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# Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia

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ABSTRACT

This study evaluated the use of therapeutic baths containing essential oils of *Lippia sidoides* (pepper rosemary) and *Mentha piperita* (peppermint) on the hematological parameters of Nile tilapia parasitized by the monogeneans *Cichlidogyrus tilapiae*, *Cichlidogyrus thurstonae*, *Cichlidogyrus halli*, and *Scutogyrus longicornis*. A total of 320 juvenile fish were distributed into 16 tanks of capacity 100 L (20 fish per tank), divided into 4 treatments in quadruplicates: fish exposed to a bath of *L. sidoides* at 20 mg $L^{-1}$; fish exposed to *M. piperita* at 40 mg $L^{-1}$; fish exposed only to a water bath; and fish exposed to water + DMSO (dimethyl sulfoxide) bath. The fish were subjected to 3 baths for 10 min, at intervals of 24 h between treatments. After the third bath, parasitological and hematological analyses were performed. The parasite prevalence in fish treated with essential oils was seen to have decreased by 70%. The efficacy attained among fish treated with *L. sidoides*, in comparison with control water and water + DMSO, was 1.96% and 14.16%, respectively; and among fish treated with *M. piperita*, it was 33.33% and 41.63%, respectively. The total numbers of red blood cells (RBC) and thrombocytes were lower in fish treated with *L. sidoides*. Glucose concentration and neutrophil count were significantly higher in fish treated with *L. sidoides*. Because of the efficacy and positive hematological results, we suggest that baths of *M. piperita* at 40 mg $L^{-1}$ should be used as anthelmintic action.

*Statement of relevance:* Authors believe on the use of essential oils to treat ectoparasites of cultured fish and consequently no damages for hematological profile of Nile tilapia were found.



## 1. Introduction

In aquaculture, phytotherapeutics for use among farmed aquatic animals may present several advantages, such as reduced environmental impact, biodegradability, lower residue levels in animals, low toxicity, and several modes of action resulting in low likelihood of causing resistance and low cost for farmers (Soares and Tavares-Dias, 2013; Chagas et al., 2014). A recent review has shown that the use of plant extracts in aquaculture has been responsible for increased immune response and hematological and biochemical improvements (Bulfon et al., 2015).

*Lippia sidoides*, commonly known as rosemary pepper, is a type of bush found in northeastern Brazil that presents antiseptic and antimicrobial properties (Costa et al., 2002). Species of *Lippia* have been exploited in several fields such as veterinary medicine, microbiology, parasitology, zootechny, and aquaculture because of their bioactive potential and ease of use on a large scale (Soares and Tavares-Dias, 2013).

The genus *Mentha*, known as peppermint, has been exploited for its flavor and is used in medicine as an antimicrobial and antioxidant agent (Tsai et al., 2013). It produces essential oil containing menthol and several components used in the pharmaceutical and natural product industry (Kumar and Patra, 2012). Menthol is the main component after oil extraction (Freire et al., 2011; Tsai et al., 2013).

A study by Moghaddam et al. (2013) showed that *Mentha piperita* had antifungal activity against three species of fungi: *Dreschlera spicifera*, *Fusarium oxysporum* f.sp. *ciceris* and *Macrophomina phaseolina*. Similar data were obtained by Freire et al. (2011), who demonstrated the inhibitory potential of this essential oil against the fungi *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Colletotrichum gloesporioides*, *Colletotrichum musae*, *Fusarium oxysporum*, and *Fusarium semitectum*.

Compared with the use of plant extracts for immunostimulant effects, they have been little used against parasites, especially against monogeneans (Bulfon et al., 2015). The anthelmintic activity of oils or plant extracts has been evaluated mainly against monogenean parasites

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of goldfish (*Carassius auratus*) (Steverding et al., 2005; Wang et al., 2009; Wang et al., 2010a, 2010b, 2011).

The efficacy of seed extracts from *Piper guineense* against the monogenean parasites *Gyrodactylus* and *Dactylogyrus* of goldfish has been studied *in vitro* and *in vivo* (Ekanem et al., 2004). Monogenean helminths are one of the most important parasites affecting fish farming and are found mainly in the gills and skin (Jerônimo et al., 2011).

Several natural substances can be used in aquaculture, but studies involving the use of extracts or essential oils in therapeutic baths against fish parasites are scarce (Reverter et al., 2014). The present study evaluated therapeutic baths using *L. sidoides* and *M. piperita* against monogenean gill parasites in Nile tilapia (*Oreochromis niloticus*), *in vitro* and *in vivo*.

## 2. Material and methods

### 2.1. Essential oil extraction

Essential oils used in this study were obtained from the leaves of *L. sidoides* and *M. piperita* cultured in the Section of Medicinal Plants of EMBRAPA Western Amazon situated in Manaus, AM (03°06′23.04″S and 60°01′35.14″W). Mean altitude is 50 m and mean air temperature is 25.6 °C with annual rainfall of 2200 mm. Plants were collected in the morning and the material processed in the Medicinal Plants and Phytochemistry Laboratory of Embrapa Western Amazon, Manaus, Brazil. Oil extraction was performed by the hydrodistillation method using a Clevenger-like equipment. After that, the oils were maintained refrigerated at −18 °C in dark glasses. Briefly, for chemical composition analysis a gas chromatograph Agilent (Palo Alto, USA) 7890A equipped with capillary column HP-5 (5%-diphenyl-95%-dimethyl silicon 30 m × 0.32 mm × 0.25 μm) was used. Temperature was programmed in 60 to 240 °C, a 3 °C $min^{-1}$, and using hydrogen as carrier gas (1.5 mL $min^{-1}$). 1.0 μL of 1% essential oil solution in dichloromethane (Merck Millipore, Darmstadt, Germany) with flux division (1:100, injector at 250 °C) was injected. The mass spectrum was obtained in a system Agilent 5973 N operated in the mode electron impact (EIMS) at 70 eV, coupled in a chromatograph Agilent 6890 using the same procedure of injection and temperature cited above. Retention indices were calculated from the retention times of the compounds and those of a serial of n-alkanes ($C_7$–$C_{26}$). Constituent identification was made by comparison of the mass spectrum obtained with the data of spectral library (Wiley 6th Ed.) and by the indices of the retention calculated and compared to published values (Adams, 2007).

### 2.2. In vitro immobilization assay

The monogenean parasites *Cichlidogyrus tilapiae* Paperna, 1960, *Cichlidogyrus thurstonae* Ergens, 1981, *Cichlidogyrus halli* Price and Kirk, 1976 and *Scutogyrus longicornis* Paperna and Thurston, 1969 were used in this assay and identified according to Douëllou (1993), Pariselle and Euzet (1995), Pariselle et al. (2003), and Thatcher (2006). The parasites were collected directly from the gills of parasitized tilapia, immediately prior to beginning assay.

Six concentrations of *L. sidoides* and *M. piperita* essential oils were used to obtain the most efficient in causing the parasite mortality. A stock solution was composed by 1 g of essential oils diluted in 9 mL of dimethyl sulfoxide (DMSO — $C_2H_6OS$) in a proportion of 1:10.

Parasitized gill filaments of Nile tilapia were separated in sterile Petri dishes 5.5 cm comprising in each concentration with three replicates. From the stock solution, essential oils were diluted in 25 mL water to obtain 320, 160, 80, and 40 mg $L^{-1}$. For the lowest concentrations of 20 mg $L^{-1}$ and 10 mg $L^{-1}$, 10 μL in 50 mL and 100 mL water divided in 8 mL in each Petri dish was diluted. Two controls were used, one of them diluting 80 μL DMSO in 25 mL water and the other only water.

For the lowest concentrations the parasites were observed each 15 min and for the highest concentrations (160 and 320 mg $L^{-1}$) the observation was continuous. Parasites were considered dead when detected the absence of movement when stimulated with a needle or body wrinkling.

### 2.3. Toxicity test

The toxicity test aimed to evaluate the tolerance of the fish to exposure to the oil. For each concentration, three fish were used in 3 L of water and the oil solution was added in order to observe their behavior. Water quality was measured before and after the treatment. In situations of abnormal behavior such as agitation, anoxia, intense swimming or tipping over, the fish was immediately transferred to another bucket and the time was registered. Fish handling and samples collection were approved by Ethic Committee of Federal University of Santa Catarina (CEUA/UFSC PP00869).

### 2.4. In vivo assay

A total of 320 healthy juvenile Nile tilapia (weight of 9.76 ± 0.48 g and length of 8.47 ± 0.18 cm) from the same spawning were acquired from tilapia fish farmer and acclimatized for 7 days prior to distribution into 16 circular tanks of capacity 100 L.

There were 4 treatments and 4 replicates: fish exposed to *L. sidoides* bath at 20 mg $L^{-1}$; fish exposed to *M. piperita* bath at 40 mg $L^{-1}$; fish exposed to water; and fish exposed to water + dimethyl sulfoxide (DMSO).

During the assay, 50% of the water was renewed and the fish were fed three times a day with commercial dry ration for omnivorous fish, containing 28% crude protein. The water quality parameters did not alter among the treatments: temperature 27.67 ± 0.99 °C, dissolved oxygen 6.83 ± 0.92 mg $L^{-1}$, pH 5.86 ± 0.65 measured using a multiparameter portable Hanna HI9829® (Hanna Instruments Brazil, SP), and ammonia 3.00 ± 1.01 mg $L^{-1}$, nitrite 0.04 ± 0.01 mg $L^{-1}$ and nitrate 0.70 ± 0.22 mg $L^{-1}$ measured by colorimetric kit Alfakit® (Alfakit, SC, Brazil).

Each treatment consisted of three baths of 10 min at intervals of 24 h. The therapeutic solution was distributed at the edge of the tank: 14 mL of stock solution of *L. sidoides*; 28 mL of *M. piperita* solution; water alone; and water + 224 mL of DMSO. After the third bath, 10 fish from each replicate were collected for parasitological and hematological analysis.

### 2.5. Parasitological and hematological analyses

Fish were anesthetized in clove oil solution (75 mg.$L^{-1}$), euthanized and the gills of 5 fish per replicate were collected for immediate parasitological analysis and the other 5 bathed in water 60 °C and fixed in alcohol 70% for posterior parasite counting. Monogenean quantification followed the method of Jerônimo et al. (2011).

The efficacy was calculated according to the formula: EF = MNPC − MNPT × 100 / MNPC (EF: efficacy, MNPC: mean number of parasites in control fish, MNPT: mean number of parasites in treated fish) (Dotta et al., 2015). Prevalence, mean intensity, and mean abundance of parasites were calculated according to Bush et al. (1997).

After fish were anesthetize the blood was withdrawn from the caudal vein with syringes containing a drop of EDTA 10% and used for blood smears stained with May Grunwald/Giemsa/Wright, hematocrit percentage, hemoglobin rate and calculated the hematimetric parameters: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (CHCM) (Ranzani-Paiva et al., 2013). For total erythrocyte count (RBC) 5 μL of the blood was diluted in 1 mL of fixative Dacie solution for posterior counting (Blaxhall and Daisley, 1973). Total number of thrombocytes and leukocytes (WBC) were calculated by the indirect method (Ranzani-Paiva et al., 2013). After hematocrit determination the capillary was broken at the level of white blood cells and the plasma