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Antifungal activity of paracloacal gland secretion of Caiman yacare (DAUDIN, 1802)

The objective of this study was to evaluate the in vitro antifungal activity of ethanolic and aqueous extracts obtained from Caiman yacare paracloacal gland (PG) against Candida albicans (ATCC 10231), as well as a bibliographical survey on the chemical composition of exudates of PG. The PG were collected from the disposal generated during the slaughter of C. yacare by regularized industry. Two extracts were made from these glands, one ethanolic and the other aqueous. The Minimal Inhibitory Concentration (MIC) of the substances were determined by dilution of the extract in series using the microdilution technique in the culture medium Sabouraud broth, carried out in a 96-well microplate visually read after 48 hours of incubation, confirmed by the method using 0.01% aqueous resazurin dye. The ethanolic extract had MIC at the concentration of 25 µg / L. The Minimum Fungicidal Concentration (MFC) was determined by subcultures of MIC in Sabouraud agar medium. The ethanolic extract presented MFC at a concentration of 50 µg / mL. The aqueous extract showed no antifungal activity at the concentrations tested. This work is the first work to assess an activity of the PG secretion and reveals pharmacological potential in a local product previously discarded.

Palavras-chave: Pantanal; Caiman; Candida albicans; Crocodilians; Yeasts.

Atividade antifúngica da secreção de glândulas paracloacais de Caiman yacare (DAUDIN, 1802)

Este estudo teve como objetivo avaliar a atividade antifúngica in vitro dos extratos etanólico e aquoso do exsudado obtido a partir glândula paracloacal (PG) de Caiman yacare frente a Candida albicans (ATCC 10231), bem como foi realizado um levantamento bibliográfico sobre a composição química de exsudatos de PG. As PG foram coletadas do descarte gerado durante o abate de C. yacare por frigorífico regularizado. Dessas glândulas foram confeccionados dois extratos, um etanólico e outro aquoso. A Concentração Inibitória Mínima (MIC) das substâncias, foram determinadas por meio da diluição do extrato em série através da técnica de microdiluição no meio de cultura caldo Sabouraud, realizado em placa de 96 poços com leitura verificada após 48 horas de incubação, confirmada pelo método visual utilizando corante de solução aquosa de resazurina a 0,01%. O extrato etanólico apresentou MIC na concentração de 25 µg / mL. A Concentração Fungicida Mínima (MFC) foi determinada por subculturas da MIC em meio de cultura ágar Sabouraud. O extrato etanólico apresentou MFC na concentração de 50 µg / mL. O extrato aquoso não apresentou atividade antifúngica nas concentrações testadas. Esse trabalho é o primeiro a aferir uma atividade da secreção da PG e revela potencial farmacológico em um produto local até então descartado.

Keywords: Pantanal; Jacaré; Candida albicans; Crocodilianos; Leveduras.

Topic: **Uso de Recursos Naturais**

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INTRODUCTION

The launch of new antibiotics decreased in last years, and reports of resistance to antibiotic therapy increased (GUIMARÃES et al., 2010). Among pathogens, there are microorganisms able to resist the human defense mechanisms, in addition, the incorrect use of chemotherapeutic favors the emergence of resistant microorganisms (GIAMARELLOU, 2006; TORTORA et al., 2012).

Bioprospection of essential oils from plants and zoological products is growing worldwide (BAYDAR et al., 2004; INOUYE et al., 2006), therefore, due to high biodiversity, Brazil can occupy a protagonist role in the scenario (KATE et al., 1999). A potential starting point for bioprospection can be the traditional communities, that even before the advent of antibiotic therapy, treated their patients through the use of local flora and fauna (ALBERNAZ et al., 2010; BARROS et al., 2012; MENALE et al. 2016; OLIVEIRA et al., 2010).

By-products obtained from parts of crocodilians, are frequently described by traditional knowledge, being the species *Paleosuchus palpebrosus* and *Caiman crocodiles* with greater presence (ALVES et al., 2010). The use of fresh fats of *Caiman latirostris*, *Melanosuchus niger* and *P. palpebrosus* for treatments of rheumatism (ALVES et al., 2008); use of fresh fat from *C. crocodilus* for treatment of pneumonia (BARROS et al., 2012); the use of fat of *C. latirostris* as healing (SOUTO et al., 2011) or for treatment of asthma (OLIVEIRA et al., 2010).

There are some scientific results regarding the use of by-products obtained from crocodilians, such as the anti-inflammatory and antimicrobial action of *Crocodylus niloticus* oil. (BUTHELEZI et al., 2012), antibacterial activity of the serum protein profile of *Alligator mississippiensis* (MERCHANT et al., 2003) Amoebicidal effect of *A. mississippiensis* serum (MERCHANT et al., 2004), a high antimicrobial activity of the leukocyte extract of *A. mississipiensis* (MERCHANT et al., 2006), antimicrobial and anti-inflammatory activities in *C. niloticus* oil (BUTHELEZI et al., 2012), antimicrobial and anti-inflammatory activity of the blood extracts of *Crocodylus siamensis* (PATA et al., 2011; KOMMANEE et al., 2012; KOMMANEE et al., 2014).

In the Pantanal, there is the cultivation of *Caiman yacare* (Jacaré do Pantanal), which represents a diversification of zootechnical and economic activities in the region. The main products commercialized are leather and meat, however, there are efforts to valorize by-products, such as the production of hamburgers, sausages and mortadella using less noble meats (FERNANDES et al., 2014; MORAIS et al., 2013; ROMANELLI et al., 2002).

The pharmacological potential of crocodilian by-products can be a promising area, adding economic value to the *C. yacare* productive chain. During the slaughter there is generation of discard of viscera, and between them there is Paracloacal Gland (PG). The exudate of PG from the crocodilians has many molecules on their chemical composition, and some of them can exert the antifungal effect in cloaca.

Finally, yeasts of *Candida albicans* is the cause of most fungal infections, which is considered an opportunistic infection that affects predisposed individuals, usually those with inefficient immune systems or who have been subjected to prolonged antibiotic treatment (LIMA et al., 2006; ZHANG et al., 2002).

In this work, it was evaluated the fungicidal activity of *C. yacare* PG exudate against *Candida albicans* (ATCC 10231), as well as a bibliographic survey on the chemical composition of PG exudate from many species of crocodilians.

MATERIAL AND METHODS

The research was carried out in partnership with the company regularly authorized by the current standard, which carries out the slaughter of *C. yacare* (SISBio 58681-1). We collected 30 PG from *C. yacare* measuring over 1.30 meters in length. The PG were submitted to mechanical asepsis with washing in running water and chemical asepsis with three times of 5 minutes baths in 70% alcohol, a bath with 2.0% sodium hypochlorite for 5 minutes and three baths with sterile distilled water for 5 minutes.

Glandular secretion was extracted by manual compression of the PG capsule in a mortar (WELDON et al., 1989; WILLIAMS et al., 1989). The exudate was drained and preserved in a sterile refrigerated bottle. For the antifungal test, two extracts were prepared, one ethanolic and one aqueous.

The exudates were added in two flasks containing, respectively, ethanol (99.8%) and sterile distilled water, in the proportion of 50% exudate and 50% diluent. The flasks were shaken daily and, after 14 days, the two solutions were filtered on filter paper and 1 mL of the filtrate was dried in vacuo. The sample was considered free of diluent when the weight did not decrease among successive weighing.

The extracts of PG were respectively resuspended in ethanol and distilled water at the final concentration of 800 µg / mL (ALVARENGA et al., 2007; BAYDAR et al., 2004; NASCIMENTO et al., 2007; OLIVEIRA et al., 2011; SANTOS et al., 2010).

For the evaluation of the antifungal activity, a lineage of *Candida albicans* (ATCC 10231) was used, which was suspended in 0.85% saline solution, comparing with the MacFarland 0.5 scale, as a standardization of inoculum density, which represents 1, 5 x 10^6 Colony Forming Units (UFC / mL) (CLSI, 2012).

The antifungal activity in the Minimum Inhibitory Concentration (MIC) of the elaborated extracts was determined by the broth microdilution method (CLSI, 2008; 2012) adapted. Aliquots of 100 µL of the PG extract suspension were added to 100 µL of Sabouraud broth culture medium in the first well of the 96 well plate "lines" and the serial (1: 2) dilution was performed, resulting in concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg / mL. Subsequently, 10 µL of standardized inoculum 1.5 x 10^6 (UFC / mL) was added to each well (CLSI, 2008; 2012).

The antibiotic Itraconazole 100 µg / mL was used as antifungal control. The control of diluent effect was carried out by ethanol 2% (v/v) assay. Sterility control of the medium was carried out by observing the culture medium Sabouraud broth without any interference.

The microplates were incubated for 48 hours at 37° C, after the incubation period 20 µL of 0.01% aqueous resazurin solution was added to each well and reincubated for 6 hours at 37° C, after the incubation time with resazurin the microplates were analyzed.

Resazurin was used because the aqueous and ethanolic extract of PG acquired a cloudy coloration that makes difficult the optical reading, therefore the chromatic conversion of resazurin is the most suitable

for oils extracted from PG. Classically, the resazurin indicates absence of microorganism growth when keep the blue color into the well at the end of the protocol (ALVES et al., 2008) and the MIC was defined as the lowest concentration of the tested solution that prevented the growth of the microorganism (NATECHE, 2006; PALOMINO et al., 2002).

After visual reading of the microplate, 10 µL were removed from the last three concentrations of microplates wells that keep the blue resazurin color, and seeded in Petri dishes containing Sabouraud agar, and then the plates were incubated for an additional 48 hours at 37° C for determination of Minimum Fungicidal Concentration (MFC). The concentrations with growth in the Petri dish was considered as fungistatic action (ELLOF, 1998), and the concentration with absence of growth was considered MFC.

The antimicrobial activity was classified as good activity when MIC <100 µg / mL and MIC moderated between 100 and 500 µg / mL (HOLETZ et al., 2002). The assays were performed in triplicate.

RESULTS

The PG is located in the distal part of the digestive system, inserted in the wall of the cloaca, exhibit an oval shape, pink color, with an aperture to cloaca lumen. The largest glands belonging to males, surrounded by capsular musculature. The exudate has a golden yellow color, is viscous, with a strong odor that disperses easily in the environment.

The yield of the ethanolic extract was 43.00% and the yield of the aqueous extract was 83.03% of the volume of the dried filtrate under vacuum.

The aqueous extract exhibit resazurin conversion from blue to pink on all tested concentrations of 400 to 0.78 µg / mL. The ethanolic extract exhibit no resazurin conversion on 400 to 25 µg / mL, considering MIC of 25 µg / mL for *C. albicans.* Wells with concentrations below 25 µg / mL exhibit resazurin conversion. Ethanolic and sterile control exhibit no resazurin conversion.

The agar incubation of ethanolic extract assay exhibit death of 99,9% of *C. albicans* at MFC of 50 µg / mL, and the concentration of 25 µg / mL was considered fungistatic once it presents a few growth of *C. albicans* (Table 1). In ethanol control the inoculum remained viable throughout the experiment period.

Table 1: MIC and MFC (µg / ml) of extracts of PG of C. yacare.

Extracts	**Microorganism**	
	Candida albicans	
	MIC	MFC
AEPGO	NC	NC
PGOEE	25	50

AEPGO – Aqueous Extract of Paracloacal Gland Oil; PGOEE - Paracloacal Gland Oil Ethanol Extract; NC – Did not get results in the tested Concentrations.

DISCUSSION

The knowledge of traditional communities in the search for bioactive molecules may represent a promising starting point for pharmaceutical research (PINTO et al., 2002; SANT'ANA, 2002), so that about

50% of current medicines were developed from natural resources. In this sense, there is a growing interest in the pharmaceutical industry for bioprospecting these natural resources (JUNIOR, 2010).

Adding value to caiman´s products and by-products helps make zootechnical activity even more feasible, a form of incentive to maintain a renewable resource rather than exploit and destroy existing populations, promoting population interests to produce and preserve the biome in which the species is inserted (BLAKE et al., 1975; MOURÃO, 2000).

This article characterizes an antifungal activity evidenced by in vitro tests against the yeasts *C. albicans*. The results are consistent with those found in crocodile by-products, such as in *C. niloticus* oil, with ample antibacterial activity and marked fungicidal activity against *C. albicans* (BUTHELEZI et al., 2012); Antibacterial and antifungal activity against *C. albicans* by leukocytes extracts of *C. siamenses* (PATA et al., 2011) and a wide antimicrobial activity of leucocyte extract from *A. mississippiensis* (MERCHAN et al., 2006).

The good antimicrobial activity was obtained from a crude oil with low solubility in culture medium. In order to improve the quality of the experiments with essential oils, solvents, detergents, or emulsifying agents, such as ethanol, are used to facilitate their solubility in the culture medium (NASCIMENTO et al., 2007). In this study the ethanolic control did not present inhibitory activity against the inoculum result similar to other studies (ALVARENGA et al., 2007; BAYDAR et al., 2004; NASCIMENTO et al., 2007; OLIVEIRA et al., 2011; SANTOS et al., 2010).

The absence of antifungal activity of aqueous extract, in comparison to ethanolic extract, can be explained by the polarity of the solvent, once water and ethanol solubilizes different molecules according to hydrophobicity affinity.

Based on the bibliographical references consulted about the chemical composition of the paracloacal secretion, we found 234 different substances, described by 8 articles that deal with chemical compositions in several species of crocodilians. In *A. mississippiensis* were described 27 molecules, *Alligator sinensis* 55 molecules, *C. crocodilus* 60 molecules, *C. latirostris* 41 molecules, *C. yacare* 23 molecules, *Crocodylus acutus* 68 molecules, *P. palpebrosus* 43 molecules and *Paleosuchus trigonatus* 36 molecules (DUNN et al., 1993; GARCÍA-RUBIO et al., 2002; KRUCKERT et al., 2006; MCDANIEL et al., 1998; WELDON et al., 1989; WELDON et al., 1988, 1989; WHEELER et al., 1999). Among the molecules there were esters, alcohol, carboxylic acids, ketones, aldehydes and hydrocarbons. The esters were the most frequently described molecule, followed by ketone and alcohol. *C. yacare* presented the lowest number of molecules described (Table 2). Later works will describe other substances present in the ethanolic extract and pointed out the substance responsible for the antifungal activity.

Table 2: Molecules found at paracloacal glands of *Alligator mississippiensis, Alligator sinensis, Caiman crocodilus, Caiman latirostris, Caiman yacare, Crocodylus acutus, Paleosuchus palpebrosus and Paleosuchus trigonatus.*

ÉSTERES	*A. sinensis*	*A. mississippiensis*	*C. crocodilus*	*C. latirostris*	*C. yacare*	*P. palpebrosus*	*P. trigonatus*	*C. acutus*
10-Heptadecenyl acetate	+							
11-Octadecenyl acetate	+							
3,7-Dimethyl-6-octen-1-yl (acetato de citronelilo)			+			+		
3-Dodecenyl acetate	+							

4,8-Heptadecadienyl acetate	+											
4-Tridecenyl acetate	+											
5,9-Octadecadienyl acetate	+											
5-Hexadecenyl acetate	+											
7-Hexadecenyl formate												+
8-Heptadecenyl acetate	+											
8-Pentadecenyl acetate	+											
9-Octadecenyl acetate	+											
Acetate dihidrofarnesil				+								
Butanoato de citronelilo				+		+						
Cholesteryl formate												+
Citronellyl acetate												+
Citronellyl butyrate												+
Citronellyl format												+
Citronellyl butanoate						+						
Citronellyl pentanoate						+						
Decyl 3-methylbutanoate		+										
Decyl acetate		+							+			
Decyl tetradecanoate***		+										
DHF 3-methylbutanoate				+								
DHF acetate				+								
DHF butenoate				+								
DHF dodecanoate				+								
DHF heptadecenoate				+								
DHF hexadecenoate				+								
DHF hexanoate				+								
DHF octadecanoate				+								
DHF octadecenoate				+								
DHF octanoate				+								
DHF pentadecenoate				+								
DHF propanoate				+								
DHF tetradecanoate				+								
DHF tetradecenoate				+								
DHF tridecanoate				+								
DHF undecanoate				+								
Dodecyl 3-methylbutanoate		+										
Dodecyl acetate	+	+							+			+
Dodecyl butanoate		+										
Dodecyl dodecanoate		+										
Dodecyl formate												+
Dodecyl hexadecanoate		+										
Dodecyl hexanoate		+										
Dodecyl octadecanoate		+										
Dodecyl oleate												+
Dodecyl pentanoate											+	
Dodecyl tetradecanoate		+				+						
Eicosenyl acetate												+
Eicosenyl formate												+
Eicosyl acetate												+
Heptadecenyl butyrate												+
Heptadecadienyl acetate												+
Heptadecadienyl formate												+
Heptadecenyl acetate									+			
Heptadecenyl acetate												+
Heptadecenyl butanoate									+			
Heptadecenyl butyrate												+
Heptadecenyl formate												+
Heptadecyl acetate												+
Heptadecyl butanoate									+			
Heptadecyl butyrate												+
Heptadecyl formate												+
Hexadecadienyl acetate		+										
Hexadecenyl acetate		+							+			+
Hexadecenyl butanoate									+			

Hexadecenyl butyrate								+
Hexadecenyl propanoate					+			
Hexadecyl acetate	+	+				+	+	+
Hexadecyl butanoate						+	+	
Hexadecyl caproate								+
Hexadecyl caprylate								+
Hexadecyl formate								+
Hexadecyl pentanoate							+	
Hexadecyl propanoate					+			
Hexadecyl propanoate						+		
Citronellyl hexanoate				+				
Methyl palmitate								+
Nonadecyl acetate								+
Nonyl acetate							+	
Octadecadienyl acetate		+						+
Octadecadienyl butyrate								+
Octadecenyl acetate		+						+
Octadecenyl butanoate						+		
Octadecenyl butyrate								+
Octadecenyl formate								+
Octadecyl acetate	+	+				+		+
Octadecyl butanoate						+		
Octadecyl butyrate								+
Octadecyl formate								+
Octyl Oleate								+
Pentadecadienyl acetate								+
Pentadecane								+
Pentadecenyl acetate	+							+
Pentadecenyl butanoate						+		
Pentadecenyl butyrate								+
Pentadecenyl formate								+
Pentadecyl acetate	+	+				+	+	+
Pentadecyl butanoate						+	+	
Pentadecyl butyrate								+
Pentadecyl caproate								+
Pentadecyl formate								+
Pentadecyl pentanoate							+	
Tetradecenyl acetate								+
Tetradecenyl butanoate						+		
Tetradecenyl butyrate								+
Tetradecyl 3-methylbutanoate		+						
Tetradecyl acetate		+					+	+
Tetradecyl butanoate						+	+	
Tetradecyl butyrate								+
Tetradecyl dodecanoate		+			+			
Tetradecyl formate								+
Tetradecyl hexadecanoate		+						
Tetradecyl pentanoate						+		
Tetradecyl propanoate					+	+		
Tetradecyl tetradecanoate		+						
Tridecyl acetate	+					+	+	+
Tridecyl butanoate						+	+	
Tridecyl butyrate								+
x,y-Heptadecadienyl acetate	+							
x,y-Hexadecadienyl acetate	+							
x,y-Octadecadienyl acetate	+							
x,y-Tetradecadienyl acetate	+							
x-Pentadecenyl acetate	+							
z,w-Heptadecadienyl acetate	+							

z,w-Pentadecadienyl acetate	+							
ETANOL	***A. sinensis***	***A. mississippiensis***	***C. crocodilus***	***C. latirostris***	***C. yacare***	***P. palpebrosus***	***P. trigonatus***	***C. acutus***
10-Heptadecen-1-ol	+							
11-Octadecen-1-ol	+							
2,3-Dihidrofarnesol			+					
3,7-Dimetil-6-octen-1-ol (citronelol)				+		+	+	
8-Heptadecen-1-ol	+							
Decanol						+	+	
3-Dodecen-1-ol	+							
Dodecanol	+						+	
u,v-Heptadecadiendien-1-ol	+							
Heptadecadienyl alcohol								+
Heptadecanol	+							
Heptadecenol							+	
x,y-Heptadecdien-1-ol	+							
Heptadecyl alcohol								+
z,w-Heptadecadien-1-ol	+							
Hexadec-5-an-1-ol	+							
Hexadec-9-an-1-ol	+							
Hexadecanol	+						+	
Hexadecanol						+		
Hexadecenyl alcohol								+
x,y-Hexadecadien-1-ol	+							
Hexadecyl alcohol								+
Nonanol						+		
Nonenol							+	
5,9-Octadecadien-1-ol	+							
9-Octadecen-1-ol	+							
Octadecanol	+					+		
Octadecenol							+	
Octadecenyl alcohol								+
x,y-Octadecadien-1-ol	+							
Octadecyl alcohol								+
Octanol							+	
8-Pentadecen-1-ol	+							
Pentadecanol							+	
Pentadecenol						+		
x, y-Pentadecadien-1-ol	+							
x-Pentadecen-1-ol	+							
z, w-Pentadecadien-1-ol	+							
Pentadecyl alcohol								+
5-Tetradecen-1-ol	+							
Tetradecanol	+					+	+	
4-Tridecen-1-ol	+							
Tridecanol	+					+	+	
Tridecenol							+	
Tridecyl alcohol								+
Undecanol						+	+	
α-Tocoferol		+						
ÁCIDO CARBOXÍLICO	***A. sinensis***	***A. mississippiensis***	***C. crocodilus***	***C. latirostris***	***C. yacare***	***P. palpebrosus***	***P. trigonatus***	***C. acutus***
z,9- Octanoic acid	+							
Oleic acid								+
Heptadecanoic acid	+							
Hexadecanoic acid	+	+				+	+	
Myristic acid								+
Octadecanoic acid		+						
Palmitic acid								+
Pentadecanoic acid	+					+		+
Stearic acid								+
Tetradecanoic acid	+							
Z-9- hexadecenoic acid	+							
Z-9- octadecanoic acid	+							

CETONA	***A. sinensis***	***A. mississippiensis***	***C. crocodilus***	***C. latirostris***	***C. yacare***	***P. palpebrosus***	***P. trigonatus***	***C. acutus***
3-Ethylheptadecan-2-one			+					
3-Ethylheptan-2-one			+		+			
3-Ethylheptan-4-one			+	+	+			
3-Ethylnon-5-en-4-one			+	+	+			
3-Ethylnonan-2-one			+	+	+			
3-Ethylnonan-4-one			+	+	+	+	+	
3-Ethylnonane-2,4-dione			+	+				
3-Ethylnonane-4,6-dione			+		+		+	
3-Ethylpentadecan-2-one			+	+	+		+	
3-Ethyltridecan-2-one			+				+	
3-Ethylundecan-2-one							+	
3-Etil-undecan-2-ona			+					
3-Etil-undecane-2,4-diona			+					
5-Ethyldec-2-en-4-one			+	+				
5-Ethylhept-2-en-4-one			+	+	+			
5-Ethylheptane-2,4-dione			+	+				
5-Ethylnon-2-em-4-one			+	+	+	+	+	
5-Ethylnonan-4-one			+	+	+	+		
5-Ethylnonane-2,4dione			+	+	+	+		
5-Ethyloct-2-em-4-one				+		+		
5-Ethyloctan-4-one			+	+				
5-Ethylpendadec-2-en-4-one			+		+			
5-Ethylpentadecane-2,4-dione								
5-Ethyltridec-2-en-4-one			+	+	+			
5-Ethyltridecane-2,4-dione			+					
5-Ethylundec-2-en-4-one			+	+	+	+		
5-Ethylundecan-4-one								
5-Ethylundecan-6-one			+	+	+	+	+	
5-Etil-undecane-2,4-diona			+	+	+			
7-Ethyldec-4-en-6-one			+	+				
7-Ethyldodec-4-em-6-one			+	+				
7-Ethylheptadecane-4,6-dione			+					
7-Ethylpentadec-4-en-one			+					
7-Ethyltridec-4-en-6-one			+	+	+			
7-Ethyltridecan-6-one			+					
7-Ethyltridecane-4,6-dione			+	+	+			
7-Ethylundec-4-en-6-one			+	+	+	+	+	
7-Ethylundecane-4,6-dione			+	+	+			
7-Etil-heptadec-4-en-6-ona			+	+	+			
Heptan-3-one				+				
ÁLDEIDOS	***A. sinensis***	***A. mississippiensis***	***C. crocodilus***	***C. latirostris***	***C. yacare***	***P. palpebrosus***	***P. trigonatus***	***C. acutus***
2-Ethyldecanal			+	+				
2-Ethyldodecanal			+	+	+		+	
2-Ethyloctanal			+	+				
2-Ethyltetradecanal			+	+	+	+	+	
HYDROCARBONETOS	***A. sinensis***	***A. mississippiensis***	***C. crocodilus***	***C. latirostris***	***C. yacare***	***P. palpebrosus***	***P. trigonatus***	***C. acutus***
Cholesta-3,5-diene								+
Cholesterol		+		+				+
Sesquiterpeno				+				

DHF = Dihidrofarnesol; Table according description in: (DUNN et al., 1993; GARCÍA-RUBIO et al., 2002; KRUCKERT et al., 2006; MCDANIEL; DUNN; WELDON, 1998; WELDON; SCOTT; TANNER, 1989; WELDON; SHAFAGATI; WHEELER, 1988, 1989; WHEELER; IBRAHIM; WELDON, 1999).

The ethanolic extract of PG exudate of *C. yacare* had a MIC of 25 µg / mL and MFC of 50 µg / mL against *C. albicans*. In accordance with this result it is possible to affirm a antimicrobial function exerted by PG on *C. yacare* cloaca, which probably protects cloaca and neighboring structures from fungal infections. This statement makes sense since the natural history of *C. yacare* is closely associated with water and PG may be a natural passive defense of the organism.

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REFERENCES

ALBERNAZ, L. C.; DE PAULA, J. E.; ROMERO, G. A.; SILVA, M. DO R.; GRELLIER, P.; MAMBU, L.; ESPINDOLA, L. S.. Investigation of plant extracts in traditional medicine of the Brazilian Cerrado against protozoans and yeasts. **Journal of Ethnopharmacology**, v. 131, n. 1, p. 116–121, 2010. **DOI**: http://doi.org/10.1016/j.jep.2010.06.011

ALVARENGA, A. L.; SCHWAN, R. F.; DIAS, D. R.; SCHWAN-ESTRADA, K. R. F.; BRAVO-MARTINS, C. E. C.. Atividade antimicrobiana de extratos vegetais sobre bactérias patogênicas humanas. **Rev. Bras. Pl. Med.**, p.86-91, 2007.

ALVES, R. R. N.; VIEIRA, W. L. S.; SANTANA, G. G. Reptiles used in traditional folk medicine: conservation implications. **Biodiversity and Conservation**, v. 17, n. 8, p. 2037–2049, 1 jul. 2008. **DOI**: http://doi.org/10.1007/s10531-007-9305-0

ALVES, R. R. N.; ROSA, I. L. Trade of Animals Used in Brazilian Traditional Medicine: Trends and Implications for Conservation. **Human Ecology**, v. 38, n. 5, p. 691–704, 2010.

BARROS, B. F.; VARELA, A. M. S.; PEREIRA, M. H.; VICENTE, L… Medicinal use of fauna by a traditional community in the Brazilian Amazonia. **Journal of Ethnobiology and Ethnomedicine**, v. 8, n. 1, p. 1–19, 2012. **DOI**: http://doi.org/10.1186/1746-4269-8-37

BAYDAR, H.; SA, O.. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. **Food Control**, v. 15, p. 169–172, 2004. **DOI**: http://doi.org/10.1016/S0956-7135(03)00028-8

BLAKE, D. K.; LOVERIDGE, J. P.. The role of commercial crocodile farming in crocodile conservation. **Biological Conservation**, v. 8, n. 4, p. 261–272, 1975. **DOI**: http://doi.org/10.1016/0006-3207(75)90004-X

BUTHELEZI, S.; SOUTHWAY, C.; GOVINDEN, U.; BODENSTEIN, J.; TOIT, D. K.. An investigation of the antimicrobial and anti-inflammatory activities of crocodile oil. **Journal of Ethnopharmacology**, v. 143, n. 1, p. 325–330, **DOI**: http://doi.org/10.1016/j.jep.2012.06.040

CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard - third edition. Clinical and Laboratory Standards Institute, n. April, p. 1-25, 2008.

CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standar- Ninth Edition. Anais..2012

DUNN, B. S. J.; WELDON, P. J.; HOWARD, R. W.; MCDANIEL, C. A.. A Lipids From the Paracloacal Glands of the Chinese Alligator (Alligator sinensis). **American Oil Chemist's Society**, v. 28, n. 1, p. 75–78, 1993. **DOI**: http://doi.org/10.1007/BF02536366

ELLOF, J. N.. A sensitive and quick Microplate Method to Determine the Minimum Inhibitory Sensisitive and Concentration of Plant Extracts for Bacteria. **Planta Medica**, v. 64, n. 2, p. 711–713, 1998. **DOI**: http://doi.org/10.1055/s-2006-957563

FERNANDES, V. R. T.; SOUZA FRANCO, M. L.; MIKCHA, J. M; DE SOUZA, V. L.; GASPARINO, E.; COUTINHO, M. E.; TANAMATI, A.; DEL VESCO A. P.. Yacare caiman (Caiman yacare) trim hamburger and sausage subjected to different smoking techniques. **Journal of the Science of Food and Agriculture**, v. 94, n. 3, p. 468–472, fev. 2014. **DOI**: http://doi.org/10.1002/jsfa.6270

GIAMARELLOU, H.. Treatment options for multidrug-resistant bacteria. **Future drugs**, v. 4, n. 4, p. 601–18, 2006. **DOI**: http://doi.org/10.1586/14787210.4.4.601

GARCÍA-RUBIO, S.; ATTYGALLE, A. B.; WELDON, P. J.; MEINWALD, J.. Reptilian chemistry: volatile compounds from paracloacal glands of the American crocodile (Crocodylus acutus). **Journal of chemical ecology**, v. 28, n. 4, p. 769–781, 2002. **DOI**: http://doi.org/10.1023/A:1015288726605

GUIMARÃES, D. O.; MOMESSO, L. S.; PUPPO, M. T.. Antibióticos: Importância terapêutica e perspectivas para a descoberta e desenvolvimento de novos agentes. **Química Nova**, v. 33, n. 3, p. 667–679, 2010.

HOLETZ, F. B.; PESSINI, G. L.; SANCHES, N. R.; CORTEZ, D. A. G.; NAKAMURA, C. V.; FILHO, B. P. D.. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. **Memórias do Instituto Oswaldo Cruz**, v. 97, n. 7, p. 1027–1031, out. 2002. **DOI**: http://doi.org/10.1590/S0074-02762002000700017

INOUYE, S.; UCHIDA, K.; MARUYAMA, N.; YAMAGUCHI, H.; ABE, S. et al.. A Novel Method to Estimate the Contribution of the Vapor Activity of Essential Oils in Agar Diffusion Assay. **Japanese Journal of Medical Mycology**, v. 47, 2006.

JUNIOR, N. L. S.. A regulamentação de acesso a recursos genéticos e repartição de benefícios: Disputas dentro e fora

do Brasil. **Ambiente & Sociedade**, v. 14, n. 1, p. 229–244, 2010. **DOI**: http://doi.org/10.1590/S1414-753X2011000100013

KATE, K. T and LAIRD, S. A., 1999.. The commercial use of biodiversity: Access to gentic resources and benefit-sharing. 1ª ed. UK: Earthscan. 168p.

KOMMANEE, J.; PREECHARRAM, S.; DADUANG, S.; TEMSIRIPONG, Y.; DHIRAVISIT, A.; YAMADA, Y.; THAMMASIRIRAK, S.. Antibacterial activity of plasma from crocodile (Crocodylus siamensis) against pathogenic bacteria. **Annals of clinical microbiology and antimicrobials**, v. 11, n. 1, p. 22, jan. 2012. **DOI**: http://doi.org/10.1186/1476-0711-11-22

KOMMANEE, J.; Phosri, S.; Daduang, S.; Temsiripong, Y.; Dhiravisit, A.; Thammasirirak, S.. Comparisons of anti-inflammatory activity of crocodile (Crocodylus siamensis) blood extract. **Chiang Mai Journal of Science**, v. 41, n. 3, p. 627–634, 2014.

KRUCKERT, K.; FLACHSBARTH, B.; SCHULZ, S.; HENTSCHEL, U.; WELDON, P. J.. Ehtyl-branched Adehydes, ketones, and Diketones from Caimans (Caiman and Paleosuchus; Crocodylia, Reptilia). **Natural Products**, v. 69, n. 6, 2006. **DOI**: http://doi.org/10.1021/np0600797

LIMA, I. DE O.; OLIVEIRA, R. DE A. G.; LIMA, E, DE O.; FARIAS, N. M. P.; SOUZA, E. L.. Atividade antifúngica de óleos essenciais sobre espécies de Candida. **Brazilian Journal of Pharmacognosy**, v. 16, n. 2, p. 197–201, 2006.

MCDANIEL, C. J.; Dunn, B. J.; Weldon, P. J.. Citronellol and Citronellyl Esters from the Paracloacal Glands of the Broad-Snouted Caiman, Caiman latirostris (Crocodylia, Reptilia). **Z. Naturforsch**, p. 932–934, 1998. **DOI**: http://doi.org/10.1515/znc-1998-9-1026

MENALE, B.; DE CASTRO, O.; CASCONE, C.; MUOIO, R.. Ethnobotanical investigation on medicinal plants in the Vesuvio National Park (Campania, Southern Italy). **Journal of Ethnopharmacology**, v. 192, p. 320–349, 2016. **DOI**: http://doi.org/10.1016/j.jep.2016.07.049

MERCHANT, M.; THIBODEAUX, D.; LOUBSER, K.; ELSEY, R. M.. Amoebacidal effects of serum from the American alligator (Alligator mississippiensis). Journal of Parasitology, v. 90, n. 6, p. 1480–1483, 2004. **DOI**: http://doi.org/10.1645/GE-3382

MERCHANT, M. E.; ROCHE, C.; ELSEY, R. M.; PRUDHOMME, J.. Antibacterial properties of serum from the American alligator (Alligator mississippiensis). **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 136, n. 3, p. 505–513, nov. 2003. **DOI**: http://doi.org/10.1016/S1096-4959(03)00256-2

MERCHANT, M. E.; LEGER, N.; JERKINS, E.; MILLS, K.; PALLANSCH, M. B.; PAULMAN, R. L.; PTAK, R. G.. Broad spectrum antimicrobial activity of leukocyte extracts from the American alligator (Alligator mississippiensis). **Veterinary immunology and immunopathology**, v. 110, n. 3–4, p. 221–8, 15 abr. 2006. **DOI**: http://doi.org/10.1463/1948020

MORAIS, C. S. N.; MORAIS, J. N. N.; VICENTE-NETO, J.; RAMOS, E. Z.; ALMEIDA, J.; ROSEIRO, C.; SANTOS, C.; GAMA, L. T.; BRESSAN, M. C.. Mortadella sausage manufactured with Caiman yacare (Caiman crocodilus yacare) meat, pork backfat, and soybean oil. **Meat Science**, v. 95, n. 2, p. 403–411, out. 2013. **DOI**: http://doi.org/10.1016/j.meatsci.2013.04.017

MOURÃO, G.. DE M. Utilização econômica da fauna silvestre no Brasil: O exemplo do jacaré-do-pantanal. EMBRAPA, v. 5, p. 1–4, 2000.

NASCIMENTO, P. F. C.; NASCIMENTO, A. C.; RODRIGUES, C. S.. Atividade antimicrobiana dos óleos essenciais : uma abordagem multifatorial dos métodos. **Brazilian Journal of Pharmacognosy**, v. 17, n. 1, p. 108–113, 2007. **DOI**: http://doi.org/10.1590/S0102-695X2007000100020

NATECHE, F. et al. Application of the resazurin microtitre assay for detection of multidrug resistance in Mycobacterium tuberculosis in Algiers. **Journal of Medical Microbiology**, v. 55, n. 7, p. 857–860, 2006. **DOI**: http://doi.org/10.1099/jmm.0.46513-0

OLIVEIRA, E. S.; TORRES, D. F.; BROOKS, S. E.; ALVES, R. R.N.. The medicinal animal markets in the metropolitan region of Natal City, northeastern Brazil. **Journal of Ethnopharmacology**, v. 130, n. 1, p. 54–60, 2010. **DOI**: http://doi.org/10.1016/j.jep.2010.04.010

OLIVEIRA, M. M. M..; BRUGNERA, D. F.; CARDOSO, M. G.; GUIMARÃES, L. G. L.; PICCOLI, R. H.. Rendimento , composição química e atividade antilisterial de óleos essenciais de espécies de Cymbopogon. **Rev. Bras. Pl. Med.**, v. 13, n. 1, p. 8–16, 2011. **DOI**: http://doi.org/10.1128/AAC.76.8.2763267-224422.678002

PALOMINO, J. C. MARTIN, A.; CAMACHO, M.; GUERRA, H.; SWINGS, J.; PORTAELS, F.. Resazurin Microtiter Assay Plate : Simple and Inexpensive Method for Detection of Drug Resistance in Mycobacterium tuberculosis. **Antimicrobial Agents and Chemotherapy**, v. 46, n. 8, p. 2720–2722, 2002. **DOI**: http://doi.org/10.1128/AAC.46.8.2720-2722.2002

PATA, S.; YARAKSA, N.; DADUANG, S.; TEMSIRIPONG, Y.; SVASTI, J.; ARAKI, T.; THAMMASIRIRAK, S.. Characterization of the novel antibacterial peptide Leucrocin from crocodile (Crocodylus siamensis) white blood cell extracts. **Developmental and comparative immunology**, v. 35, n. 5, p. 545–53, maio 2011. **DOI**: http://doi.org/10.1016/j.dci.2010.12.011

PINTO, A. C.; SILVA, D. H. S.; BOLZANI, V. DA S.; LOPES, N. P.; EPIFANIO, R. DE A.. Produtos naturais: Atualidade, desafios e perspectivas. **Química Nova**, v. 25, n. SUPPL. 1, p. 45–61, 2002. **DOI**: http://doi.org/10.1590/S0100-40422002000800009

ROMANELLI, P. F.; CASERI, R.; FILHO, J. F.. Processamento da carne do jacaré (Caiman crocodilus yacare). **Ciência e Tecnologia de Alimentos**, v. 22, n. 1, p. 70–75, 2002. **DOI**: http://doi.org/10.1590/S0101-20612002000100013

SANT'ANA, P. J. P.. As parcerias para a bioprospecção no Brasil. **Parcerias Estratégicas**, v. 15, p. 111–127, 2002.

SANTOS, A. P.; ZATTA, D. T.; MORAES, W. F.; BARA, M. T. F.; SILVA, M. DO R. R.; PAULA, J. R.. Composição química , atividade antimicrobiana do óleo essencial e ocorrência de esteróides nas folhas de Pterodon emarginatus Vogel ,

Fabaceae. **Brazilian Journal of Pharmacognosy**, 2010. **DOI**: http://doi.org/10.1590/S0102-695X2010005000052

SOUTO, W. M. S. et al. Parallels between zootherapeutic practices in ethnoveterinary and human complementary medicine in northeastern Brazil. **Journal of Ethnopharmacology**, v. 134, n. 3, p. 753–767, 2011. **DOI**: http://doi.org/10.1016/j.jep.2011.01.041

TORTORA, G. J.; CASE, C. L.; FUNKE, B. R. Microbiologia. 10. ed. Artmed: Porto Alegre, 2012. VERDADE, L. M.. A exploração da fauna silvestre no Brasil: jacarés, sistemas e recursos humanos. **Biota Neotrópica**, v. 4, n. 2, p. 1–12, 2004. **DOI**: http://doi.org/10.1590/S1676-06032004000200002

WELDON, P. J.; SCOTT, T. P.; TANNER, M. J.. Analysis of Gular and Paracloacal Gland secretions of the American Alligator (Alligar mississippiensis) by Thin-Layer Chromatography. **Journal of chemical ecology**, v. 16, n. 1, p. 3–12, 1989. **DOI**: http://doi.org/10.1007/BF01021263

WELDON, P. J.; SHAFAGATI, A.; WHEELER, J. W.. Lipids from the paracloacal glands of the American alligator (Alligator mississippiensis). **Lipids**, v. 23, n. 7, p. 727–729, jul. 1988. **DOI**: http://doi.org/10.1007/BF02535676

WELDON, P. J.; SHAFAGATI, A.; WHEELER, J. W.. Lipids on the paracloacal gland secretions of Dwarf (Paleosuchus palpebrosus) and Smooth-fronted (P.trigonatus) Caimans. **Biochemical Systematics and Ecology**, v. 17, n. 5, p. 431–435, 1989. **DOI**: http://doi.org/10.1016/0305-1978(89)90061-6

WILLIAMS, P. A Mitchell, W.; Wilson, G. R.; Weldon, P.. J.Bacteria in the Gular and paracloacal glands of the American alligator (Alligator mississippiensis; Reptilia, Crocodila). **Letters in Applied Microbiology**, v. 10, p. 73–79, 1989. **DOI**: http://doi.org/10.1111/j.1472-765X.1990.tb00268.x

WHEELER, J. W.; IBRAHIM, A.; P. WELDON, J.. 2,3-Dihydrofarnesyl and citronellyl esters in the paracloacal gland secretions of the brown caiman (Caiman crocodilus fuscus) from Costa Rica. **Biochemical Systematics and Ecology**, v. 27, n. 1, p. 27–32, jan. 1999. **DOI**: http://doi.org/10.1016/S0305-1978(98)00054-4

ZHANG, Z.; ELSOHLY, H. N.; JACOB, M. R.; PASCO, D. S.; WALKER, L. A.; CLARK, A. M.. Natural products inhibiting Candida albicans secreted aspartic proteases from Lycopodium cernuum. **Journal of Natural Products**, v.65, n.7, p.979-985, 2002. **DOI**: http://doi.org/10.1007/BF025374956