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Chemical characterization and potential use of reptile fat from sustainable programs

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ABSTRACT

Reptile meats and fats are used for their medicinal properties and nutritional values perceived through the culture of native peoples, though often with no scientific basis. Providing scientific information about potential medicinal and nutritional use of reptile fats would be a strategy for the full use of wild animals, supporting the sustainable use and conservation of biodiversity. The objective of this study was to characterize and chemically compare the fat and oil of individuals of Argentine Black and white tegu (*Salvator merianae*) and Broad-snouted caiman (*Caiman latirostris*) from sustainable use and conservation programs. In addition, we evaluated the microbiological characteristics and the antimicrobial activity of the oils obtained by different methods. We used two methodologies to obtain oils, one by fusion extraction and the other by drying-decantation (traditional hunter's method). We obtained the chemical and microbiological characterization of fat and oil of *C. latirostris* and *S. merianae*. All the oil samples presented less than 10 CFU/ml of all the microorganisms tested. *C. latirostris* and *S. merianae* oil showed nutritional quality parameters that indicate its potential use. Furthermore, *S. merianae* oil showed antimicrobial activity against *Staphylococcus aureus* and *Candidas tropicalis*. No inhibition occurs for the rest of the microorganisms analyzed. *C. latirostris* oil did not show antimicrobial activity, although the lipid profile does indicate some anti-inflammatory potential. This study demonstrates the potential application of the tested oils and confirms the pharmacological basis for the traditional therapeutic use of *S. merianae* oil.

Keywords: *Caiman latirostris*; *Salvator merianae*; Lipids; Oil; Sustainable use.

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SIGNIFICANCE STATEMENT

Reptile use is related to the cultural heritage of several regions and human ethnic groups. The consumption of reptiles is usually driven by their medicinal properties and perceived nutritional values, generated through the culture of native peoples, often with no scientific basis. This paper aims to evaluate the chemical and microbiological characteristics of lipid deposits and oil obtained traditionally and experimentally from Argentine Black and white tegu (*Salvator merianae*) and Broad-snouted caiman (*Caiman latirostris*), from individuals coming from sustainable use and conservation programs. In addition, the paper aims to determine and validate the antimicrobial and anti-inflammatory capacity of these oils, reported by ethnopharmacology. The results show the potential use of the reptile oils analyzed and confirm the pharmacological basis for their traditional therapeutic use.

INTRODUCTION

Wildlife has been historically used by humans (Alves et al. 2008a; Janssen 2021) and in several situations species have been exploited in non-sustainable rates. This overexploitation contributed to the reduction of populations of several species, until international and national legislation put a stop to this situation. Currently, fauna faces additional threats such as loss of habitat due to the constant growth of human populations (Larriera 2011; Meng et al. 2021). Human need for food and shelter will continue to grow as populations increase, demanding more wood, minerals, fossil fuels and, above all, more land suitable for intensive agriculture (Larriera 2011).

Faced with these situations, the main challenge for conservationists is to find solutions that conserve natural ecosystems and the species that inhabit them in the long term. Incorporating natural ecosystems into production systems may be a sound tool for the conservation of natural habitats, since the production of wildlife commodities is of high interest to humans (Larriera 2011; Larriera and Imhof 2006). Through the sustainable use of wildlife, and more specifically of key or economically important species, monetary benefits can act as an incentive for the conservation of other species associated with the same habitats (Larriera et al. 2008).

The use of reptile by-products, in addition to leather, is associated with the cultural heritage of local and indigenous peoples, both in Argentina and in the rest of Latin America (Aguirre et al. 2006; Cawthorn and Hoffman 2016). Just as these animals can be perceived as sacred, they also have a negative image due to legends or popular beliefs, which often restrict their consumption (Alves et al. 2012). However, the use of reptile meat and fats is encouraged by their medicinal properties and nutritional values perceived and generated through the culture of native peoples, although often with no scientific basis (Gorzula and Señaris 1998).

The reptile taxa with the highest number of species used by humans in the world are snakes (60 species), followed by lizards (51), turtles (43) and crocodiles (11) (Alves et al. 2013). There is a historical tendency for dwellers to use lizard, caiman and snake fat and oil for medicinal purposes (Alves et al. 2008b; Alves et al 2012; Fitch et al. 1982; Gorzula and Señaris 1998; Shim-Prydon and Camacho-Barreto 2007; Teixeira et al. 2020) to treat diseases such as asthma, bronchitis, ear and throat pain, among others, and as amulets to protect against the evil eye and snake bites (Alves et al. 2007; 2008b; Costa-Neto 1999; Ferreira et al. 2009; Lindsey et al. 1999; Shim-Prydon and Camacho-Barreto 2007). Other examples are the eggs of the Orinoco crocodile (*Crocodylus intermedius*), which have been used as a folkloric remedy for asthma. In Bolivia, the meat of *Tupinambis teguixin* is used in soups to treat asthma and a dried ring made from the tail is used to treat tooth-ache. Moreover, the oil of *Crotalus durissus* or "cascabel" is used as a general remedy for coughs, a dry throat and as an expectorant (Gorzula and Señaris, 1998).

Consequently, oils obtained from reptile fats represent important alternatives for the treatment of human aliments in several parts of the world, especially in underdeveloped regions (Alves et al. 2007). Caiman fat and snake oil are commonly sold online as “alternative medicine”. Despite that, research on their potential use and efficacy as food and therapeutic material is scarce. Providing scientific information to support the sustainable medicinal and food use of reptiles may be an efficient strategy to support biodiversity conservation (Alves and Albuquerque 2013).

The efficacy of oils and fats may be affected by the conditions under which these animal by-products are obtained, stored and prepared, which are usually precarious and can lead to the transmission of zoonotic diseases caused by bacteria, fungal viruses and parasites (Mendoza-Roldán et al. 2020; Rahman et al. 2020), and the loss of their chemical properties. Therefore, studies on the traditional uses of animal resources must consider both conservation issues, the sanitary quality of the products to be consumed, and the culture aspects related to the knowledge of the people about these products.

In this study we characterize and make a chemical and microbiological comparison of the fat and

oil of individuals of Argentine Black and white tegu (*Salvator merianae*) and the Broad-snouted caiman (*Caiman latirostris*) coming from sustainable use and conservation programs. In addition, we evaluate the microbiological characteristics and antimicrobial activity of the oils according to the different methods used to obtain these products.

MATERIAL AND METHODS

Origin of the lipid deposit samples

It is worth mentioning that caiman populations in Argentina are not endangered. The individuals of *Caiman latirostris* used in this study came from the sustainable use and conservation program "Proyecto Yacaré/Yacarés Santafesinos", based on the technique of ranching or collection of eggs from natural populations in the province of Santa Fe (Law 11820), registered at the Dirección de Fauna Silvestre de la Nación (resolutions Nos. 283/00 and 03/04), complying with CITES regulations and following the recommendations of the Crocodile Specialists Group (CSG/SSC/IUCN) (Figure 1A).

The eggs are collected in the wild with the participation of local people, who are fundamental to the protection of these animals. The nests are then transferred to artificial incubators until hatching; of the individuals raised in captivity, a certain percentage (around 10%) is reintroduced into the wild, and the rest is destined for the commercialization of by-products such as meat and leather. From the slaughter of the caimans destined for production, we obtained the visceral fat and fat body of ten individuals, which we kept at -20°C for further processing.

Regarding *Salvator merianae* (ex *Tupinambis merianae*) (Figure 1B), the populations that inhabit the province of Santa Fe, as well as other provinces of Argentina, are abundant and sustainably harvested under the "Tupinambis" hunting program, registered with the Dirección de Fauna Silvestre de la Nación and with the Ministerio de Medio Ambiente of the Province of Santa Fe (Resolutions 516/93, 216/96, 1437/00 and 485/19). This program is regulated by an annual quota of approximately 1,500,000 hides and a hunting season restricted to certain months of the year.

In this case, local people participate actively and receive an economic benefit from the hides delivered to the program. From the contact with hunters linked to the program, we were able to obtain lipid deposits from eight individuals, which we kept at -20°C until they were processed. We also collected samples of oils stored by hunters in different hunting seasons (2019, 2020 and 2021). We obtained a single sample for each year, because only one hunter had oil from different years stored in separate containers.

Evaluation of the quality of the fat

The fat samples were processed according to the experimental protocol proposed by Bligh-Dyer (1959), with modifications of the official technique carried out in the framework of the International Thematic Network CYTED (208RT 0343). The method is based on obtaining fatty acid methyl esters, which are identified by comparing the relative retention times with commercial standards (AOCS 2007). For the analysis, we used a 2014 Shimatzu gas-liquid chromatograph with a flame ionization detector, an automatic injection system and a 100 m capillary column.

Nutritional quality was evaluated through four parameters according to fatty acid composition data: total saturated fatty acids, total polyunsaturated fatty acids, the n-6/n-3 ratio and the Atherogenic Index (Ulbricht and Southgate 1991):

$$IA = \frac{(C12:0 + 4(C14:0) + C16:0)}{(MUFA + PUFA)}$$

These parameters are based on the functional effects of different fatty acids, allowing for a better evaluation of the nutritional quality of lipids in foods.

Obtaining the oils

Oil production is generally carried out using solvent extraction or fusion techniques (Buthelezi et al. 2012; Días et al. 2013; Ferreira et al. 2009; Vera Candioti et al. 2021). Based on previous studies of the work team (Vera Candioti et al. 2021) and considering the prior knowledge of the local people who use the oil, we extracted the oils from the fat of Broad-snouted caiman and Argentine Black and white tegu using two methodologies: fusion and drying-decantation. The fusion extraction method, tested by Vera Candioti et al. (2021), involves the following steps: the fat is placed in a glass container and heated under constant stirring while the temperature is controlled so that it does not exceed 80°C (Figure 2A). As the fat begins to melt, the oil is transferred to another container, avoiding the passage of the unmelted tissue. For this operation we used a reticulated mesh to retain the solid material, which we returned for later fusion. Once the fusion process was finished, the resulting solid tissue was placed in a mesh of porous tissue and pressed to extract more oil, which we collected and mixed with the other portions of oil collected.

The drying-decantation method is the one usually applied by hunters; they store the fat in containers and subsequently dry it at room temperature and obtain the oil through decantation (Figure 2B). After a

Figure 1. Reptiles used to obtain the fat and oil samples used in this study. a- Individuals of captive Broad-snouted caiman (*Caiman latirostris*) from the sustainable use and conservation program "Proyecto Yacaré", Santa Fe, Argentina. b- Wild individual of Argentine Black and white tegu (*Salvator merianae*) in Santa Fe, Argentina. Author: Sofia E. Pierini

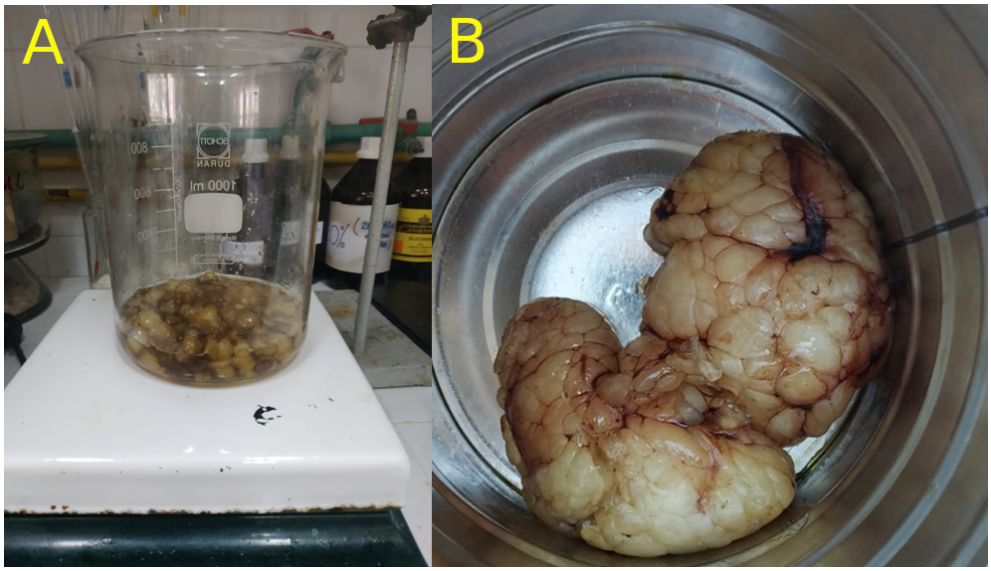

Figure 2. Oil extraction processes. a- Oil extraction from Argentine Black and white tegu (*Salvator merianae*) fat using fusion method. b- Oil extraction of oil from Broad-snouted caiman (*Caiman latirostris*) visceral fat and fat body using the drying and decantation method.

few weeks using this method at temperatures above 28°C, we obtained approximately 100 g of oil. This results in a considerable volume of oil over time.

Evaluation of oil quality

Nutritional quality

Oil samples, like fats, were analyzed using the method for obtaining fatty acid methyl esters, which allows us to evaluate their nutritional quality (see section Evaluation of the quality of the fat).

Microbiological analysis

In Argentina, there are no microbiological indicators for reptile fat intended for human consumption. We therefore decided to perform the usual determinations for the microbiological analysis of other species in order to establish their sanitary quality. We used the techniques recommended by the ICMSF (International Commission on Microbiological Specifications for Foods): yeast and mold count, Aerobic Mesophilic Bacteria count, Total Coliforms count, *Escherichia coli* determination, *Staphylococcus aureus* determination, *Salmonella* spp. determination and sulfite-reducing clostridia determination.

Antimicrobial analysis

The “well diffusion method" was used to determine the antimicrobial activity of the oils. The culture media were APC (Biokar) and HyL (Biokar), and the target microorganisms were *Pseudomonas* sp. (Ps.) (Gram-negative), *Sthaphylocossus aureus* (Gram-positive), *Saccharomyces cerevisiae*, *Candida tropicalis*, *Penicillium roqueforti* and *Aspergillus niger*. These microorganisms belong to the collection of the chair of ”Microbiología General y Principios de Biotecnología" of the Departamento de Ingeniería de Alimentos y Biotecnologia de la Facultad de Ingeniería Química de la Universidad Nacional del Litoral. The diameter of the well was 0.6 cm. Bacteria were incubated for 24 hours at 37°C, while yeasts and molds were incubated for 96 hours at 30°C. We tested both pure oil and a 10-1 dilution.

Statistical analysis

To compare the fatty acid ratios of the lipid deposits between *C. latirostris* and *S. merianae*, we conducted an analysis of variance (ANOVA) test for each fatty acid. In addition, we compared *S. merianae* oils obtained and stored in different seasons. In all cases, the assumptions of normality and homogeneity of variances were checked (analyzed graphically and with the Shapiro Wilk test). In cases in which we detected differences ($p < 0.05$) we performed subsequent comparisons using the DGC test. The data were analyzed with InfoStat software, version 2018 (Di Rienzo et al. 2019).

RESULTS

Chemical characterization of fat and oil

The fatty acid profile of the fat body of *Salvator merianae* is different from the lipid deposits of *Caiman latirostris*, showing a higher percentage of palmitic acid (C16:0), linoleic (C18:3 n-3) and arachidonic (C20:4). It is worth mentioning that the lipid deposits (fat body and visceral fat) of individuals of *C. latirostris* in captivity, coming from the management plan of the species, were similar to each other (Table 1).

We obtained the fatty acid profiles of the oils (Table 2), both from *C. latirostris* oil (obtained by fusion) and from *S. merianae*, stored at room temperature for 2 years, 1 year and recently collected. The fatty acid profiles of the oils collected and stored from *S. merianae*, in different seasons (2019, 2020 and 2021), showed certain differences.

Microbiological analysis

We analyzed the sanitary quality of the *C. latirostris* and *S. merianae* oils obtained both by fusion and decanting from different seasons; Aerobic Mesophilic Bacteria count, Total Coliform count, *Escherichia coli* determination, *Staphylococcus aureus*, *Salmonella* spp. and sulfite-reducing clostridia were less than 10 CF/ml of oil in each sample.

Antimicrobial analysis

In the analysis of the all tested oils obtained of *C. latirostris* by fusion and decanting we found no antimicrobial activity for any of the organisms studied. Also, *S. merianae* oils obtained by fusion and by decanting, in 2019, no showed antimicrobial activity for any of the organisms studied. However, for *S. merianae* oils obtained by decantation in 2020 and 2021, we observed inhibition halos of 1.2 and 1.4 cm, respectively, when we tested their antimicrobial activity against *S. aureus*. For the rest of the microorganisms tested, no inhibition was observed.

The results of the antimicrobial activity of the diluted *S. merianae* oils obtained by decantation in 2020 and 2021 were similar to those we recorded for the undiluted oils when we tested their antimicrobial activity against *S. aureus* (inhibition halos of 1.1 and 1.2 cm, respectively). However, in this case the antimicrobial activity was not maintained for the same length of time, because the halos were eventually invaded after 24 h. Although they were perceived as a clearer area, they did not remain free of microbial growth.

For the inhibitory activity tests of the diluted oils against *C. tropicalis*, the antimicrobial activity manifested itself with inhibition halos between 2 and 2.8 cm, for all the samples tested. This antimicrobial activity did not persist throughout the test as the halos were invaded after 24 hours.

DISCUSSION

The lipid composition of the fats of *C. latirostris* and *S. merianae* shows that these are tissues with a high content of unsaturated fatty acids, mainly polyunsaturated fatty acids (PUFA). Regarding PUFA, it is important to mention the percentages obtained of C18:2 and C18:3 n-3, because they are essential fatty acids. In the fat of *S. merianae* we observed a high content of n-3, showing that these animals' fat has a better fatty acid profile than that of *C. latirostris*, which could be an intrinsic characteristic of the species. Even so, we must consider that the samples of the Argentine Black and white tegu came from the wild, where the diet is much more var-

ied, with a greater amount of food sources of these essential fatty acids, which is reflected in their fatty tissues (Caldironi and Manes 2006; Geiser and Learmonth 1994). On the other hand, we obtained the fats from Broad-snouted caiman from captive individuals fed a diet with a regular composition of food items (Simoncini et al. 2020).

The Fatty Acid (FA) profiles of the oils collected from *S. merianae* showed certain differences in different seasons (years 2019, 2020 and 2021) that can be attributed to the variability of the samples obtained from wild animals. As mentioned, the composition of animal tissues reflects the diet they consume. Therefore, in wild individuals, environmental conditions and the availability of prey in each season may influence the FAs of the oils obtained in different years (Geldenhuys et al. 2015; Isaksson et al. 2015; Speake et al. 1999).

The differences obtained show that the variability of the FAs of oils between years are not due to the storage process, since there is no pattern that indicates that the oil with the longest storage time is lower in quality. Even the oil stored since 2019 has the highest percentages of C20:4 and C20:5 when compared to the other seasons. Since these FAs (C20:4 and C20:5) are among the PUFAS most prone to loss of desaturation, this shows that storage at room temperature does not affect the lipid profile, which validates the extraction technique (drying-decantation) and storage (at room temperature) of the oils used by local people.

The atherogenic index (AI) is a biomarker related to the nutritional quality of a diet, since it is related to pro- and anti-atherogenic fatty acids. In our study, we found an AI of 0.28 for caiman oil, similar to the value reported by Vera Candioti et al. (2021), of between 0.27 and 0.29. In the case of the Argentine Black and white tegu oil, we recorded an AI of between 0.51 and 0.71, and in both types of oil the values did not exceed the recommended maximum limit of 1, so the oils are suitable for human consumption with no risk of developing chronic non communicable diseases (Zhu et al. 2018).

Regarding the n-6/n-3 ratio, the recommendation for consumption should be between 5:1 and 10:1 (Yang et al. 2015). However, these ratios are far from the current diets of Western countries, which contain a large amount of n-6 fatty acids (Palou et al. 2008). The ratio of n-6/n-3 fatty acids is critical in terms of physiological effects, as high ratios might promote inflammatory diseases, cardiovascular diseases and cancer; in addition to causing a decrease in insulin sensitivity in muscle and favoring the accumulation of lipids in tissue (Simopoulos 2008, 2016). In *S. merianae* oil we observed low values of n-6/n-3, close to 2:1, which were the best oil values for 2020 compared to the rest of the oils evaluated (Table 2).

Added to this, the n-3 values of *S. merianae* oil indicate that this oil has potential anti-atherogenic as well as anti-inflammatory properties. On the other hand, as already described for *C. latirostris* oil, it presented a PUFA n-6/n-3 ratio with values close to 14:1, while the recommended intake values are between 5:1 and 10:1. Nonetheless, although in caiman oil this ratio is not optimal, it has a ratio of one half of the oils consumed by human populations, such as sunflower oil and corn oil. Unfortunately, there is no pure fat or oil that has a balanced amount of essential fatty acids, good oxidative stability and optimum nutritional characteristics, but these reptile oils could be potentially used for food consumption (Vera Candioti et al. 2021). These parameters are essential to evaluate the nutritional quality of fats and oils (Vera Candioti et al. 2021); our data indicate a high potential of these oils that could be used as raw material for developing food and as dietary supplements, or simply consumed as an addition to meals.

Another important aspect to evaluate is the microbiological characteristics of the oils, since they may be used for human consumption and/or cosmetics. That is why it is important to evaluate whether the processing of fat, in order to obtain oil, eliminates existing natural microorganisms, mainly those that cause pathologies in humans, resulting in a health hazard for consumers (Soto Varela et al. 2016). In our study, the oils of both species, obtained with the fusion and drying-decantation technique, showed zero or very low values of the presence of microorganisms, far below the limit allowed in this type of substance.

The fusion technique, in which we exposed the fat to temperatures below 80° C, did not register any microorganisms, as expected, since we anticipated that they are eliminated at such temperatures, if any existed in the sample. What is striking is that the technique used by local people, where the fat is only dried in their homes at room temperature and subsequently stored in containers to produce oil by decantation, has also proven to be a safe method for obtaining and storing it without the risk of containing microorganisms.

Regarding the antimicrobial activity of reptile oils, studies report varied results. Some oils such as those from lizards (*Tupinambis merianae*) (Ferreira et al. 2009) and snakes (*Boa constrictor*, *Spilotes pullatus*) showed no activity (Falodun et al. 2008), although others did have synergistic effects when tested in association with certain antibiotics (Dias et al. 2013; Oliviera et al. 2014). With respect to crocodile oil (*Crocodylus niloticus*), there are studies that show high antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Candida albicans* (Buthelezi et al. 2012). Our study showed no antimicrobial activity from the oil of Broad-snouted caiman obtained by fusion or decantation. In con-

trast, we recorded antimicrobial activity, more specifically against *S. aureus* and *C. tropicalis*, in the oil of *S. merianae* obtained by decantation, but not in the oil obtained by fusion.

It is worth mentioning that we observed a bactericidal effect against *S. aureus* in the pure oil and, with dilution, a bacteriostatic effect against *S. aureus* and a fungistatic effect on *C. tropicalis*, where the halo was invaded. The result against *S. aureus* perhaps indicates a specificity of the oil against Gram-positive bacteria. This antimicrobial potential may be due to the proportion of certain fatty acids, such as C18:1, C18:2 and C18:3, in the oil. These fatty acids may show antibacterial activity by affecting the endogenous synthesis of bacterial fatty acids (Zheng et al. 2005), so a higher content of unsaturated fats may indicate greater therapeutic efficacy.

It is important to note that we only recorded antimicrobial activity in the oils obtained by decantation, since in many cases the method of obtaining the oil, such as subjecting the fat to heat, might deteriorate its antibacterial capacity. These results support the traditional use of hunters and rural settlers, who apply fat from the Broad-snouted caiman to wounds in horses or cattle to avoid infections. Other popular uses of these fats are when people suffer blows and apply caiman fat as an anti-inflammatory and when they place a piece of caiman fat on their forehead to relieve severe headaches (Alves et al. 2012).

Even though *C. latirostris* oil did not report microbial activity, its use in traditional medicine is widespread, and its application may be based on the oil's anti-inflammatory and healing potential, which has been proven in oils from other crocodilian species (Buthelezi et al. 2012; Li et al. 2012). This characteristic can be estimated by the levels of certain fatty acids, such as palmitic (C16:0), oleic acid (C18:0) and linoleic acid (C18:2), which have shown to be efficient in accelerating wound healing (Cardoso et al. 2004). These fatty acids are present in considerable proportions in the oils of Broad-snouted caiman and Argentine Black and white tegu we evaluated, so the proinflammatory effect of these acids may be responsible for promoting wound healing process (Pereira et al. 2008).

CONCLUSION

This study demonstrates the potential use of the reptile oils tested and confirms the pharmacological basis for the traditional therapeutic use of *S. merianae* oil. Further studies are needed to evaluate these natural products from animals, prior to their use and as possible new pharmaceutical formulations.

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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 declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CONTRIBUTION STATEMENT

Conceived of the presented idea: PMLL, FEV, MSS, MAG, CIP.
Carried out the experiment: PMLL, FEV, MSS.
Carried out the data analysis: PMLL, FEV, MSS.
Wrote the first draft of the manuscript: PMLL, FEV, MSS.
Review and final writing of the manuscript: PMLL, FEV, MSS, MAG, CIP.
Supervision: MAG, CIP.

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Table 1. Fatty acid profiles of lipid deposits from individuals of *C. latirostris* (captivity) and *S. merianae* (wild). Data are presented as mean ± standard deviation. Different letters show differences (p value < 0.05 highlighted in bold)

Fatty acids	Fat body of *S. merianae*	Fat body of *C. latirostris*	Visceral Fat *C. latirostris*	P
C12:0	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.0606
C14:0	0.85 ± 0.20 ^{a}	0.38 ± 0.05 ^{b}	0.41 ± 0.02 ^{b}	**<0.0001**
C14:1	0.00 ± 0.00 ^{a}	0.04 ± 0.02 ^{b}	0.07 ± 0.02 ^{c}	**0.0171**
C15:0	0.29 ± 0.12 ^{a}	0.05 ± 0.03 ^{b}	0.07 ± 0.01 ^{b}	**<0.0001**
C16:0	27.15 ± 1.61 ^{a}	17.37 ± 1.02 ^{b}	19.31 ± 0.77 ^{b}	**<0.0001**
C16:1 7c	0.00 ± 0.00 ^{a}	0.24 ± 0.04 ^{b}	0.28 ± 0.02 ^{b}	**0.0396**
C16:1 9c	2.96 ± 1.22 ^{a}	3.15 ± 0.35 ^{a}	4.31 ± 0.37 ^{b}	**0.0041**
C17:0	0.64 ± 0.18 ^{a}	0.12 ± 0.02 ^{b}	0.14 ± 0.02 ^{b}	**<0.0001**
C17:1 10c	0.00 ± 0.00 ^{a}	0.03 ± 0.02 ^{b}	0.06 ± 0.01 ^{b}	**0.0031**
C18:0	7.08 ± 0.97 ^{a}	5.36 ± 0.34 ^{b}	5.26 ± 0.18 ^{b}	**0.0001**
C18:1 9c	34.81 ± 4.01	34.32 ± 0.60	33.61 ± 0.39	0.5691
C18:1 11c	2.73 ± 1.49	2.35 ± 0.13	2.10 ± 0.06	0.3544
C18:1 12c	0.00 ± 0.00	0.01 ± 0.02	0.01 ± 0.02	0.8959
C18:2	11.56 ± 1.42 ^{a}	32.56 ± 0.84 ^{b}	29.74 ± 1.26 ^{c}	**<0.0001**
C18:3 (n-6)	0.28 ± 0.06 ^{a}	0.11 ± 0.08 ^{b}	0.10 ± 0.02 ^{b}	**0.0005**
C18:3 (n-3)	7.27 ± 2.94 ^{a}	0.89 ± 0.60 ^{b}	0.96 ± 0.77 ^{b}	**<0.0001**
C20:0	0.00 ± 0.00	0.07 ± 0.09	0.07 ± 0.08	0.9745
C20:1 8c	0.00 ± 0.00	0.04 ± 0.06	0.04 ± 0.05	0.8963
C20:1 11c	0.51 ± 0.13 ^{a}	0.19 ± 0.24 ^{b}	0.14 ± 0.17 ^{b}	**0.0216**
C20:2	0.00 ± 0.00	0.13 ± 0.17	0.11 ± 0.14	0.1484
C20:3 (n-6)	0.13 ± 0.16	0.22 ± 0.04	0.19 ± 0.03	0.0712
C20:3 (n-3)	0.24 ± 0.05 ^{a}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	**<0.0001**
C20:4	1.14 ± 0.56 ^{a}	0.60 ± 0.06 ^{b}	0.83 ± 0.10 ^{b}	**0.0165**
C20:5	0.15 ± 0.08 ^{a}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	**<0.0001**
C22:0	0.04 ± 0.09	0.02 ± 0.01	0.02 ± 0.02	0.6701
C22:4	0.00 ± 0.00	0.06 ± 0.08	0.10 ± 0.11	0.4730
C22:5	0.27 ± 0.12 ^{a}	0.06 ± 0.04 ^{b}	0.09 ± 0.02 ^{b}	**0.0003**
C22:6	0.24 ± 0.07 ^{a}	0.07 ± 0.05 ^{b}	0.12 ± 0.03 ^{b}	**0.0003**
C24:1	0.41 ± 0.36 ^{a}	0.14 ± 0.10 ^{b}	0.12 ± 0.09 ^{b}	**0.0471**
[1]SFA	36.10 ± 3.65 ^{a}	23.36 ± 1.09 ^{b}	25.29 ± 0.88 ^{c}	**<0.0001**
[2]MUFA	41.42 ± 7.21	40.51 ± 0.48	40.72 ± 0.46	0.6503
[3]PUFA	21.45 ± 5.45 ^{a}	33.64 ± 1.00 ^{b}	31.07 ± 1.17 ^{b}	**<0.0001**
[4]UFA	62.87 ± 12.66 ^{a}	74.15 ± 0.95 ^{b}	71.80 ± 0.94 ^{c}	**<0.0001**
[5]IA	0.48 ± 0.19 ^{a}	0.25 ± 0.01 ^{b}	0.29 ± 0.01 ^{c}	**<0.0001**
[6]n-3	8.02 ± 3.20 ^{a}	1.04 ± 0.55 ^{b}	1.21 ± 0.77 ^{b}	**<0.0001**
[7]n-6	13.41 ± 2.32 ^{a}	32.72 ± 0.90 ^{b}	29.93 ± 1.27 ^{c}	**<0.0001**
n6/n3	1.67 ± 0.72 ^{a}	39.34 ± 17.97 ^{b}	34.68 ± 19.06 ^{b}	**0.0066**

[1] Σ Saturated = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0

[2] Σ Monounsaturated = C14:1 + C16:1 cis-7 + C16:1 cis-9 + C17:1 + C18:1 cis-9 + C18:1 cis-11 + C18:1 cis-12 + C20:1 cis-8 + C20:1 cis-11 + C24:1

[3] Σ Polyunsaturated = C18:2 + C18:3 n-6 + C18:3 n-3 + C20:2+ C20:3 n-6 + C20:3 n-3 + C20:4+ C20:5 + C22:4 + C22:5 + C22:6

[4] Σ Unsaturated = C14:1 + C16:1 cis-7 + C16:1 cis-9 + C17:1 + C18:1 cis-9 + C18:1 cis-11 + C18:1 cis-12 + C18:2 + C18:3 n-6 + C18:3 n-3 + C20:1 cis-8 + C20:1 cis-11 + C20:2+ C20:3 n-6 + C20:3 n-3 + C20:4+ C20:5 + C22:4 + C22:5 + C22:6 + C24:1

[5] IA = (C12: 0 + 4 (C14: 0) + C16: 0) / (MUFA + PUFA)

[6] Σn-3 PUFA =C18:3 n-3 + C20:3 n-3 + C20:5 + C22:5 + C22:6

[7] Σn-6 PUFA = C18:2 + C18:3 n-6 + C20:3 n-6 +20:4 + 22:4 n-6

Table 2. Fatty acid profiles of *C. latirostris* oils (obtained by fusion) and comparison between *S. merianae* oils (obtained by decantation) stored in different seasons; data are present as mean ± standard deviation. Different letters show differences (p value < 0.05 highlighted in bold).

	C. latirostris	***S. merianae***			
Fatty acids	**Year 2021**	**Year 2019**	**Year 2020**	**Year 2021**	**P**
C14:0	0.38±0.02	1.25±0.04^a	1.41±0.20^a	1.03±0.12^b	**0.0318**
C15:0	0.00±0.00	0.29±0.03	0.42±0.07	0.29±0.07	0.3026
C16:0	19.33±0.41	26.59±0.44^a	32.87±0.64^b	28.69±0.05^c	**0.0034**
C16:1 9c	4.25±0.12	2.90±0.01^a	3.75±0.45^b	2.87±0.19^a	**0.0034**
C17:0 ISO	0.00±0.00	0.53±0.02^a	0.61±0.04^b	0.54±0.01^a	**0.0017**
C17:0	0.13±0.01	0.62±0.07	0.72±0.04	0.67±0.01	0.4166
C 18:0	5.56±0.32	8.93±0.12^a	9.61±0.80^a	8.00±0.05^b	**0.0368**
C18:1 9c	33.50±0.52	37.78±0.86	35.52±0.85	37.63±0.59	0.2604
C18:1 11c	2.04±0.05	2.30±0.22^a	2.84±0.52^a	3.37±0.20^b	**0.0302**
C18:2	29.67±0.33	11.94±0.41^a	7.26±2.15^b	7.91±0.09^b	**0.0005**
C18:3 (n-6)	0.13±0.02	0.27±0.05	0.34±0.09	0.41±0.09	0.2721
C18:3 (n-3)	1.02±0.12	4.31±0.22^a	3.03±0.42^a	6.44±0.69^b	**0.0130**
C20:1 11c	0.27±0.07	0.57±0.01^a	0.52±0.04^b	0.60±0.03^a	**0.0429**
C20:2	0.30±0.04	0.31±0.03^a	0.13±0.05^b	0.18±0.05^b	**0.0192**
C20:3(n-6)	0.24±0.03	0.00±0.00	0.00±0.00^a	0.00±0.00	**-**
C20:3 (n-3)	0.00±0.00	0.19±0.01^a	0.13±0.05^b	0.22±0.03^a	0.2000
C20:4	0.95 ± 0.28	0.53±0.06^a	0.21±0.013^b	0.41±0.03^a	**0.0007**
C20:5	0.00±0.00	0.21±0.03^a	0.06±0.01^b	0.08±0.00^b	**0.0242**
C22:4	0.28±0.02	0.00±0.00	0.00±0.00	0.00±0.00	**-**
C22:5	0.13±0.02	0.12±0.02	0.07±0.02	0.05±0.01	0.9868
C22:6	0.15±0.02	0.07±0.02^a	0.13±0.04^b	0.04±0.01^a	**0.0375**
[1]SFA	25.44±0.41	38.25±0.59^a	45.55±0.80^b	39.23±0.02^a	**0.0069**
[2]MUFA	40.44±0.62	43.55±0.62	42.63±0.33	43.91±0.23	**0.3613**
[3]PUFA	33.80±0.39	17.94±0.58^a	11.35±1.07^b	15.56±0.95^c	**0.0048**
[4]UFA	74.65±0.35	61.49±0.10^a	53.98±0.26^b	58.41±0.62^a	**0.0111**
[5]n-3	2.30±0.11	4.90±0.09^a	3.41±0.71^a	6.800.76	**0.0156**
[6]n-6	31.49±0.32	13.04±0.45^a	7.95±0.19^b	8.77±0.19^b	**0.0006**
n-6/n-3	13.73±0.60	2.66±0.41^a	2.33±0.32^b	1.30±0.12^c	**0.0031**
Ratio PUFA/SFA	1.33±0.03	0.47±0.04^a	0.25±0.08^b	0.55±0.02^c	**0.0120**
[7]AI	0.28±0.01	0.51±0.01^a	0.71±0.01^b	0.54±0.01^c	**0.0040**

[1] Σ Saturated = C14:0 + C15:0 + C16:0 + C17:0 +C17:0 ISO+ C18:0
[2] Σ Monounsaturated =C16:1 cis-9 + C18:1 cis-9 + C18:1 cis-11 + C20:1 cis-11
[3] Σ Polyunsaturated = C18:2 + C18:3 n-6 + C18:3 n-3 + C20:2+ C20:3 n-6 + C20:3 n-3 + C20:4+ C20:5 + C22:4 + C22:5 + C22:6
[4] Σ Unsaturated = C16:1 cis-9 + C18:1 cis-9 + C18:1 cis-11 + C18:2 + C18:3 n-6 + C18:3 n-3 + C20:1 cis-11 + C20:2+ C20:3 n-6 + C20:3 n-3 + C20:4+ C20:5 + C22:4 + C22:5 + C22:6
[5] Σn-3 PUFA =C18:3 n-3 + C20:3 n-3 + C20:5 + C22:5 + C22:6
[6] Σn-6 PUFA = C18:2 + C18:3 n-6 + C20:3 n-6 +20:4 + 22:4 n-6
[7] AI = (C12:0 + 4 (C14:0) + C16:0) / (ΣMUFA + ΣPUFA)