number of metabolites in the phytochemical prospecting tests, including phenols, phlobabenic tannins, anthocyanins, anthocyanidins, flavonoids, saponins and alkaloids.

Little has been researched about its properties, but Kushima [21] reported that species of this genus have popular use in the treatment of gastrointestinal disorders (ulcers, gastritis, inflammation). In the current study, extracts from *Davilla* spp. demonstrated potential in the control of fish parasites, in preliminary in vivo studies, in Tambaqui juveniles, in addition to its effectiveness in cytotoxicity tests against *A. salina* nauplii.

The genus Duranta (Verbenaceae) is most recognized for its ornamental use. There are bushes commonly cultivated for their purplish or violet flowers, known as violeteira, pingo-de-ouro, earring-of-oxum, duranta, among others. According to popular literature, the fruits are toxic due to the presence of steroids and saponins (triterpenes). In the prospection carried out in this work, the presence of phenols and tannins was detected in its extract. Furthermore, it showed maximum efficacy against the microcrustacean *A. salina*, for the evaluated concentrations. Therefore, as well as other genetic resources of the Rondônia state flora, it is suggested that its potential as a phytotherapic is the object of further studies.

Conclusion

Both the phytochemical prospection and the evaluation of cytotoxicity against *Artemia salina*, showed that several genetic resources of the Rondônia flora have the potential to be used in the control of animal parasites, constituting a viable alternative from the environmental, economic and social point of view.

Acknowledgments

For financial support: FAPERO; logistical: Federal University of Rondônia, Presidente Médici campus; and academic: DAAB (Agricultural Development Research Group of the Brazilian Amazon, UNIR Presidente Médici campus) and RON (Herbário Rondoniense, UNIR Porto Velho campus).

Consent for publication

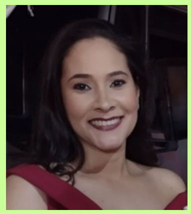
The authors read and approved the final manuscript.

Competing interest

The authors declare no conflict of interest. This document only reflects their point of view and not that of the institution to which they belong.

Author details

Érica Eloy Silva



Bachelor in Fisheries Engineering, from the Federal University of Rondônia, having participated in scientific initiation projects, focusing on health and bioprospecting, Extension projects and technological initiation in the area of fish technology. He is currently a professor of aquaculture at the Universidade Estadual Paulista Júlio de Mesquita Filho, Campus de Jaboticabal (CAUNESP) with a research line for the reproduction of native fish (breeder nutrition.

Rodrigo Vieira Alves Amaral

Technician in Chemistry at the Federal University of Rondônia - Presidente Médici Campus, Brazil. Completed a degree in Industrial Chemistry from the State University of Paraíba in (2011), a master's degree in Chemical Engineering from the Federal University of Campina Grande in (2013) and a PhD in Chemical Engineering from the Federal University of Campina Grande in 2013 and a PhD in Chemical Engineering from the Federal University of Campina Grande in (2017) with an emphasis on desalination, reuse of water for irrigation and physical-chemical analysis in water and plants.

Weder Carlos de Souza Tomicha

Graduating in Fishing Engineering, Federal University of Rondônia, Campus Presidente Médici -RO. improvement in Pisciculture field day by the Support Service for Micro and Small Companies of Rondônia (2019), improvement in Fish Farming Workshop by Animal Nutrition (2018), improvement in Food and Nutritional Sovereignty by the Federal University of Rondônia (2013).

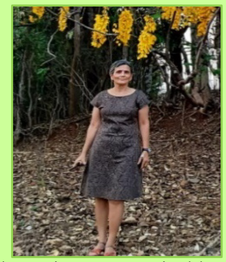
Ricardo Henrique Bastos de Souza

Graduated in Fishing Engineering from the Federal University of Recôncavo da Bahia (2010). Master in Animal Science from the State University of Santa Cruz (2013). He´s currently studying for a PhD in Animal Science at the Federal University of Mato Grosso do Sul (2019 - 2023). Worked as Fishery Resources Analyst at Fundação Instituto of Fisheries of the State of Rio de Janeiro (2012 - 2014). He is currently an assistant professor at the Federal University of Rondônia. He has experience in Fisheries Resources and Fisheries Engineering working in the area of nutrition of aquatic organisms.

Raul Dirceu Pazdiora

Associate Professor Level III at the Federal University of Rondônia- Department of Zootechnics, Presidente Médici Campus, Brazil. He has a degree in Veterinary Medicine from the Federal University of Santa Maria (2005), a Master's in Animal Science (Animal Production) from the same institution (2008) and a Doctorate in Animal Science (Animal Production) from the São Paulo State University. Júlio de Mesquita (UNESP), Jaboticabal campus (2011). He has experience in Veterinary Medicine and Zootechnics, with an emphasis on Animal Production-Beef and Dairy Cattle, working mainly on the following topics: feed evaluation, ruminant nutrition, genetic improvement and animal management.

Creuci Maria Caetano



Graduated in Biological Sciences (1981), with Master in Cellular Biology (1995) and PhD in Biological Sciences (2001) from the State University of Maringá (UEM), Paraná. Pedagogue (UEM; 1994), with a perfection in Science and Citizenship - Ethos Science of the State Faculty of Campo Mourao, Paraná (1996), Specialization in Control and Environmental Management (UEM, 1999) and International Postgraduate Course in Cultural Heritage, Sustainable Tourism and Cultural Landscape (Fondo Verde; 2017). Her research interest is Plant Cytogenetics, Applied Ethnobotany, Conservation and Use of Phytogenetic Resources (RFG). She has been linked with the International Institute of Phytogenetic Resources (IPGRI) (now Bioversity International), the National University of Colombia and the Federal University of Rondônia Foundation, in the Western Brazilian Amazon, Presidente Médici campus, Department of Fishery Engineering.

References

[1] Jennings S, Kaiser M, Reynolds J. Marine fisheries ecology. 1st ed. United Kingdom: Blackwell Science; 2001

[2] Miller G. Ciência Ambiental. 2nd ed. São Paulo, Brasil: Tradução. Thomson; 2007

[3] Pavanelli G, Takemoto R, Eiras J. Parasitologia de peixes de água doce do Brasil. 1st ed. Maringá, Brasil: EDUEM; 2013.

[4] Feitosa HN, Saturnino KC, Dirceu Pazdiora R, Ziemniczak HM, Paiva Medeiros S, Caetano Nunes Pazdiora BR. Histopathological analysis of *Colossoma macropomum* liver, treated with bioproducts used by controlling parasites. *Magna Scientia UCEVA* 2022;2:109–16. *https://doi.org/10.54502/msuceva.v2n1a11*

[5] Tavares Dias M, Ferreira J, Affonso E, Ono E, Martins M. Toxicity and effects of copper sulfate on parasitic control and hematological response of tambaqui *Colossoma macropomum*. Boletim Do Instituto de Pesca 2011;7:355–65. *https://www.pesca.agricultura.sp.gov.br/37_4_355-365.pdf*

[6] Maciel P, Affonso E, Onaka E. Infestação massiva por Neoechinorhynchus sp. (Acanthocephala: Neoechinorhynchidae) em tambaquis, *Colossoma macropomum*, jovens de piscicultura de Itacoatiara-AM, Brasil. X Encontro Brasileiro de Patologistas de Organismos Aquáticos, 2008, Búzios, Rio de Janeiro, Rio de Janeiro, Brasil: INPA; 2008. https://repositorio.inpa.gov.br/handle/1/25552

[7] Godoi MMI de M, Engracia V, Lizama M de LAP, Takemoto RM. Parasite-host relationship between the tambaqui (*Colossoma macropomum* Cuvier 1818) and ectoparasites, collected from fish farms in the city of Rolim de Moura, State of Rondônia, Western Amazon, Brazil. *Acta Amazon* 2012;42:515–24. *https://doi.org/10.1590/S0044-59672012000400009*

[8] Boijink C, Inoue L, Chagas E, Chaves F. Boas práticas de manejo na piscicultura para conservação da qualidade ambiental: Uso de produtos naturais como anti-helmíntico em tambaqui. Manaus, AM: 2011. *https://ainfo.cnptia.embrapa.br/digital/bitstream/item/48954/1/Doc-88Cheila-A5.pdf.*

[9] Rodrigues B R, Meurer F, Rogério Boscolo W. Aditivos en la nutrición de peces. *Revista Colombiana de Ciencia Animal-RECIA* 2015;7:228. *https://doi.org/10.24188/recia.v7.n2.2015.286*

[10] Matos F. Introdução à fitoquímica experimental. 1st ed. Fortaleza, Brasil: Edições UFC; 2007.

[11] Nascimento J, Melo A, Lima E, Silva T, Veras Filho J, Santos E, et al. Estudo fitoquímico e bioensaio toxicológico frente a larvas de *Artemia salina* Leach. de três espécies medicinais do gênero Phyllanthus (Phyllanthaceae) . *Rev Ciênc Farm Básica Apl* 2008;29:145–50. *https://rcfba.fcfar.unesp.br/index.php/ojs/article/view/481/479*.

[12] Silva N, Fernandes Júnior A. Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of Venomous Animals and Toxins Including Tropical Diseases* 2010;16:402–13. *https://doi.org/10.1590/S1678-91992010000300006*

[13] Barros JD, Filho SS, Castro V, Torres VM, Higino JS, Monteiro de Melo AF. Estudo toxicológico pré-clínico agudo e determinação da CL50 do extrato bruto seco da vitex agnus castus Linn. *Revista Eletrônica de Farmácia* 2011;7. *https://doi.org/10.5216/ref.v7i3.9893*

[14] Bories C, Loiseau P, Cortes D, Myint S, Hocquemiller R, Gayral P, et al. Antiparasitic Activity of *Annona muricata* and *Annona cherimolia* Seeds. *Planta Med* 1991;57:434–6. *https://doi.org/10.1055/s-2006-960143*

[15] Moghadamtousi S, Fadaeinasab M, Nikzad S, Mohan G, Ali H, Kadir H. *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *Int J Mol Sci* 2015;16:15625–58. *https://doi.org/10.3390/ijms160715625*

[16] Embrapa Amazonia Occidental. Anais da IX Jornada de Iniciação Cientifica da Amazônia Ocidental.Documentos 100. Manaus, AM: 2012. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/950196/1/IXJornadaIC.pdf.

[17] Jorge LIF, Ferro V de O, Koschtschak MRW. Diagnose comparativa das espécies *Chenopodium ambrosioides* L. (erva-de-santa-maria) e *Coronopus didymus* (L.)Sm (mastruço): principais características morfo-histológicas e químicas. *Revista Brasileira de Farmacognosia* 1986;1:143–53. *https://doi.org/10.1590/S0102-695X1986000200005*

[18] Tapondjou LA, Adler C, Bouda H, Fontem DA. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *J Stored Prod Res* 2002;38:395–402. *https://doi.org/10.1016/S0022-474X(01)00044-3*

[19] Ketzis JK, Taylor A, Bowman DD, Brown DL, Warnick LD, Erb HN. *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Ruminant Research* 2002;44:193–200. *https://doi.org/10.1016/S0921-4488(02)00047-0*

[20] Williams AR, Ropiak HM, Fryganas C, Desrues O, Mueller-Harvey I, Thamsborg SM. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. Parasit *Vectors* 2014;7:518. https://doi.org/10.1186/s13071-014-0518-2

[21] Dugani A, Dakhil B, Treesh S. Protective effect of the methanolic extract of *Malva parviflora* l. leaves on acetic acid-induced ulcerative colitis in rats. *Saudi Journal of Gastroenterology* 2016;22:225. *https://doi.org/10.4103/1319-3767.182459*