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Zootherapeutic practices in the Amazon Region: chemical and pharmacological studies of Green-anaconda fat (*Eunectes murinus*) and alternatives for species conservation

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ABSTRACT

The treatment of diseases with animal resources or their derivatives is a traditional practice worldwide, representing a wide field of research for the elaboration of strategies of management and conservation of the fauna, and contributing to the search for sustainable therapy alternatives. This study presents the therapeutic applications of animal fats to the treatment of several diseases in Pimenteiras do Oeste, state of Rondônia, Brazil. Twelve animals including: mammals, fishes, reptiles and birds are reported as a source of medicinal lard for the treatment of respiratory illnesses (asthma, flu, bronchitis, cough), rheumatism, earache and as a healing agent (dislocation and wounds). The ethnopharmacological focus of the study was on Green-anaconda (*Eunectes murinus*) fat, which stands out for its frequent local use and the lack of previous chemical studies. The chemical composition of *E. murinus* fat was analyzed by gas and liquid chromatography, both coupled to mass spectrometry. The main fatty acids identified were oleic, linoleic and palmitic acids, which were also predominant in the composition of the triglycerides. Pharmacological analysis of Green-anaconda fat showed a significant anti-inflammatory effect, which is related to its use by traditional communities. Having confirmed the pharmacological potential of Green-anaconda fat, its fatty acid composition was used as a parameter in the search for vegetable oils from the Amazon Region with a similar composition. This comparative analysis can be of help by proposing therapeutic alternatives for the Amazonian population. The use of plant sources can contribute to the conservation of the aforementioned species.

Keywords: Ethnopharmacology; Chromatography and Mass Spectrometry Analysis; Oleic Acid; Linoleic Acid; Palmitic Acid; Snakes.

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SIGNIFICANCE STATEMENT

A survey was conducted with the population of Pimenteiras do Oeste, state of Rondônia, Brazil, regarding the medicinal use of animal fat. Green-anaconda (*Eunectes murinus*) was selected for further studies for being the second most cited taxon in the survey and due to the lack of specific chemical and pharmacological studies of this species. We show that *E. murinus* fat is mainly composed of oleic, linoleic and palmitic acids and that it has anti-inflammatory and antinociceptive activity. Based on the composition of the fatty acids present in *E. murinus* fat, some oils extracted from Amazonian plants are presented as alternatives for the treatment of wounds and as antinociceptive and anti-inflammatory agents, favoring the preservation of *E. murinus* and giving the population an accessible alternative. This use would also minimize the impact of extractivism of Green-anaconda fat in the Amazon region, which is regulated by environmental laws.

INTRODUCTION

Over time, human beings have benefited from natural resources such as plants (Cragg and Newman 2013) and animals (Costa-Neto 2005) for their basic and medicinal needs. Archeological records of different forms of interaction and use of animals are known worldwide, the oldest written records being from China, India, Egypt, and Mesopotamia which detailed the use of animals for therapeutic purposes by diverse populations and cultures (Alves et al. 2013a; Lev 2003). From the traditional knowledge associated with the medicinal use of animals and their derivatives, also called zootherapics, it is possible to obtain information that will lead to interdisciplinary scientific studies on biologically active constituents within the scope of ethnopharmacology (Alves and Rosa 2007a; Alves and Albuquerque 2013).

Even today, traditional communities around the world have important knowledge about the fauna and its therapeutic applications (Fischer et al. 2018). In Brazil, the first written records of the therapeutic use of animals by indigenous groups were provided by Portuguese colonists, Jesuits and naturalists from the 1500's (Martius 1939). This knowledge has been transmitted over generations, especially in places with difficult access to health care (Santos and Lima 2009). More than 350 animal species are used for medicinal purposes in Brazil (Alves and Rosa 2007b; Ferreira et al. 2009a). Among these, fishes (93 species), mammals (66), reptiles (57), and birds (47) are the most used vertebrates; insects (39), molluscs (18) and crustaceans (17) are the most used invertebrates (Alves et al. 2013d). The selection of species for medicinal use is related to several factors such as geographic distribution, occurrence in a given environment and ease of obtaining (Alves et al. 2020). Mammals, birds, fish, reptiles and amphibians are used to treat a variety of diseases (respiratory, rheumatic, arthritis and skin problems). Various parts of the animals are used, such as fat, bone, skin, tail, liver, bile ("bile"), head, rattle (rattlesnakes), secretions and urine.

Although medicines derived from animals for human and veterinary use have already been reviewed by several authors (Alves and Alves 2011; Alves and Rosa 2006; Schmeda-Hirschmann et al. 2014), there is still much to be studied. In the Amazon Region, fats are frequently used as zootherapics by the *cabocla* population (MEB 1993). However, there is a lack of ethnopharmacological studies that address the traditional use of medicinal fat in Brazil and worldwide. These studies are fundamental for a better understanding of the pharmacological importance of these zootherapics, followed by new policies for sustainable resource management and species conservation.

Wild animals predominate among the species used for medicinal purposes (Alves et al. 2008), negatively affecting species populations and increasing their risk of extinction (Souto 2011). Much of the threat to biodiversity lies in the destruction of habitats and the unsustainable use of resources (Whiting et al. 2011). Trade in products from these animals for traditional medicine is a threat to the conservation of several species (Ferreira et al. 2013; Whiting et al. 2011, 2013), especially in relation to the most heavily exploited species (Alves et al. 2013c). Special care must be taken to avoid overexploitation of wild animals, with strategies that allow bioprospecting for medicinal use in a sustainable manner so that biodiversity is conserved (Alves and Albuquerque 2013). In addition, considering that the sanitary conditions where these animal products are prepared and stored are notoriously precarious (Alves et al. 2013d), their use can lead to the transmission of zoonotic diseases caused by harmful germs such as viruses, bacteria, and fungi (Rahman et al. 2020). Thus, it is necessary to seek alternatives to the zootherapeutic practices used by traditional communities. Studies on traditional uses of animal resources should consider conservation biology and public health policies, with the sustainable management of natural resources that have been highlighted by bioprospection being of paramount importance (Ferreira et al. 2009b). In the specific case of fats, these alternatives may come from domestic animals or vegetable oils.

The present study was conducted to understand the composition and pharmacological data of fats for medical use. It focused on the traditional knowledge associated with the use of animal fat for the treatment of various diseases in Pimenteiras do Oeste, state of Rondônia, Brazil, based on an ethnopharmacological approach related to the chemical composition of Green-anaconda fat (*Eunectes murinus*: Squamata), which stands out for its frequent local use. Due to its widespread use by the population, Green-anaconda fat is sold as a natural healing product in some markets along the Amazon Region. Although its cicatrizing activity has been recently demonstrated (Souza et al. 2017), as far as we know, there are no studies demonstrating its antinociceptive and anti-inflammatory activities. Also, there are no studies on the chemical profile of *E. murinus* fat, which may contribute to the understanding of its healing and anti-inflammatory activity. This knowledge can be useful for the selection of vegetable oils that can replace the medicinal applications of Green-anaconda fat.

MATERIAL AND METHODS

Characterization of the study area

The municipality of Pimenteiras do Oeste is located in the state of Rondônia, 178 meters above sea level, with the following geographical coordinates: latitude 13°28'57" south and longitude 61°02'48" west (Figure 1). It has an area of 6,014.7 Km^2, its population, estimated in 2019 by the IBGE, was 2,169 inhabitants, with 54% of the urban population representing the smallest municipality in the state in terms of population (IBGE 2019). Pimenteiras do Oeste is located on the banks of the Guaporé River, bordering Bolivia (Pinheiro and Sahr 2016). According to older residents, the region was occupied by *quilombolas* (called "maroons" in English) (populations of escaped African and Afro-descendant slaves) from Vila Bela da Santíssima Trindade, in the state of Mato Grosso, who were forced to flee towards the North of Brazil following the course of the Guaporé River (Jesus 2014). The region was colonized by these *quilombolas*, with the culture of ex-slaves thus becoming in contact with indigenous cultures and forming families of new identity. Today there are populations of remaining *quilombolas*, indigenous and riverine communities, and workers from other regions (Leocádia 2018). The region occupies a transition strip between three important biomes: Amazon Region, Pantanal and Cerrado (Figure 1), thus having great biodiversity represented by the richness of its ecosystems and by a genetically diverse fauna and flora (Fernandes et al. 2010).

Survey of zootherapeutic data

Zootherapeutic data were obtained with a semi-structured questionnaire and by informal conversations with the research subjects, with some points being redefined according to the progress of the interview (Vinuto 2014). The subjects reported their knowledge about the use of animal fat for the treatment of diseases, listing the animals used, the indications for use and the mode of use.

The snowball sampling technique was used in order to access the population using medicinal fat. In this non-probabilistic sampling technique, people initially chosen are used as informants, indicating new participants for the research (Baldin and Munhoz 2012; Penrod et al. 2003). The selection of people to be interviewed for the research started with the search of key informants, people respected in the community such as presidents of local associations and health agents. The key informants took us to local specialists, *quilombolas*, riverside dwellers and people with experience in the use of animals and medicinal plants, such as healers, patriarchs, and matriarchs. The data were analyzed qualitatively based on the popular names of the cited animals, the parts of the animals used for treatment and the indications for use. The free listing method was used to determine the Salience Index (SI), a technique used to identify cultural domains in the order of importance (Bisol 2012; de Oliveira et al. 2011). The SI was calculated with the aid of the ANTHROPAC 4.0 software (Analytic Technologies, USA).

Animal species were classified using "taxonomic clues" (Bitencourt et al. 2014; Lima et al. 2011), i.e., by comparing popular names with the scientific names of the species found in surveys of fauna carried out in the study region, for Environmental Licensing with the State Secretary for Environmental Development - SEDAM / RO: 1 - Environmental Control Plan (PCA); 2 - Environmental Plan for Conservation and Use of the Surroundings of the Artificial Reservoir (PACUERA) of the Small Hydroelectric Power Plant (PCH) – Cascata Chupinguaia; 3 - PACUERA Hydroelectric Cachimbó Alto; and 4 - Environmental Control Plan for the Veado Preto Hydroelectric Plant. This identification technique avoided the collection and sacrifice of animals, with the exception of the Green-anaconda used in ethnopharmacological studies, being mandatory to obtain the animal *in situ*. Exotic domestic animals were classified according to the list of animal species introduced in the national territory of the Ministry of Agriculture, Livestock and Supply, made available through Normative Instruction N° 19/18 (MAPA 2018). The conservation status of the wild animal species (Table 1) follows the Red Book of Brazilian Fauna Threatened with Extinction (ICMBIO 2018).

In order to guarantee the ethical principles of research with human beings within the scope of the associated traditional knowledge, the legal represen-

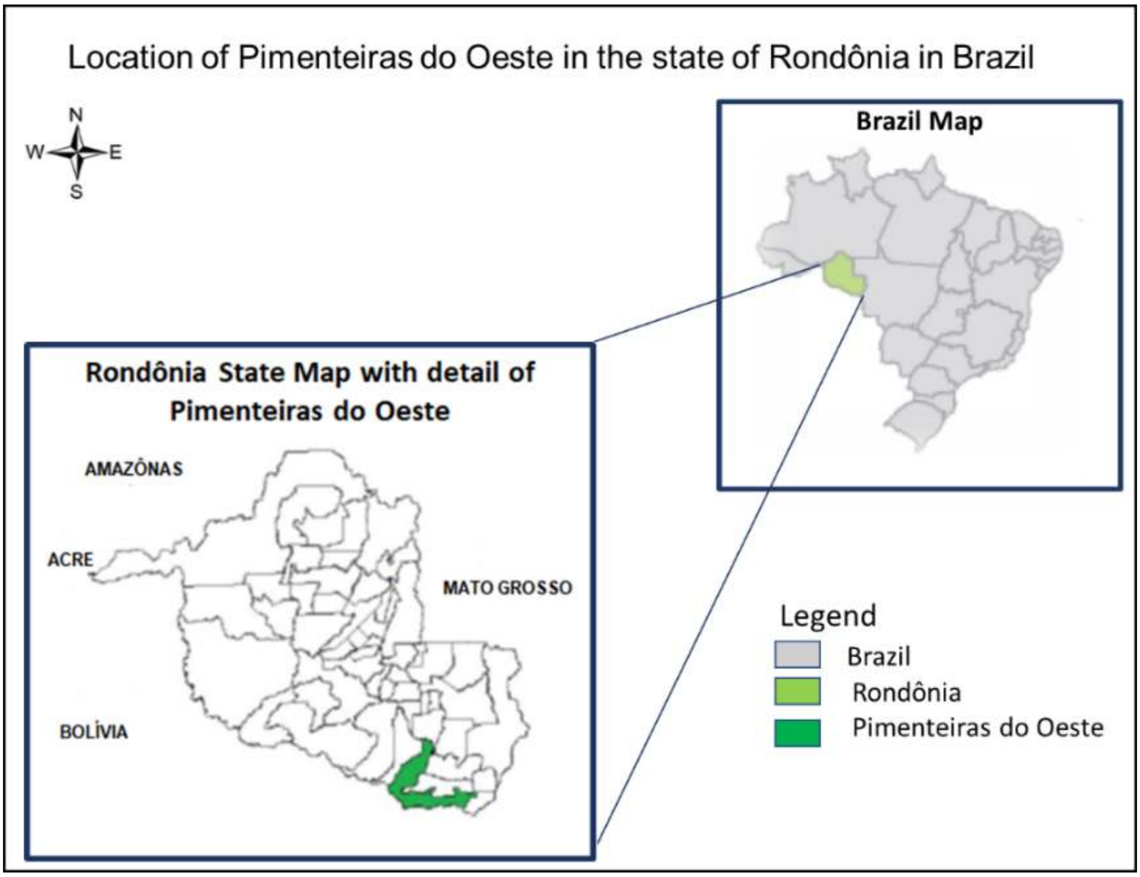

Figure 1. Location of Pimenteiras do Oeste in the state of Rondônia, Brazil.

tatives of two associations of rural producers in Pimenteiras do Oeste, as well as the research subjects, gave written informed consent to participate in the study and received a guarantee of confidentiality about personal information. All ethical procedures were in accordance with Law No. 13,123/2015 and Decree N° 8,772/2016, and the study was registered in the Brazilian National System for Access to Genetic Heritage and Associated Traditional Knowledge (SisGen 2017), under number A020866.

Eunectes murinus capture and fat extraction

Eunectes murinus was captured in the municipality of Cabixi, south of Rondônia, 68 km from Pimenteiras do Oeste, with the authorization of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through the Biodiversity Authorization System (SISBIO), under number 8830-1, issued on December 6, 2017. Active capture was carried out by searching the microenvironments of these animals, such as holes in the ground and debris from fallen logs. The captured specimen was euthanized with an overdose of thiopental. Fat was immediately removed from the ventral region of the snake and the animal was fixed in 70% alcohol and deposited in the vertebrate collection of the Museu Nacional/UFRJ under number MNRJ 26905. Fat was prepared according to the procedure used by the local population, which consists of heating the fat in a water bath and storing it in bottles for later use. The process yielded a product of semi-solid consistency, which was stored under refrigeration for future analysis.

Fatty acid composition of *Eunectes murinus* fat determined by GC-MS

Fatty acids were determined indirectly using the corresponding methyl esters, according to the method described by ISO-5509:1978. Approximately 1.0 g of the fat was weighed in a tube, 10.0 mL of n-heptane were added with stirring, followed by 0.50 mL of 2.0 mol/L NaOH solution in methanol with further stirring for 20 seconds. After phase separation, the supernatant was collected and evaporated for further analysis. For gas chromatography coupled to mass spectrometry (GC-MS) analysis, 1 mg of the supernatant previously obtained was dissolved in 1 mL dichloromethane. GC-MS was performed using a

SHIMADZU GC-2010 Plus chromatograph, GCMS-QP2020 SHIMADZU mass analyzer equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 μm) with helium gas at a flow speed of 1mL/min. The injector and detector temperature were 270°C and 200°C, respectively. The column temperature was programmed under the following conditions: 50°C for 1 min, followed by an increase to 200°C at the rate of 25°C/min, with the temperature remaining at 200°C for 10 min and then increasing at a rate of 10°C /min to 300°C, where it remained for 10 min. The spectra were obtained in the range of 40 to 700 m/z. The results were compared with data from the NIST11.lib and NIST11s.lib libraries (National Institute of Standards and Technology).

Triglyceride composition of *Eunectes murinus* fat determined by HPLC-ESIMS

The triglyceride profile was determined by high-performance liquid chromatography combined with electrospray mass spectrometry (HPLC-ESIMS) using a Thermo Scientific LCQ FLEET system. The sample was prepared by dissolving 3.0 mg of Green-anaconda fat in 1.0 mL of 50 mM sodium acetate in methanol:isopropanol (90:10). HPLC was performed on a Thermo Fisher Scientific Dionex UltiMate™ 3000 chromatograph equipped with a RS3000 degasser, LPG3400RS pump, DAD3000 detector, PCC3000RS oven, and WPS3000TRS automatic injector. All HPLC analyses were done using a Core-shell C-18 (Phenomenex®) - 50 x 3.0 mm, 2.6 μm column, with a UV absorption detector at 257 nm. The elution gradient used was 50 mM sodium acetate in methanol (eluent A) and isopropanol (eluent B), programmed as follows: 90% A for 5 min, followed by a linear gradient to 90% B in 25 min, and then maintained for 5 min. The equilibrium time for each analysis was 10 min, the flow rate 0.2 mL/min, injection volume 10 μL, temperature 30°C, and sample concentration 3.0 mg/mL (La Nasa et al. 2013). The mass spectrometer used was a Thermo Scientific LCQ FLEET with an electrospray interface in positive mode. ESI-MS spectra were obtained in an m/z range of 100 to 1500. MS measurements were carried out using He as the collision gas in the ion trap and N_2 as the sheath and auxiliary gas in the source. For ESI in the positive ion mode, the following parameters were used: capillary temperature 350°C, sheath gas flow 19 a.u, and spray voltage 4.0 kV. MS spectra were acquired in the m/z range of 50–1,500. For MSn experiments, target precursor ions were fragmented using 35% collision energy. An isolation width of 2 Da was used with a 30 ms activation time for the MS/MS experiments.

Pharmacological analyses of *Eunectes murinus* fat

The pharmacological tests were conducted on adult male Swiss mice (*Mus musculus*), weighing 25 to 35 g, obtained from the Central Biotery of State University of Feira de Santana (UEFS). All experimental procedures were approved by the Ethics Committee of Animal Use of the State University of Feira de Santana (CEUA-UEFS), under protocol number 001/2019. The experiments were carried out at the Laboratory of Pharmacology of Natural and Bioactive Products (LAFAR), State University of Feira de Santana (UEFS). *E. murinus* fat, the vehicle (0.9% NaCl) and the standard were diluted with 0.2% Tween 80 as a solubilizing agent and administered intraperitoneally (ip).

Assessment of pharmacological behavior

The pharmacological behavioral screening was performed based on the methodology proposed by Almeida and Oliveira (2006), in which comparative criteria are used for a series of behaviors normally exhibited by animals. This experiment aims to observe possible changes induced by the snake fat on the activities of the Central Nervous System by analyzing the parameters established in the standard protocol. For this purpose, three groups of five mice each received doses of the *E. murinus* fat (100, 200 and 500 mg/kg), and a control group, also consisting of five animals received only the vehicle (0.9% saline). The drug was administered in a volume of 0.1 ml/10 g animal weight. Behavioral parameters, including sedation, ptosis, decreased walking, hyperactivity, seizure and loss of corneal reflex, were observed at 30, 60, 120 and 240 min, in addition to the possibility of death for up to 72 hours after treatment.

Evaluation of motor activity in the rota-rod

The Rota-Rod apparatus model EFF - 411 – INSIGHT, Brazil, was used for this test. The apparatus consists of a non-slip swivel bar divided into four equal segments 5 cm in diameter, rotating at 7 rpm. In order to avoid misinterpretation of the results due to the natural inability of the animals to maintain equilibrium and movement on the rotating bar, the mice were pre-selected 24 hours before the test, with those remaining on the rotating bar for 180 seconds in up to three attempts being considered able to participate. The following day, the preselected mice were divided into five groups of six animals each as follows: control group (which received the 0.9% NaCl vehicle), experimental groups (treated with EMF at the doses of 75, 150 or 300 mg/kg), and t standard group (animals treated with 1.5 mg/kg diazepam). At 30, 60 and 120 minutes after the administration of the substances, the groups were submitted to the test and the time spent by each animal on the rotating bar was recorded (Dunham and Miya 1957; Mattei and Franca 2006).

Acetic acid-induced writhing test

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This test is based on the induction of a nociceptive response by peritoneal irritation after intraperitoneal (ip) administration of acetic acid (0.8%), with the nociceptive effect being characterized by abdominal contortions followed by hind limb extensions (Koster et al. 1959). The test was applied to five groups of six animals each: control (animals treated with vehicle), experimental groups (animals treated with fat at the doses of 75, 150 or 300 mg/kg), and one standard group (animals treated with indomethacin at the dose of 10 mg/kg). Thirty minutes after ip administration of the treatments, the animals received 0.8% acetic acid solution (0.1 mL/10 mg, ip) and were placed in individual polyethylene boxes for the recording of the total number of abdominal contortions over a period of 10 minutes.

Formalin test

This test is used to assess antinociceptive and anti-inflammatory activity in mice. For this test, the same groups, route of administration and treatments as used for the abdominal contortion test were employed. Thirty minutes after treatment the animals were injected with 20 μL of 2.5% formalin into the subplantar region of the right posterior paw. After formalin administration, the animals were placed in individual observation boxes and the time, in seconds, spent licking/biting the injected paw to occur was considered to be indicative of nociception (Dubuisson and Dennis 1977; Hunskaar et al. 1985).

Carrageenan-induced paw edema

This test was also used to assess the anti-inflammatory activity of the fat and followed the methodology recommended by Yesilada and Kupeli (2007), with some modifications. The experiment is based on the variation of the volume of the hind legs of the animals after the application of an inflammatory stimulus. The same groups, route of administration and treatments as described for the previous tests were employed. Thirty minutes after ip administration of treatments, the animals were injected into the subplantar region of the right posterior paw with 50 μL of 2.5% carrageenan diluted in saline solution (Nsonde Ntandou et al. 2010). An equal volume of saline solution (0.9% NaCl) was applied to the left hind paw. Paw thickness was then measured at 30, 60, 120, 180 and 240 minutes after the administration of carrageenan using a digital plethysmometer. Edema was evaluated by the difference in volume between the paw treated with carrageenan and the one that received only saline. The mean value of the anti-inflammatory activity of the animals treated with Green-anaconda fat was compared to control and to the values obtained for the animals treated with indomethacin.

RESULTS AND DISCUSSION

Ethnopharmacological survey

Twenty people from two associations of rural producers in Pimenteiras do Oeste (6 women and 14 men) participated in the survey, most of them being over 50 years of age. Within the group that answered the questionnaire, the use of animal fat proved to be a common practice, with 12 animals recorded as a source of medicinal fat (Table 1). The animals belonged to 4 groups: mammals, fish, reptiles and birds (Figure 2). Mammals and reptiles had the largest number of species used for medicinal purposes (4 species each), followed by fish (3 species). Taken together, the three classes concentrated almost 100% of the animals mentioned in the study. Regarding insects, an absence of citations was expected in the present zootherapeutic study on medicinal lard, since insects are not characteristic for the production and storage of fat, which basically occurs during the larval life stage, changing to protein and keratin formation during adulthood.

Among the vertebrates, mammals and reptiles cover the largest number of species used as zootherapics in the world, followed by birds, fish and amphibians, (Alves and Alves 2011; Alves et al. 2013e, 2020), similarly to the results presented here. In a review on the use of medicinal animals in Latin America (Alves and Alves 2011), mammals had the highest number of cited species (130), followed by birds (122), fish (110), reptiles (95), and insects (54), partially contrasting to the present study, where birds showed a low diversity of medicinal species. In the survey carried out by Silva (2010) in the municipality of Sumé - Paraíba, the largest number of citations involved mammals (16), insects (13), birds (12) and reptiles (9). According to Oliveira et al. (2010), the diversity of local fauna, and its availability and accessibility, directly influence the taxonomic groups and products used in traditional medicine in any region.

The use of the free listing in a questionnaire al-

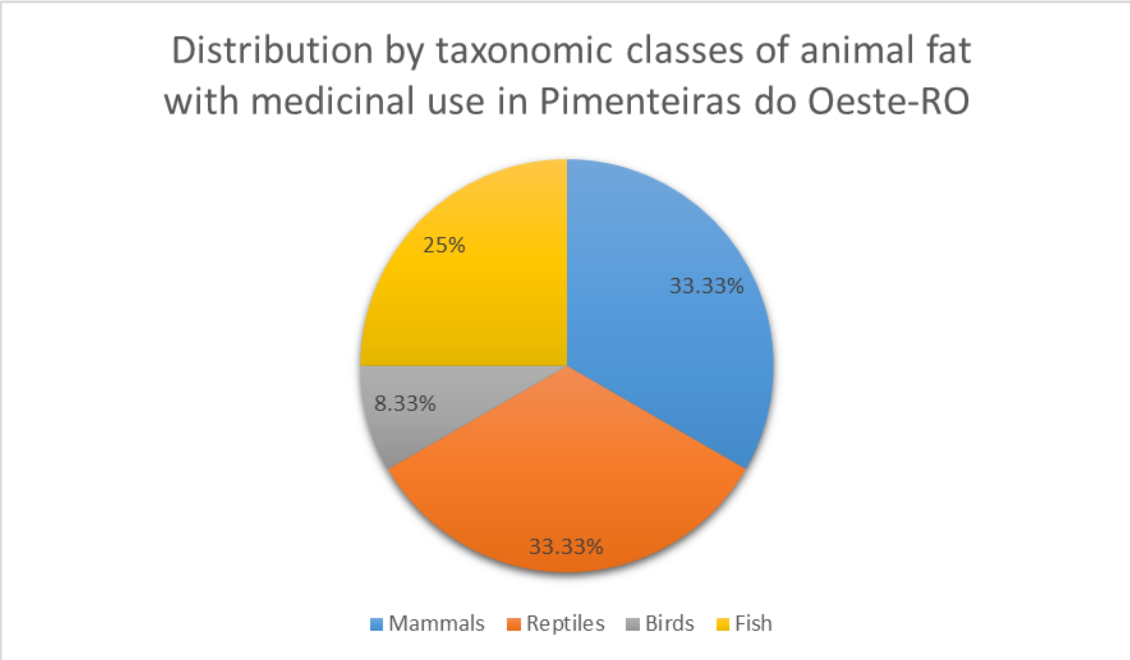


Figure 2. Distribution by taxonomic class of animal fat with medicinal use in Pimenteiras do Oeste, state of Rondônia, Brazil.

lows information about the main constituents of a domain by a group of people. In the free listing, people tend to quote terms in order of familiarity; the more they know, the more they will quote (Quinlan 2005). The most cited animal by the interviewees was the stingray (ray), with 16 citations, which had the highest salience index (SI = 0.554) (Table 1), demonstrating its enormous cultural importance among the research subjects. Respondents use its fat to treat bronchitis and asthma and as an expectorant. Silva (2008) mentions the use of stingray fat in the Amazon Region for rheumatism, skin burns and wounds. Although possibly referring to the species of the same genus, or at least a species of the same family (*Potamotrygon* spp.; Potamotrygonidae), the uses mentioned differed between the states of Amazonas (Rio Negro) and Rondônia (Rio Guaporé), Brazil. In the Tocantins river, the fat of different species of ray is used to asthma, cough, cold, earaches, skin burns and hernia (Begossi and Braga 1992).

The electric eel (*Electrophorus electricus*; Gmnotyformes) showed outstanding cultural importance (SI = 0.457), being indicated to treat ear pain and bronchitis, data that differ from the indications reported in other studies carried out in Northeast Brazil, such as treatment of strokes, torsions and insect bites (Alves and Alves 2011; Coutinho 2010), as well as in the state of Amazonas and Tocantins, where it was mentioned for the treatment of rheumatism, wound and stroke (Silva 2008). The third most mentioned fish species was the trahira (*Hoplias*: Characiformes), used for the treatment of earache, as also reported in other surveys from the Amazon region (Begossi and Ramires 2013; Silva 2008).

The second most mentioned animal in the present study was the Green-anaconda (13 citations), but without a high salience index (SI = 0.101). Possibly, this low SI is related to the prohibition of hunting wild animals, which intimidates the local populations, leading them to omit mention of their use. This also contributed to the fact that many local experts refused to participate in the study. Green-anaconda fat is used for the treatment of "joint pain" (rheumatism), "breakage" (disruption of bone structure), muscle strain, wounds and stroke. In a study conducted in the municipalities of Santa Isabel do Rio Negro and Barcelos, Rio Negro (state of Amazonas, Brazil), the Green-anaconda was the most cited animal, also being indicated for the treatment of inflammatory processes - muscle distension, disruption of bone structures, rheumatism and swelling, in addition to being used for respiratory problems, as well as for circulatory problems, such as stroke (Silva 2008). In other studies, fat from snakes such as Boa constrictor (Squamata), and rattlesnakes (*Crotalus atrox*; Squamata) has also been mentioned for the treatment of rheumatism, lung disease, thrombosis, boils, tuberculosis, sore throat, and osteoarthritis (Alves and Alves 2011; Coutinho 2010).

The second most cited reptile (10 citations) was the caiman (*Caiman yacare*; Crocodylia), with an SI of 0.299, third in the common consensus. Its fat is used for the treatment of bronchitis and cough, and as an expectorant. In the study by Silva (Silva 2008), the caiman was also the second most cited animal, with fat being used for the treatment of strokes, childhood epileptic seizures, rheumatism, and respiratory diseases (tuberculosis, flu and asthma). In another study, it was reported that the fat of the broad-snouted caiman (*Caiman latirostris*; Crocodylia) is used for the treatment of chilblains, stroke and snake bites (Marques 1995). Another important medicinal reptile used as zootherapic in Brasil are the lizards. Their uses can be quite different across the country. For example, in Pimenteira do Oeste they are used against earache and "full chest", but on an island in the coast of São Paulo they are used to treat snake bites, rheumatism, asthma, tetanus and skin thorns (Begossi 1992).

Regarding mammals, the capybara (*Hydrochaeris hydrochaeris*; Rodentia) was mentioned by 9 respondents (SI = 0.104), its fat being indicated for the treatment of respiratory problems (*e.g.*, flu, cough, bronchitis) and rheumatism, as mentioned in the review by Alves and Alves (2011) on the use of medicines of animal origin in Latin America and in the study by Silva (2008).

Among birds, chickens had 4 citations, with low cultural importance as sources of medicinal lard (SI 0.05), possibly due the vast animal biodiversity in the triple frontier region between the Amazon-Pantanal-Cerrado biomes. In addition, in the Brazilian Amazon Region, there is no tradition of raising domestic animals in local communities, which often feed on fishing and hunting. Similarly, in the study carried out in municipalities close to Rio Negro (state of Amazonas, Brazil), chickens as well as other domestic animals were sporadically mentioned, with less emphasis (Silva 2008). These data differ from those for the northeast region, where chickens had a large number of citations in the works of Alves et al. (2012) and Silva (2010). Castillo and Ladio (2019) investigated the therapeutic value of animals for rural farmers living in the central highlands of Chubut, Argentina. In this study, the chicken was one of the most cited animals. Fat is also used to treat burns and sore throat. In the review by Alves and Alves (2011) on the use of medicinal animals, chicken fat was used to treat respiratory diseases, like cough, asthma and bronquitis, in different regions of the country, similar to the indication reported in Pimenteira do Oeste for the treatment of influenza.

The use of fats extracted from wild cats is not so common, or could be less mentioned by the informants, as these animals are considered vulnerable and are on the red list of threatened animals. However, it is worth mentioning that in this research, the main medicinal uses of the jaguar (*Phantera onca*) are in accordance with the reports in the literature and were for bone pain, inflammation, musculoskeletal problems, arthritis and rheumatism (Alves et al. 2013b).

In the present study, the major therapeutic applications of medicinal fats were for the treatment of respiratory diseases (asthma, flu, bronchitis, cough), rheumatism, healing of dislocation and wounds, and ear pain (Table 1).

The routes of fat administration cited by the interviewees were: topical – application to the site with friction and massage, and oral - ingestion of a few drops of fat. These routes of administration were also mentioned by populations studied in different regions of Brazil (Alves and Rosa 2006; Souto et al. 2018), India (Borah and Prasad 2017), and Spain (Benítez 2011). According to Alves et al. (2013d), fats, body secretions and oils obtained from medicinal animals are ingested or used as an ointment. In some cases, animal fats can also be used as a vehicle, and not as an active ingredient.

Table 1. Animal fats used medicinally in Pimenteiras do Oeste, state of Rondônia: popular names, scientific names, number of citations, salience index, method of use and indications of the disease to be treated and conservation status.

Class	Common Name	Scientific name	NC	SI	Administration	Indications	Conservation Status
Fish	Stingray/Ray (Arraia)	*Potamotrygon* sp.[2,4]	16	0.554	Oral (2 to 7 drops / day), can be taken pure, mixed with coffee, or with honey	Bronchitis/asthma/expectorant	Least concern
	Trahira (Traíra)	*Hoplias malabaricus*[1,2,3,4]	5	0.285	Drip 2 drops in the ear	Earache	Least concern
	Electric eel (peixe elétrico)	*Electrophorus Electricus*[2]	3	0.457	Oral (take 2 to 3 drops/day and drip in the ear 2 to 3 drops/day	Bronchitis/earache	Least concern
Reptiles	Caiman (Jacarés)	*Paleosuchus* sp.[3] *Caiman* sp.[2,4]	10	0.299	Oral (2 to 6 drops or a teaspoon)	Bronchitis/expectorant/cough	Least concern
	Green-anaconda (Sucuri)	*Eunectes murinus*[2,3]	13	0.101	Pass the fat on the spot. Take 3 drops 3 times a day	Wound/rheumatism	Least concern
	Tortoises (Tartarugas)	*Podocnemis expansa*[2,3]	5	0.121	Pass the oil on the site	Wound/hydration	almost threatened
	Lizards (Lagartos)	*Ameiva ameiva*[2,3,4] *Tupinambis* sp.[2,3,4] *Tupinambis teguixin*[1] *Tropidurus oreadicus*[1]	2	0.138	Dripping in the ear/rubbing on the chest	Earache / full chest	Least concern
Mammals	Capybara (Capivara)	*Hydrochoerus hydrochaeris*[1,2,3]	9	0.104	Oral (3 drops/day) can be taken pure, mixed with coffee, or with honey	Flu /cough /bronchitis/rheumatism	Least concern
	Paca (Paca)	*Agouti paca*[2,3,4]	2	0.058	Smell/Take a few drops of the bile	Inflammation/infection	Least concern
	Jaguar (Onça)	*Panthera onca**[1,2,3,4] *Puma concolor**[1,2,3,4]	1	0.075	Apply to site	rheumatism	Vulnerable
	Sheep (Carneiro)	*Ovis aries*[5]	2	0.058	Apply tosite	Pain	without risk
Birds	Chicken (Galinha)	*Gallus gallus domesticus*[5]	4	0.05	Take a few drops	Flu	without risk

Legend: NC - Number of citations; SI - Salience Index; Survey of species in the study region (Pimenteira do Oeste – Rondonia) used for the "taxonomic clues". 1. PACUERA - Environmental Plan for Conservation and Use of the Surroundings of the Artificial Reservoir of the Cachimbo Alto Hydroelectric Plant; 2. PCH Cascata Chupinguaia Environmental Control Plan; 3. Environmental Control Plan for the Microcentral Hidrelétrica Veado Preto; 4. PACUERA - Environmental Plan for Conservation and Use of the Surroundings of the Artificial Reservoir of the PCH Cascata Chupinguaia; 5. List of animals from the Ministry of Agriculture, Livestock and Supply - MAPA.

* List of Endangered Brazilian Fauna Species - classified as vulnerable (VU).

Conservation Status of the animal species

According to the participants in the present study, due to the ban on hunting, the use of animals, or part of them, for the treatment of disease is not as common as in the past. The region where the interviews were conducted is on the border between Brazil and Bolivia, where IBAMA and the National Force act directly. The fear of the population of having a problem with the environmental agencies certainly made it difficult to obtain information for the present research. Some subjects even promptly denied participation, which was respected based on the right of refusal granted to possible participants. The semi clandestine or clandestine nature of the trade in medicinal animals is the main cause of the scarcity of information on zootherapics. Therefore, users and traders are normally unwilling to provide information due to most medicinal animals captured in the wild being protected by law, some of which appear on official lists of threatened species (Alves and Rosa 2010).

In most cases, animals are hunted or killed for food, for subsistence purposes or to defend against a threat and not for zootherapeutic use. In all cases, once the animal is killed, certain residues that would be discarded can be used for medicinal purposes, as is the case with fats for example. This occurs more often with some animals that are usually unpleasant, such as the stingray, which can sting a person causing a lot of pain and promote health disorders, such as infection and tissue necrosis. Green-anaconda, on the other hand, can feed on domestic animals and even humans (although it is not common), generating economic loss and life risk (Miranda 2016).

According to the "Red Book of Threatened Brazilian Fauna" (ICMBIO 2018), the wild species mentioned in the survey (Table 1) are of least concern, except for the jaguar (vulnerable) and the turtle (almost threatened). However, only for the domestic animals mentioned (sheep and chickens) there is no concern with conservation.

Ethnopharmacological studies focusing on the use of animals can be especially important to confirm or clarify their eventual therapeutic usefulness. These must be coupled with considerations on species conservation and public health policies (Alves and Albuquerque 2013). Since in the present study the use of Green-anaconda medicinal fat was one of the most prominently cited, the data on chemical composition and biological activity related to the main traditional uses raised will be approached in a broad ethnopharmacological perspective, considering the risks for the conservation of the species and for human health.

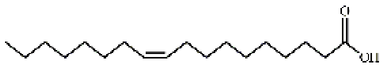
Although reptiles are among the animals most used as zootherapics in traditional medicine and among the most studied from a chemical and biological point of view, most studies were done for snake venom (Alves and Albuquerque 2013). Herpetofauna medicinal includes a total of 284 reptiles, with snakes (123 species) in greater number, followed by lizards (71), turtles (76) and crocodilians (14). However, due to the relatively small number of published studies on the subject, it is possible that the number of species of medicinal reptiles used is greater. Despite the intensive use for medicinal purposes, there is little information about the magnitude of the extraction and its impact on the conservation of the species involved, since its exploration requires a careful strategy that allows for sustainability of the species explored (Alves et al. 2008a, 2013e).

Fatty acid composition of *Eunectes murinus* fat

The chemical composition of *E. murinus* fat was determined by GC-MS. Analysis of the mass spectra permitted the identification of 10 constituents, which corresponded to 95.56% of the total methyl esters derived from the fatty acids present in the lard (Table 2). The predominant fatty acids were palmitic and stearic acids (saturated fatty acids), oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid). Unsaturated fatty acids are the major constituents and correspond to 66.31%, with oleic acid (37.35%) being the main component, followed by linoleic acid (26.04%). Saturated fatty acids represent 29.25% of the constituents, with palmitic acid being the major constituent (19.49%).

A study carried out in the market of Belém, Iquitos, in the Peruvian Amazon, permitted the identification of several animal oils widely used in traditional medicine for the treatment of rheumatism and inflammation. Of the six oils/fats analyzed, only two showed antimicrobial activity, but five exhibited anti-inflammatory activity by topical application (Schmeda-Hirschmann et al. 2014). The fatty acid profile of these six oils/fats was determined by CG-MS, mainly consisting of oleic, palmitic and linoleic acids. A similar composition was also observed in the study of the fat of different species of snakes bred in captivity and commercially acquired - rattlesnakes (*Crotalus atrox*; Squamata), rat-snakes (*Elaphe obsoleta*; Squamata), pythons (*Python regius*; Squamata), true boas (*Boa constrictor*; Squamata), and Gaboon-vipers (*Bitis gabonica*; Squamata). Oleic acid was the main component, followed by palmitic acid. The main fatty acids found in the fat of the Teiú lizard (*Tupinambis merianae*; Squamata), collected in the municipality of Crato, state of Ceará, Brazil, were oleic (41.11%), palmitic (31.47%), linoleic (15.89%) and stearic (11.53%) acids (Ferreira et al. 2009a).

Table 2. Fatty acids present in *Eunectes murinus* fat identified by gas chromatography coupled to mass spectrometry (GC/MS) in increasing order of retention time (RT).

Fatty acid	Chemical structure	**RT (min)**	**% Area**
Lauric acid (12:0)		7.744	0.35
Miristic acid (14:0)		9.282	1.53
Pentadecanoic acid (15:0)		10.424	0.33
Palmitoleic acid (16:1)		11.644	1.28
Palmitic acid (16:0)		11.998	19.49
Margaric acid (17:0)		14.218	0.37
Linoleic acid (18:2)		16.233	26.04
Oleic acid (18:1)		16.467	37.35
10-Octadecenoic acid (18:1)		16.646	1.64
Stearic acid (18:0)		17.369	7.18

Although the composition of fatty acids can vary in proportion depending on the species, feed, habitat and methods of fat extraction (McCue 2008; Tougan et al. 2018), most studies show that oleic, linoleic and palmitic acids are components often found in the highest proportions. These fatty acids show anti-inflammatory and healing activities. It has been demonstrated that oleic and linoleic acids have a pro-inflammatory effect indicated by the increase in the mass of the wound, in the content of proteins and DNA and in the migration of neutrophils to the wound tissue and the air sacs. These fatty acids can also stimulate neutrophils to release VEGF-α, a major regulator of angiogenesis and vasculogenesis, and cytokines IL-1β and CINC-2α/β (cytokine-induced neutrophil chemoattractors in inflammation). This can be an important mechanism for the effect of linoleic and oleic acids in accelerating the wound healing process (Pereira et al. 2008). N-hexadecanoic acid (palmitic acid) competitively inhibits phospholipase A2. Considering that phospholipase A2 inhibition may be one of the ways to control inflammation, its inhibition could explain the anti-inflammatory activity of palmitic acid (Aparna et al. 2012).

Triglyceride composition of *Eunectes murinus* fat

The triglyceride profile of *Eunectes murinus* fat was obtained by HPLC-ESIMSMS in positive mode. The mass spectra produced adducts with sodium as the base peak. From the analysis of the fragmentation patterns, it was possible to identify some of the triglycerides and to differentiate the structures of the isobaric isomers (Table 3).

The identification of triglycerides was sometimes difficult because some compounds with different fatty acid compositions may be isobaric. In such cases MSMS analysis helped the confirmation of the nature of the compounds. Twenty-four molecular triglyceride species were detected. The fatty acid composition obtained by GCMS analysis was taken as refer-

Table 3. Triglyceride composition of *Eunectes murinus* fat.

ECN	RT	MS[1] Precursor Ion (nominal mass)	Triglycerides (proposed compositions)	Formula (M+Na)$^+$
42	14.85	**875.7**	PLLn	$[C_{55}H_{96}O_6 + Na]^+$
42	15.04	**901.6**	LLL	$[C_{57}H_{98}O_6 + Na]^+$
44	15.50	**825.6**	OLaO / LaSL / MLP	$[C_{51}H_{94}O_6 + Na]^+$
44	15.84	**851.6**	PaOPa / OML / PPaL / PLnP	$[C_{53}H_{96}O_6 + Na]^+$
44	16.39	**877.6**	LPL	$[C_{55}H_{98}O_6 + Na]^+$
44	16.69	**903.7**	OLL	$[C_{53}H_{98}O_6 + Na]^+$
46	17.15	**827.6**	PMO / SLaO	$[C_{51}H_{96}O_6 + Na]^+$
46	17.63	**853.6**	PLP / MSL	$[C_{53}H_{98}O_6 + Na]^+$
46	17.91	**879.7**	LOP	$[C_{55}H_{100}O_6 + Na]^+$
46	18.18	**905.6**	OOL	$[C_{57}H_{102}O_6 + Na]^+$
46	18.55	**931.6**	LOEd / LEcL	$[C_{59}H_{104}O_6 + Na]^+$
48	19.01	**855.7**	POP / PPaS	$[C_{53}H_{100}O_6 + Na]+$
48	19.27	**881.7**	OPO	$[C_{55}H_{102}O_6 + Na]^+$
48	19.61	**907.7**	OOO	$[C_{57}H_{104}O_6 + Na]^+$
48	19.84	**933.6**	OLEc / OEdO	$[C_{59}H_{106}O_6 + Na]^+$
50	20.67	**883.6**	OPS	$[C_{55}H_{104}O_6 + Na]^+$
50	20.86	**909.7**	OOS / OPEc	$[C_{57}H_{104}O_6 + Na]^+$
50	21.12	**935.6**	LEO / OOEc / SLEc / ODP	$[C_{59}H_{108}O_6 + Na]^+$
52	22.15	**911.7**	Não identificado	$[C_{57}H_{106}O_6 + Na]^+$

ECN (Equivalent Carbon Number) RT (Retention Time)
Fatty acids: O-oleic; L-linoleic; Ln-linolenic; S-stearic; P-palmitic; Pa-palmitoleic; La-lauric; M-myristic; E-eicosanoid; Ec-eicosenoic; Ed-eicosadienoic; D-docosanoic.

ence for the identification. However, it was possible to observe that oleic, palmitic and linoleic acids were predominant in the composition of triglycerides. Among the triglycerides identified, 14 contained oleic acid, 11 contained palmitic acid, and 11 linoleic acid (Table 3). In a previous study carried out with reptiles, it was shown that triglycerides represent about 90% of stored lipids, with a predominance of oleic, palmitic and linoleic acids and small amounts of myristic, eicosadienoic and eicosatrienoic acids in their composition (Hadley and Christie 1974). The high concentration of oleic acid in relation to linoleic acid in triglycerides may reflect a variation in the diet of the animals, or that the animal has a highly active fatty acid desaturation system.

Pharmacological evaluation of *Eunectes murinus* fat

Before starting pharmacological tests to evaluate the anti-nociceptive and anti-inflammatory activity of *Eunectes murinus* fat, tests were carried out to assess whether the fat would cause behavioral and motor coordination changes in mice. This test makes it possible to assess whether *E. murinus* fat has any toxic effects and ensures that the subsequent tests would not be compromised by any of these effects. In the first 4 hours of observation after the treatments, the results showed that the *E. murinus* fat did not cause behavioral changes in the mice regarding ptosis, sedation, decreased touch response, loss of corneal reflex, ambulation, cycling, cleaning, elevation, or climbing. There were no changes in motor coordination in any of the conditions evaluated. No deaths were recorded within up to 72 h of observation. The list of evaluated behaviors and the motor coordination test data are presented in Additional File 1 (Additional File 1 and 2).

Anti-nociceptive and anti-inflammatory activities

Acetic acid-induced writhing test

The abdominal contortion test is based on the induction of a nociceptive response caused by peritoneal irritation after intraperitoneal administration of 0.8% acetic acid, such nociceptive effect being characterized by abdominal contortions followed by extensions of the hind limbs (Koster et al. 1959). The administration of fat at doses of 75 mg/kg, 150 mg/kg and 300 mg/kg produced a dose-dependent reduction in

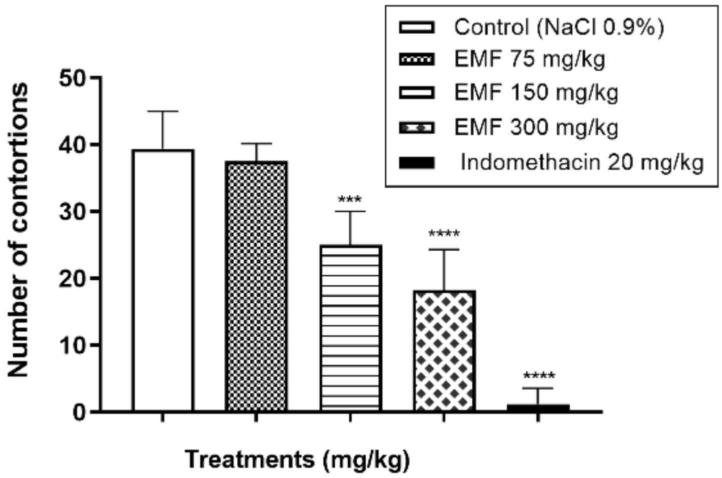


Figure 3. Antinociceptive effect of Eunectes murinus fat (EMF) on the number of abdominal contortions induced by 0.8% acetic acid in mice ($n = 6$). Results are expressed as mean ± SEM (ANOVA followed by Dunnet's test, $p < 0.05$). *** 0.001 **** 0.0001 Significant after analysis of variance.

the number of contortions when compared to the negative control (Figure 3), with the dose of 300 mg/kg inducing a more expressive reduction of 51.28%.

Formalin test

This test is used to measure the effectiveness of antinociceptive agents. An important characteristic of rodents is that the animals have two phases of nociceptive behavior, which seem to involve two different stimuli. The neurogenic phase (phase 1) starts immediately after the application of formalin, lasting 0 to 5 minutes, a period involving stimuli from local nociceptors. In the inflammatory phase (phase 2), there is a period of sensitization during which inflammation occurs (Dubuisson and Dennis 1977). This phase seems to have two components of sensitization, a peripheral one and a central one (Le Bars et al. 2001). This phase starts 15 to 30 minutes after the injection and last 20 to 40 minutes. The behavior observed in this phase is the result of the action of inflammatory mediators such as prostaglandins, serotonin, histamine and bradykinin. In this test, analgesic drugs with a mechanism of action on the central nervous system inhibit both phases, while drugs that act only on the peripheral system inhibit only the second phase (Oliveira et al. 2008). Treatment with Green-anaconda fat (75 mg/kg and 300 mg/kg) in the formalin test reduced the time spent licking or biting the paws by the animals in both the neurogenic (Figure 4A) and inflammatory (Figure 4B) phases compared to control animals that received only the vehicle. The licking or biting times of animals treated with Green-anaconda fat were similar to those of animals treated with indomethacin at a dose of 20 mg/kg (positive control), suggesting an anti-inflammatory activity of Green-anaconda fat.

Carrageenan-induced edema test

In the paw edema test, carrageenan was used to induce an inflammatory process in mice. Significant edema reduction was observed starting one hour after treatment with *E. murinus* fat (300 mg/kg) and continuing a long time. This effect was similar to that of indomethacin (20 mg /kg), the standard drug used in the test (Figure 5).

It has been shown that fat and oil from different animal species are used to treat a variety of diseases, but there are still few studies that confirm their pharmacological properties. In the review by Mishra et al. (2020) different therapeutic uses for animal fat and oil are mentioned, for example, the anti-inflammatory activity of bird oil and crocodile (which also has antimicrobial activity). Sheep fat that is indicated for cracking skin, burns, burns and skin infections. Different species of Python (*Python sebae sebae, Python molurus* and *Python tigris*) are used in Africa for the treatment of rheumatism, boils, keloids and broken bones (Ugwudike et al. 2013). Oil of shark, lizards and several other animals that are also used as medicines like those obtained from *Bubalus bubalis* (buffalo wax), *Ancer ancer* (goose), *Camellia japonica* (camel), *Sus scrofa* (pig), *Vulpes* sp. (Fox), *Capra rentalus* (goat), *Equus caballus* (horse wax), *Moschus moschiferus* (musk deer), *Ovis* (lamb), *Spalax leucodon ehrenbergi* (mole), worm, fish etc (Mishra et al. 2020).

Our results showed that *Eunectes murinus* fat has potential antinociceptive and anti-inflammatory ac-

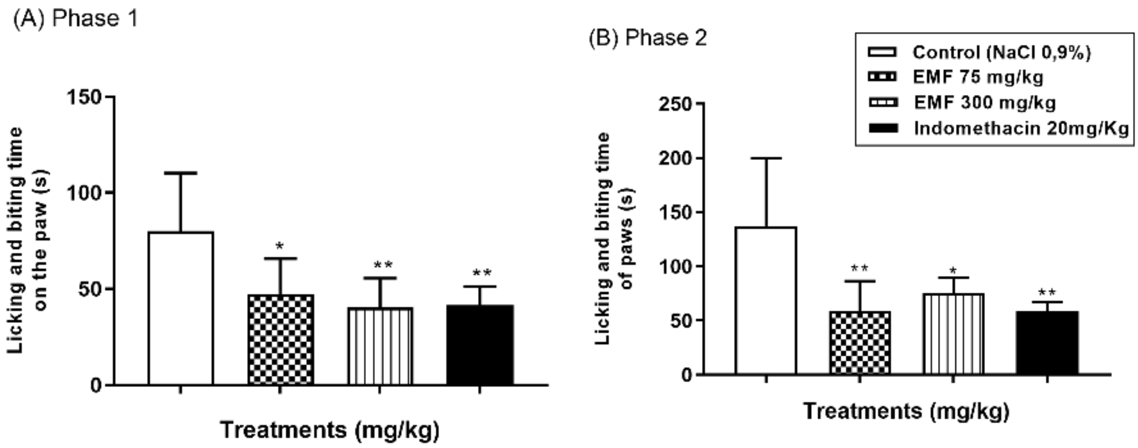


Figure 4. Anti-inflammatory effect of *Eunectes murinus* fat (EMF) on the time (s) spent licking and biting the paws in Phase 1 (A) and Phase 2 (B) of the formalin test applied to mice ($n = 6$). * 0.05 ** 0.01 Significant after analysis of variance (ANOVA) followed by the Dunnett test ($p < 0.05$).

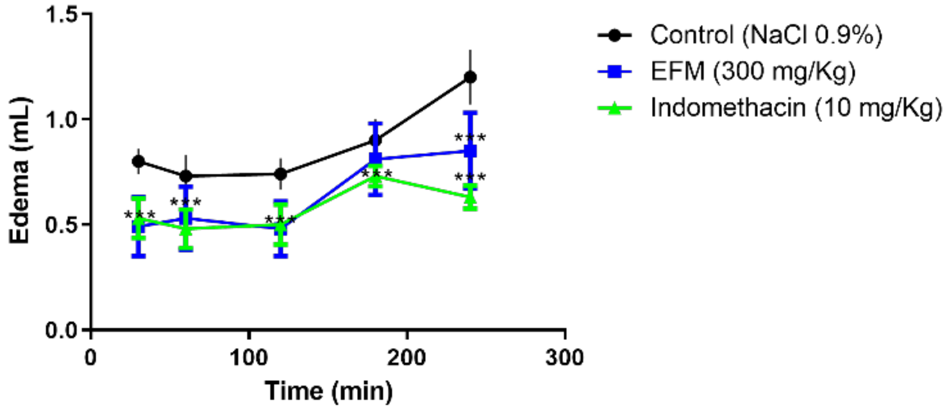


Figure 5. Effect of *Eunectes murinus* fat (EMF), 300 mg/kg. ip, on carrageenan-induced paw edema in mice. The asterisks represent the level of significance in relation to the control. *** $p < 0.001$. (Two-way ANOVA-Bonferroni).

tivities. As far as we know, these activities have not been previously described. Souza et al. (2017) demonstrated that Green-anaconda (*E. murinus*) fat had a more efficient healing action than the nitrofural ointment (industrialized), when evaluating the total epithelial healing time and the proportional daily reduction of a dorsal incision in mice. In a study carried out with *B. constrictor* fat in Nigeria, a dose-dependent inflammation-reducing action was demonstrated. However, significant anti-inflammatory activity was observed only at a dose of 400 mg/kg (Falodun et al. 2008).

In a study with the fat of lizards (*Tupinambis merianae*), rattlesnakes (*Crotalus durissus*), iguanas (*Iguana iguana)*, boa (*Boa constrictor*) and armadillos (*Euphractus sexcinctus*), better results were observed for the reduction of chronic inflammation than of acute inflammation in ear edema in mice (Ferreira et al. 2014). In the case of paw edema, only the lard of *C. durissus* and *B. constrictor* showed anti-inflammatory activity. Research with the lard of the Australian bird *Dromaius novaehollandiae* (Struthioniformes) also showed inflammatory inhibition in mice, with 70% inhibition (Yoganathan et al. 2003). In the study by Schmeda-Hirschmann et al. (2014) using lard of electric eels (*Electrophorus electricus)*, stingrays (*Potamotrygon motoro*), boa (*Boa constrictors*), yellow-footed tortoises (*Chelonoidis denticulata*; Testudines), black-caimans (*Melanosuchus niger*; Crocodylia) and botos (*Inia geoffrensis*; Cetacea) showed topic anti-inflammatory activity. In ear edema induced by arachidonic acid

(AA) and induced by tetradecanoylforbol acetate (TPA), stingray fat was the one with the higher anti-inflammatory activity. It should be noted that its composition differed from that of the other animals due to a higher content of palmitic acid (26.26%), followed by oleic acid (25.44%), while for the other animals the highest content was of oleic acid.

Alternatives to the use of medicinal fats from wild animals

One of the major concerns related to the use of animals by traditional medicine is the direct impacts caused by overexploitation, mainly in the case of multiple uses (in addition to the medicinal purpose), increasing the pressure on the natural populations of animal species, in addition to the indirect impacts caused by habitat degradation and climate change, leading to the decline or extinction of species in recent years. Thus, conservation measures must consider the medicinal use of wild animal species in conjunction with other causal factors and consider alternatives for the use of these species (Alves and Albuquerque 2013).

Another issue worth mentioning about zootherapy is the health risk associated with the consumption of products from wild animals, whether as food or medicine, which can facilitate the transmission of severe and generalized zoonoses (Van Vliet et al. 2017). In a review by Alves et al. (2013e) cases of severe extra-gastrointestinal infections by *Salmonella arizona* were attributed to the ingestion of a folk medicine prepared from the rattlesnake. The review also describes a case of human sparganosis in Vietnam, caused (among others) by the ingestion of plerocercoid larvae in raw or undercooked meat of reptiles (or amphibians). In addition, in the case of animals at the top of the food chain, there may be a large accumulation of heavy metals, especially in animal fat deposits. In the Amazon, activities such as illegal mining increase this risk, mainly through artisanal gold mining, as demonstrated in the study by Albuquerque et al. (2020), with fish species from the Lower Amazon.

Although the *E. murinus* fat used by the population is subjected to a heating process for extraction and purification, which can reduce the microbial load of possible pathogens, there will always be a risk of contamination from handling to application. The risk is higher in cases of ingestion than in topical use (the most common in the population). These factors encourage the use of vegetable oils as substitutes for wild animals, including Green-anaconda. However, this substitution may not be easy for populations to understand. According to the study by Ferreira et al. (2016), replacing wild animal products with plants and domestic animals is difficult due to cultural heritage. The replacement proposal must consider the history of the use of medicinal animals, seeking to understand the reasons why they did not use the vegetables that are easier to obtain and do not result in punishments, as in the case of illegal hunting (Alves and Albuquerque 2013; Ferreira et al. 2016). However, the medical systems found in cities tend to absorb elements from other cultural systems due to intercultural adaptations. The populations of cities that benefit from traditional products more easily accept the incorporation of other products, even if they are not part of the same *corpus* (Ferreira et al. 2016) which could facilitate the substitution of oils of animal origin by vegetables.

In general, plant species are more studied than animal species, they are legal products, with increasing importance in the bioeconomy and with the risks mentioned above minimized. There are many data in the literature demonstrating that they are indispensable in the human diet as suppliers of essential fatty acids and fat-soluble bioactive compounds. The pharmacological effects of vegetable oils have already been well described and are related to their influence on the inflammatory cascade, their anti-inflammatory and curative properties, the reduction of oxidative stress, neuroprotection and cardiovascular protection (Ahmad et al. 2019; Alkhalaf et al. 2019; Ferreira et al. 2012; Morais et al. 2017; Nagy and Tiuca 2017; Di Pasquale 2009; Zhao et al. 2005).

Sunflower oil (*Helianthus annuus* L.; Asterales) is one of the most widely used vegetable oils in the world as a food and for medicinal applications. Many studies on its pharmacological properties are available in the literature (Al-Snafi 2018), with the oil being widely used in topical formulations for the treatment of skin wounds and burns. The major fatty acid components of sunflower oil are oleic and linoleic acids (Pérez-Vich et al. 1998; Zanoschi et al. 1991). Other widely used oils are those of andiroba (*Carapa guianensis* Aubl.; Sapindales), with reported anti-inflammatory properties (Silva 2018) and of copaiba (*Copaifera* spp.; Fabales). Copaiba oil is widely used in folk medicine as a healing and local anti-inflammatory, being administered orally and by topical application in natura or in ointments. Although extremely useful as anti-inflammatories, the copaiba and andiroba oils are characterized by the presence of bioactive terpenes, greatly differing from other vegetable oils rich in fatty acids (Maciel et al. 2002; Montes et al. 2009). The oil of *pequi* (*Caryocar brasiliense* A.St.-Hil.; Malpighiales), a fruit widely consumed in the Brazilian Cerrado, is rich in unsaturated fatty acids, with oleic acids standing out with a content of approximately 56% and palmitic acids with a content of 35%, followed by smaller quantities of myristic, stearic palmitoleic, linoleic and linolenic fatty acids (De Lima et al. 2007). Its use is indicated by the traditional population for the treatment of respiratory diseases, gastric ulcers, muscular and rheumatic pain, due to its anti-inflammatory and healing effects (Matos 2007). According to the study by Bezerra et al. (2015), pequi oil plays a beneficial role in wound healing, promoting faster tissue repair and reducing the inflammatory characteristics.

The use of Amazonian vegetable oils could be a more sustainable and easily accessible alternative to animal fat used in the treatment of wounds and as an anti-inflammatory by Amazonian communities (Serra et al. 2019). The challenge is to find the best vegetable oils that are easy to obtain and prepare, with a fatty acid composition similar to that of animal fats. Considering the Amazonian biodiversity, this process will require extensive scientific research in order to find the most sustainable products, as well as wide interaction with the community in order to disseminate the knowledge acquired and offer alternatives to the use of animal fats.

In order to propose Amazonian vegetable oils as possible alternatives to the medicinal use of *Eunectes murinus* fat, a literature search was carried out to identify the composition of fatty acids of different species. Table 4 shows the fatty acid composition of 24 plant species in the Amazon Region with the potential for treating inflammation and healing due to their high fatty acid content. Among the oils of the plant species reviewed, the pulp oil of *tucumã* (*Astrocaryum vulgare* Mart.; Arecales) was the most similar to the Green-anaconda oil, mainly containing closely similar proportions of oleic, linoleic, palmitic and stearic fatty acids (Bora et al. 2009). A study of A. vulgare M. pulp oil collected in French Guiana showed that this oil would have the potential to prevent various inflammatory diseases, an activity that could be attributed to its high oleic acid content (Bony et al. 2012). Of the species shown in Table 4, *açaí* oil (*Euterpe oleracea* Mart.; Arecales) was the one with the highest oleic acid content, 75.79%. Despite having a slightly different composition compared to Green-anaconda oil, with 21.26% palmitic acid and 2.94% palmitoleic acid and the absence of linoleic acid, it also has anti-inflammatory and antinociceptive activity (Favacho et al. 2011). It is interesting to note that the main components of the oils of the species listed in Table 4 are oleic, linoleic and palmitic acids. Most oils contain a low amount of linolenic acid (ω-3), less than 1%, with *cupuaçu* oil (*Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum.; Malvales) being the only one that contains a significant amount, 17.98% (Rogez et al. 2004).

Considering that the substitution of medicines obtained from wild animals by derivatives of domestic animals may be a favorable option from the point of view of conservation, mainly for products of animal origin marketed in cities, the by-products of the meat and meat products industry would be an alternative (Ferreira et al. 2016). For the specific case of the anti-inflammatory activity of Green-anaconda fat, we can mention the use of sheep fat and fish oil as possible alternatives for the population of Pimenteiras do Oeste, since these species are part of the list of animals used by this population for medical purposes (Table 1). It has been shown that sheep fat (*Ovis aries*; Artiodactyla) can be used as an anti-inflammatory agent during the first phase of the healing process, effectively contributing to the acceleration of the tissue repair process (Martins et al. 2011) and to prevent cartilage damage (Cimen et al. 2017), also representing a possible alternative to the use of an wild animal. Fish oils, which are rich in long-chain ω-3 polyunsaturated fatty acids, can also be considered. There is already plenty of evidence about the benefits of a fish-rich diet against inflammatory diseases, particularly rheumatoid arthritis and osteoarthritis, dry eyes, and macular degeneration (Barkoot et al. 2018; Cleland et al. 2003; Weitz et al. 2010).

The investigation of the influence of fatty acids present in animal and vegetable oils used for the treatment of diseases is necessary in view of the vast Amazonian biodiversity and the large number of traditional populations that use these resources. Knowledge of this therapeutic arsenal and the ethnopharmacological understanding of these uses can contribute to the search for the best ways to conserve species.

Table 4. Fatty acid composition of Amazonian plant species.

Plant species	Fatty acids (%)												
	Saturated												
	C6:0	**C8:0**	**C10:0**	**C12:0**	**C13:0**	**C14:0**	**C15:0**	**C16:0**	**C17:0**	**C18:0**	**C20:0**	**C22:0**	**C24:0**
Açaí (Euterpe oleracea) (Favacho et al. 2011)	-	-	-	-	-	-	-	21.26	-	-	-	-	-
Araticum (Annona crassiflora) (Lopes et al. 2012)	3.17	2.32	0.34	0.38	-	1.97	-	10.78	-	6.83	0.54	0.13	-
Babaçu (Orbinya speciosa) (da Ponte et al. 2017)	0.2	13.7	14.7	57.5	-	9.6	-	2.3	-	0.4	-	-	-
Bacaba-semente(Oenocarpus distichus) (Mambrim and Barrera-Arellano 1997)	-	-	-	-	-	-	-	24.7	-	3.5	0.5	-	-
Bacaba-polpa (Oenocarpus distichus) (Cunha et al. 2019; Mambrim and Barrera-Arellano 1997)	-	-	-	-	-	-	-	23.4	-	3.2	0.2	-	-
Bacuri (Platonia insignis) (Rogez et al. 2004)	-	-	-	-	-	2.16	-	37.68	-	6.76	-	-	-
Castanha-do-Pará(Bertholletia excelsa) (de Araújo et al. 2007)	-	-	-	-	-	0.05	-	13.85	-	10.25	-	-	-
Coco(Cocos nucifera) (da Ponte et al. 2017)	-	1.9	3.5	38.6	-	22.2	-	13.6	-	3.1	-	-	-
Coquinho-Azedo(Butia capitata) (Lopes et al. 2012)	6.73	1.42	0.41	3.64	-	3.55	-	18.81	-	2.00	0.16	0.07	-
Cubiu (Solanum sessiliflorum) (Marx et al. 1998)	-	-	-	0.18	-	0.01	1.12	1.00	-	0.09	-	-	0.01
Cumaru (Dipteryx odorata) (de Araújo et al. 2007)	-	-	-	-	-	-	-	6.60	-	4.50	-	4.33	3.93
Cupuaçu (Theobroma randiflorum) (Rogez et al. 2004)	-	-	-	-	-	0.12	-	55.22	-	3.12	-	-	-
Inaja (Maximiliana maripa) (Da Cruz Rodrigues et al. 2010)	-	-	-	3.70	-	7.60	-	20.10	-	3.50	3.20	-	-
Macaúba-polpa (Acrocomia intrumescens) Ref. 9	-	0.05	0.03	0.24	-	0.16	-	19.56	-	2.48	0.22	0.02	-
Macaúba-semente (Acrocomia intrumescens) (Venancio et al. 2016)	-	3.96	3.35	41.95	-	9.39	-	7.86	-	2.01	0.12	0.07	-
Mari (Poraqueiba paraenses) (Da Cruz Rodrigues et al. 2010)	-	-	-	-	-	-	-	20.80	0.10	6.40	1.10	-	-
Muru muru (Astrocaryum murumuru) (Mambrim and Barrera-Arellano 1997)	-	2.7	2.0	51.6	-	25.8	-	6.0	-	2.9	-	0.1	-
Patawa (Oenocarpus bataua) (Da Cruz Rodrigues et al. 2010)	-	-	-	-	-	0.10	0.30	13.30	0.10	4.10	0.60	-	-
Pataua (Jessenia batana) (Mambrim and Barrera-Arellano 1997)	-	-	-	-	-	-	-	11.3	-	3.9	0.1	-	-

Pequi-polpa (Caryocar brasiliense) (De Lima et al. 2007)	-	-	-	0.04	-	0.13	-	35.17	-	2.25	0.23	-	-
Pequi-semente (Caryocar brasiliense) (De Lima et al. 2007)	-	-	-	-	-	0.46	-	43.76	-	2.54	0.20	-	-
Piquiá (Caryocar villosum) (Marx et al. 1997)	-	-	-	-	-	33.5	-	0.59	-	-	-	-	-
Pupunha(Bactris gasipaes) (de Araújo et al. 2007)	-	1.90	2.24	27.25	-	22.47	-	13.52	-	3.97	-	-	-
Sapucaia (Lecythis pisonis) (de Araújo et al. 2007)	-	-	-	-	-	-	-	12.7	-	5.1	-	-	-
Sumaúma (Ceiba pentandra) (de Araújo et al. 2007)	-	-	-	-	-	0.1	-	22.4	-	3.4	-	-	-
Tucumã-polpa (Astrocaryum vulgare) (Bora et al. 2009)	-	-	-	-	0.18	-	-	13.86	0.27	9.80	0.82	0.18	0.18
Tucumã-semente (Astrocaryum vulgare) (Bora et al. 2009)	-	1.93	1.95	50.16	0.06	24.44	-	6.21	-	2.34	-	0.10	-
Uricuri-polpa (Attalea phallerata) (de Araújo et al. 2007)	-	-	-	7.3	-	11.0	-	21.9	-	-	-	-	-
Uricuri-semente (Attalea phallerata) (de Araújo et al. 2007)	-	-	-	36.4	-	16.6	-	10.2	-	-	-	-	-

Plant species	Fatty acids (%)						
	Monounsaturated					Polyunsaturated	
	C16:1n9	C17:1n10	C18:1n9	C18:1n7	C 20:1n11	C18:2n9,12	C18:3n9,12,15
Açaí (Euterpe oleracea) (Favacho et al. 2011)	2.94	-	75.79	-	-	-	-
Araticum (Annona crassiflora) (Lopes et al. 2012)	0.23	-	66.90	0.24	0.68	1.55	2.83
Babaçu (Orbinya speciosa) (da Ponte et al. 2017)	-	-	1.4	-	-	0.6	-
Bacaba-semente(Oenocarpus distichus) (Mambrim and Barrera-Arellano 1997)	1.1	-	49.7	-	-	18.7	1.8
Bacaba-polpa (Oenocarpus distichus) (Cunha et al. 2019; Mambrim and Barrera-Arellano 1997)	0.8	-	57.1	-	-	14.0	0.6
Bacuri (Platonia insignis) (Rogez et al. 2004)	0.46	-	46.72	-	-	2.72	3.18
Castanha-do-Pará(Bertholletia excelsa) (de Araújo et al. 2007)	0.45	-	30.50	-	-	44.90	-
Coco(Cocos nucifera) (da Ponte et al. 2017)	-	-	13.2	-	-	2.72	3.18
Coquinho-Azedo(Butia capitata) (Lopes et al. 2012)	0.48	-	48.07	-	0.10	11.00	3.13
Cubiu (Solanum sessiliflorum) (Marx et al. 1998)	0.08	-	1.53	-	-	0.16	0.13
Cumaru (Dipteryx odorata) (de Araújo et al. 2007)	-	-	47.35	-	-	21.56	5.50
Cupuaçu (Theobroma randiflorum) (Rogez et al. 2004)	0.56	-	18.8	-	-	3.08	17.98
Inaja (Maximiliana maripa) (Da Cruz Rodrigues et al. 2010)	0.10	-	52.40	-	-	8.90	0.20
Macaúba-polpa (Acrocomia intrumescens) Ref. 9	2.61	-	55.43	-	-	12.73	0.88
Macaúba-semente (Acrocomia intrumescens) (Venancio et al. 2016)	0.10	-	28.33	-	-	3.89	0.15
Mari (Poraqueiba paraenses) (Da Cruz Rodrigues et al. 2010)	0.30	-	67.60	-	-	3.40	0.10
Muru muru (Astrocaryum murumuru) (Mambrim and Barrera-Arellano 1997)	-	-	5.7	-	-	3.0	0.1
Patawa (Oenocarpus bataua) (Da Cruz Rodrigues et al. 2010)	0.70	-	76.70	-	-	3.90	0.10
Pataua (Jessenia batana) (Mambrim and Barrera-Arellano 1997)	0.6	-	77.7	-	-	4.9	0.5
Pequi-polpa (Caryocar brasiliense) (De Lima et al. 2007)	1.03	-	55.87	1.90	0.27	1.53	0.45

Pequi-semente (Caryocar brasiliense) (De Lima et al. 2007)	1.23	-	43.59	1.38	0.04	5.51	0.09
Piquiá (Caryocar villosum) (Marx et al. 1997)	0.10	-	29.50	-	-	0.52	-
Pupunha(Bactris gasipaes) (de Araújo et al. 2007)	-	-	20.9	-	-	-	-
Sapucaia (Lecythis pisonis) (de Araújo et al. 2007)	0.3	-	33.0	-	-	48.6	0.3
Sumaúma (Ceiba pentandra) (de Araújo et al. 2007)	0.3	-	22.1	-	-	35.0	-
Tucumã-polpa (Astrocaryum vulgare) (Bora et al. 2009)	0.17	0.06	46.81	-	0.20	26.13	0.93
Tucumã-semente (Astrocaryum vulgare) (Bora et al. 2009)	0.06	-	8.36	-	-	4.16	-
Uricuri-polpa (Attalea phallerata) (de Araújo et al. 2007)	-	-	47.5	-	-	-	-
Uricuri-semente (Attalea phallerata) (de Araújo et al. 2007)	-	-	20.9	-	-	-	-

CONCLUSION

The record of zootherapeutic knowledge of the population of Pimenteiras do Oeste, state of Rondônia, Brazil about the use of animal fat in the treatment of diseases revealed that the fat of different animals can be used for the same purpose, especially for diseases of the respiratory system and musculoskeletal disorders such as rheumatism and joint inflammation. In the present study, we demonstrated that *Eunectes murinus* fat is mainly composed of oleic (37.53%), linoleic (26.04%) and palmitic (19.49%) acids. Pharmacological analyses showed that this fat has antinociceptive and anti-inflammatory activity. As an alternative to the use of fats from wild animals, vegetable oils with a high fatty acid content could be proposed, such as tucumã (*Astrocaryum vulgare*) pulp oil, with a composition like that of Green-anaconda, and açaí (*Euterpe oleracea*) which contains 75.79% oleic acid. However, complementary studies must be carried out to identify and characterize the anti-inflammatory potential of each fatty acid, as well as its mechanism of action. This could contribute to the indication of new sources of fatty acids for the treatment of diseases and, thus, to prevent the use of endangered species of wild animals.

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DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

CONTRIBUTION STATEMENT

Conceived the presented idea: DRO, LWT
Carried out the experiment: CFA, CVRPF, AFS, MLR
Carried out data analysis: CFA, DRO, PP, CVRPF, AFS, MLR, AJRS, LWT
Wrote the first draft of the manuscript: CFA, DRO, LWT
Review and final writing of the manuscript: CFA, DRO, LWT
Supervision: DRO, LWT

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Additional Files

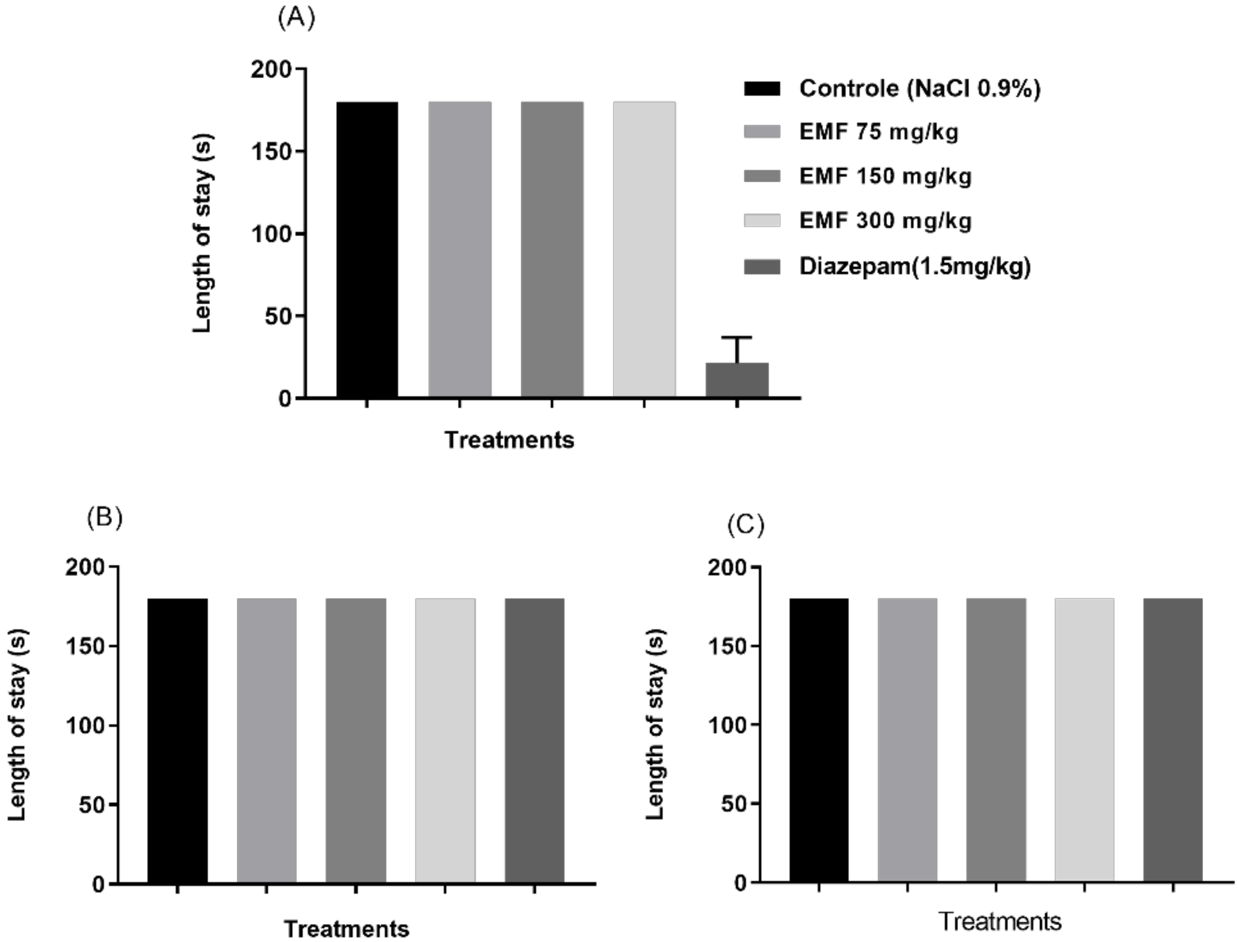
Add File 1. Symptoms and responses evaluated in pharmacological behavioral screening of male mice (*Mus musculus*) treated with *Eunectes murinus* fat (100, 200 and 500 mg/kg).

PHARMACOLOGICAL ACTIVITY	Quantification of effects (unfilled) no effect, (-) decreased effect, (+) present effect, (++) intense effect				
	up to 30‘	1h	2h	3h	4h
1 – Central Nervous System					
a - Stimulant					
Hyperactivity					
Irritability					
Aggressiveness					
Tremors					
Convulsions					
Piloerection					
Intense vibrissae movement					
Others					
b - Depressor					
Hypnosis					
Ptosis					
Sedation					
Anesthesia					
Ataxia					
Straightening reflex					
Catatonia					
Analgesia					
Decreased touch response					
Loss of corneal reflex					
Loss of atrial reflex					
c - Other behaviors					
Ambulation					
Excessive yawning					
Cleaning					
Rise					
Climb					
Vocalize					
Shaking your head					
Abdominal contortions					
Abduction of hind legs					
Cycling					
Stereotype					
2 - Autonomous NS*					
Diarrhea					
Cold					
Increased defecation					
Forced breathing					
Tearing					
Urination					
Salivation					
Cyanosis					
Muscle tone					
Force to grab					
3 - Deaths					

Additional notes: The table is empty because none of the symptoms /responses were observed in any of the evaluated times, but it shows all the evaluations performed. *NS -Nervous System

Route rod

The data presented in the graphs below show that the Eunectes murinus fat does not alter the animals' motor coordination when submitted to the rod route test (Add File 2 A, B and C).



Add File 2. Effect of the *E. murinus* fat on the motor coordination of mice ($n = 6$) on the rotating bar at 30 min (A), 60 min (B) and 120 min (C). Results are expressed as mean ± EPM (ANOVA followed by Dunnet's test ($p < 0.05$).

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