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ANIMAL SCIENCES

Sedative and anesthetic efficacy of the essential oils from the Brazilian native plants *Pilocarpus pennatifolius* and *Cordia verbenacea* in Nile Tilapia

CARLOS HERMINIO M. FORTES, BERNARDO BALDISSEROTTO, FREDERICO D. FLEIG & BERTA MARIA HEIZNMANN

Abstract: This study aimed to evaluate the effectiveness of essential oils (EO) of *Pilocarpus pennatifolius* (PPOL) and *Cordia verbenacea* (CVOL) as sedatives and/or anesthetics in juvenile Nile tilapia (*Oreochromis niloticus*). The main constituents of PPOL were 2-undecanone (57.2%) and 2-Tridecanone (28.3%), and of CVOL α-pinene (34.8%), which were determined by gas chromatography. PPOL led juveniles to sedation at concentrations of 2 (703.8s), 10 (407.4s), 20 (225.5s), 50 (190.2s), and 80 (163.5s) mg L^{-1}. CVOL also induced sedation at these concentrations, with times of 504.9, 294.2, 142.8, 113.8, and 135.6 s, respectively. Both EOs at 200 mg L^{-1} induced deep anesthesia within 507.5 (PPOL) and 1267.7 s (CVOL). Exposure to PPOL at 2, 20, and 80 mg L^{-1} for 48 h resulted in fish sedation, while CVOL lead fish to sedation at the two lowest concentrations and anesthesia at the highest one. Possible stress responses were evaluated during anesthetic recovery and long exposure,-and PPOL did not cause behavioral or glucose levels changes in all evaluations. However, CVOL increased glucose levels-at the highest concentrations studied (80 and 200 mg L^{-1}), indicating that this oil may have caused stress in fish.

Key words: glucose, stress, *Oreochromis niloticus*, 2-undecanone, α-pinene.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus*, is cultivated worldwide in tropical and sub-tropical areas (9% of all world fish production in 2018), with Brazil being one of the main producers (FAO 2022). This species accounted for 64.6% of Brazilian fish production (IBGE 2021). The common aquaculture practices related to intensive fish production induce stress that can affect fish growth, reproduction, and general physiological status (Brijs et al. 2018, Jerez-Cepa & Ruiz-Jarabo 2021, Shinde & Ganesh 2022). Therefore, it might be necessary in some situations to use sedatives and/or anesthetics in basic production procedures, such as transport and biometrics, to minimize impacts on the well-being and quality of life of fish (Javahery et al. 2012, Souza et al. 2019). Anesthetics of synthetic origin, such as MS-222 (tricaine methanesulfonate), benzocaine and others can lead to hypoxia, loss of mucus, tissue irritation, and other undesirable adverse effects (Barbas et al. 2017, Zahl et al. 2012, Sneddon 2012). Consequently, many studies focused on anesthetics of natural origin, more precisely essential oils (EOs), which originated from the secondary metabolism of plants (Souza et al. 2019). These mixtures of plant constituents have low rates of poisoning for fish and humans (Aydin & Barbas 2020), are biodegradable (Boaventura et al. 2020, Figueiredo et al. 2008), have often

good availability, low cost, and are easy to use (Akbulut et al. 2010, Figueiredo et al. 2008). Some of them are considered ideal anesthetics, as they quickly immobilize the fish and provide an uneventful recovery (Aydin & Barbas 2020). The investigation of new herbal anesthetics for the aquaculture industry have become increasingly important, as they minimize suffering, distress, damage, and pain in fish (Bodur et al. 2018). In addition, they can concomitantly bring other benefits to fish health, as they can strengthen the immune system (Bandeira et al. 2017, Santos et al. 2020), act as antimicrobial (Yostawonkul et al. 2019, Abdelkhalek et al. 2020), and antioxidant (Cantanhêde et al. 2021, Silva et al. 2021).

Brazilian biodiversity has many riches yet to be established, especially because Brazil has the largest equatorial and humid tropical forest in the world (Pinto et al. 2002). Furthermore, of the almost 32.000 species of angiosperms described worldwide, 19.000 (57%) are native to Brazilian territory (Simões et al. 2017). Thus, many plant families stand out for having species that grow in Brazilian territory and present interesting pharmacological properties to be studied. For example, *Pilocarpus pennatifolius* (Rutaceae), which is native to South America (Brazil, Argentina, Uruguay, and Paraguay) (Do Carmo et al. 2018) and is it popularly known in Brazil as jaborandi, white agouti, agouti cinnamon (Souza et al. 2003, Santos & Moreno 2004). Plants of this family have several classes of known secondary metabolites (Groppo et al. 2008) with antimicrobial potential (Kuete et al. 2008, Lv et al. 2015, Venugopala et al. 2013), antioxidant (Islam et al. 2014, Kassim et al. 2013), anti-inflammatory (Cho et al. 2012, Asgarpanah & Khoshkam 2012, Silva et al 2013a), and antitumoral activities (Bisi et al. 2017).

Cordia verbenacea (Boraginaceae) is also native to South America (Bayeux et al. 2002) found along the entire Brazilian coast (Dutra et al. 2016), within the Atlantic Forest and in the low-lying areas of the Amazon (Melo et al. 2021, Faria et al. 2023) to southern Brazil (Bayeux et al. 2002). It is popularly known as "erva-baleeira" (Michielin et al. 2009, Faria et al. 2023), "erva-da-praia", and "maria-miraculosa" (Dutra et al. 2016). This plant species has analgesic (Vettorello et al. 2021), anti-inflammatory (Passos et al. 2007, Martim et al. 2021), antimicrobial (Meccia et al. 2009, Martim et al. 2021), and antiallergic activities (Passos et al. 2007), among others (Matias et al. 2013, Vázquez-Sánchez et al. 2018, Melo et al. 2021, Pereira et al. 2021). It was one of the first herbal medicines from Brazilian flora approved for use in humans (Calixto 2019).

According to Barbas et al. (2021), there is a need to develop new anesthetics based on EOs for use in aquaculture, especially because they are very close to what is expected of an ideal anesthetic for the fish production. They main desired characteristics are a good availability, safety for the environment, fish, and for the handler (ease of use). Despite the known pharmacological and biological activities of *P. pennatifolius* and *C. verbenacea*, there are still no studies related to the sedative and anesthetic potential of their EOs in *O. niloticus*. Thus, this study sought to evaluate the sedative and/or anesthetic potential and possible stressful effects of both EOs on this fish species.

MATERIALS AND METHODS

Collect of the plants, extraction and analysis of the chemical composition of the essential oils

Fresh leaves of *Pilocarpus pennatifolius* were collected in the city of Santa Maria (S 29° 11' 52"; W 53° 16.8' 56"), Rio Grande do Sul state, southern Brazil. The identification of the species was carried out by Dr. Frederico Dimas Fleig, Department of Forestry Sciences at Universidade Federal de Santa Maria (UFSM) and voucher

material was deposited in the herbarium of the Department of Biology at UFSM, SMDB n° 23007. The EO of the leaves of *Cordia verbenacea* was purchased from Laszlo Aromatologia Eireli (Brazil).

The EO extraction from fresh leaves of *P. pennatifolius* was carried out by hydrodistillation for three hours, in triplicate, in a modified Clevenger apparatus (Farmacopeia Brasileira 1988, European Pharmacopoeia 2007). After obtaining the EO, it was stored and sealed in an amber glass bottle at -4°C. Both EOs were qualitatively analyzed by gas chromatography in an Agilent 7890A hyphenated system, equipped with a 5975C series mass selective detector. For analysis, the parameters were: DB5-MS fused silica capillary column (5% phenylmethylsiloxane, 30 m x 0.25 mm, film thickness: 0.25 µm); 1:50 split injection mode; Carrier gas: He (1 mL min^{-1}); 40°C oven heating program, for 4 min (Ti), 40-320°C at 4°C/min; inlet, detector and interface temperature: 250 °C. The identification of the constituents was performed by comparison with the fragmentation patterns of the mass spectra and the Kovats retention indices (KI), determined through a calibration curve of a homologous series of n-alkanes (C8-C40) , with literature data and the equipment library (NIST 2008, 2024, Adams 2011, Silva et al. 2015, Garlet et al. 2019). The compounds were quantified using gas chromatography with flame ionization detection on an Agilent 7890A GC. The inlet/detector temperature was 300°C, with the analysis parameters previously mentioned, with the exception of the splitless injection.

Fish maintenance

The present study was approved by the UFSM Animal Welfare and Ethics Committee, under number 9303280623 and is registered in SISGEN under number AE4FE76. One hundred and eighty four juveniles (5.86 ± 1.03 g and 6.26 ± 1.06 cm n = 8) of *O. niloticus* were purchased from a fish farm in Santa Maria and transported to the Fish Physiology Laboratory at UFSM. Fish were placed in 250 L tanks with constant aeration, protected from light, at 26 °C, fed with commercial feed (36% CP; Supra Water Line | Alisul Alimentos S.A., Carazinho, Brazil), supplied until satiety four times a day. Daily, 10% of the water in the tanks was replaced 30 minutes after feeding, to remove feces and food remains. Daily dissolved oxygen levels (6.51 ± 0.19 mg L^{-1}) and temperature (25 .91 ± 1.53 °C) were measured with a YSI55 oximeter, and pH with a pH meter (7.18 ± 0.45, DMPH-2, Digimed, Brazil).

Sedative and/or anesthetic induction and recovery

Fish were individually placed in aquariums (11.5 cm high x 12.5 cm wide x 17.5 cm long) containing 1 L of aerated water. After 96 hours of completing the experiments, the fish were euthanized by immersion in eugenol (100 mg L^{-1}) and subsequently sectioning the spinal cord (Balko et al. 2018). The EOs were previously diluted in 95% ethanol (1:10) and then tested at the following concentrations (n= 8 each concentration): 2, 10, 20, 50, 80, and 200 mg L^{-1}. The experiments were carried out in triplicate and the concentrations were chosen after pilot tests with a concentration range varying from 25 to 400 mg L^{-1}. The objective was to evaluate lower concentrations of PPOL and CVOL in juveniles of *O. niloticus* considering the importance of sedative concentrations for the fish transport in aquaculture (Becker et al. 2012, Ribeiro et al. 2016, Ventura et al. 2020). Ethanol (2000 µL L^{-1}) was used as negative control and eugenol (2 and 50 mg L^{-1}) as positive control. Anesthetic and/or sedative induction and recovery were evaluated using the steps described by Tarkhani et al. (2017) with some adaptations: N - normal; S2 - deep sedation (loss of reaction to external stimuli);

S3a - partial loss of balance (tactile response only to pressure on the caudal peduncle); S3b - total loss of balance (loss of ability to swim, fish overturned); S4 - anesthesia (loss of reflexes; fish do not respond to pressure stimuli on the caudal peduncle); and R - recovery (fish regains balance and returns to normal swimming). When the animals reached the S4 stage, or within a maximum time of 30 min, they were transferred to recovery aquaria, which contained 1 L of aerated water. To determine recovery times, the time elapsed until the fish returned to normal swimming behavior was observed. Each animal was used only once, and induction and recovery times were measured with a digital stopwatch.

Long-term exposure

The fish were exposed individually in aerated 1 L aquariums and all at the same time to each EO for up to 48 h and were observed for 5 min at times 0 (800 µL L^{-1} ethanol only), 10, 20 and 30 min, 1, 2, 3, 6, 12, 24, and 48 h, to check possible adverse effects and mortality. The concentrations tested were 2, 20, and 80 mg L^{-1} (n= 8 each concentration) as they are sedative concentrations (see results), aiming to evaluate if the fish reached deep anesthesia (S4) or adverse effects. Stimulation was applied to the caudal peduncle with a glass rod in specimens that appeared to be in S4. After 48 h of exposure to these EOs concentrations, fish were placed in aerated 10 L recovery aquariums (one for each concentration/ EO) for observation for additional 24 h. Eugenol was not used in this protocol, as we did not want to compare adverse effects and/ or mortality.

Determination of blood glucose levels

Blood puncture in fish was performed via intracardiac route with a sterile hypodermic needle and syringe (1mL, U100, 0.45x13, 26Gx1/2"). Fish were contained with a damp cloth and glucose was determined using a digital glucometer (G-TECH Free[1]®, South Korea) and analytical strips (G-TECH Free[1]®, South Korea). The measurements were carried out on fish exposed to each EO and on fish not subjected to any previously described handling (basal group) (n=8 each EO/ concentration and basal group). The blood collection was performed immediately after recovery from sedation or anesthesia and at the end of 48 h exposure.

Statistical analysis

The minimum significance level was 95% ($p< 0.05$), and data obtained were presented as mean ± standard deviation. Homoscedasticity and normal distribution of data were analyzed using the Levene and Shapiro-Wilk tests, respectively. Comparisons between different concentrations of each EO were performed using the Kruskal-Wallis test for non-parametric data followed by Dunn's test, using Prism version 9.0® software. To construct the dose response curves, it was used the "log (agonist) vs. Response – Find E Canything" available in the software, where the EC50 was indicated as a parameter, that is, considering the concentration of the agonist (X) that gives an average response between minimum and maximum. Accordingly, the equation below was used:

$$Y = \text{Minimum} + (\text{Maximum-Minimum}) / (1 + 10\text{^}(\text{LogEC50-X}))$$

RESULTS

Yield and chemical composition of the essential oils

Extraction by hydrodistillation for 3 h in a Clevenger apparatus in triplicate used 600 g of fresh crushed leaves of *P. pennatifolius* in each unit. This resulted in an average yield of 0.1 ± 0.007 % and average density of 0.6 ± 0.008 g mL^{-1}. It was

possible to identify 99.8% of the composition of PPOL and 45.4% of the CVOL (Table I). The main compounds of the PPOL were 2-undecanone (57.2%), 2-tridecanone (28.3%), germacrene D (10.4%), 2-undecanol (1.5%), damascone (1.3%), spathulenol (0.6%), and undecenol acetate (0.5%). The CVOL presented as main constituents α-pinene (34.8%), α-humulene (3.8%), spathulenol (2.8%), elemene (2.7%), and limonene (1.3%).

Sedative and/or anesthetic induction and recovery

Fish exposed to 2 and 50 mg L^{-1} eugenol reached sedation (S2) in 262.8 ± 11.8 s and deep anesthesia (S4) in 143.7 ± 10.5 s, respectively. Fish recovered from S2 at 2 mg L^{-1} within 293.3 ± 35.5 s and from S4 at 50 mg L^{-1} within 600.9 ± 56.3 s. Fish exposed to ethanol did not show any visible alteration compatible with central nervous system depression.

Essential oil of P. pennatifolius

Fish exposed to the PPOL at concentrations of 2, 10, 20, 50, and 80 mg L^{-1} only reached the sedation stage (S2) and those exposed to 200 mg L^{-1} reached the deep anesthesia stage (S4). Fish exposed to 2 mg L^{-1} took longer to reach S2 stage compared to those exposed to 50, 80, and 200 mg L^{-1}. Time to reach the S2 stage was longer in fish exposed to 10 mg L^{-1} compared to 80 and 200 mg L^{-1}, and in those exposed to 20 mg L^{-1} was longer than in fish submitted to 200 mg L^{-1}. The recovery time was longer in fish exposed to the highest concentrations (80 and 200 mg L^{-1}) [Table II].

Table I. Chemical composition of the essential oils of *Pilocarpus pennatifolius* and *Cordia verbenacea*.

			%	
RI[a] E[b]	**RI[a] L[c]**	**Compounds**	***P. pennatifolius***	***C. verbenacea***
929	939	α-pinene	-	34.8
1027	1028	Limonene	-	1.3
1292	1291	2-Undecanone	57.2	-
1301	1303	2-Undecanol	1.5	-
1388	1392	Elemene	-	2.7
1388	1387	Damascone	1.3	-
1453	1452	α-Humulene	-	3.8
1479	1480	Germacrene D	10.4	-
1494	1494	2-Tridecanone	28.3	-
1504	1504	Undecenol acetate	0.5	-
1574	1571	Spathulenol	0.6	2.8
Identified components			99.8	45.4
Unidentified components			0.2	54.6

[a]RI = Retention index; [b]Experimental; [c] Literature ADAMS (2011) and NIST (2024).

Table II. Time (s) to reach the stages of sedation (S2), anesthesia (S4) and anesthetic recovery (R) in juvenile tilapia (*Oreochromis niloticus*) individually exposed to the essential oils of *Pilocarpus pennatifolius* and *Cordia verbenacea* at concentrations of 2 to 200 mg L^{-1}. (n=8 each) by Mean ± Standard deviation.

	Concentrations (mg L^{-1})					
	Essential oil of *Pilocarpus pennatifolius*					
Stages	**2**	**10**	**20**	**50**	**80**	**200**
S2	703.8 ± 117.9^{a}	407.4 ± 84.5^{ab}	225.5 ± 37.7^{ac}	190.2 ± 28.1^{bcd}	163.5 ± 31.7^{cd}	31.8 ± 16.8^{d}
S4	-	-	-	-	-	507.5 ± 119.8
R	98.4 ± 73.9^{b}	141 ± 94.5^{b}	214.7 ± 172.6^{b}	208.8 ± 184.4^{b}	609.6 ± 187.8^{a}	600.4 ± 57^{a}
	Essential oil of *Cordia verbenacea*					
S2	504.9 ± 58.4^{a}	294.2 ± 93.7^{ab}	142.8 ± 96.7^{bc}	113.8 ± 60.6^{be}	135.6 ± 10.5^{bd}	73.1 ± 51.8^{cde}
S4	-	-	-	-	-	1265.7 ± 171.8
R	1143.6 ± 331.3^{b}	1254.1 ± 305^{b}	1033.8 ± 334.7^{b}	1214.9 ± 225.3^{b}	1490.4 ± 123.8^{ab}	1758.3 ± 46.31^{a}

Mean ± standard deviation. Different letters on the same line indicate a significant difference between concentrations (n=8 each concentration) by Kruskal-Wallis test followed by Dunn's test; (-) indicates stage not reached; (S2) sedation; (S4) anesthesia; (R) recovery.

Essential oil of C. verbenacea

Fish exposed to CVOL at concentrations of 2, 10, 20, 50, and 80 mg L^{-1} reached only the sedation stage (S2) and those exposed to 200 mg L^{-1} reached the deep anesthesia stage (S4). Fish exposed to 2 mg L^{-1} presented the longest time to reach the S2 stage. The time to reach the S2 stage was longer in fish exposed to 10 mg L^{-1} compared to 200 mg L^{-1}. Recovery time was longer in fish exposed to 200 mg L^{-1}, but did not differ from 80 mg L^{-1} (Table II).

Long-term exposure

Essential oil of P. pennatifolius

After 10 min, 25% of the juveniles exposed to 2 mg L^{-1} were in S2 and after 20 min, all fish were in S2. After 6 h of exposure, 62.5% were still in S2 and after 12 h only 25% remained in S2. All juveniles exposed to 20 mg L^{-1} were in S2 from 10 min to 6 h exposure, and after 12 h, 87.5 % remained in S2. From 24 to 48 h, all fish exposed to both concentrations showed normal behavior and no adverse effects, clinical signs, or behavior that were outside the normality of the species were observed. Fish exposed to 80 mg L^{-1} were 100% in S2 from 10 to 30 min, and after 1 h, 75 % were in S3b and 25 % still in S2. Between 2 and 3h, all fish were in S3b and within 6 and 12 h they were all in S3a. After 24 h of exposure 100 % were in S2 and at 48 h 62.5 % were in S2 and the remaining returned to normal behavior, with no adverse effects, clinical signs, or behavior that were outside the normality (Figure 1a).

Essential oil of C. verbenacea

At 10 min up to 2 h, 100% of the juveniles exposed to 2 mg L^{-1} were in the S2 stage and after 2 h, all fish returned to normal behavior. All juveniles exposed to 20 mg L^{-1} were in the S2 stage from 10 min to 24 h of exposure, and at 48 h, all fish showed normal behavior. All fish exposed to 80 mg L^{-1} were in the S2 stage from 10 to 30 min, at 1 h 100% in the stage S3a, at 2 h 75% were in the stage S3b and 25% in the stage

S3a, and at 3 h 100% were in the S4 stage. From 6 to 24 h 100% were in the S2 stage and at 48 h, all returned to normal behavior. No adverse effects, clinical signs, or unusual behaviors were observed throughout the experiment (Figure 1b).

Blood glucose levels in *O. niloticus* juveniles

Blood glucose levels immediately after recovery from sedation or anesthesia with the EOs

Blood glucose levels were not affected significantly by sedation or anesthesia with the PPOL (Figure 2a). Fish anesthetized with the highest concentration of the CVOL showed higher blood glucose levels than those from the basal group and those sedated with 2, 10, and 20 mg L^{-1}. Fish sedated with 80 mg L^{-1} presented higher blood glucose levels than those sedated with 10 mg L^{-1} (Figure 2b).

Blood glucose levels after 48 h of exposure to the EOs

Blood glucose levels were not affected significantly in fish exposed for 48 h to the sedative concentrations of the PPOL (Figure 3a), but fish exposed to 80 mg L^{-1} CVOL showed higher blood glucose levels than those from the basal group and exposed to 2 mg L^{-1} (Figure 3b).

The concentration-response curves for the EOs

There is a significant negative relationship concentration-response for both EOs for the S2 stage, i.e., the time to reach sedation decreases with the increase of EO concentration. This decrease in time to reach S2 was progressive for the PPOL, but for the CVOL the time stabilized at concentrations higher than 20 mg L^{-1}. Deeper sedation and anesthesia stages (S2, S3a, S3b, and S4) were observed only at 200 mg L^{-1}, so no relationship could be calculated for these stages. There is a positive relationship concentration-response for recovery, with a sharp increase in the time to recover at concentrations higher than 50 mg L^{-1} (Figures 4a, b).

DISCUSSION

The water quality parameters observed in this study were within the ideal range for juvenile Nile tilapia (Teixeira et al. 2017, Obirikorang et al. 2020, Zahran et al. 2021).

Blood glucose levels in the basal group were like the results found in other studies (Navarro et al. 2016, Teixeira et al. 2017, Ferreira et al. 2021).

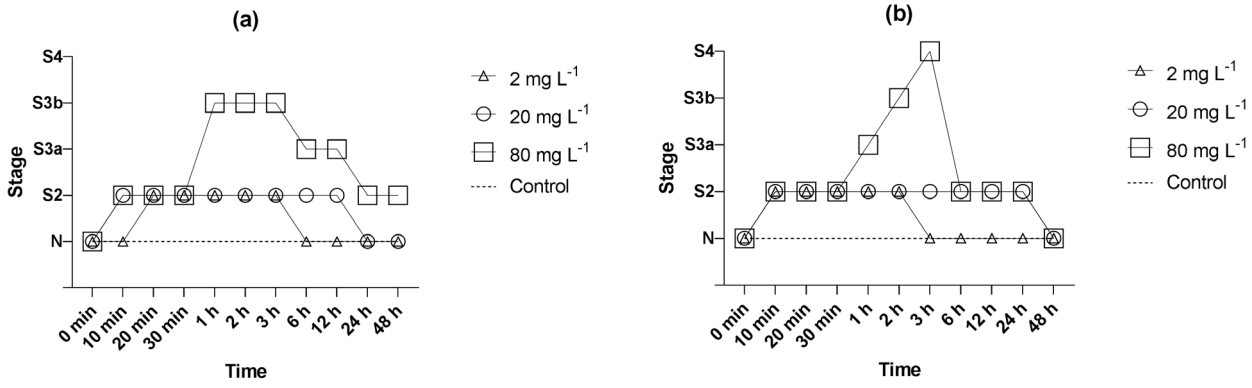

Figure 1. Stages of anesthesia observed over time in Nile tilapia (*Oreochromis niloticus*) exposed to essential oil of *Pilocarpus pennatifolius* (a) and *Cordia verbenacea* (b). N - Normal behavior, S2 - sedation, S3a - partial loss of balance, S3b - total loss of balance, S4 - anesthesia (n=8 each concentration).

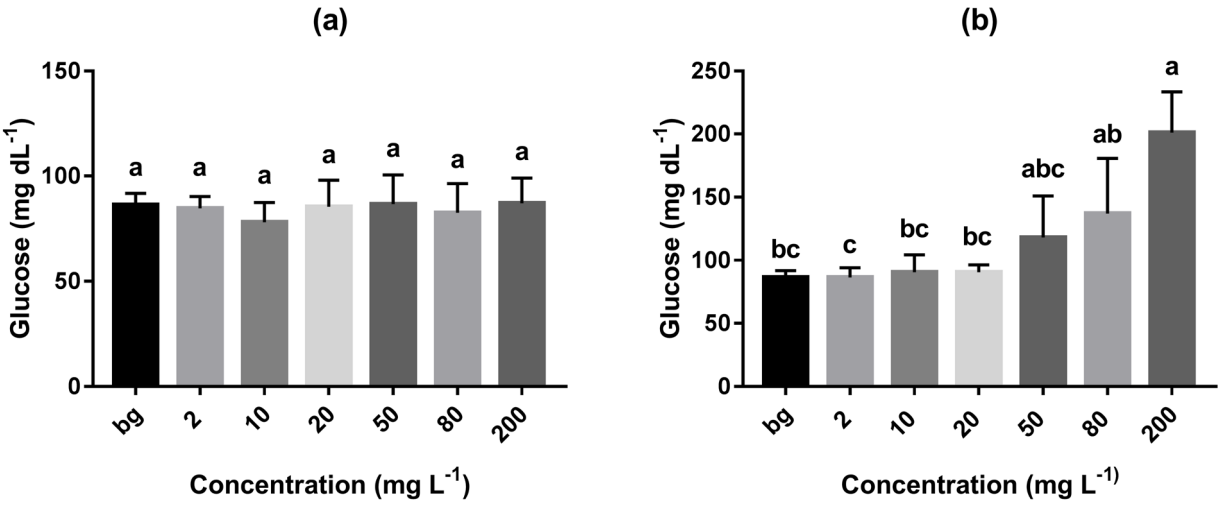

Figure 2. Blood glucose levels in juvenile Nile tilapia (*Oreochromis niloticus*) immediately after recovery from sedation or anesthesia with EOs from *Pilocarpus pennatifolius* (a) and *Cordia verbenacea* (b) at concentrations of 2 to 200 mg L^{-1}. Different letters on the same column indicate a significant difference between concentrations and basal group (bg) (n=8 each) by Kruskal-Wallis test followed by Dunn's test.

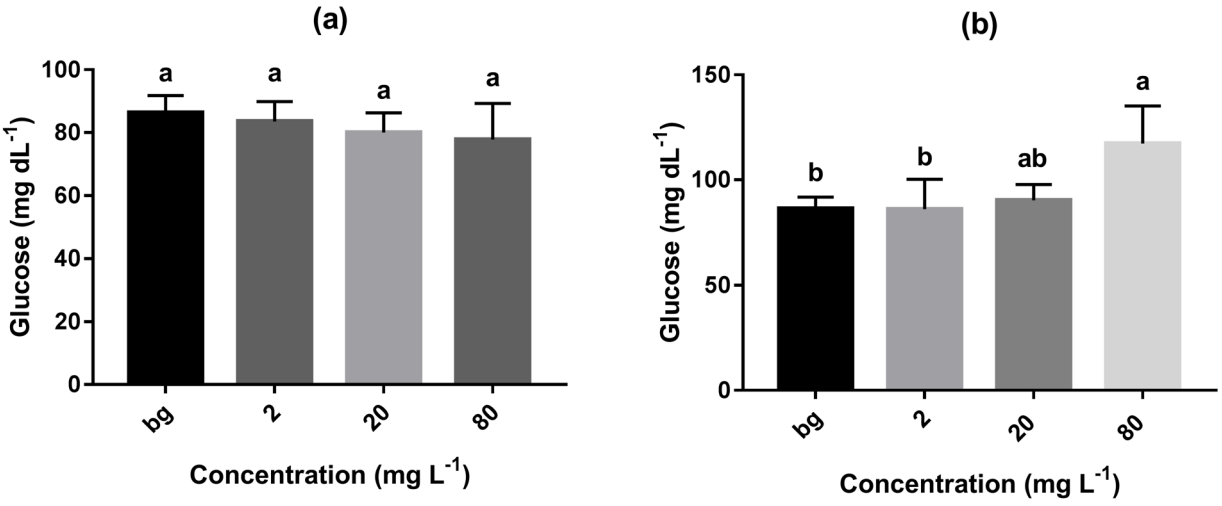

Figure 3. Blood glucose levels in juvenile Nile tilapia (*Oreochromis niloticus*) after 48 h of exposure to the EOs of *Pilocarpus pennatifolius* (a) and *Cordia verbenacea* (b) at the sedative concentrations of 2, 20, and 80 mg L^{-1}. Different letters on the same column indicate a significant difference between concentrations and basal group (bg) (n=8) by Kruskal-Wallis test followed by Dunn's test.

Essential oil of *P. pennatifolius*

The main compounds of this EO were 2-undecanone (57.2%), 2-tridecanone (28.3%), and germacrene D (10.4%) and consequently, we observed that 85.5% of the constituents are aliphatic ketones. Santos et al. (2004), which identified 99.3% of the EO constituents obtained from dried powdered leaves of the same plant species, found a predominance of tridecane (56.8%), pentadecane (25.5%), γ-muurolene (6.6%), spathulenol (2.6%), β-elemene (2.1%), β-caryophyllene (1.2%) and eugenol (0.3%).

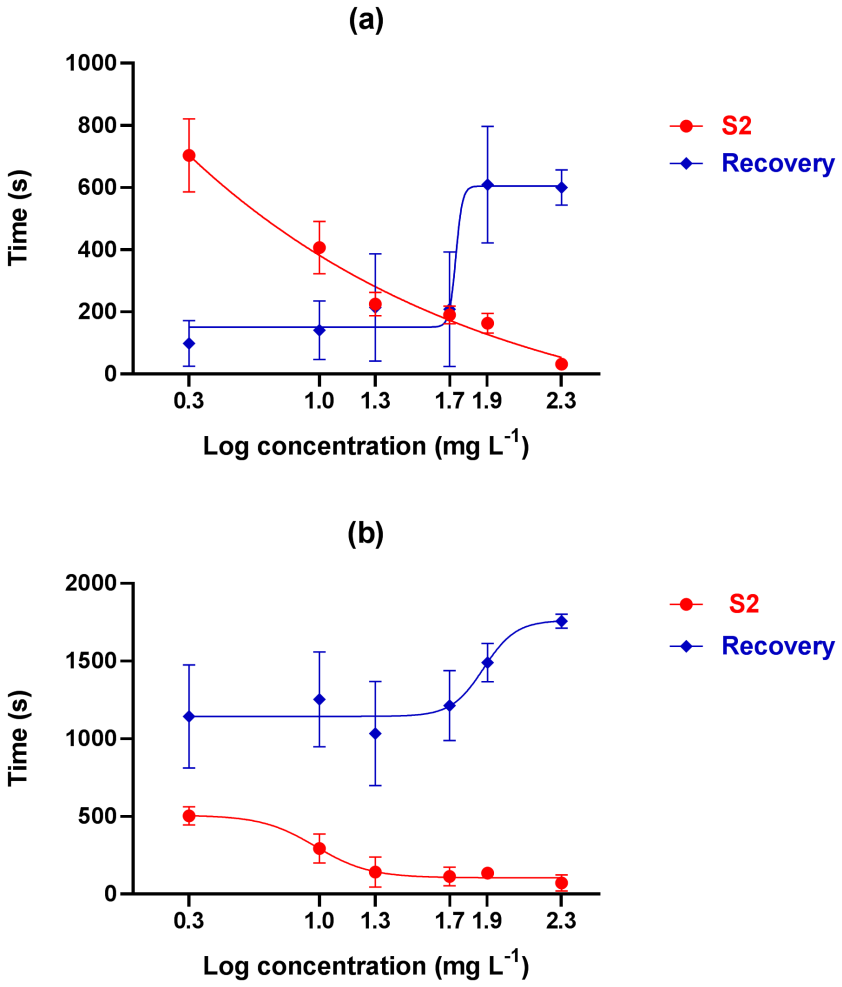

Figure 4. Concentration-response to reach sedation (S2) and recovery in *Oreochromis niloticus* exposed to the essential oils of *P. pennatifolius* (a) and *C. verbenacea* (b). The S3a, S3b, and S4 stages were reached only in fish exposed to 200 mg L^{-1}, so this analysis was not made for these stages. Concentrations: 2 mg L^{-1} (log = 0.3); 10 mg L^{-1} (log = 1.0); 20 mg L^{-1} (log = 1.3); 50 mg L^{-1} (log = 1.69), 80 mg L^{-1} (log = 1.9), and 200 mg L^{-1} (log = 2.3). The "log (agonist) vs. Response – Find E Canything" available in the software, considering the concentration of the agonist (X) that gives an average response between minimum and maximum, according to the equation: Y = Minimum + (Maximum-Minimum) / (1 + 10 ^ (LogEC50-X))

Thus the major constituents are saturated hydrocarbons (82.3%), with a carbon chain similar in length to the ketones described in our study, which also indicated the presence of spathulenol in the EO, but at 0.6%. At this point, it is worth noting that in our work the essential oil was obtained from crushed fresh leaves. Since the low stability of several components of essential oils is well known in the literature (Mutlu-Ingok et al. 2020), it is possible that the process of drying leaves, before extracting essential oils by Santos et al. (2004), has promoted the degradation of the major components, which give rise to hydrocarbons. However, to confirm this hypothesis, additional studies must be carried out.

The PPOL was an excellent sedative and anesthetic for juvenile Nile tilapia, as anesthetic induction and recovery were smooth at all concentrations evaluated and there was no mortality. Germacrene D was identified in the EO of young (9.1%) and old (9.2%) leaves of *Nectandra megapotamica*, and these EOs presented sedative (30 µL L^{-1}/1.3-3.2 min) and anesthetic effects (from 150 µL L^{-1}/5.6-8.0 min) in *Centropomus parallelus* (Tondolo et al. 2013). Furthermore, germacrene D was present in the composition of *Hyptis mutabilis* EO, which showed sedative and anesthetic effects in *Rhamdia quelen* (Silva

et al. 2013b). *Pilocarpus pennatifolius* EO also did not cause an increase in blood glucose levels in fish exposed for short or long periods and no adverse and behavioral effects visible in the evaluations. The concentrations of 20 and 50 mg L^{-1} induced sedation (S2) at 225.5 and 190.2 s and fish recovered within 214.7 and 208.8 s, respectively. Fish at 200 mg L^{-1} showed deep anesthesia (S4) in 507.5 s and returned to normal behavior in 600.4 s. These results are very promising for sedation (S2) and anesthesia (S4) in Nile tilapia, especially because no changes in glucose levels were found compared to the control, nor adverse effects, clinical signs, and abnormal behavior were observed. Furthermore, most major compounds present in the EOs in the current study were not previously described in any other EO or extract with sedative and/or anesthetic activity. The EOs that showed sedative and/or anesthetic effect in Nile tilapia have as main compounds linalool, geranial, pulegone and eugenol [Table III]. It is worth highlighting that these other EOs presented disadvantages in terms of stress, toxicity, mortality, among others. In this sense, the PPOL stands out, as it

Table III. Essential oils that induced sedation and/or anestesia in Nile tilapia (*Oreochromis niloticus*) juveniles.

Stage	Concentration	Plant	Main compound present in the EO	Reference
S2	2 mg L^{-1}	*Pilocarpus pennatifolius*	2-Undecanone (57.2%)	Present study
S4	200 mg L^{-1}			
S2	2 mg L^{-1}	*Cordia verbenacea*	α-Pinene (34.8%)	Present study
S4	-			
S2	10 or 25 µL L^{-1}	*Ocimum basilicum*	Linalool (53.3%)	Netto et al. (2017)
S4	400 µL L^{-1}			
S2	20 µL L^{-1}		Methylchavicol (66.5%)	Ventura et al. (2020)
S4	-			
S2	10 or 25 µL L^{-1}	*Cymbopogum flexuosus*	Geranial (50.1%)	Netto et al. (2017)
S4	600 µL L^{-1}			
S2	10 µL L^{-1}	*Hesperozygis ringens*	Pulegone (72.3%)	Ferreira et al. (2022) Pinheiro et al. (2016)
S4	450 and 600 µL L^{-1}			
S2	-	*Ocimum americanum*	Linalool (74.3%)	Rucinque et al. (2021)
S4	500 µL L^{-1}			
S2	-	*Lippia alb*	Linalool (74.1%)	Rucinque et al. (2021)
S4	500 µL L^{-1}			
S2	20 µL L^{-1}		Linalol (47.6%)	Hohlenwerger et al. (2016)
S4	-			
S2	5 mg L^{-1}	*Ocimum gratissimum*	Eugenol (73.6%)	Ferreira et al. (2021)
S4	90 and 150 mg L^{-1}			
S2	10 – 40 µL L^{-1}	*Aloysia triphylla*	Geranial (28.9%)	Teixeira et al. (2017)
S4	300 µL L^{-1}			

All EOs were extracted by hydrodistillation in a Clevenger apparatus.

did not result in any clinical signs or changes in stress levels.

The lowest concentrations of the PPOL are the most interesting for use in procedures that require only sedation, such as transport. Concentrations of 2 and 20 mg L^{-1} in the long exposure protocol kept the fish sedated for more than 5 h and for more than 11 h, respectively. Furthermore, there were no adverse effects or increase in blood glucose levels. None of the concentrations tested in long exposure achieved anesthesia.

Essential oil of *C. verbenacea*

The main compounds found in the CVOL were α-pinene (34.8%), α-humulene (3.8%) and spathulenol (2.8%). In this sense, the descriptions in the literature for the highest percentages of constituents in this EO are sesquiterpenes, such as ß-caryophyllene (25.4%), bicyclogermacrene (11.3%) and δ-cadinene (9.4%), followed by the monoterpene, α -pinene (9.5%) (Rodrigues et al. 2012). Furthermore, the same authors also identified spathulenol (5.7%), linalool (0.5%) and germacrene D (4.5%). Thus, the EO under study differed from those described in the literature, as it was predominantly composed by α-pinene (34.8%). However, it did not differ from Carvalho et al. (2004), which also found α-pinene as the major constituent (29.6%). It is worth mentioning that both authors mentioned above also identified spatulenol in the composition of the CVOL.

The CVOL at 2, 10, 20, 50, and 80 mg L^{-1} caused only sedation in Nile tilapia juveniles. As the concentration increased, the time to reach sedation was reduced, but at all concentrations studied, the recovery time was above 1000 s. This could be an indication that this EO is causes persistent depression in the central nervous system of fish. The concentration of 200 mg L^{-1} of CVOL may be used for stunning during the slaughter process or some other procedure that requires longer-lasting anesthesia. In procedures such as medication and vaccine applications that require anesthesia for faster recovery, PPOL at of 200 mg L^{-1} can be suggested. However, the indications from some researchers that the recovery time should be lesser than or close to 300 s (Keene et al. 1998, Roubach et al. 2001, Obirikorang et al. 2020) would not be valid for these EOs.

However, if we evaluate some synthetic anesthetics and even those of natural origin in fish, many are not close to this recovery value (Priborsky & Velisek 2018). One cannot rule out the possibility of using a natural anesthetic just because of the long recovery time. Therefore, more studies would be needed at physiological and pharmacological levels. In the long exposure evaluation at 2 mg L^{-1} CVOL, from 10 min to 2 h the fish remained at S2 and in the following evaluations the fish returned to normal behavior, so this concentration could only be used to transport juvenile tilapia over short distances. The blood glucose levels in fish exposed to this concentration did not differ from the basal group, thus being an indication that the fish were not stressed by this EO. At 20 mg L^{-1}, the juveniles were in the S2 stage between 10 min up to 24 h, and blood glucose levels did not change, so this concentration could be used for long distance transportation. The concentration of 80 mg L^{-1} in long exposure led to stages S3a, S3b, and S4, and increased blood glucose levels, indicating stress in juvenile Nile tilapia. This increase in blood glucose levels was also seen in fish anesthetized with 200 mg L^{-1} CVOL and consequently fish may have been stressed by anesthesia.

CONCLUSIONS

The EOs of PPOL and CVOL showed promising sedative and anesthetic effects. Furthermore, PPOL did not change blood glucose levels, so we recommend its use at concentrations of 2, 10, and 20 mg L^{-1} for sedation and 200 mg L^{-1} for anesthesia of *O. niloticus*. It is worth noting that the anesthetic induction with this EO was very calm, safe, and did not visibly alter the behavior of the fish or cause mortality. The CVOL also followed the same pattern, but the lower concentrations studied apparently were safer and can be used for sedation of juvenile tilapia, as they did not cause an increase in glucose levels compared to the basal group. However, since the higher concentrations lead to glucose rise, CVOL is not recommended for anesthesia of the fish species studied.

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CARLOS HERMINIO M. FORTES[1]
https://orcid.org/0000-0001-7111-2235

BERNARDO BALDISSEROTTO[1,2]
https://orcid.org/0000-0002-8770-0100

FREDERICO D. FLEIG[3]
https://orcid.org/0000-0003-1683-3157

BERTA MARIA HEIZNMANN[1,4]
https://orcid.org/0000-0002-6509-949X

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

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

[3]Universidade Federal de Santa Maria, Departamento de Ciências Florestais, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

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

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

Author contributions

First author: CHMF; Conceptualization, Methodology and Formal Analysis: CHMF, BMH and BB; Validation, Data Collection, Data Analysis, Writing - Original Draft, Writing - Review and Editing, visualization: CHMF; Resources: BMH, BB and FDF; Writing – Review & Editing, Supervision, Funding Acquisition: BMH and BB.