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SHORT COMMUNICATION

Anthelminthic potential of the *Ficus insipida* latex on monogeneans of *Colossoma macropomum* (Serrasalmidae), a medicinal plant from the Amazon

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Abstract

Introduction Herbal therapy is a potentially beneficial alternative for fish aquaculture, since it may be cheaper and more effective than chemotherapy. The aim of this study was to investigate the in vitro efficacy of *Ficus insipida* latex on monogeneans of *Colossoma macropomum* gills.

Materials and methods To evaluate the anthelmintic activity, four concentrations of *F. insipida* latex (250, 500, 750 and 1000 µL/L) and exposure time to cause immobilization on monogeneans were used. In addition, two control groups, being one with water from the breeding tank and the other with breeding water from the tank + 70% ethyl alcohol were used.

Results At the concentration of 250 µL/L of *F. insipida* latex, the immobilization of the monogeneans occurred after 4 h of exposure, while at the concentration of 500 µL/L, it occurred after 2 h. At the concentrations of 750 and 1000 µL/L of *F. insipida* latex, the immobilization of the monogeneans occurred after 1 h and 30 min, respectively. After exposure to 250 µL/L of *F. insipida latex*, 100% of immobilization of monogeneans was observed within 4 h, to 500 and 750 µL/L, 100% immobilization occurred within 4 h and to 1000 µL/L, 100% mortality occurred after 2 h.

Conclusions Thus, we recommended the use of 1000 µL/L of *F. insipida* latex for therapeutic baths in of *C. macropomum* against monogeneans, after previous test of toxicity.

Keywords Fish farming · Freshwater fish · Parasites · Treatment

Herbal therapy has been used by mankind for thousands of years. Even today it is used by almost 80% of the human populations in developing countries that is dependent on medicinal plants for healthcare. Among the medicinal plants, recent investigations have indicated that *Ficus* species (common fig tree) are cultivated for over 11,000 years, possibly before cereal grains [1]. *Ficus insipida* Willd, 1806 (Moraceae) is a medicinal plant with wide distribution in the Neotropical region, being found in Mexico, Ecuador, Colombia, Venezuela, Peru, Paraguay, Bolivia, and Brazil. Different parts of this medicinal plant has been used (e.g., leaves, fruits, roots, and latex) for treatment of diseases. It has ethnobotanical importance due to its use by indigenous populations from the Amazon to treat intestinal helminthiasis [2–4]. Phytotherapy proves to be a good alternative for the treatment of many diseases, especially due to low cost [2].

Ficus species have a latex within their vascular system, providing protection and self-healing against physical aggression in the environment. Latex consists of a cytoplasmatic fluid of lactiferous tissues that contains the usual organelles of plant cells, such as nuclei, mitochondria, vacuoles and ribosomes, among others. The latex of *Ficus* spp. is an aqueous suspension of a complex mixture of molecules found in specialized secretory cells of the plants, known as laticifers [1, 5], which synthesize and store diverse metabolites in considerable amounts, namely, terpenoids, alkaloids, tannins, sterols and proteins (Table 1). Hence, it has been demonstrated anthelmintic activity of the *F. insipida* latex against helminths *Ascaris lumbricoides, Ancylostoma duodenale*, *Necator americanus, Trichuris trichiura*, and

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Table 1 Chemical composition of the latex of *Ficus* species

Species	Compound class	Compound name	References
Ficus carica	Aldehydes	Pentanal, hexanal, heptanal, benzaldehyde and, octanal	[5]
	Alcohols	1-Butanol-3-methyl, 1-butanol-2-methyl, 1-pentanol, 1-hexanol, phenylethyl alcohol, and phenylpropyl alcohol	
	Ketones	6-Methyl-5-hepten-2-one	
	Monoterpenes	α-Thujene, α-pinene, β-pinene, limonene, eucalyptol, terpinolene, cis-linalool oxide, linalool, and epoxylinalol	
	Sesquiterpenes	α-Guaiene, α-bourbonene, β-caryophyllene, trans-α-bergamotene, α-caryophyllene, τ-muurolene, germacrene D, cardinene, and α-calacorene	
	Miscellaneous	Methyl salicylate, quinolone and psoralen	
	Organic acids	Oxalic, citric, malic, quinic, shikimic and fumaric	
Ficus carica	Coumarins	Bergapten, 40, 50-dihydropsoralen, marmesin, umbelliferone, and psoralen	[1]
	Flavonoids and anthocyanins	Rutin, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rhamnoglucoside	
	Triterpenoids, sterols and acyl steryl glucosides	Baurenol, lupeol, 24-methylenecycloartanol, w-taraxasterol ester, b-sitosterol, 6-*O*-linoleyl-b-D-glucosyl-b-sitosterol, 6-*O*-oleyl-b-D-glucosyl-b-sitosterol, 6-*O*-palmitoyl-b-D-glucosyl-b-sitosterol, 6-*O*-stearyl-b-D-glucosyl-b-sitosterol, betulol, lanosterol, lupeol acetate, a-amyrin, and b-amyrin	
Ficus carica	Phytosterol	Betulol, lupeol, lanosterol, lupeol acetate, β-Amyrin, β-sitosterol and α-amyrin	[20]
	Amino acids	Leucine, tryptophan, phenylalanine, lysine, and histidine, asparagine, alanine, glutamine, serine, glycine, ornithine, tyrosine, cysteine, tryptophan, cysteine and tyrosine	
	Fatty acids	Myristic, pentadecylic, palmitic, margaric, cis-10-heptadecenoic, stearic, oleic, elaidic, linoleic, arachidic, heneicosylic, behenic, tricosylic, and lignoceric	
	Volatile compounds	–	
Ficus insipida	Amino acids	Aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, 1-2-cysteines, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, tryptophan and ammonia	[21]
Ficus insipida	Proteases	Ficins	[19]
Ficus sur	Triterpene	3-Acetoxy-α-amyrin and 3-acetoxy-β-amyrin	[22]
Ficus carica	Phenolic and flavonoids	–	[23]
Ficus benghalensis	Serine protease	Benghalensin	[24]

Strongyloides stercoralis in human [3], and O*xyurus syphacia obvelata* and *Aspiculuris tetraptera* in rats [2]. However, no study has investigated the anthelminthic potential of *Ficus* latex in fish parasites, although the phytotherapy also has proved to be a good alternative for the treatment of diseases in fish farming, mainly due to the efficacy of many medicinal plants [6]. We need, therefore, to search for new natural drugs yielding pharmacologically active principles to use as an alternative to conventional chemotherapies that are commonly used in fish farming of *Colossoma macropomum* Cuvier, 1818 (tambaqui). Development of anthelmintic resistance in helminths to conventional products gives a clear indication that control programs based exclusively on their use are, therefore, not sustainable.

Colossoma macropomum, one of the largest fishes with scales from the Amazon basin, has frequently problems with monogenean infections, including *Anacanthorus spathulatus*, *Notozothecium janahuachensis*, *Mimarotecium boegueri* and *Linguadactyloides brikmanni* [7–10], ectoparasites with direct life cycle, whose transmission can be favored by intensive culture [10, 11]. These parasites are a problem frequent in the intensive fish farming due to practices used that make fish susceptible to diseases outbreaks in a result of high density of stocking and low water quality, leading to a decrease in animal welfare [7, 8, 10, 11]. Hence, monogeneans can cause great economic losses in farmed fish due to mortalities in fish farming [10–12]. However, the number of antiparasitic drugs that are effective against monogeneans consistently and safely, and that can be used in a wide variety of environmental conditions is very limited. Therefore, in fish farming, managing and controlling infections by monogeneans pose a constant challenge, since these tasks are greatly complicated by the limited availability of licensed anthelmintic drugs with varying degrees of effectiveness [9–11]. It is, therefore, important to know the anthelmintic potential of medicinal plants such as *F. insipida*, since this plant has bioactive compounds, is biodegradable, has low cost and has a wide availability in the Amazon region. Thus, the present study investigated the in vitro efficacy of *F. insipida* latex in monogeneans of *C. macropomum* gills.

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Latex of *F. insipida* was obtained through incisions around the stem of the tree [13], in July 2018 in Macapá, State of Amapá, Brazil (0°1′44.53″N–51°4′5.77″0). Exsiccates of *F. insipida* were deposited in the Amapaense Herbarium (Voucher: Gonzales 001-HAMAB) of the Institute of Scientific and Technological Research of the State of Amapá, Brazil (IEPA), in Macapá, State of Amapá, Brazil. Samples of *F. insipida* latex were collected in a dark-colored sterile hermetic container containing ethyl alcohol (70%) in a ratio of 10:1 (V:V) and refrigerated at 4° C to avoid oxidation of the components and losses of enzyme activity [14, 15], and used within 5 days.

To evaluate the anthelmintic activity of *F. insipida* latex concentrations and exposure time to cause immobilization in monogeneans of *C. macropomum* (102.3 ± 27.1 g and 18.1 ± 2.1 cm), in vitro trials were carried out. Gills of 15 fish naturally parasitized by monogeneans were used. Fish were euthanized by spinal cord section and the gills then used for exposure to *F. insipida* latex in Petri dishes (5.5 cm). The treatments used in three replicates were: two control groups, one with water from the breeding tank and the other with breeding water from the tank + 70% ethyl alcohol, and four concentrations of *F. insipida* latex (250, 500, 750 and 1000 μL/L). All these concentrations of *F. insipida* latex were diluted in water from the breeding tank to a volume of 5 mL using Petri dishes. Under a stereomicroscope, fields of view containing ± 20 monogeneans were selected in each repetition, and after submerging the branchial arches in the different concentrations of anthelmintic solutions, the parasites were observed under a microscope at 15-min intervals to count the number of live and dead monogeneans. The parasites were considered dead when they were detached from the gill tissue or when they were attached to the gill tissue, but had completely lost their mobility [7]. The efficacy of each treatment was estimated as proposed by Zhang et al. [16]. We recorded the time it took to kill 100% of the monogeneans and hypothesized that a treatment was effective if 100% parasite mortality was achieved within 2 h.

Continuous monitoring is required for diagnosing and controlling parasitic infections within *C. macropomum* aquaculture. The main substances that have been used for treatment and parasite control, even they have not been satisfactorily effective, are chemical products that are highly toxic to fish, humans, and environment [7–10]. Use of chemotherapeutics needs to be reduced and replaced by other effective measures for diminishing disease outbreaks [6], Thus, recently, the use of medicinal plants or their bioactive compounds and nanoproduct of medicinal plants, for controlling diseases that compromise the production and productivity of *C. macropomum*, has been increasing. Herbal therapy is a potentially beneficial alternative for *C. macropomum* aquaculture, since it may be cheaper and effective [6–9].

We identify *A. spathulatus, N. janahuachensis, M. boegueri* and *L. brikmanni* in gills of *C. macropomum* of this study. In both control groups (water of tank and water of tank + ethyl alcohol), the immobilization of the monogeneans occurred only after 4 h and 30 min of exposure, respectively. At the concentration of 250 μL/L of *F. insipida* latex, the immobilization of the parasites occurred after 4 h of exposure, while at the concentration of 500 μL/L, it occurred after 2 h of exposure. In concentrations of 750 and 1000 μL/L of *F. insipida* latex, the immobilization of the parasites occurred after 1 h and 30 min, respectively (Table 2). After exposure to 250 μL/L of *F. insipida latex*,

Table 2 In vitro efficacy of the concentrations of *Ficus insipida* latex in monogeneans of *Colossoma macropomum* in different exposure times

Exposure time	Treatments	Live parasites	Mortality (%)
0 h	Water of tank	26.3 ± 9.3	0
30 min	Water of tank	26.3 ± 9.3	0
1 h	Water of tank	26.3 ± 9.3	0
2 h	Water of tank	26.3 ± 9.3	0
4 h	Water of tank	24.0 ± 5.3	8.7
6 h	Water of tank	6.7 ± 2.9	74.5
7 h	Water of tank	0	100
0 h	Ethyl alcohol	28 ± 2.9	0
30 min	Ethyl alcohol	27.6 ± 3.0	1.4
1 h	Ethyl alcohol	26.6 ± 2.3	5.0
2 h	Ethyl alcohol	24.6 ± 3.6	12.1
4 h	Ethyl alcohol	21 ± 2.9	25.0
6 h	Ethyl alcohol	6.6 ± 2.3	76.4
7 h	Ethyl alcohol	0	100
0 h	250 μL/L	23 ± 2.1	0
30 min	250 μL/L	23 ± 2.1	0
1 h	250 μL/L	23 ± 2.1	0
2 h	250 μL/L	23 ± 2.1	0
4 h	250 μL/L	6 ± 0.8	73.1
5 h	250 μL/L	0	100
0 h	500 μL/L	23.3 ± 4.7	0
30 min	500 μL/L	23.3 ± 4.7	0
1 h	500 μL/L	23.3 ± 4.7	0
2 h	500 μL/L	17.6 ± 3.0	24.4
4 h	500 μL/L	0	100
0 h	750 μL/L	21.3 ± 1.2	0
30 min	750 μL/L	21.3 ± 1.3	0
1 h	750 μL/L	16.3 ± 2.3	61.3
2 h	750 μL/L	9 ± 3.7	57.7
4 h	750 μL/L	0	100
0 h	1000 μL/L	27.6 ± 4.0	0
30 min	1000 μL/L	20.6 ± 4.4	25.3
1 h	1000 μL/L	13.3 ± 4.4	51.8
2 h	1000 μL/L	0	100

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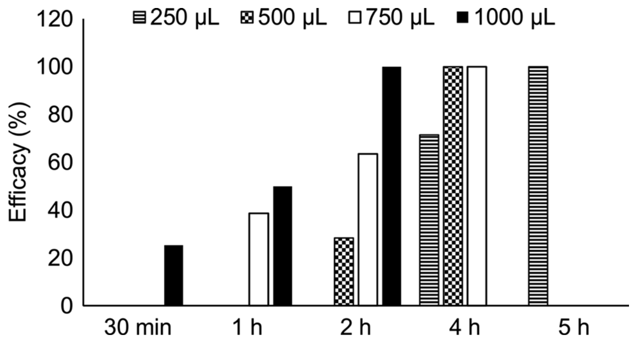

Fig. 1 In vitro efficacy of *Ficus insipida* latex in monogeneans of *Colossoma macropomum* in different exposure times

100% of mortality of monogeneans was observed within 4 h. At concentrations 500 and 750 µL/L, 100% mortality of the parasites occurred within 4 h of exposure, while at 1000 µL/L, this occurred after 2 h of exposure (Fig. 1). Anthelmintic efficacy of the *F. insipida* latex, varying from 26 to 68%, has been reported also for helminths of human [3], as well as for helminths of rats, which varied from 8.4 to 38.6%, due to the proteolytic activity of the latex [2].

Anthelmintic activity of latex from other *Ficus* species was also studied. *Ficus carica* latex when used in concentration of 3 mL/kg/day, during three consecutive days, was effective against *Aspiculuris tetraptera* (41.7%), but this had no efficacy in eliminating *Aspiculuris tetraptera* (2.6%) and *Vampirolepis nana* (8.3%) of rats [2]. Comparative studies of in vitro activity of *Ficus religiosa*, *Ficus elastica* and *Ficus benghalensis* (250 and 500 µL) showed that *F. benghalensis* latex took longer time to cause immobilization and mortality of *Pheretima posthuma*, a worm of soil [17], indicating, therefore, differences among species. Wanderley et al. [18] studied the in vitro proteolytic activity of *Ficus benjamina* latex and observed inhibition of larval development of *Haemonchus contortus*. Such anthelmintic activities of these *Ficus* species are attributed to different bioactive compounds present in the latex (Table 1). Proteolytic enzymes such as fucins are one of the compounds responsible for the anthelmintic activity of *Ficus* species [2–4, 19], because such enzymes may cause destruction of the cuticle, changes in muscle cells and swelling of the epithelial cells of helminths [2, 3, 18]. In addition, anthelmintic effects of tannins also have been attributed to latex of *Ficus* spp., because these compounds have the capacity to bind free protein available in the digestory tube for larval nutrition of helminths, reducing nutrient availability, which result in starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation leading to death [17].

To conclude, as 1000 µL/L of *F. insipida* latex showed in vitro efficacy against monogeneans of *C. macropomum*, thus this concentration should be assayed in therapeutic baths for this fish, but after previous test of toxicity. Furthermore, it should be investigated the agronomic sustainability of such concentration of the *F. ficus* latex for use in fish farming.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical disclosures This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA), and authorization from Ethics Committee in the Use of Animal of the Embrapa Amapá (#013/2018) was carried out.

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