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Research Paper

# Anti-inflammatory activity of animal oils from the Peruvian Amazon

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ABSTRACT

*Ethnopharmacological relevance:* Animal oils and fats from the fishes *Electrophorus electricus* and *Potamotrygon motoro*, the reptiles *Boa constrictor*, *Chelonoidis denticulata* (*Geochelone denticulata*) and *Melanosuchus niger* and the riverine dolphin *Inia geoffrensis* are used as anti-inflammatory agents in the Peruvian Amazon. The aim of the study was to assess the topic anti-inflammatory effect of the oils/fats as well as to evaluate its antimicrobial activity and fatty acid composition.

*Materials and methods:* The oils/fats were purchased from a traditional store at the Iquitos market of Belen, Peru. The topic anti-inflammatory effect was evaluated by the mice ear edema induced by arachidonic acid (AA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) at the dose of 3 mg oil/ear. Indomethacine and nimesulide were used as reference anti-inflammatory drugs. The application resembles the traditional topical use of the oils. The antimicrobial effect of the oils/fats was assessed by the microdilution test against reference strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteritidis*. The fatty acid composition of the oils/fats (as methyl esters) was determined by GC and GC–MS analysis after saponification.

*Results:* All oils/fats showed topic anti-inflammatory activity, with better effect in the TPA-induced mice ear edema assay. The most active drugs were *Potamotrygon motoro*, *Melanosuchus niger* and *Geochelone denticulata*. In the AA-induced assay, the best activity was found for *Potamotrygon motoro* and *Electrophorus electricus* oil. The oil of *Electrophorus electricus* also showed a weak antimicrobial effect with MIC values of 250 μg/mL against *Escherichia coli* ATCC 25922 and *Salmonella enteritidis*-MI. The main fatty acids in the oils were oleic, palmitic and linoleic acids.

*Conclusions:* Topical application of all the oils/fats investigated showed anti-inflammatory activity in the mice ear edema assay. The effect can be related with the identity and composition of the fatty acids in the samples. This study gives support to the traditional use of animal oils/fats as ant-inflammatory agents in the Peruvian Amazon. However, new alternative should be encouraged due to the conservation status of several of the animal sources of the crude drugs.



## 1. Introduction

Latin American traditional medicine is a blend of the practices from Amerindian cultures and the introductions from the European conquerors and settlers. Healers and users, mainly in medium and large human settlements employs plants and herbal drugs that are easily accessible, can be collected from the wild, grown in small gardens or be purchased in markets or shops. Much less is known on the use of animals in therapeutic practices. The use of animal drugs is often overseen in Latin America. This fact is associated with the habitat loss of several animal species formerly integrated in the colonial or pre-hispanic tradition, the extinction of many of them and the substitution of their former indications of use for other drugs. The use of animal-derived drugs for human and veterinary medicine has been revised by several authors in Brazil (Alves and Rosa 2006; Alves and Rosa, 2007; Alves and Alves, 2011; Souto et al., 2011a, 2011b; Alves et al., 2012). Much less is known for other South American countries, including Peru.

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Studies on the use of natural resources in healing practices in the Peruvian Amazon include the assessment of antimalarial activity of plants from the Nanay river (Loreto Department) against *Plasmodium falciparum* and on the ferriprotoporphyrin inhibition assay (Ruiz et al., 2011). An ethnobotanical survey was carried out at Suni Miraño, in the same Department (Jovel et al., 1996). The authors focused the work on plants and tested the extracts on different microorganisms using the disk diffusion assay. The shamanic practices in Iquitos and nearby areas was described by Luna (1984), including the diet restrictions associated with the apprenticeship to become a shaman and the process of plant teaching. At present, ayahuasca still play a relevant role in the healing practices of Peruvian Amazon. Other studies undertaken with plants collected near Iquitos, the Loreto Department or Pucallpa, comprises the screening for wound healing plants (Villegas et al., 1997) and the evaluation of risks associated with the use of oje, an anthelmintic preparation obtained from *Ficus insipida* latex (Hansson et al., 2005). On spite of the diversity of medicinal resources used in the Peruvian Amazon, little has been done on the therapeutic practices involving animal drugs. A visit to the Mercado Belén in Iquitos, offers a challenging perspective on the use of natural resources in the area.

**Fig. 1.** Plant macerates in alcohol, aphrodisiac preparates and animal oils on sale at a traditional shop in the Belen Market, Iquitos, Peru.

**Fig. 2.** Animal oils selling place at the Belen Market, Iquitos, Peru.

The Belen market at Iquitos (3°45′07″ S latitude, 73°16′06.1″ W longitude) is a commercialization center for all kind of local food and traditional medicine resources produced, collected and gathered from the Peruvian Amazon area. Along with aromatic and medicinal plants, wildlife meat, turtle eggs, plant and animal drugs are sold in small stands (Figs. 1 and 2).

As we did not find information on the use of animal drugs in Amazonian Peru, a survey was undertaken to identify the most common animal drugs commercialized in the traditional market of Iquitos. The animal oils and fats are trading products from fishers and hunters and are sold without controls regarding environmental issues. The oils are stored either in plastic or glass bottles and kept at room (environmental) temperature, which means an average of 30 °C or higher during the summer months of December through February.

Following our studies on South American pharmacopeias, we now report the bioactivity and composition of the most commonly traded animal oils at the Iquitos market.

## 2. Materials and methods

### 2.1. Animal oils

Sellers of animal oils and animals used as medicine at the Iquitos Belen market were interviewed by two different individuals (G S-H and G. V.-A) and the information was compared. Sellers were asked about the products offered, their uses, origin, obtention/preparation of the products, administration and storage. The responses for the general uses were consistent for all six sellers, including the larger shops with medicinal plants preparations and animal oils as well as for small shops offering mainly unprocessed animals or its parts. The oils were purchased by one of the authors (G. V.-A.), accompanied by a biologist (Javier del Águila Chávez, Universidad Científica del Perú, Iquitos) for the association of the oil sources with the taxonomic identity of the animals. The oil/fat samples were purchased at the "Pasaje Paquito", Mercado Belén, Iquitos, Peru, in October, 2012, Puesto de Venta el Otoronguito. Small samples were obtained as follows: *Electrophorus electricus* (6.96 g), *Potamotrygon motora* (7.15 g), *Boa constrictor* (6.93 g), *Chelonoidis denticulata* (*Geochelone denticulate*) (6.99 g), *Melanosuchus niger* (6.94 g) and *Inia geoffrensis* (3.15 g). The oil samples for analysis were stored in amber glass containers and used for fatty acid analyses, antimicrobial activity and topical anti-inflammatory effect in animals.

### 2.2. Antiinflammatory activity

Two inflammatory agents, namely arachidonic acid (AA) and phorbol 12-myristate 13-acetate (TPA) were used to estimate the probable anti-inflammatory action mechanism of the oils under study. The reference drugs used were indomethacin and nimesulide against TPA and AA, respectively.

All animal experiments were performed according to the ethical guidelines suggested by the "International Norms for the Biomedical Investigation with Animals", elaborated by the Council of International Organizations (1990) and the bio-ethics norms of the Commission of the Chilean Public Health Institute and Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Adult male CF-1 mice (20–25 g), obtained from a stock maintained at the Chilean Public Health Institute, were used to assess the anti-inflammatory effect. All animals were housed in a climate- and light-controlled room with a 12 h light-dark cycle, fasted overnight before the day of the assays, with free access to water. For each of the samples under study, the anti-inflammatory

activity was evaluated in groups of 8 treated and 16 control mice. After 5 min of sample treatment, mice received 5 μg of TPA or AA (Sigma; St. Louis, MO), as pro-inflammatory agents, dissolved in 20 μL of acetone (solvent does not interfere with the assay). Control subjects only received TPA or AA at the same concentration. Both, the sample and the TPA or AA, were applied to the inner (10 μL) and outer (10 μL) surfaces of the right ear. The left ear only received acetone. Mice were sacrificed by cervical dislocation (after 6 h of TPA and 1 h AA), and a 6 mm diameter section of the right and left ears were cut and weighed. The weight difference between both ear sections correspond to the edema value (Lloret and Moreno, 1995). Topical anti-inflammatory effect ($E$) was evaluated according to the following equation: $\% E = [W_c - W_s/W_c] \times 100$; where $W_c$ and $W_s$ are the differences in median values of the weights of the right and the left ear sections of the control ($W_c$) and the treated animals ($W_s$) respectively (Delporte et al., 2003).

The significance of the results ($p$) was determined using Kruskall–Wallis test. Mann Whitney test was used for the individual comparisons. The differences were considered significant for $p \leq 0.05$

### 2.3. Antimicrobial activity

#### 2.3.1. Microorganisms

The antibacterial activity of the oils was assessed against the following bacteria: methicillin-sensitive *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, and the clinical isolated *Escherichia coli*-121 (Laboratorio Hospital Marcial Quiroga, San Juan, Argentina) and *Salmonella enteritidis* MI (MI-Instituto Malbrán, Buenos Aires, Argentina). Bacteria were grown on Mueller Hinton broth medium.

#### 2.3.2. Antibacterial activity

The MIC values were determined using the microbroth dilution method according to the protocols of the CLSI (Clinical and Laboratory Standards Institute) (2008). All tests were performed in Mueller Hinton broth (MHB), and cultures of each strain were prepared overnight. Microorganism suspensions were adjusted in a spectrophotometer with sterile physiological solution to give a final organism density of 0.5 McFarland scale ($1–5 \times 10^5$ CFU/ml). Stock solutions of oils in DMSO were diluted to give serial two-fold dilutions that were added to each medium to obtain final concentrations ranging from 125 to 1000 μg/ml. The final concentration of DMSO in the assay did not exceed 1%. The antimicrobial agent Cefotaxime (Argentina Pharmaceutica) was included in the assays as positive control. The plates were incubated for 24 h at 37 °C. The MIC values were defined as the lowest oil concentrations showing no visible bacterial growth after the incubation time. Tests were done in triplicate.

### 2.4. Fatty acid analysis

The fatty acid composition of the oils was investigated by gas chromatography of the corresponding methyl esters (FAME). A sample of the oil (100 mg) was saponified with 15 mL of alcoholic KOH for 20 min. After addition of 15 mL of 20% $BF_3$ in MeOH, the reaction mixture was refluxed for 2 h. After cooling, water and 5 ml of petroleum ether (PE) were added. The methyl esters dissolved in the PE fraction. The mixture was analyzed by gas chromatography and the FAME were identified by comparison of the retention times with known FAME mixtures. In addition, GC–MS analysis was undertaken to cross-check the information provided by GC. GC analysis was carried out using a Shimadzu GC-9 A instrument with a capilar Supelco SP 2330 column (30 m × 0.25 mm i.d.). To reconfirm the identity of the methyl esters, GC–MS analysis was undertaken using a Perkin Elmer Turbo Mass equipment (USA) with a capilar Elite 5 MS column (column length 30 m, internal diameter 0.25 mm, Cat. N9316282). The chromatographic conditions were as follows. Time: 45 min. Delay time: 0.0 min; initial temperature: 80 °C for 2 min. Ramp 1: 4.0 °C/min to 200 °C hold for 0.0 min. Ramp 2: 6.0 °C/min to 250 °C hold for 6.676 min.

The standard used (Supelco) contained the following fatty acids (as methyl esters): caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0); tridecanoic acid (C13:0), myristic acid (C14:0), myristoleic acid (C14:1n9c); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1n9c); heptadecanoic acid (C17:0); stearic acid (C18:0), elaidic acid (C18:1n9t); oleic acid (C18:1n9c); linoleic acid (C18:2n6c); arachidic acid (C20:0); cis-11-eicosenoic acid (C20:1); linolenic acid (C18:3n3); behenic acid (C22:0) and erucic acid (C22:1n9).

## 3. Results and discussion

### 3.1. Fat/oil sources, uses and taxonomy

According to the sellers, the animal oils and fats are obtained by melting the animal fat-rich tissues under the fire at low temperature. For the stingray oil, the liver is placed under the sun or slightly heated under a water bad ("baño Maria") to obtain the crude drug. The animal sources of the oil/fat are common in the Iquitos area and most of them can be found/purchased at the wild meat or fish shops in the market. The animal oil seller was asked to describe the animals from which the oils were obtained for a better approximation to the taxonomic identity. The skin from the boa allowed unequivocal identification of the fat source. The turtle "motelo" (only one species with this name), the ray *Potamotrygon motora* and the electric eel are common in the Peruvian Amazon, being well-known by the local customers. The "black lizard" *Melanosuchus niger* is the only source of the oil and the only species with this common name. On spite of official controls on endangered species, Iquitos market offers almost all products from the Amazon region, including animal drugs and meat of wild animals. The status of the riverine dolphin *Inia geoffrensis* is as protected species. The users have empirical ways to recognize the fat/oil sources, including odor, color and consistency.

#### 3.1.1. Fishes

*Electrophorus electricus* Linneo, 1766 (syn.: *Electrophorus multivalvulus* Nakashima, 1941; *Gymnotus electricus* Linnaeus, 1766; *Gymnotus regius* Chiaje, 1847) (Anguila) The oil is rubbed against rheumatism, bone pain, osteoporosis. It is applied to the affected area as a massage.

*Potamotrygon motoro* (Müller & Henle, 1841) (syn.: *Potamotrygon alba* Castex, 1963). (Raya). The oil is recommended for bronchial diseases, cough and asthma. Some 5 oil drops are placed in a cup with water or juice and taken before meals, until the disease symptoms improved. It is also appreciate to relieve tendinitis. The oil can be mixed with beehoney and a small teaspoon is orally taken during one or several days (or up to one month) until the health improves. It can also be mixed with "lagarto" fat.

#### 3.1.2. Reptiles

*Boa constrictor* Linneus, 1758 (Boa amarilla). The fat is applied in massage to the affected zone for rheumatism, osteoporosis, facial paralysis, broken bones and bruises.

*Chelonoidis denticulata* (Linnaeus 1766) (syn.: *Geochelone denticulata* Linnaeus, 1766) (Motelo). The fat of the terapine is used for

wrinkles and skin spots. The fat/oil is topically applied overnight. Other animal parts with traditional uses include the penis of the land turtle motelo. Women who want to bear a male child eats the penis while man can place the penis in an alcoholic beverage and take it for a better sexual performance.

*Melanosuchus niger* Spix, 1825 (Lagarto Negro). For bronchial diseases, the oil is used in the same way as the "raya" (*Potamotrygon motoro*) oil. It is also used for rheumatis, arthritis by rubbing and massage of the painful areas.

#### 3.1.3. Mammals

*Inia geoffrensis* (Blainville, 1817) (Bufeo colorado). The oil obtained from the freshwater dolphin is used as a perfume to attract the beloved, for both sexes. Some 2–3 drops of the oil are added to the perfume used and applied as an additive. The used should go close to the beloved and make contact. It is believed, that the perfume is more effective if the user is under dietary restrictions. In the container where the oil was displayed for sale, the female genital parts were included. According to the seller, giving more power to the oil. All the mentioned oils/fats are used to relieve rheumatism, in massages.

### 3.2. Antiinflammatory activity

The oils were assessed in vivo using two different models of inflammation. As the oils are used undiluted, the experiments were performed at a single dose, using the animal drugs as sold in the markets and following their indications of use. Oils were evaluated topically at the dose of 3 mg/ear in mice with a weight of 25 g. Two experimental protocols were used. Compounds that are active in the AA assay are dual inhibitors of COX-1 and 5-LOX, while the active products in the TPA study are inhibitors of COX-2 and $_{i}$NOS. The results are summarized in Table 1.

### 3.3. Antimicrobial activity

The antibacterial activity of the oils was evaluated by the broth microdilution method against a panel of Gram-positive and Gram-negative bacteria. The most relevant results are shown in Table 2. The oils of the turtle *Chelonoidis denticulata* (*Geochelone denticulata*), the black alligator *Melanosuchus niger*, the stingray *Potamotrygon motora* and the yellow boa *Boa constrictor*, have to be regarded as inactive as antimicrobial agents, with MICs values $>1000$ μg/mL. However, the oils of the electric eel *Electrophorus electricus* and the dolphin *Inia geoffrensis* presented better activities, with a MIC of 250 μg/ml against *Salmonella enteritidis*-MI, and reduce (100%) growth of *Escherichia coli* ATCC 25922 with MICs values of 250 and 500 μg/ml, respectively. It should be considered, however, that the oils are applied topically and not diluted for use. The most relevant results are shown in Table 2.

### 3.4. Fatty acids composition

The fatty acids composition of the different oils was assessed by GC analysis using a commercial standard of fatty acids methyl esters (Supelco) as reference. The identity of the fatty acids methyl esters was reconfirmed by GC–MS analysis, comparison with databases and reinterpretation of the mass spectral data and retention times. Results are presented in Table 3.

From the fatty acid content of the reptile fats, oleic acid was the main compound, accounting for 52.11% of *Boa constrictor*, 39.86 for *Geochelone denticulata* and 36.14% for *Melanosuchus niger* oils, respectively. Palmitic, linoleic and stearic acid contributed with 25.56, 9.19 and 6.42% for *Boa constrictor*, 31.67, 5.66 and 5.92 for *Geochelone denticulata* and 20.58, 5.80 and 7.32% for *Melanosuchus niger*, respectively.

A different picture is observed for the oil from the fishes *Potamotrygon motoro* and *Electrophorus electricus*, where the palmitic, oleic and linoleic acid content was 26.26%, 25.44% and 9.72%, respectively for *Potamotrygon motoro* and 22.55%, 29.93% and 10.71% for *Electrophorus electricus*. Large differences in the composition was observed for the low molecular weight fatty acids of *Electrophorus electricus*. oil. The content of caprylic (10.30%) and capric acid (4.98%) was much higher than in any other of the analyzed fats/oils. In addition, two unidentified compounds were detected, accounting for about 10.6% of the total. The oil from the dolphin *Inia geoffrensis* showed higher palmitoleic acid content (14.34% vs. 2.40–6.91%), lower oleic acid (17.53% vs. 25.44–52.11%) and contained elaidic acid (6.90%), accounting for a different fatty acid profile than that observed for the reptile and fish oils.

While the oil from *Crocodilus niloticus* showed as main fatty acids palmitic (15.436%) and oleic acid (19.593%) (Buthelezi et al., 2012), the main fatty acids from the Amazonian black alligator fat were oleic (36.14%), palmitic (20.58%), stearic (7.32%) and palmitoleic acid (6.91%). The crocodile fat was obtained from animals from a farm in South Africa while the samples sold at Iquitos are from wild living animals hunted at the Loreto Department, Peru. All oils showed topical anti-inflammatory activity. Generally, they were more active against TPA than AA. In the assay where the inflammation was induced by TPA, the most active oils where *Potamotrygon motoro* and *Melanosuchus niger*. In the assay where the inflammation was induced by AA, the most active were *Potamotrygon motoro* and *Electrophorus electricus.* It is important to note that the most active topical anti-inflammatory oil, in both assays, was the fish (ray) *Potamotrygon motoro*.

**Table 1**
Topical effects of animal oils and fats used as anti-inflammatory agents in Iquitos, Peru, assessed by the mice ear edema induced by arachidonic acid (AA) and *O*-tetradecanoyl-phorbol-13-acetate (TPA).

| Oil/fat source | mg/25 g mouse | $\%E_{AA} \pm$ SEM | $\%E_{TPA} \pm$ SEM |
|---|---|---|---|
| *Boa constrictor* | 3 | 13.8* ± 4.5 | 35.5* ± 4.4 |
| *Electrophorus electricus* | 3 | 25.5* ± 4.9 | 42.7* ± 4.8 |
| *Geochelone denticulata* | 3 | 16.7* ± 5.3 | 52.7* ± 5.2 |
| *Inia geoffrensis* | 3 | 20.5* ± 8.8 | 48.6* ± 5.4 |
| *Melanosuchus niger* | 3 | 10.5* ± 2.5 | 54.1* ± 5.1 |
| *Potamotrygon motoro* | 3 | 36.8* ± 5.1 | 60.0* ± 5.8 |
| IND | 0.5 | n.d | †92.9 ± 3.2* |
| NIM | 1 | †49.3 ± 4.0 | n.d |

$E_{AA}$ topical anti-inflammatory effect against AA; $E_{TPA}$ topical anti-inflammatory effect against TPA; IND indomethacin; NIM nimesulide; n.d not determined.

* $p \leq 0.05$.

**Table 2**
Antibacterial activity of *Electrophorus electricus* and *Inia geoffrensis* oils used in the traditional medicine of Iquitos, Peru. Results are presented as MICs in μg/ml.

| Microorganism | MIC (μg/ml) | | |
|---|---|---|---|
| | *Electrophorus electricus* | *Inia geoffrensis* | Cefotaxime |
| Gram (−) | | | |
| *Escherichia coli* ATCC 25922 | 250 | 500 | 0.5 |
| *Escherichia coli*-121 | >1000 | >1000 | 5 |
| *Salmonella enteritidis*-MI | 250 | 250 | 0.05 |
| Gram (+) | | | 0.1 |
| *Staphylococcus aureus* methicillin-sensitive ATCC 25923 | 1000 | >1000 | 0.5 |
| *Staphylococcus aureus* methicillin-resistant ATCC 43300 | 1000 | >1000 | 0.5 |

**Table 3**
Fatty acid composition (as methyl esters) of animal oils commercialized at the Iquitos market as anti-inflammatory/anti-rheumatic by topic application/massage. Each value is an average of triplicate analyses with its standard deviations. Tr: traces; –: not detected.

| Compound | Rt (min) | Fishes | | Reptiles | | | Mammals |
|---|---|---|---|---|---|---|---|
| | | *Electrophorus electricus* | *Potamotrygon motora* | *Boa constrictor* | *Geochelone denticulata* | *Melanosuchus niger* | *Inia geoffrensis* |
| Caprylic acid (C8:0) | 9.54 | 10.30 ± 0.55 | – | – | – | – | – |
| Unknown | 11.68 | 5.29 ± 0.07 | – | – | – | – | – |
| Capric acid (C10:0) | 13.90 | 4.98 ± 0.07 | – | – | – | – | – |
| Unknown | 13.41 | 5.29 ± 0.05 | – | – | – | – | – |
| Lauric acid (C12:0) | 18.48 | – | – | 0.13 ± 0.02 | 3.83 ± 0.14 | 0.18 ± 0.01 | – |
| Myristic acid (C14:0) | 22.78 | 0.42 ± 0.03 | 1.47 ± 0.09 | 1.12 ± 0.06 | 4.15 ± 0.14 | 2.10 ± 0.11 | 3.67 ± 0.50 |
| Myristoleic acid (C14:1n9c) | 24.12 | Tr | 0.30 ± 0.02 | Tr | 0.22 ± 0.01 | 1.01 ± 0.11 | 2.63 ± 0.03 |
| Pentadecanoic acid (C15:0) | 24.81 | Tr | 0.91 ± 0.04 | Tr | 0.34 ± 0.03 | 1.12 ± 0.05 | 1.54 ± 0.21 |
| Palmitic acid (C16:0) | 26.81 | 22.55 ± 0.16 | 26.26 ± 0.21 | 25.56 ± 0.09 | 31.67 ± 1.26 | 20.58 ± 0.35 | 22.73 ± 0.06 |
| Palmitoleic acid (C16:1n9c) | 27.80 | Tr | 4.40 ± 0.13 | 2.40 ± 0.08 | 6.34 ± 0.12 | 6.91 ± 0.07 | 14.34 ± 0.40 |
| Heptadecanoic acid (C17:0) | 28.62 | 0.87 ± 0.04 | 1.06 ± 0.01 | 0.34 ± 0.01 | 0.23 ± 0.01 | 1.65 ± 0.01 | 2.42 ± 0.18 |
| Stearic acid (C18:0) | 30.43 | 3.22 ± 0.11 | 7.25 ± 0.09 | 6.42 ± 0.02 | 5.92 ± 0.16 | 7.32 ± 0.01 | 6.61 ± 0.19 |
| Elaidic acid (C18:1n9t) | 31.03 | – | – | – | – | – | 6.90 ± 0.02 |
| Oleic acid (C18:1n9c) | 31.29 | 29.93 ± 0.34 | 25.44 ± 0.62 | 52.11 ± 0.97 | 39.86 ± 0.04 | 36.14 ± 0.89 | 17.53 ± 0.03 |
| Linoleic acid (C18:2n6c) | 32.61 | 10.71 ± 0.14 | 9.72 ± 0.12 | 9.19 ± 0.05 | 5.66 ± 0.16 | 5.80 ± 0.03 | 7.71 ± 0.06 |
| Arachidic acid (C20:0) | 33.90 | – | | 0.18 ± 0.02 | 0.20 ± 0.02 | | – |
| Linolenic acid (C18:3n3) | 34.36 | 0.43 ± 0.09 | 2.92 ± 0.01 | 0.62 ± 0.19 | 0.15 ± 0.01 | 2.03 ± 0.03 | 3.13 ± 0.09 |
| cis-11-eicosenoic acid (C20:1) | 34.85 | – | 1.75 ± 0.03 | 0.38 ± 0.11 | 0.27 ± 0.02 | 1.13 ± 0.01 | 1.21 ± 0.01 |
| Behenic acid (C22:0) | 38.20 | – | – | – | – | – | – |
| Erucic acid (C22:1n9) | 39.16 | – | – | – | – | – | 1.15 |
| Total% | | 93.99 | 81.48 | 98.45 | 98.84 | 85.94 | 91.57 |

In a review on animal crude drugs used in Latin America, Alves and Rosa (2006) refers to the use of *Electrophorus electricus* (Linnaeus, 1766) (Electrophoridae) to treat rheumatism, osteoporosis, insect and snake bites, among others. The river ray *Potamotrygon motoro* (Müller & Henle, 1841) (Potamotrygonidae) is cited for asthma, bronchopulmonar diseases and burns. The fat of the gymnotidae *Electrophorus electricus* and the stingray *Urotrygon microphthalmum* (Delsman, 1941) are reported by Alves and Rosa (2006) for rheumatism and asthma and pain relief, respectively.

For the Boidae *Boa constrictor* (Linnaeus, 1758), rheumatism, lung diseases, boils, swelling, pain asthma are some of the indications of use. The Testudinidae *Chelonoidis denticulata* (Linnaeus, 1766), is recommended for pains, rheumatism, asthma, while the Alligatoridae *Melanosuchus niger* (Spix, 1825) is used for infection, sore throat, as anti-inflammatory, asthma and rheumatism. The river dolphin *Inia geoffrensis* is not cited, but the Dephinidae *Sotalia fluviatilis* (Gervais and Deville, 1853) and *Sotalia guianensis* (Van Bénéden, 1864), for the same uses: asthma, rheumatism, general anti-inflammatory, antifungal and to treat cancer. In a study undertaken in northeastern Brazil, Alves et al. (2012) describe the use of the turtle *Phrynops geoffroanus* (Schweigger, 1812) fat as an anti-inflammatory, for sore throat and flu and the fat from *Boa constrictor* (Linnaeus, 1758) for rheumatism, anti-inflammatory and to heal broken bones. The same uses are described for the boidae *Boa constrictor* by Alves and Rosa (2006).

The fat or oil from *Inia geoffrensis* (Blainville, 1817) has been described as used to treat asthma, rheumatism, headache womb disorders and sore throat in fishing communities in northeastern Brazil (Alves and Rosa, 2006).

The uses described in the Iquitos market are representative of the Peruvian Amazon. They are the same or very similar as the uses described in Brazil, with convergence of use as anti-rheumatic and to relief pain (Alves et al., 2009).

Massage is a relevant part of the treatments using animal oils in the Peruvian Amazon. Sensory stimulation including massage (Lund, 2000) has a positive effect on the perception of pain. Studies in human patients have shown that massage therapy reduced pain, anxiety, depression and sleep disturbance (Field et al., 2007). Swimming exercise decreased acute pain by activation of serotonergic and opioidergic pathways (Mazzardo-Martins et al., 2010). The effect of the animal oils in patients can be explained in part by its anti-inflammatory effect, combined with the massage associated with its administration. Other therapeutic practices that reduces pain include manual acupuncture (Cidral-Filho et al., 2011). The involvement of interleukin-10 in the anti-inflammatory effect of acupuncture in a rodent model of peritonitis was reported by Duarte da Silva et al. (2011). The animal studies and clinical data suggest that the way traditional drugs are administered might have a role in their effect.

The antimicrobial activity of the African crocodile (*Crocodilus niloticus*) was reported by Buthelezi et al. (2012). At 150 μL oil/mL, the oil reduced growth of *Staphylococus aureus* and *Klebsiella pneumoniae* by 58.4 and 54.0%, respectively. The activity is higher than that found for the oil of the South American species *Melanosuchus niger*, which showed MICs values $>1000$ μg/mL towards *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis*.

The anti-inflammatory activity of products of animal origin has been reported in several investigations that have used the model of edema induction in mice ears. Yoganathan et al., (2003) evaluated the anti-inflammatory activity of the *Dromaius novae-hollandiae* (emu) oil using TPA as the inflammatory agent. Researchers obtained a 70% edema inhibition in the mice treated. Falodun et al. (2008) showed the efficacy of *Boa constrictor* fat, since it lowered in 65%, 42% and 63% the edemas, in doses of 12, 6 and 3 mg/ear, respectively. Ferreira et al. (2010), showed the topical anti-inflammatory effect of the *Tupinambis merianae* oil when using TPA, AA and phenol as inflammatory agents. These authors concluded that, due to the composition of the fatty acids in the oils, these have anti-inflammatory effects because of the inhibition of the AA pathway and its metabolites. And so, the production of pro-inflammatory mediators is reduced. Ferreira et al. (2014) recently reported the anti-inflammatory effect of *Boa constrictor* oil on the *Croton* oil-induced ear edema in mice at the dose of 13 mg oil/ear. The *Boa constrictor* fat sample from Iquitos, evaluated at 3 mg/ear in mice, was obtained from an animal from the Amazon forest while the study of Ferreira et al. (2014) was undertaken with a sample from Joao Pessoa (Parahyba State) at the

northeastern Atlantic coast of Brazil. Given the different geographical origin of the samples, the comparative study of anti-inflammatory, antimicrobial and fatty acids composition of the Peruvian samples give an insight into the activity and chemical composition of Peruvian Amazon animal oils. In 2012, Buthelezi et al. ascertained the topical and oral anti-inflammatory effect of *Crocodylus niloticus* oil, reducing the edema by 60.8% three hours after oral administration and 57.5% twelve hours after topical administration. These results prove the relevance of evaluating a greater amount of natural animal origin products to treat inflammatory pathologies.

The model of ear edema induction in mice, using different inflammatory agents (TPA and AA), is widely used to identify the probable topical anti-inflammatory effect of a substance under study and its probable action mechanism (Gábor, 2003). The topical administration of TPA provokes an acute edema with leukocyte infiltration, acting through the triggering of the protein kinase C (PKC), which is $Ca^{2+}$ and phospholipids dependent. Active PKC acts at different levels, activating the nuclear factor kappa B (FN-κB). This transcription factor promotes the expression of several pro-inflammatory agents, such as cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS), inflammatory cytokines such as interleukins IL-1, IL-2, IL-6, IL-8 and the tumor necrosis factor (TNF-α), which is another pro-inflammatory and host defense cytokine. Its excessive production leads to chronic pro-inflammatory diseases, which brings as consequence the formation of prostanoids and the increased production of free radicals (Ban et al., 2009). By contrast, the anti-inflammatory response to AA is quicker and is produced by an increased activity of myeloperoxidase and elastase, due to neutrophils' quick arrival after applying this inflammatory agent (Ban et al., 2009). The topical administration of AA provokes a short lasting edema, which has a quick start associated to the increased levels of prostaglandins (PGs), thromboxane TXB2 and leukotriene LTB4, with a slight rise of LTC4 levels. The vasodilation begins several minutes after applying AA. After 10 min, the vascular permeability increases, developing the edema, which reaches its maximum point after 1 h and practically disappears after 6 h, even though a residual erythema remains. According to the administered dose of AA in the mice ear, its metabolism may lean towards a COX or a 5-lipoxigenasa (5-LO) pathway. In low concentrations, COX metabolites prevail, while in high doses 5-LO metabolites prevail. AA barely stimulates epidermal mitotic activity and DNA synthesis; therefore, there is no epidermal hyperplasia. The biochemical study shows that AA is quickly metabolized (approx. 15 min), allowing the formation of eicosanoids, mainly $PGE_2$, $LTC_4$ y $LTD_4$ (Inoue et al., 1988; Griswold et al., 1991).

Das (2008) observed that fatty acids may participate directly in the anti-inflammatory process, as part of the immune response of antigens, in which phospholipase $A_2$ ($PLA_2$) generates AA in the plasmatic membrane. Subsequently, AA is oxidized into prostaglandins, leukotrienes and thromboxanes, which are responsible for the visible symptoms of the inflammation (Griswold et al., 1987). However, numerous researchers have demonstrated that fatty acids possess anti-inflammatory effects, supporting their use to treat cutaneous inflammations. Recently, Cardoso et al., (2011) proposed that oleic acid can modulate inflammatory and immune responses in skin lesions. This fact results in differential wound repair and suggests that monounsaturated fatty acid n-9 may be a useful tool in the treatment of cutaneous wounds, especially in cases of skin burns and diabetic or pressure ulcers. Calder (2005) indicated that the administration of fatty acids generated a partial substitution in the plasmatic membrane of AA for another type of fatty acid, which triggers the decrease of AA and its by-products, which, in turns, would lessen the inflammatory response.

Regarding our results, all the oils evaluated showed topical anti-inflammatory activity and were most active against TPA (Table 1). This may be related to the composition of the oils, since unsaturated fatty acids which are the main components (Table 3) are known to show anti-inflammatory activity (Calder, 2005; Das, 2006; Saraiva et al., 2011). Henry et al. (2002) showed that at 100 ppm, oleic acid, one of the main fatty acid in the oils, moderately inhibited *in vitro* the enzyme COX-1. Oh et al. (2009) demonstrated that oleic acid reduces lipopolysaccharide-induced expression of iNOS and COX-2 in microglial cells. These results suggest that oleic acid shows anti-inflammatory effect by inhibiting ROS, p38, MAPK and Akt/IKK/NF-kB signaling pathways in LPS-stimulated BV2 microglia.

Previously, Fritsche (2008) proved that linoleic acid, another main component of the oils, is the precursor of the acid 13- hydroxyl-octadecadienoic (13-HODE) pathway 15-LOX. This acid inhibits the production of the superoxide anion, reduces the migration of polymorphonuclear lymphocytes, regulates the action of leukotrienes and prostanoids. Linoleic acid also inhibits in vitro the enzymes COX-1 and COX-2 (Ringbom et al., 2001; Henry et al., 2002) and reduces the gene expression of NF-κB due to the triggering of the PPAR-γ signals and TNF-α in human monocytic cells THP-1. This lowers the levels of IL-6, IL-1 and TNF-α (Zhao et al., 2005). Some studies have reported that linoleic acid is pro-inflammatory, since it is a precursor of AA through the enzyme Δ6-desaturase (Calder, 2005). However, Ziboh et al. (2000) proved that there is a low amount of this enzyme in the epidermis, and so, linoleic acid would not increase the production of AA in the skin. The results allow us to conclude that part of the topical anti-inflammatory activity of the oils evaluated is due mainly to oleic and linoleic acid.

## 4. Conclusions

A survey at the Belén Market in Iquitos, Peruvian Amazon, allowed the identification of several animal oils extensively used in traditional medicine to treat rheumatism and inflammatory conditions. Six of the most frequently used animal oils/fats were assessed as topic anti-inflammatory agents by the TPA an AA-induced inflammation as well as for antimicrobial effect. Two out of the five oils showed some antimicrobial activity and all of them presented anti-inflammatory effect by topical application, as used in traditional medicine. While our findings suggest a pharmacological basis for the therapeutic use of the animal oils, other factors should also be considered, including the massage associated with its application. In the best of our knowledge, this is the first report on the bioactivity and composition of zootherapeutics in the Peruvian Amazon. Further studies are required to document other medicinal products from the Peruvian fauna and to disclose its pharmacological potential.

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