Antimicrobial potential of anaconda body fat oil (Eunectes murinus), in Santarém city, Pará state, Brazil

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Abstract

The use of natural substances from animals and plants has always been the subject of research, because it is made in a rustic way and through popular knowledge, built by everyday experiences and without scientific approval. The oil from the body fat of the snake - or better known as lard of anaconda, extracted from the anaconda snake (Eunectes murinus) is a product widely used in traditional communities and is marketed in markets for therapeutic purposes in several diseases. Because of the low cost and popular beliefs, many people use zootherapeutic means to treat themselves. Therefore, the importance of therapeutic proof of these products. Thus, the aim of this study was to verify whether the lard of anaconda made in the traditional way has antimicrobial potential in certain strains of pathogenic bacteria and fungi. For that, the evaluation of the lard’s antimicrobial potential was carried out using the disk diffusion method, adapted from the manual M7-A6 standardized by the Clinical and Laboratory Standards Institute, against the strains of Staphylococcus aureus, Escherichia coli, Candida albicans and C. krusei. The observed results showed that there was no presence of bacterial or yeast growth inhibition halos, indicating that the anaconda lard oil did not inhibit the growth of fungi and bacteria in any of the strains used. Therefore, the present study allows us to conclude that the anaconda lard oil (E. murinus), did not present any inhibitory antimicrobial potential, against the tested bacterial and fungal strains.

Keywords: traditional knowledge, zootherapy, snake, bacteria.

Resumo

O uso de substâncias naturais provenientes de animais e plantas sempre foi alvo de pesquisas, por ser feito de forma rustica e através de saberes populares, construídos por experiências no cotidiano e sem aprovação científica. O óleo da gordura corporal da serpente – ou mais conhecida como banha de sucuriju, extraída da serpente sucuriju (Eunectes murinus) é um produto muito utilizado em comunidades tradicionais e é comercializada em mercados com finalidade terapêutica em diversas moléstias. Por conta do baixo custo e de crenças populares, muitas pessoas utilizam de meios zooterápicos para se tratar por si mesmo. Daí a importância da comprovação científica. O óleo da lardura corporal é um produto adaptado do manual M7-A6 padronizado pelo “Clinical and Laboratory Standards Institute”, frente às cepas de Staphylococcus aureus, Escherichia coli, Candida albicans e C. krusei. Os resultados observados demonstraram que não houve a presença de halos de inibição de crescimento bacteriano ou levaduriformes, indicando que o óleo da banha de sucuriju não inibiu o crescimento de fungos e bactérias em nenhuma das cepas utilizadas. Portanto, o presente estudo permite concluir que o óleo da banha de sucuriju (E. murinus), não apresentou nenhum potencial antimicrobiano inibitório, frente às cepas bacterianas e fúngicas testadas.

Palavras-chave: conhecimento tradicional, zooterapia, serpente, bactérias.

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**Introduction**

Fungi and bacteria are agents that promote health problems in both humans and animals. Bacteria have been living in the terrestrial environment for approximately 3.5 billion years, surviving temperature fluctuations, lack of nutrients, ultraviolet radiation and other external factors. These agents have a high adaptive and transmutation capacity that is linked to their genomic structure, which gives them the exchange of genes between bacteria (EmyInumaru et al., 2019). Thus, bacteria began to promote infectious processes that were only discovered in the 19th century when research related to these pathogens began (Sousa et al., 2019).

Therefore, different substances and drugs were produced, such as antimicrobials that are natural substances (antibiotics) or synthetic (chemotherapy) that act on microorganisms leading to inhibition of their growth or causing their destruction (Sáez-Llorens et al., 2000). Due to the great increase in the resistance of pathogenic microorganisms to multiple drugs, due to the indiscriminate use of antimicrobials, there is a concern for the search for new therapeutic alternatives (Novais et al., 2003, Antunes et al., 2006; Oliveira et al., 2006, 2007).

Natural products are an extremely viable alternative, since they have always been important for the discovery of new drugs, being suppliers of active ingredients and because they are also a more economical alternative in disease control for developing countries, where most drugs are imported (Xu & Lee 2001). In Brazil, several species of animals have been used for medicinal purposes since colonization, representing a therapeutic alternative widely disseminated throughout the country (Alves & Rosa 2006, 2007).

In view of the varied uses of animals, the use specifically for medicinal purposes is known as Zootherapy (Marques 1994; Alves & Rosa 2005). This may be understood as the use of medicines made from animal body parts, products of their metabolism (such as body secretions and excrement) or materials built by them, such as nests and cocoons (Costa-Neto & Alves 2010).

Among reptiles, the anaconda snake (*E. murinus*), is an example of an animal species widely used by the riverside population to combat some types of diseases. This animal's fat is used as antibiotics for inflammation and respiratory processes, as well as in the treatment of bone and muscle diseases (Silva, 2008).

The knowledge of the antimicrobial potential of natural products is necessary for a better understanding of their properties, proof of their effectiveness and the development of more complex drugs, which are difficult to adapt (Daferera et al., 2003). Thus, the aim of this study was to verify whether the lard of anaconda made in the traditional way has antimicrobial potential in certain strains of pathogenic bacteria and fungi.

**Materials and method**

**Specimen**

The specimen used in this research came from the region of Lago Grande do Curuai, which has communities spread over the floodplain and terra firme area, located in Santarém city, Pará state, under authorization SISBIO 14018-11. The preparation of the product was handmade: the fat located in the peritoneum region was removed and placed in a container to be prepared through cooking and the supernatant stored in small plastic containers. In the laboratory, the oil was placed in a bain-marie at 43º for 10 minutes to obtain its liquefaction, conditioned and kept in eppendorf tubes for further analysis. For the preparation of the solution, 100 mg of the sample was solubilized in 900 ml of Dimethyl Sulfoxide (DMSO - Merck, Darmstadt, Germany), to obtain an initial concentration of 100 mg/ml.

**Disk diffusion method**

The evaluation of the antimicrobial potential of the anaconda lard was carried out through a qualitative biological assay, in triplicate, using as positive controls the antibiotic chloramphenicol (10 µg) for *Staphylococcus aureus* and *Escherichia coli*; nystatin (250 µg) for *Candida albicans* and *C. krusei*. The test was performed using the disk diffusion method, adapted from the manual M7-A6 standardized by the Clinical and Laboratory Standards Institute (2003).
In Petri dishes containing 10 ml of Müller-Hinton agar (MH), the following microbial strains were inoculated with a swab on the surface of the growth medium: *S. aureus* (ATCC 25923), *E. coli* (ATCC 29922), *C. albicans* (ACLI) and *C. krusei* (ACLI). The inoculants have a concentration of 108 CFU/ml, originating from a previous culture in MH and SDA medium (Sabouraud Dextrose Agar) for 24 hours. The inoculum concentration was adjusted using a 0.5 MacFarland scale. Subsequently, on Whatman paper discs no. 4 (diameter 5 mm) sterilized, 10 µl of solutions at 100 mg/ml of lard were applied. Then, each plate received five paper disks, one being the positive control receiving the antibiotic or antifungal, a negative control containing DMSO and the assay triplicate. After the distribution of these discs, the plates were incubated at 37ºC for 24 and 48 h, respectively, for bacteria and yeast. After incubation, the reading of the bacterial or yeast growth inhibition halos was performed.

**Results and discussion**

There was no presence of bacterial or yeast growth inhibition halos, indicating that the anaconda body fat oil did not inhibit the growth of fungi and bacteria in any of the strains used (Figure 1A, B, C and D), revealing that this isolated product has no efficacy in the control of microorganisms, and its use by traditional medicine on diseases caused by infectious agents, has no pharmacological basis.

![Antimicrobial assay results](image1.png)

**Figure 1.** Photographic records of the antimicrobial assay of the anaconda body fat oil (*Eunectes murinus*) against *Staphylococcus aureus* isolates (A), *Escherichia coli* (B). R1, R2, R3 (triplicate): Anaconda lard + DMSO; C - (negative control): DMSO; C + (positive control): chloramphenicol. *Candida krusei* (C), *Candida albicans* (D). R1, R2, R3 (triplicate): Anaconda lard + DMSO; C - (negative control): DMSO; C + (positive control): nystatin.
In this study, the ineffectiveness in microbial control using anaconda body fat oil was observed, corroborating the study by Ferreira et al. (2009) who performed a work using the fixed oil extracted from the body fat of representatives of herpetological fauna, such as that of Tupinambis merianae, where the results showed that the oil tested in isolation did not present antibacterial activity, with clinical relevance, against the strains standard and multidrug-resistant E. coli and S. aureus, similar results were observed by Sales (2012) with Rhinella jim. where he demonstrated that the oil administration alone did not present clinically relevant antimicrobial activity. Similar results were observed in the study by Cabral (2012) with Leptodactylus macrosternum and L. vastus, where the oils tested alone did not show significant inhibitory action on bacterial and fungal growth against most of the tested strains, except for the Pseudomonas aeruginosa ATCC strains 15442 and C. krusei ATCC 6258, respectively. Furthermore, Oliveira (2013) using Spilotes pullatus, found that the oil did not inhibit the growth of fungi and bacteria used.

In contrast, presenting different results from this study, other studies have demonstrated the antimicrobial action of natural products from reptiles. Falodun et al. (2008) and Ferreira et al. (2011) showed that the fat of the Boa constrictor snake has antibacterial and antibiotic modulating activity, respectively. Dias (2013) indicated that the fixed oil of the chelonian Phrynops geoffroanus when analyzed separately against the strains of fungi and bacteria tested, showed antifungal activity from the clinical point of view for C. krusei ATCC 6258.

Ciscotto et al. (2009) describe the bactericidal and antiparasitic activity of L-amino-acid oxidase from the venom of the snake Bothrops jararaca, corroborating with Morais et al. (2009) who report the anticoagulant activity of the B. jararaca venom’s antithrombin, similar results were observed by Liu et al. (2008) who demonstrated an anti-tumor activity of extracts from the lizard Gekko japonicus widely used in traditional Chinese medicine. The researches, previously mentioned, disagree with the present study, which did not identify the antimicrobial potential of anaconda body fat oil (E. murinus) on the strains of bacteria and fungi evaluated.

Dias & Monteiro (2010) describe that due to a worrying reality with the control of bacteria and fungi, which is the resistance developed to existing drugs, with loss of their effectiveness. For Duarte (2006) the development of new drugs, as well as the bioprospecting for new antimicrobial substances from natural sources has been increasing the interest of pharmaceutical companies.

Conclusion

The body fat oil of anaconda (E. murinus), did not present any inhibitory antimicrobial potential in relation to the tested bacterial and fungal strains.

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