

Full Length Research Paper

Antibacterial, total phenols, antioxidant, and fatty acids of the lyophilized body fat of *Podocnemis expansa* (Schweigger, 1812) from farm in Acre State, Brazil

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Received 4 June, 2020; Accepted 15 June, 2020

***Podocnemis expansa* (Podocnemididae) is a chelonian of suborder Pleurodira and is popularly known as Amazon turtle. The animal's body fat was removed from the farm in Xapuri city in Acre State, Brazil. The fat was lyophilized and the oil was extracted by the Soxhlet method using hexane, as a solvent. The fatty acids majority, identified and quantified by gas chromatography-flame ionization detector, were: Arachidonic (25.2%), oleic (ω 9, 17.6%), palmitic (ω 7, 13.5%), stearic (11.9%) and linoleic (ω 6, 9.5%). These, are essential for human health and can be helpful in the treatment of cardiovascular diseases, arthritis, psoriasis, improving symptoms of depression, Alzheimer's disease, among others therapies and pathologies. The total phenolic compounds (377.52%) and the antioxidant activity (88.64%) determined in this investigation indicate its high potential in preventing lower risks of cardiovascular and other chronic diseases. Therefore, the results demonstrate the pharmacological, therapeutic and nutraceutical potential of the main chemical constituents of these substances for prospecting and developing biologically active and functional molecules to expand scientific investigations and the commercial chemical industry.**

Key words: Chelonians, fatty acids, biodiversity, chromatography, Amazon turtle.

INTRODUCTION

Brazil has 36 species of turtles, including 29 freshwater species, 5 marine species and 2 terrestrial species. Of

these, 17 species can be found in Amazon and are distributed in 5 families: 2 belonging to the suborder

Pleurodira (Podocnemididae and Chelidae) and 3 belonging to suborder Cryptodira (Kinosternidae, Geoemydidae and Testudinidae).

Among these species, *Podocnemis expansa* stands out, which has been extensively investigated in different areas of knowledge and lines of research (Piceli et al., 2016; Da Costa Araújo et al., 2019; Mesak et al., 2019; Bing et al., 2019; Carmargo et al., 2020) and its zootherapeutic, biological, nutraceutical and functional use, as well as other specimens of the biodiversity of exotic, native, wild and global domestic fauna, have also been extensively studied (Williams et al., 2016; Barboza et al., 2016; Borah and Prasad, 2017; de Queiroz Dias et al., 2018; Jugli et al., 2019). In addition to these studies, Alves et al. (2017), in a critical review of trends in the medicinal use of edible wild vertebrates in Brazil, point to the need to understand the multiplicity and trends in the therapeutic uses of Brazilian vertebrates and indicate that reptiles stand out for presenting greater plasticity in the treatment of multiple health conditions.

The Amazon turtle *P. expansa* (Schweigger, 1812) is a chelonian of the suborder Pleurodira, Podocnemididae family, popularly known as the Amazon turtle and is largest freshwater chelonian in South America, reaching up to 107.0 cm in length (Carvalho, 2012; Oliveira-Júnior et al., 2009). *P. expansa* has a wide distribution from the eastern Andes to the Orinoco basin and drains from the Amazon River in Colombia, Venezuela, Guyana, Northeastern Peru, Eastern Ecuador, Northern Bolivia and Northern Brazil. In Brazil, this occurs in the states of Amazonas (AM), Pará (PA), Roraima (RR), Rondônia (RO) (Fachín-Terán et al., 1995), Acre (AC), Amapá (AP), Mato Grosso (MT), Goiás (GO) (Bataus, 1998; Vogt, 2008) and Tocantins (TO) (Bonach, 2003), in the basins of the Amazonas and Tocantins-Araguaia rivers and basins of the North Atlantic.

According to Vogt (2001), these animals suffer from the direct action of the man who consumes his eggs and the meat of adults. It is estimated that approximately 50 million eggs were collected annually in hydrographic basins near Tefé-AM (Bates, 1892). According to Rodrigues (2005), in addition to food consumption, turtles are also affected by the indirect impact of human action, as their nesting environments are altered or destroyed, through the removal of gallery forests, silting, pollution and poisoning. The practice was regulated in Brazil since 1988 (Brazil, 1988) and, currently, it is guided by IBAMA No. 07 (Brazil, 2015), which establishes and standardizes the categories of use, management of wild fauna in captivity and defines, within IBAMA, the authorization procedures for the established categories.

According to Ferreira et al. (2009), freshwater turtles

are included among many animals where your meat, blood, body fat, eggs and their shells are sought as a zootherapeutic. Oil, lard and meat became products, valuable goods, sources of income, presenting other benefits (Ferrarini, 1980; Gilmore, 1986; IBAMA, 1989). According to Ferrarini (1980) and Ferreira (1786), the chelonian body fat was used for cosmetics and soap making, and the most sought-after species were the amazonian tortoise *P. expansa* and the tracajá *Podocnemis unifilis*, the fat was still used for cooking and to preserve meat and chicks for later consumption.

Thus, considering the importance of investigating the use of fixed oils of reptile origin and its respective by-products from breeding in captivity duly authorized and in line with Brazilian legislation, this research aims to characterize the oils of *P. expansa* bodily landmarks and contribute to the knowledge of Brazilian Amazonian biodiversity, adding value to the management and management of the commercial activity of aquatic turtles in the Amazon.

MATERIALS AND METHODS

Sampling location and obtaining

The research was approved by the Animal Ethics Commission of the Federal University of Acre - UFAC under No. 23107.018920/2018-29, registered in the Biodiversity Authorization and Information System, SISBIO with No. 60093-2 and in National System of Genetic Heritage Management and Associated Traditional Knowledge - SisGen under number A85C938.

P. expansa specimen, amazon turtle was identified by the authors following Ferrara et al. (2017), animals of this species have a head containing a large interparietal shield, presence of interorbital groove, adult females have an ontogenetic variation, which makes the head dark brown with advancing age, as well as the carapace is flattened and wider in the posterior region and has brown, gray or olive green color and the adults have plastron with yellow, cream or brown color.

The research samples were obtained through a donation by the owner of the Três Meninas farm, at 125 km of BR-317, in Xapuri, Acre, Brazil (10°0'56.783 "S 67°9'46.76" W), Figure 1, as well as signing a letter of consent to transport the animals. The specimens were captured in May 2018, then they were accommodated in metal cage and transported in a body car to the slaughter and material collection site. The linear measurements of the total length (CT), carapace width (LC), plastron length (CP) and plastron width (LC) were determined, all in cm, with the aid of a caliper (precision degree 0.1 cm), the total individual weight was also determined, and these measures were used to estimate the age of the animals and to evaluate the body development, according to Morselli et al. (2016).

The animals were slaughtered in the farm following the Normative Resolution No. 37, of February 15, 2018 of CONCEA (Brazil, 2018), according to Santos et al. (2008) exceptionally, it is accepted with restricting the stunning by rapid cooling (-2°C) until

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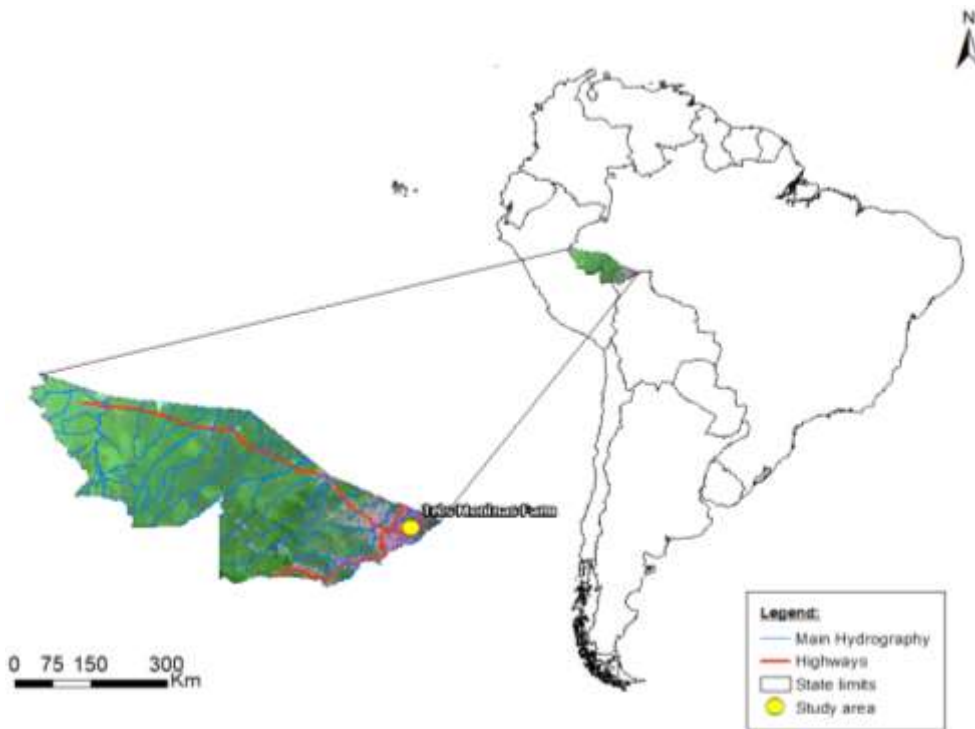


Figure 1. Location map of the study area where Três Meninas Farm is located in Xapuri, 2018, localized state of Acre, Brazil.

the loss of orientation and opercular movements, followed by maintenance in immersion in ice water for defined periods, according to the protocols compatible by age and size, then the animals were beheaded. After slaughter and boning, the meat and body fat were separated and frozen at a temperature of -4°C , packed in plastic bags and a styrofoam box for 2 h, and then in an electrolux horizontal freezer model H222 (Elextrolux of Brazil, Manaus, Brazil) at a temperature of -4°C for three days.

The analyzed material was obtained through the lard removed from one of the animals classified as younger than the others, for having a weight of 9 kg and carapace length of 40 cm, carapace width 48 cm. According to Gaspar and Silva (2009), the fat deposit in turtle meat is found mainly between the muscles, which are recognized in the present study as body fat. This body fat was transported to the Chemistry Laboratory of the Federal University of Roraima - UFRR, by the service of transportation of biological material from a national aviation agency, for chemical and biological analyzes, with the proper authorizations foreseen in the law of access to genetic resources in national territory.

Lyophilization and extraction of oil from body fat

The equipment used in drying the material was a freeze dryer manufactured by LIO TOP model L101 (LIOBRAS, São Carlos, Brazil). This equipment has a laboratory scale, with a cooling chamber that works at a temperature below -50°C coupled to a vacuum pump, it has an acrylic chamber with stainless steel shelf with three round shelves where samples are stored for the lyophilization process. The body fat was frozen in a -50°C freezer for 48 h. Then, it was inserted into the lyophilizer with a temperature pre-stabilized at -56°C immediately closed and placed in vacuum. After, awaited temperature and pressure stability with reading

below $900\ \mu\text{Hg}$, temperature below -50°C and maintained for a period of 72 h. So, after 72 h in the lyophilizer, the body fat lost all water and we called lyophilized body fat from *P. expansa* (LBFPE). The LBFPE was crushed in a domestic blender and sieved between 20 and 40 Mesh. The extraction of oil from LBFPE was performed by the Soxhlet method, using hexane, as a solvent, called OLBPE - fixed oil from lyophilized body fat from *P. expansa*.

Total phenolic compounds in LBFPE

The determination of total phenolic compounds (TPC) was made, according to the proposal by Wolfe et al. (2003) modified where methanolic extracts were prepared from the extraction of 4.0 g of LBFPE with 35 mL of 80% (v/v) methanol acidified with 0.5% (v/v) hydrochloric acid, in falcon tubes and subsequently, were placed in a water bath with boiling water for 30 min, the supernatant being separated and over the remaining material, $2 \times 35\ \text{mL}$ were added again and treated in the same conditions as the previous one. Subsequently, the fractions were added and centrifuged with centrifuge model Q222T108 (QUIMIS, Diadema, Brazil) at 3400 rpm for 30 min. The samples were placed in amber glasses and were stored in a refrigerator at 2°C until the moment of analysis. According to Singleton et al. (1999), to make the readings, gallic acid (GA) was used as a reference standard, using the Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

The method involves the reduction of the Folin-Ciocalteu reagent by the phenolic compounds present in the sample with the formation of the blue complex. An aliquot (0.1 mL) of the extracts was transferred to a 10 mL test tube and 3 mL of distilled water was added followed by 0.25 mL of Folin-Ciocalteu reagent. The mixture was left to stand for 3 min and finally 2 mL of the 7.5% (w/v) sodium carbonate solution (Na_2CO_3) was added. A blank test was

also used under the same conditions, so that 0.1 mL of distilled water was used in place of the samples. They were incubated in a bath at 37°C for half an hour and the readings were made in a spectrophotometer at 765 nm, being expressed the quantification of total phenols in the extracts as mg GA100 g⁻¹ sample.

Determination of LBFPE's antioxidant activity

The determination of antioxidant activity in the different extracts of LBFPE was evaluated by the extinction absorption method of the radical 1,1-diphenyl-2-picryl hydrazil (DDPH). The DDPH method was developed by means of molecular absorption spectrophotometry in the visible ultraviolet measured at 515 nm (Miranda and Fraga, 2006) in the Shimadzu model UV-1800 equipment (Shimadzu, Kyoto, Japan). The technique consists of preparing an incubation of 300 µL of the methanolic extract with 2.7 mL of the 0.06 mm DPPH solution, leaving it for 60 min in incubation and dark to be able to read it later at 515 nm. The calibration curve was made by preparing diluted standards from the mother concentration of 60 µM in the range between 10 and 50 µM and at the same time the blank was made with methanol.

Fatty acids profile from LBFPE

The fatty acids were analyzed indirectly with the identification of methyl esters corresponding to the oil (0.2 g) OLBPE extracted from LBFPE and saponified by reflux for 30 min. In a solution of sodium hydroxide and methanol, described by Hartman and Lago (1973). Subsequently, the oil was dissolved in a cryogenic tube with a capacity of 2 mL, ~ 1 mg of the oil in 100 µL of a solution of sodium hydroxide (NaOH) 1 mol L⁻¹ in ethanol/water (95:5). After vortexing for 10 s, the oil was hydrolyzed in a microwave oven Electrolux model MT030 (Electrolux of Brazil, Manaus, Brazil), at 30% power for 4 min, and left to cool. After cooling, 400 µL of 20% hydrochloric acid (HCl), 1 g of NaCl and 600 µL of ethyl acetate were added and vortexed for 10 s and rest for 5 min. An aliquot of 300 µL of the organic layer was removed, placed in a microcentrifuge tube and dried by evaporation, thus obtaining free fatty acids.

The free fatty acids were methylated with 100 µL BF₃ (boron-methanol trifluoride solution) by heating for 10 min in a water bath at 60°C, extracted in 500 µL of hexane and fractionated by Gas Chromatography.

The samples were submitted to HP7820A Gas Chromatograph (Agilent Technologies, Santa Clara, United States) equipped with flame ionization detector, and the EZ Chrom Elite Compact program (Agilent Technologies, Santa Clara, USA) was used for data acquisition. A Supelcowax-10 column (30 m × 0.2 mm × 0.2 µm) (Supelco, Pennsylvania, USA) with temperature gradient was used: 150°C, 1 min, 10°C min⁻¹ to 240°C; injector (1/20 split) at 250°C and detector at 250°C. Hydrogen was used as the carrier gas (6 mL min⁻¹) and the injection volume of 1 µL of the mobile phase. Peak identification was done by comparison with FAME C₁₄-C₂₂ methyl fatty acid standards (Supelco cat. No 18917).

Antimicrobial activity of fixed oil from LBFPE

Antimicrobial activity bioassay was based on the methodology carried out by Wiegand et al. (2008), bacteria pre inoculum were prepared, in which the microorganisms were transferred from the culture medium where they were stored, to test tubes containing 3.0 mL of BHI culture medium. Then, the tubes were incubated in an oven at 37.5°C for 24 to 48 h.

The sample was previously solubilized in dimethyl sulfoxide (DMSO) at a concentration of 12.5 mg mL⁻¹. From this solution, an aliquot of 40 µL was removed and added to 960 µL of BHI culture

medium, obtaining the working solution at concentration of 500 µg mL⁻¹.

The bioassays were carried out in 96 microwell plates, in triplicate, adding 100 µL of the working solution at a concentration of 500 µg mL⁻¹ in three wells. Then, 100 µL of the inoculum of the standardized microorganism was added to each well. Each inoculum was prepared adding 500 µL of the corresponding pre-inoculum to test tubes containing sterile distilled water. The concentration was adjusted to transmittance between 74 and 75% at a wavelength of 600 nm, corresponding to the 0.5 scale of McFarland standard turbidity, that is, 10⁵ CFU mL⁻¹.

Four controls were carried out: microorganism growth control; the blank, inoculum was replaced with sterile distilled water; the positive control, working solution was replaced by a commercial antibiotic (ampicillin), and the sterility control of the culture medium, containing 100 µL of BHI culture medium and 100 µL of sterile distilled water. The microplates were incubated in an oven at 37.5°C and after 24 h the test was read in a plate reader at 492 nm. Results were calculated as percentual inhibition using the Equation 1 (Almeida et al., 2017):

$$\% \text{ inhibition} = [100 - ((SA - CSA) / (AH - AM))] \times 100 \quad (1)$$

where SA = sample absorbance; CSA = control sample absorbance; AH = absorbance in the microorganism control; AM = absorbance of the culture medium control.

The samples were tested against the following microorganisms: *Staphylococcus aureus*: ATCC 29212 (Gram-positive bacteria), *Bacillus cereus*: ATCC 11778 (Gram-positive bacteria), *Escherichia coli*: ATCC 25922 (Gram-negative bacteria), and *Salmonella typhimurium*: ATCC 14028 (Gram-negative bacteria).

RESULTS AND DISCUSSION

LBFPE and OLBPE samples

Lyophilization is the drying method that minimizes the loss of sensory characteristics and nutritional qualities of food, according to Pessoa and Teixeira (2012). Lyophilized body fat *P. expansa* (LBFPE), after being crushed in a domestic blender model LN32 (ARNO, Manaus, Brazil) and sieved with granulometric screen model BERT010 (BERTEL, Caieiras, Brazil) between 20 and 40 Mesh, is a light cream powder, as shown in Figure 2 was obtained from Amazon turtle body fat (*P. expansa*) *in natura* of 3,790 mg LBFPE 100 g⁻¹ and extraction using hexane as solvent obtained 2,188 mg fixed oil (OLBPE) 100 g⁻¹ in LBFPE.

According to Felloews (2000), this is the best method so that the product to be preserved has characteristics at the end of the process that would not be possible with other techniques (Felloews, 2000). Koroishi (2005) validated this information regarding the use of the orange juice process. The same for Ribeiro et al. (2019), studying the lyophilization and physical-chemical characterization of a blend composed of kiwi (*Actinidia deliciosa*) and passion (*Passiflora edulis*) fruits.

Determination of total phenolic in LBFPE

Quantification of the content of total phenolic compounds



Figure 2. Lyophilized body fat *P. expansa* species.

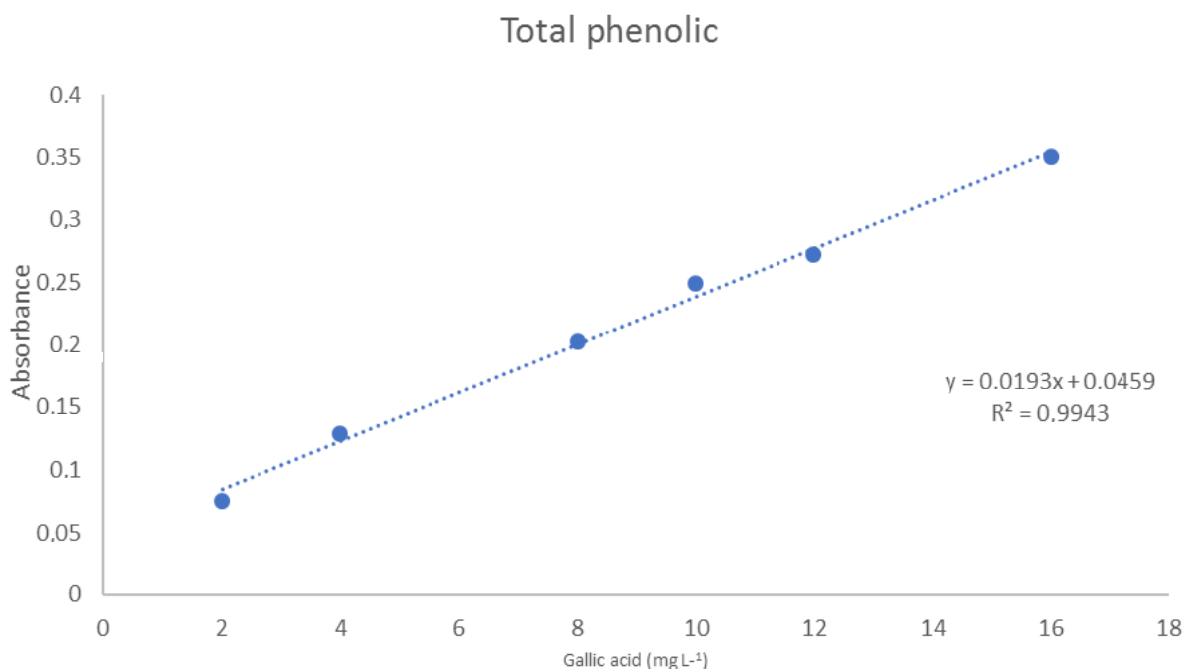


Figure 3. Linear regression analysis from the reaction between gallic acid and Folin-Ciocalteu reagent referring to lyophilized body fat from *P. expansa*, municipality of Xapuri - Acre, Brazil.

through the study of triplicates was made from the reaction determined between the reagent Folin-Ciocalteu and gallic acid according to Da Silva et al. (2013), the linear regression analysis was determined with the linear equation $y = 0.00193x + 0.0459$ with correlation coefficient r equal to 0.9943. The range of gallic acid concentration was 2 to 18 mg L⁻¹. The total phenol content of the methanol extract was expressed in mg equivalent of gallic acid per 100 g of the oil (mg EAG 100 g⁻¹), as shown in Figure 3.

Total phenol content found in the crude methanolic

extract from lyophilized body fat of turtle (*P. expansa*) was 377.52 mg GAE 100 g⁻¹. According to Morais et al. (2019) values between 100 and 500 mg of GAE 100 g⁻¹ indicate average concentration of phenols, so the body fat oil of *P. expansa* can be considered an average source of total phenolic compounds. Tan and Chang (2017) when evaluating the total phenol content in 8 different types of food with concentration values of the phenolic constituents varying between 2.04 and 201.53 mg GAE 100 g⁻¹, found answers in the suppression of the activity of α -amylase, α -glucosidase and lipase. Thus,

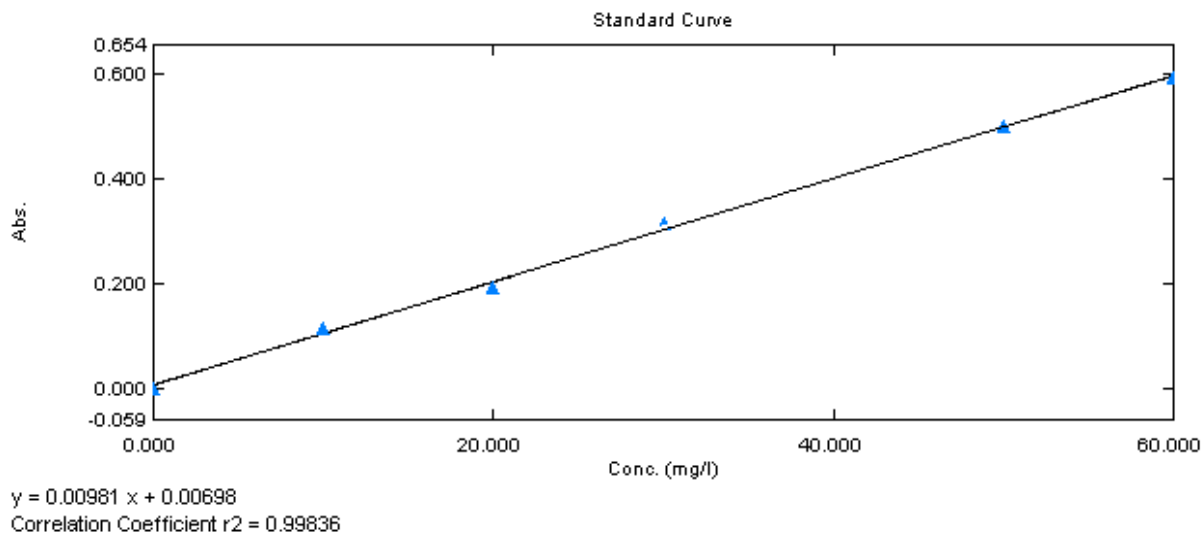


Figure 4. Linear regression of methanolic extract of the lyophilized body fat of *P. expansa*, from the municipality of Xapuri - Acre, Brazil.

from an analysis of the total levels of phenol found in this study and its widely known biological role, these results point to and indicate the need for more robust investigations into a possible therapeutic potential for the treatment of gastric, digestive, pancreatic and diabetes diseases. Another relevant aspect, considering the nutraceutical importance of these bioactive compounds, is the direction of studies applied to the food industry within the scope of the potential of the product explored in this study as a functional food. According to Oliveira and Bastos (2011), phenolic compounds are abundant in fruits, vegetables and foods derived from them, such compounds are associated with reduced risk of cardiovascular diseases, cancer and other chronic diseases.

Determination of LBFPE's antioxidant activity

The lyophilized body fat of *P. expansa* showed 88.64% of antioxidant activity. According to the DPPH calibration curve, the corresponding concentration is between 10 and 20 mg L⁻¹, that is, high antioxidant capacity (Figure 4).

According to Alves et al. (2007), the consumption of 1,1-diphenyl-2-picryl hydrazil (DPPH) by the sample is directly proportional to its antioxidant activity, therefore, from the result obtained regarding *P. expansa* body fat, the analysis was determined of linear regression, with the equation of the line $y = 0.00981x + 0.00698$ with the correlation coefficient r equal to 0.99836. The concentration ranged from 0 to 60 mg L⁻¹.

According to Azevedo et al. (2014), phenolic compounds can have positive effects on various

diseases derived from oxidative stress, such as cancer, due to the high antioxidant potential. To date, we have not found studies evaluating the antioxidant capacity of *P. expansa* oil, but the present result demonstrated a high level of antioxidant potential in the fat of this Amazonian chelonian that has been reported for centuries for use in popular medicine (Alves and Santana, 2008; Moura-Santos and Campos, 2011; Barros et al., 2012).

In this context and in view of the aforementioned, with the objective of improving knowledge about species of aquatic turtle species, studies have been widely carried out at a phylogenetic and molecular level (Viana et al., 2017) with this reptile of great ecological importance in the Brazilian Legal Amazon. The soft-shelled-turtle (*Pelodiscus sinensis*) is a commercially important aquatic species rich in collagen. Investigations conducted by the researchers Xu et al. (2016), demonstrated the antioxidant activity with the elimination of free radicals in approximately 98.9% from a new collagen extracted from *P. sinensis*. More recent studies have also demonstrated that antioxidant enzymes, peroxiredoxins, isolated soft-shelled-turtle have been shown to influence the defense against oxidative and modular stress in the immunohistochemical response *in vitro* (Zhang et al., 2019).

Fatty acid profile in OLBPE

Chromatographic profile of the major fatty acids from the analyzed lyophilized body fat of *P. expansa*, from the municipality of Xapuri - Acre, Brazil is as shown in Figure 5 and Table 1, chromatogram and fatty acids, respectively.

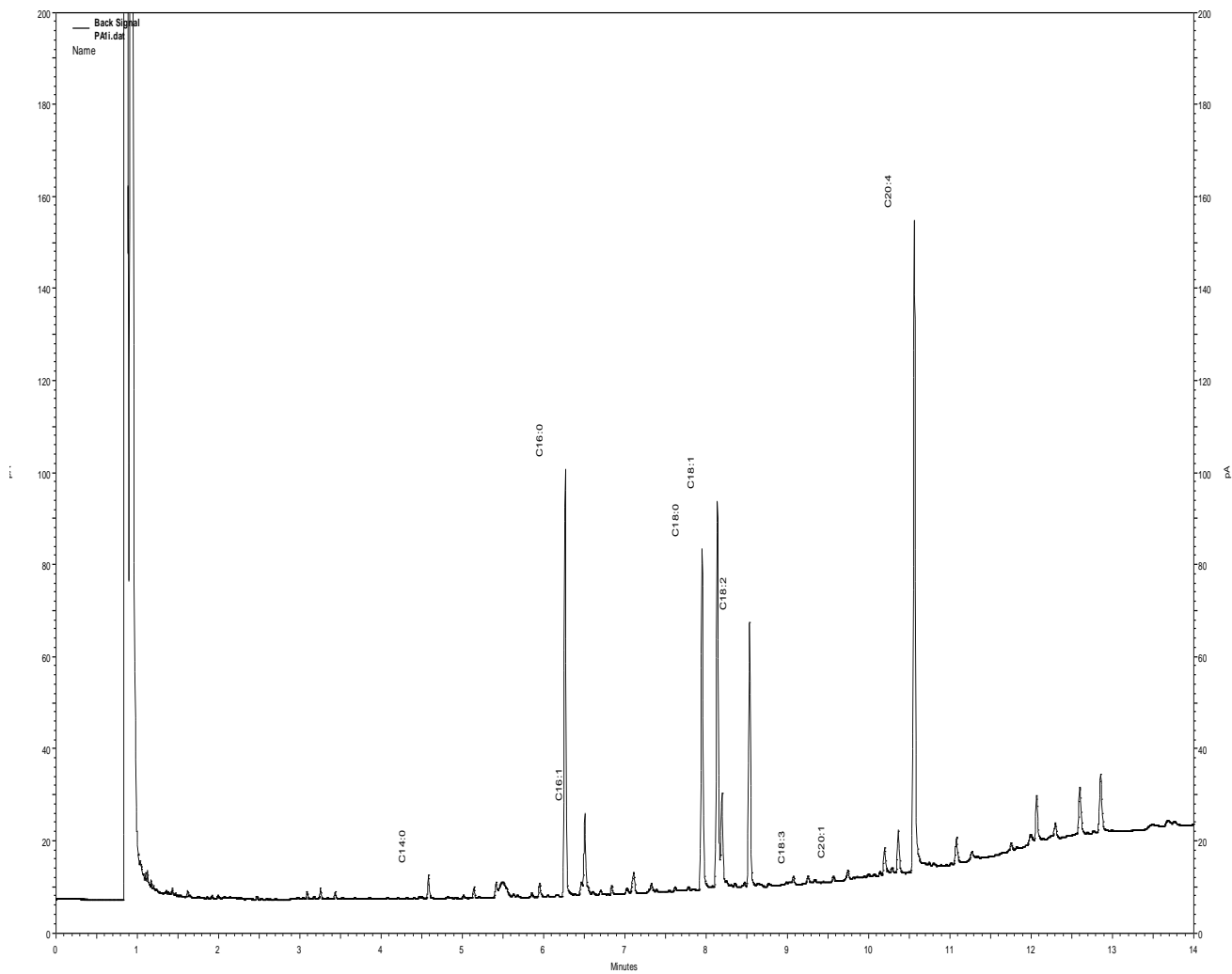


Figure 5. Chromatogram of fatty acids from lyophilized body fat of *P. expansa*.

Table 1. Majority fatty acids in lyophilized body fat of *P. expansa* from breeding Três Meninas, Xapuri - Acre, Brazil.

Fatty acids	Carbon number	Retencion time (min)	%
Myristic acid	C 14:0	4.588	0.7
Palmitic acid	C 16:0	6.271	13.5
Palmitoleic acid (ω 7)	C 16:1	6.512	3.7
Stearic acid	C 18:0	7.955	11.9
Oleic acid (ω 9)	C 18:1	8.143	17.6
Linoleic acid (ω 6)	C 18:2	8.539	9.5
Linolenic acid (ω 3)	C 18:3	9.255	0.5
Gadoleic acid	C 20:1	9.749	0.4
Arachidonic acid	C 20:4	10.567	25.2
SFA ¹			26.1
UFA ²			73.9
Others			17.0
Total acids			100

¹UFA - unsaturated fatty acids and ²SFA - saturated fatty acids.

Table 2. Antimicrobial activity of OLBFPE (250 µg mL⁻¹) from LBFPE and the standard ampicillin (250 µg mL⁻¹).

Sample	Microorganisms and % inhibition of growth			
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>B. cereus</i>
OLBFPE	17.80 ± 2.70	23.49 ± 1.47	8.68 ± 1.96	32.41 ± 1.55
Ampicilin	97.87 ± 0.52	95.39 ± 0.21	95.86 ± 0.62	90.37 ± 1.26

OLBFPE - Fixed oil from lyophilized body fat of *P. expansa*; LBFPE - lyophilized body fat of *P. expansa*.

A high arachidonic acid content was identified in sample from Xapuri - Acre, probably due to the age or stage of development of the animal (9 kg). This statement is based on the comparison with the animals studied by Gaspar and Silva (2009), which presented arachidonic acid (11.13%). This animal was raised in artificial ponds, and fed on vegetables and legumes, and supplemented with fish food. According to Rodrigues and Moura (2007), when performing comparative bromatological analyzes between free-living and captive animals, significant differences in meat composition, are noted, so the lack of balanced feed specific to the species may be responsible for the chemical difference found in meat of confined animals.

According to Araújo et al. (2013), the knowledge about the nutrition of turtles is still in its initial phase, and it is necessary to obtain a lot of information in order to properly feed these animals in captivity, and thus explore the zootechnical potential of the species in the best possible way. Using different regions than the one analyzed in the present work, Scarlato and Gaspar (2007), showed a chemical composition of the hull with very similar concentrations of myristic (3.59%), oleic (3.59%) and oleic (C 18:1) (3.59%) acids. However, according to the authors, the levels of palmitic (40.0%) and stearic (22.7%) acids, acids common in freshwater animals, presented divergent values between the body fat in the slaughtered animals and the fat present in their hooves.

Dias et al. (2013) studied the body fat of *Phrynops geoffroanus* and found a compound of different fatty acids from the animal in the present study. Polyunsaturated fatty acids act in several physiological and metabolic processes, and are important in child nutrition due to their rapid increase in the brain during the first year of life (Kus-Yamashita et al., 2016). The values obtained in the present study, regarding the percentage of unsaturated fatty acids in the fat from *P. expansa* (73.9%), are lower than those also presented by Dias et al. (2013) for the *P. geoffroanus* species (84.63%). These compounds have several applications related to the low incidence of autoimmune and inflammatory diseases such as asthma, psoriasis and type 1 diabetes (Cecconello and Marques, 2014).

One of the main fatty acids found in the youngest animal in the present study, arachidonic acid has

characteristics such as polyunsaturated fatty acid prevalent in the central nervous system (Kus-Yamashita et al., 2016). Both arachidonic and linoleic are essential fatty acids of the omega-6 family (Kus-Yamashita et al., 2016), they are important to supply the organic demand, in sufficient amounts in the diet, and studies indicate that their use brings benefits for human health, preventing cardiovascular diseases, colon cancer, immunological diseases and favoring brain and retinal development (Silva et al., 2007).

Antimicrobial activity of the oil from LBFPE

From antimicrobial activity experiments, it was possible to verify the activity of oil derived from the body fat of *P. expansa* (OLBFPE) against some bacteria and fungi. The inhibition values reached varied for Gram-positive bacteria between 17.80% for *S. aureus* and 32.41% for *B. cereus*, and for Gram-negative bacteria, between 8.6% for *S. typhimurium* and 23.49% for *E. coli* (Table 2).

The antibacterial properties of various fatty acids have been studied as shown by Nobre et al. (2002), Agoramoorthy et al. (2007) and Dias et al. (2013). According to Zheng et al. (2005), the mechanism of antimicrobial activity is related to the action of unsaturated fatty acids in the synthesis of endogenous microbial fatty acids.

Conclusion

The lyophilized body fat, of the studied species *P. expansa* from the municipality of Xapuri - Acre, Brazilian amazon can be considered an average source of total phenolic compounds, an antioxidant potential, however, low activity against the microorganisms tested. In this fat, the ω3, ω6 and ω9 acids were identified, which are essential for human health and can be helpful in the treatment of cardiovascular diseases, arthritis, psoriasis, improving symptoms of depression, Alzheimer's disease, among others, as well as other acids pharmacological interests. Thus, they are preliminary results, therefore, it is necessary to deepen our research in the search for isolation and purification of natural bioactive products and to contribute to the knowledge of Amazonian biodiversity,

adding value to the activity of commercial breeding of Amazonian aquatic turtles.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This Doctoral Program of the Biodiversity and Biotechnology Network of the Legal Amazon at the Federal University of Acre – UFAC was sponsored by CAPES. Also, Fazenda Três Meninas (Xapuri-Acre) is appreciated for donation of the specimen used in this research.

REFERENCES

- Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Brazilian Journal of Microbiology* 38(4):739-742.
- Almeida SP, Mozombite DMS, Melo Filho AA, Laranjeira AGA, Reis de Melo ACG, Martins RMG, Takahashi JA, Ferraz VP, Duarte OT, Tadei WP (2017). Chemical composition, antiacetylcholinesterase inhibition and toxicity activities of essential oil from *Hyptis dilatata* (Lamiaceae) flowers. *Journal of Medicinal Plants Research* 11(14):275-283.
- Alves RRN, Santana GG (2008). Use and commercialization of *Podocnemis expansa* (Schweiger 1812) (Testudines: Podocnemididae) for medicinal purposes in two communities in North of Brazil. *Journal of Ethnobiology and Ethnomedicine* 4(1):1-6.
- Alves RRN, Oliveira PR, Medeiros MFT (2017). Trends in medicinal uses of edible wild vertebrates in Brazil. *Evidence-Based Complementary and Alternative Medicine*. <https://doi.org/10.1155/2017/4901329>
- Araújo JC, Gadelha ES, Palha MDC, Vieira-Rosa P (2013). Minerals and vitamins for chelonians in captivity. *Publications in Veterinary Medicine and Animal Science* 7(5):312-419.
- Azevedo LFP, Faria TSA, Pessanha FF, Araujo MF, Lemos GCS (2014). Phytochemical screening and antioxidant activity of *Costusspicatus* (Jacq.) Sw. *Brazilian Journal of Medicinal Plants* 16(2):209-215.
- Barboza RRD, Lopes SF, Souto WM, Fernandes-Ferreira H, Alves RR (2016). The role of game mammals as bushmeat in the Caatinga, Northeast Brazil. *Ecology and Society* 21(2):2.
- Barros FB, Varela SA, Pereira HM, Vicente L (2012). Medicinal use of fauna by a traditional community in the Brazilian Amazonia. *Journal of Ethnobiology and Ethnomedicine* 8(37):1-19.
- Bataus YSL (1998). Estimation of population parameters of *Podocnemis expansa* (Amazonian tortoise) in Crixás - Açu River (GO) using biometric data. *Dissertation (Master in Ecology)*. Federal University of Goiás. 54p.
- Bates HW (1892). *The naturalist on the river Amazon*. Londres: John Murray. 395p.
- Bing J, You Z, Zheng Q, Tang J, Ran Y, Huang G (2019). Biological and genomic analyses of a clinical isolate of *Yarrowia galli* from China. *Current Genetics* 66:549-559.
- Bonach K (2003). Incubation time and hatch rate of Amazon turtles (*Podocnemis expansa*) under different nest managements. *Dissertation (Master in Conservation Biology)*. University of Sao Paulo. 74p.
- Borah MP, Prasad SB (2017). Ethnozoological study of animals based medicine used by traditional healers and indigenous inhabitants in the adjoining areas of Gibbon Wildlife Sanctuary, Assam, India. *Journal of Ethnobiology and Ethnomedicine* 13(39):1-13.
- Brazil. Brazilian Institute for Forestry Development (1988). Ordinance No. 133, of May 5, 1988, regulates the creation of the Amazon turtle in Pará State.
- Brazil. Brazilian Institute of the Environment and Renewable Resources (IBAMA) (2015). Normative Instruction IBAMA nº 07, of April 30, 2015. Institutes and regulates the categories of use and management of wild fauna in captivity, and defines, within IBAMA, the authorization procedures for the established categories.
- Brazil. Ministry of Science, Technology, Innovations and Communications. National Council for Animal Experimentation-CONCEA. (2018). Normative Resolution N.º. 37, of February 15, 2018. Euthanasia Practice Guideline of the National Council for Animal Experimentation Control.
- Carmargo F, Abrão NB, Queiroz TD, Arnhold E, Araújo LB, Martins DB (2020). Serum biochemistry of the Arrau turtle (*Podocnemis expansa*): Frozen analyte stability and the effects of long-term storage. *Veterinary Clinical Pathology* 49(1):42-47.
- Carvalho JL (2012). Ecotoxicology of mercury (Hg) in *Podocnemis expansa* (SCHWEIGGER, 1812) (Testudines, Podocnemididae) in turtle nests on the Monte Cristo tablelands on the margin of the tapajós river, Pará, Brazil. *Dissertation (Master in Natural Resources of Amazonia)*. Federal University of Western Pará. 75p.
- Cecconello KB, Marques FO (2014). Effects of omega-3 fatty acids in inflammatory bowel diseases: A literature review. *Science and Health Magazine* 7(2):98-108.
- Da Silva ECC, Muniz MP, Nunomura RCS, Nunomura SM, Zilse GAC (2013). Phenolic constituents and antioxidant activity of geopropolis from two species of stingless Amazonian bees. *Quimica Nova* 36(5):628-633.
- da Costa Araújo AP, Lima VS, de Andrade Vieira JE, Mesak C, Malafaia G (2019). First report on the mutagenicity and cytotoxicity of ZnO nanoparticles in reptiles. *Chemosphere* 235:556-564.
- de Queiroz Dias D, Sales DL, Andrade JC, da Silva ARP, Tintino SR, de Moraes Oliveira-Tintino CD, Ferreira FS (2018). Body fat modulated activity of *Gallus gallus domesticus* Linnaeus (1758) and *Meleagris gallopavo* Linnaeus (1758) in association with antibiotics against bacteria of veterinary interest. *Microbial Pathogenesis* 124:163-169.
- Dias DDQ, Cabral MES, Sales DL, Oliveira OP, Filho JADA, Teles DA, Souza GG, Coutinho HGM, Costa JGM, Kerntopf MR, Alves RRN, Almeida WDO (2013). Chemical composition and validation of the ethnopharmacological reported antimicrobial activity of the body fat of *Phrynosoma geoffroanus* Used in Traditional Medicine. *Evidence-Based Complementary and Alternative Medicine*. <https://doi.org/10.1155/2013/715040>
- Fachin-Terán A, Vogt RC, Gomes MFS (1995). Food habitats of an assemblage of five species of Turtles in the Rio Guaporé, Rondônia, Brazil. *Journal of Herpetology* 29(4):536-547.
- Felloows PJ (2000). *Food Processing Technology: Principles and Practice*. New York, NY: Woodhead Publishing Limited. 608p.
- Ferrara CR, Fagundes CK, Morcatty TQ, Vogt RC (2017). Amazonian turtles: Identification and distribution guide. Manaus, AM: WCS. 180p.
- Ferrari SA (1980). *Chelonian: endangered animals*. Manaus, AM: Falangola. 68p.
- Ferreira AR (1786). Memória sobre yurara-reté. [1786] In *Archivos do Museu Nacional, Rio de Janeiro*, volume XII, ano 1903, pp. 181-186. <http://www.obrasraras.museunacional.ufrj.br/0017.html>
- Ferreira FS, Brito SV, Ribeiro SC, Almeida WO, Alves RR (2009). Zooterapeutics utilized by residents of the community PoçoDantas, Crato-CE, Brazil. *Journal of Ethnobiology and Ethnomedicine* 5(21):1-10.
- Gaspar A, Silva TJP (2009). Shelf-life and acceptance of Amazon turtle meat (*P. expansa*). *Acta Amazonica* 39(3):669-674.
- Gilmore RM (1986). Fauna and ethnozoology of tropical South America, in: B.G. Ribeiro (ed.) *Suma Etnológica Brasileira*. Petropolis, RJ: Voices. pp. 189-233.
- Hartman L, Lago R (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice* 22(7):475-476.
- IBAMA (1989). *Chelonian Amazon Project - 10 years*. Brasília, BSB, Brazil, 119p.
- Jugli S, Chakravorty J, Meyer-Rochow VB (2019). Zooterapeutic uses of animals and their parts: An important element of the traditional

- knowledge of the Tangsa and Wancho of eastern Arunachal Pradesh, North-East India. *Environment, Development and Sustainability* 22:4699-4734.
- Koroishi ET (2005). Study of the lyophilization process: application for orange juice. Campinas, SP, Brazil. Dissertation (Master in Engineering Chemistry), State University of Campinas, Faculty of Chemical Engineering, 122p.
- Kus-Yamashita MMM, Mancini-Filho J, McDonald B, Ravacci G, Rogero MM, Santos RD, Waitzberg D, Reyes MS, Yehuda S, Gierke J, Cori H, Pires T, Lajolo FM (2016). Polyunsaturated Fatty Acids: Health Impacts. *European Journal of Nutrition and Food Safety* 6(3):111-131.
- Mesak C, Montalvão MF, Paixão CFC, de Oliveira Mendes B, da Costa Araújo AP, Quintão TC, Malafaia G. (2019). Amazon turtles exposed to environmental concentrations of the antineoplastic drug cyclophosphamide present mutagenic damages? If so, would such damages be reversible?. *Environmental Science and Pollution Research* 26(6):6234-6243.
- Miranda ALP, Fraga CAM (2006) Free radical scavenger activity: Determination of the antioxidant Profile of bioactive substances. In: Monge A, Ganellini CR (Ed.). *Practical Studies for Medicinal Chemistry Geneva: IUPAC.*
- Morais KS (2019). Bioactivity and antioxidant potential of *Capsicum chinense* species in the seventeenth Amazon. Boa Vista, Roraima, Brazil. Thesis (doctorate). Posgraduate Program in Biodiversity and Biotechnology of the Legal Amazon. Federal University of Roraima. 83p.
- Morselli MEP, Faria FSEDV, Ribeiro VMF, Viana MNS, Parente AF, Baginski LJ, Jardim C, Reis DBV (2016). Biometric and haematological indices in turtles from the Amazon farm in Rio Branco/AC. *Brazilian Archive of Veterinary Medicine and Animal Science* 68(6):1548-1556.
- Moura-Santos EA, Campos CECA (2011). Use of the Amazon tortoise lard *Podocnemis expansa* (Schweigger, 1812) by riverside dwellers from three communities in the Bailique Archipelago, Macapa - AP. X Congress of Ecology of Brazil, September 16 to 22, São Lourenço - MG.
- Nobre MO, Nascente PS, Meireles MC, Ferreira L (2002). Antifungal drugs for small and large animals. *Rural Science* 32(1):175-184.
- Oliveira DM, Bastos DHM (2011). Bioavailability of phenolic acids. *New Chemistry* 34:1051-1056.
- Oliveira-Júnior AA, Tavares-Dias M, Marcon JL (2009). Biochemical and hematological reference ranges for Amazon fresh water turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. *Research in Veterinary Science* 86(1):146-151.
- Pessoa JDC, Teixeira GHA (2012). Technologies for innovation in the euterpe chains - Brasilia, DF: Embrapa. 343p.
- Ribeiro VHA, Barros SL, Santos NC, Silva MA, Melo MOP, Nascimento APS (2019). Freeze-drying and physical-chemical characterization of a blend composed of kiwi and passion fruit. In: II Paraíba Congress of Agroecology & IV Technological Exhibition. Anais. Green Notebook on Agroecology and Sustainable Development, Pombal, PB: 7(8):e-6834. <https://doi.org/10.18378/cvads.v9i7.6834>
- Rodrigues MT (2005). Conservation of Brazilian reptiles: The challenges for a megadiverse country. *Megadiversity, Conservation Biology* 19(3):659-664.
- Rodrigues MJJ, Moura LSS (2007). Bromatological analysis of the meat of the Amazon tortoise, *Podocnemis expansa* (Schweigger, 1812) in natural habitat: Subsidies to optimize rational rearing. *Amazônia: Ciência e Desenvolvimento* 2(4):7-16.
- Santos ALQ, Bosso ACS, Alves-Junior JRF, Brito FMM, Pachally JR, Ávila-Junior RH (2008). Pharmacological restraint of captivity giant Amazonian turtle *Podocnemis expansa* (Schweigger, 1812) (Testudines, Podocnemididae) with xylazine and propofol. *Acta Cirúrgica Brasileira* 23(3):270-273.
- Scarlato RC, Gaspar A (2007). Centesimal nutrition of the tartaruga-da-Amazônia *Podocnemis expansa* shell bred in captivity and at slaughter age. *Food Science and Technology* 27(supl):41-44.
- Silva DRB, Miranda-Júnior PF, Soares EA (2007). The significance of long chain polyunsaturated fatty acids in pregnancy and lactation. *Brazilian Journal of Mother and Child* 7(2):123-133.
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology* 299:152-178.
- Tan Y, Chang SK (2017). Digestive enzyme inhibition activity of the phenolic substances in selected fruits, vegetables and tea as compared to black legumes. *Journal of Functional Foods* 38:644-655.
- Viana MDNS, Oliveira JDA, Agostini MAP, Erickson J, De Moraes GM, Monjeló LADS, Vogt RC (2017). Population Genetic Structure of the Threatened Amazon River Turtle, *Podocnemis sextuberculata* (Testudines, Podocnemididae). *Chelonian Conservation and Biology* 16(2):128-138.
- Vogt RC (2001). Turtles of the Rio Negro in N.L. Chao, P. Petry, G. Prang, L. Sonneschien, M. Tlusty editors. Conservation and management of ornamental fish resources of the Rio Negro Basin, Amazonia, Brazil - Project Piaba. Universidade do Amazonas, Manaus, Brasil. pp. 245-262.
- Wiegand I, Hilpey K, Hancock REW (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* 3(2):163-175.
- Williams VL, Moshoeu TJ, Alexander GJ (2016). Reptiles sold as traditional medicine in Xipamanine and Xiquelene Markets (Maputo, Mozambique). *South African Journal of Science* 112(7-8):75-83.
- Wolfe K, Wu, X, Liu, RH (2003). Antioxidant Activity of Apple Peels. *Journal of Agricultural and Food Chemistry* 51(3):609-614.
- Xu R, Li D, Peng J, Fang J, Zhang L, Liu L (2016). Cloning, expression and antioxidant activity of a novel collagen from *Pelodiscus sinensis*. *World Journal of Microbiology and Biotechnology* 32(6):100.
- Zhang Y, Mi K, Ding X, Li Y, Wang T, Dou T, Wei W (2019). Characterization of a classical 2-cysteine peroxiredoxin1 gene from Chinese soft-shelled turtle *Pelodiscus sinensis* with its potent antioxidant properties and putative immune response. *Developmental and Comparative Immunology* 101:103456.
- Zheng CJ, Yoo JS, Lee TG, Cho, HY, Kim YH, Kim WG (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters* 579(23):5157-5162.