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Effects of the fixed oil of *Butia catarinensis* Noblick & Lorenzi on the erythrocyte membrane and bacterial strains

Efeitos do óleo fixo de *Butia catarinensis* Noblick & Lorenzi sobre a membrana eritrocitária e cepas bacterianas

Efectos del aceite fijo de *Butia catarinensis* Noblick & Lorenzi sobre la membrana de eritrocitos y cepas bacterianas

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Abstract

Butia catarinensis Noblick & Lorenzi, popularly known as "beach butiá", is a species extensively used in the food area, but little explored regarding its toxicological safety and pharmacological potential. Therefore, the aim of this study was to evaluate whether the fixed oil of *B. catarinensis* (OFBc) promotes hemolysis in vitro and if it can increase the resistance of human erythrocytes to hemolysis (anti-hemolytic activity), and also to investigate whether it has antibacterial properties. The hemolytic, antihemolytic and antibacterial activities were described for the first time, respectively. For the study, human erythrocytes from healthy volunteers were isolated and incubated with different concentrations of the oil and Triton X-100 or 0, 24% NaCl as hemolysis inducers. OFBc showed low dose-dependent cytotoxicity to human erythrocytes. None of the concentrations exhibited cytoprotective effect nor inhibitory activity

against the bacteria tested. In conclusion, the cytotoxicity screening performed in this study using the *in vitro* hemolysis assay suggests that the fixed oil of *B. catarinensis* may be pharmacologically safe for use in humans.
Keywords: Arecaceae; Hemolysis; Osmotic fragility; Therapeutics.

Resumo

Butia catarinensis Noblick & Lorenzi, popularmente conhecida como butiá-da-praia, é uma espécie extensivamente utilizada na área alimentícia, mas pouco explorada quanto a sua segurança toxicológica e potencial farmacológico. Logo, o objetivo deste estudo foi avaliar se o óleo de fixo de *B.* catarinensis (OFBc) promove hemólise *in vitro* e pode aumentar a resistência dos eritrócitos humanos à hemólise (atividade anti-hemolítica), além de investigar se possui propriedades antibacterianas. As atividades hemolítica, anti-hemolítica e antibacteriana foram descritas pela primeira vez, respectivamente. Para o estudo, eritrócitos humanos de voluntários saudáveis foram isolados e incubados com diferentes concentrações do óleo e Triton X-100 ou NaCl 0, 24% como indutores de hemólise. O OFBc mostrou baixa citotoxicidade dose-dependente para hemácias humanas. Nenhuma das concentrações exibiu efeito citoprotetor e nem atividade inibitória frente as bactérias testadas. Em conclusão, a triagem de citotoxicidade realizada neste estudo através do ensaio de hemólise *in vitro*, sugere que o óleo fixo de *B. catarinensis* pode ser seguro farmacologicamente para uso em seres humanos.
Palavras-chave: Arecaceae; Hemólise; Fragilidade osmótica; Terapêutica.

Resumen

Butia catarinensis Noblick & Lorenzi, conocida popularmente como butiá-da-praia, es una especie ampliamente utilizada en el sector alimentario, pero poco explorada en cuanto a su seguridad toxicológica y potencial farmacológico. Por lo tanto, el objetivo de este estudio fue evaluar si el aceite fijo de *B. catarinensis* (OFBc) promueve la hemólisis *in vitro* y puede aumentar la resistencia de los eritrocitos humanos a la hemólisis (actividad antihemolítica), además de investigar si tiene propiedades antibacterianas. Las actividades hemolítica, anti-hemolítica y antibacteriana se describieron por primera vez, respectivamente. Para el estudio, se aislaron eritrocitos humanos de voluntarios sanos y se incubaron con diferentes concentraciones del aceite y Triton X-100 o NaCl al 0, 24 % como inductores de hemólisis. OFBc mostró una citotoxicidad dependiente de la dosis baja para los glóbulos rojos humanos. Ninguna de las concentraciones exhibió efecto citoprotector o actividad inhibitoria contra las bacterias ensayadas. En conclusión, la detección de citotoxicidad realizada en este estudio a través del ensayo de hemólisis *in vitro* sugiere que el aceite fijado de *B. catarinensis* puede ser farmacológicamente seguro para su uso en humanos.
Palabras clave: Arecaceae; Hemólisis; Fragilidad osmótica; Terapéutica.

1. Introduction

Red blood cells (RBCs) are the most abundant cells in the circulatory microenvironment whose concentration in the blood of an adult individual can reach approximately 5 X 10^6 erythrocytes in 1 μL (Dzierzak & Philipsen, 2013). The main function performed by RBCs is the transport of gases (oxygen, carbon dioxide) from the lungs to the tissues, which is vital to the survival of organisms (Kuhn et al., 2017). These cells have a greater vulnerability to lipid peroxidation, due to the high concentration of polyunsaturated fatty acids in their membranes and high oxygen tension driven by the gas-carrying function of hemoglobin. Together these conditions can lead, for example, to the occurrence of Fenton and Haber- Weiss reactions related to oxidative stress (Reddy et al., 2007; Farag & Alagawany, 2018; D'alessandro et al., 2019). Thus, agents harmful to health capable of generating reactive oxygen species (ROS) may favor the redox imbalance of erythrocytes causing oxidative tissue damage (Abreu-Naranjo et al., 2020).

In this context, xenobiotic substances of synthetic or natural origin can disrupt erythrocyte (RBC) homeostasis causing structural damage to the membrane of these cells and consequent modification in their permeability, reduction in the levels of cytoplasmic antioxidant enzymes, osmotic stress and eventually leading to hemolysis (Schön et al., 1994; Khoshbin et al., 2015; Beni et al., 2020). On the other hand, it is also known that plant components can contribute to decrease the osmotic fragility of erythrocytes under pathophysiological conditions by improving the redox state of their plasma membranes due to their antioxidant properties (Beni et al., 2020; Roggia et al., 2020).

Among the plants with antioxidant action registred in the literature, *Butia catarinensis* known as beach butia (buitá-da-praia), is a palm tree belonging to the Arecaceae family, densely exploited for food purposes (Cruz et al., 2017; Rockett et al., 2021; Rodrigues et al., 2022; Kumagai & Hanazaki, 2013). The species is native to the southern region of Brazil, occurring

mainly in the state of Santa Catarina (Kumagai & Hanazaki, 2013; Dutra et al., 2021). Additionally, scientific investigative approaches on the potential biological activities of this species are scarce, and studies on its toxicity are non-existent. In this sense, it is essential to introduce studies for its toxicological evaluation and bioactive potential in order to guarantee its safety and efficacy (Banerjee et al., 2018).

Based on the above information and considering that there is no scientific basis in the literature for the toxic or antibacterial effects of the fixed oil of *B. catarinensis*, the present study aimed to investigate for the first time its cytotoxicity and the influence of the oil on osmotic fragility in human erythrocytes.

2. Methodology

2.1 Collection, botanical identification and oil extraction

The fruits of the *Butia catarinensis* palm were collected in the Ponta da Barra community on Gravatá beach (28°28'54''S and 48°46'56"W) in the municipality of Laguna, coast of Santa Catarina - SC. The specimen (FLOR 37972) was deposited in the herbarium of the FLOR herbarium collection at the Federal University of Santa Catarina. The oil was extracted from the dried butia nuts using a bench top oil extractor (Piteba®, The Netherlands).

2.2 Hemolytic activity

The preliminary toxicity of the chemical components of *Butia catarinensis* fixed oil (OFBc) was determined using hemolysis assay on human erythrocytes according to the method recommended by Dacie and Lewis (2001) with adaptations. The red cell suspension was prepared from fresh A, B, and O blood from healthy donors. The blood was collected by venipuncture and centrifuged at 2500 rpm for 5 minutes. Afterwards, the blood plasma was discarded and the sediment was washed with 0.9% NaCl solution. This step was repeated three times, and the final sediment was resuspended in saline solution (0.9% NaCl) in order to obtain a 0.5% suspension. The blood samples were then incubated with different concentrations of OFBc 10, 100, 500, and 1000 µg/mL and allowed to stand at room temperature. After 60 min, the samples were centrifuged at 2500 rpm for 5 min. An aliquot of the supernatant was then collected and hemolysis quantified by spectrophotometry at a wavelength of 540 nm. Triton-X 100 at 1% was used as a positive control, while the negative control was composed of the 0, 5% erythrocyte solution. The percentage of hemolysis was calculated using the following formula:

$$HA\ (\%) = \frac{\text{Abs test sample}}{\text{Abs positive control}}\ X\ 100$$

2.3 Anti-hemolytic activity or osmotic fragility

The assay was based on Dacie and Lewis (2001) with modifications. Following the previous assay, after spectrophotometer reading, all supernatant was removed from samples with lower hemolytic index and the sediment was resuspended in hypotonic saline (NaCl 0, 24%). Immediately after incubation for 60 min at room temperature, the samples were again centrifuged at 2500 rpm for 5 min, and the optical density of the supernatant was measured using a spectrophotometer at 540 nm. The positive control was the 0, 5% solution of red cells in hypotonic medium (NaCl 0, 24%). The percentage of hemolysis was calculated as follows:

$$AA\ (\%) = \frac{\text{Abs test sample}}{\text{Abs positive control}}\ X\ 100$$

2.4 Investigation of the antibacterial activity

For the antibacterial assay the agar diffusion method per well was used, based on the technique developed by Moody et al. (2004), using as positive control cephalexin at 100 mg/mL. The following gram-positive bacteria were selected for the assay: *Enterococcus faecalis* UFPEDA 138, *Staphylococcus aureus* UFPEDA 02 and gram-negative: *Pseudomonas aeruginosa* UFPEDA 416, *Klebisiella pneumoniae* UFPEDA 396 and *Escherichia coli* UFPEDA 224, which were kindly provided by Prof. Dr. Keila Moreira from UFAPE - Universidade Federal do Agreste de Pernambuco.

For the determination of the antibacterial activity the microorganisms were standardized on McFarland's scale of 0, 5. The OFBc was diluted in Tween 80 and saline, obtaining decreasing concentrations of 100, 50 and 25 mg by serial dilution. After inoculating the bacteria in Petri dishes, 20 µL of the OFBc was added in 5 mm diameter wells. Subsequently this procedure, the plates were incubated in a bacteriological incubator at 37°C for 24 hours. By the end of this time, the inhibitory halos were measured using a pachymeter.

2.5 Statistical analysis

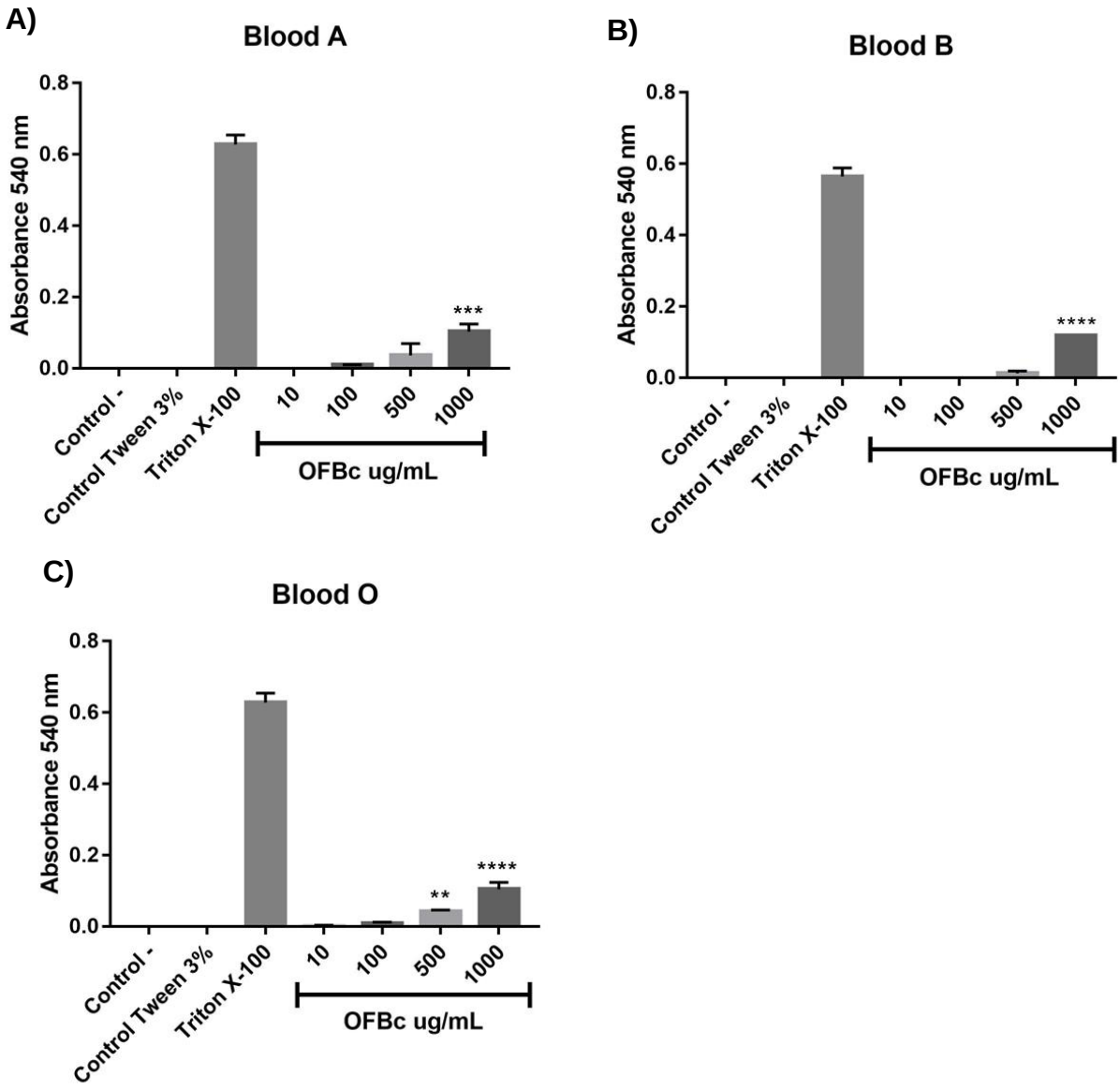
For statistical analysis, ANOVA test of variance followed by Dunnett's post-test was used, considering a significance level of $p < 0, 05$. All data were expressed as mean ± standard deviation of the mean in triplicate (n=3). The software used was GraphPad Prism version 7.0.

3. Results and Discussion

In this study, the effect of *Butia catarinensis* fixed oil (OFBc) on induction of hemolysis, erythrocyte membrane resistance, and bacterial cell inhibition was studied for the first time.

The cytotoxic study was performed *in vitro* using the RBC lysis analysis and examined for hemolytic and antihemolytic activity. The results are shown in Figure 1. The hemolytic activity of OFBc showed a dose-dependent increase, indicating that the oil (mainly at doses of 500 and 1000 µg/mL), is able to promote alterations in RBC integrity compared to the negative control group (NaCl 0, 9%).

Figure 1 - Hemolytic effect of human erythrocytes treated or not with different concentrations of *Butia catarinensis* fixed oil (OFBc). Data are represented as mean ± S.D. (n=3). Analysis by ANOVA followed by Dunnett's post-test. The significance level for rejecting the null hypothesis was $p < 0.5$ compared to the negative control (NaCl 0, 9%). A) Blood type A (*** - $p < 0.002$). B) Blood type B (**** - $p < 0.0001$). C) Blood type O (** - $p < 0.01$; **** - $p < 0.0001$).



Source: Authors.

Although there was a statistical difference between the groups treated with 500 and 1000 µg/mL doses of the oil and the negative control (Figure 1A, B, C), it should be noted that, according to the results summarized in Table 1, the percentage of lysis presented for human erythrocytes was less than 12% in all concentrations tested. That said, Rangel and collaborators (1997) state that samples with a hemolytic rate of less than 40% are considered to have low cytotoxicity. Therefore, this assay is indicative of the safety of the oil for use in humans as a potential drug source.

Table 1 - Percentage of hemolysis of human erythrocytes of blood types A, B and O treated with the fixed oil of *Butia catarinensis*.

Blood Type	Fixed Oil of *Butia catarinensis* - OFBc			
	10	100	500	1000
A	0,00%	1,00%	3,70%	6,10%
B	0,00%	0,00%	1,30%	11,80%
O	0,17%	0,90%	4,32%	10,45%

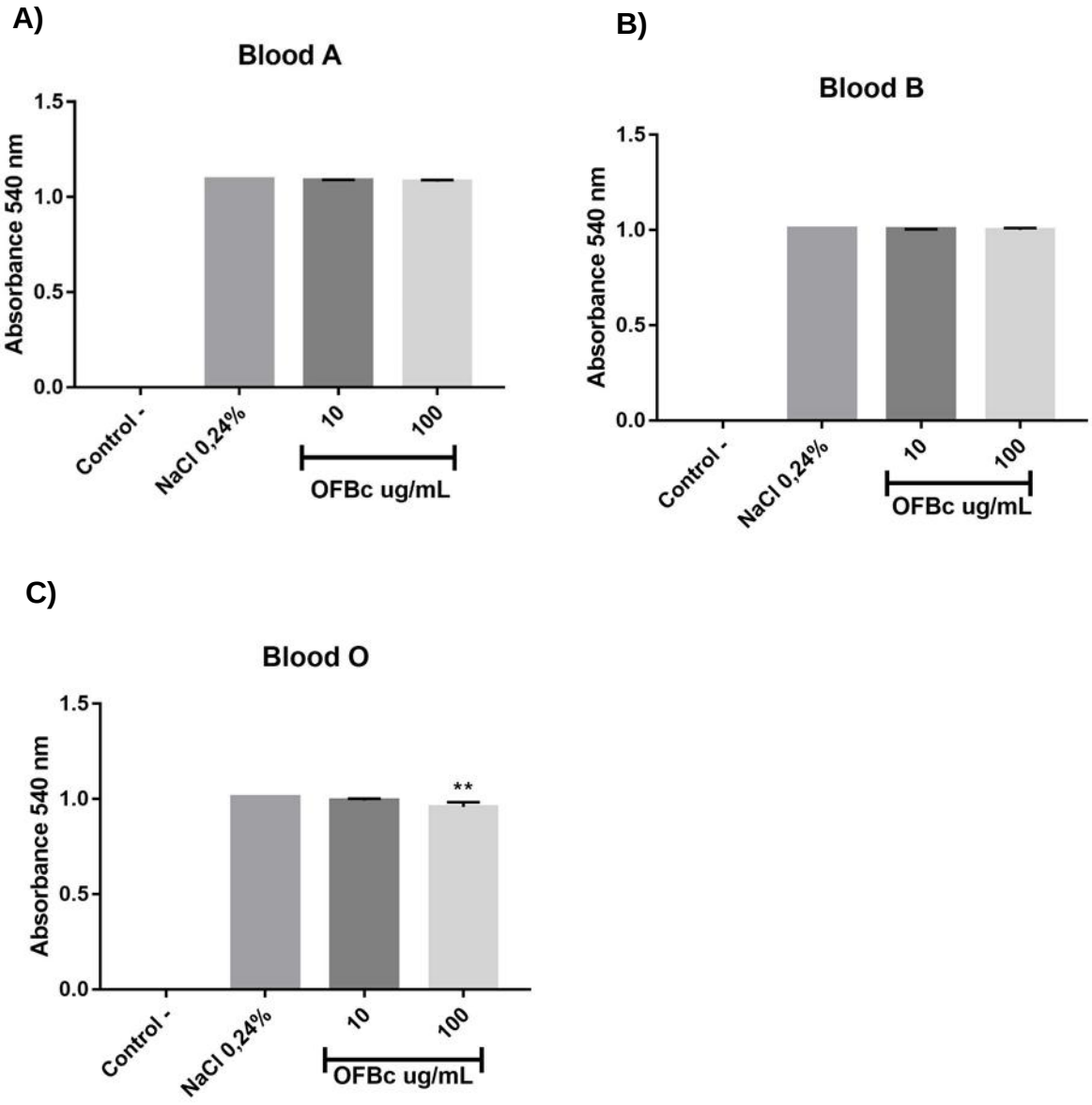
Source: Authors.

The hemolytic activity assay consists of an important technique capable of evaluating the cytotoxic effect and verifying the interaction of chemical components present in plants using RBC membranes (Farag & Alagawany, 2018; Hemthanon & Ungcharoenwiwat, 2022). In this sense, chemical compounds from vegetable oils can cause modifications in RBC morphology as consequent hemoglobin extravasation, which can result in a clinical condition of hemolytic anemia (Barros et al., 2016). Thus, toxicological screening is indispensable for the safe establishment of plant use.

From the results, it can also be observed that the concentrations with the lowest hemolytic potential were the doses of 10 and 100 µg/mL for both blood types (Table 1). These concentrations were selected for the following anti-hemolytic activity assay.

Figure 2 presents the effect of osmotic fragility of blood samples treated with OFBc at concentrations of 10 and 100 µg/mL. According to these results it is possible to observe that there was little or no reduction of osmotic stress in erythrocytes of types A, B, and O compared to the positive control group (NaCl 0, 24%). The tested concentrations were unable to reduce the percentage of hemolysis in hypotonic medium as can be seen in Table 2.

Figure 2 - Antihemolytic effect (osmotic fragility) of human erythrocytes treated and not treated with different concentrations of *Butia catarinensis* fixed oil (OFBc). Data are represented as mean ± S.D. (n=3). Analysis by ANOVA followed by Dunnett's post-test. The significance level for rejecting the null hypothesis was $p < 0.5$ compared to the positive control (NaCl 0, 24%). A) Blood type A. B) Blood type B. C) Blood type O (** - $p < 0.005$).



Source: Authors.

Table 2 - Osmotic fragility (Anti-hemolytic activity) of human erythrocytes of blood types A, B and O treated with the fixed oil of *Butia catarinensis*.

Blood Type	Fixed Oil of *Butia catarinensis* - OFBc	
	10	100
A	99,54%	98,97%
B	99,66%	99,40%
O	98,47%	94,85%

Source: Authors (2022).

According to hypothesis substantiated by Filho et al. (2014), when the plant sample under study is not able to promote significant changes in osmotic fragility, it may be suggestive of absence of interaction of plant compounds with the cell membrane. This hypothesis is corroborated by the results found in this study in the hemolytic assay, which showed low cytotoxic effect to human erythrocytes.

In this perspective, Oteiza (1994) explains that the rigidity of the erythrocyte membrane can be affected by the interaction of chemical agents with lipid components, as a result of modifications in their distribution. Thus, the osmotic fragility assay system is a useful tool to establish the toxicological safety of candidate substances as therapeutic agents in biological systems, being able to measure whether a particular compound or molecule can modify the oxidative state of the organism under disease conditions (Filho et al, 2014; Farag, et al., 2017).

Table 3 brings together the antibacterial assay results for OFBc, including the commercial antibiotic control for each bacterial species. OFBc was not able to inhibit any of the bacterial strains under study. The inhibitory effect was observed only with cephalexin, which showed inhibition for both gram-positive and gram-negative bacteria.

Table 3 - Average inhibitory halos (mm) of *Butia catarinensis* fixed oil (OFBc) against pathogenic bacteria by the agar diffusion method per well. Legend: (-) = No inhibition; (NC) = Negative control (3% Tween 80).

Species	Concentration OFBc (mg/mL)				
	100	50	25	CN	Cephalexin
Pseudomonas aeruginosa UFPEDA 416	-	-	-	-	15 ± 0,0
Klebsiella pneumoniae UFPEDA 396	-	-	-	-	17,67 ± 1,15
Enterococcus faecalis UFPEDA 138	-	-	-	-	16,67 ± 2,08
Staphylococcus aureus UFPEDA 02	-	-	-	-	18,33 ± 0,58
Escherichia coli UFPEDA 224	-	-	-	-	17,00 ± 0,0

Source: Authors (2022).

Cruz et al. (2017) evaluated extracts of *B. catarinensis* seeds produced from different extracting solvents, and observed that they showed inhibition of the growth of *Escherichia coli* CTT 1371 and *Bacillus cereus* ATCC 8739. The

authors attributed such activity mainly to the presence of phenolic compounds. In the present study, however, the oil extracted from the seeds caused no inhibitory effect on E. coli and other bacterial strains. This divergence may be explained by the difference in extractive methods used in the two studies, resulting in distinct chemical composition: predominance of phenolic compounds and fatty acids for the extracts and the oil, respectively (Busato et al., 2014; Woźniak et al., 2019). In addition, E. coli strains may show distinct resistance profiles due to the genetic variability that may exist between strains of the same species, thus obtaining different inhibition responses to the botanical samples (Morio et al., 2010).

4. Conclusion

The cytotoxicity screening performed in this study using the hemolysis assay suggests that the fixed oil of *Butia catarinensis* may be pharmacologically safe for its use in humans. Furthermore, the oil did not exhibit antibacterial activity for the investigated strains and was not able to reduce the osmotic fragility of erythrocytes in the study performed.

In conclusion, we emphasize that other pharmacological and toxicological in vitro and in vivo tests are necessary to establish the therapeutic efficacy and safety of *B. catarinensis* fixed oil. In this direction, further studies will be conducted in order to expand and contribute to the literature with information despite the pharmacology and biological safety of this species.

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References

Abreu-Naranjo, R, Paredes-Moreta, J. G., Granda-Albuja, G., Iturralde, G., González-Paramás, A. M., & Alvarez-Suarez, J. M. (2020). Bioactive compounds, phenolic profile, antioxidant capacity and effectiveness against lipid peroxidation of cell membranes of *Mauritia flexuosa* L. fruit extracts from three biomes in the Ecuadorian Amazon. *Heliyon*, 6 (10): e05211.

Banerjee, J., DAS, A., Sinha, M., & Saha, S. (2018). Biological Efficacy of Medicinal Plant Extracts in Preventing Oxidative Damage. *Oxidative Medicine and Cellular Longevity*, 2018 (1): 1-2, 7904349.

Barros, F. J., Costa, R. J. O., Cesário, F. R. A. S., Rodrigues, L. B., Costa, J. G. M., Coutinho, H. D. M., Galvao, H. B. F., & Menezes, I. R. A. (2016). Activity of essential oils of Piper aduncum anf and Cinnamomum zeylanicum by evaluating osmotic and morphologic fragility of erythrocytes. *European Journal of Integrative Medicine*, 8 (4): 505 – 512.

Beni, A. A., Rodrigues, R. F., Conte, L., Costa, I. F., Delalibera, É. A., Roehrs, M., Rampelotto, C., Emanuelli, T., & Somacal, S. (2020). Dietary supplementation with annatto food-coloring extracts increases the resistance of human erythrocytes to hemolysis. *Nutrition Research*, 76 (1): 71-81.

Busato, N. V., Silveira, J. C., Costa, A. O. S., & Junior, E. F. C. (2014). Estratégias de modelagem da extração de óleos essenciais por hidrodestilação e destilação a vapor. *Ciência Rural*, 44 (9): 1574-1582.

Cruz, P. N., Pereira, T. C. S., Guindani, C., Oliveira, D. A., Rossi, M. J., & Ferreira, S. R. S. (2017). Antioxidant and antibacterial potential of butia (*Butia catarinensis*) seed extracts obtained by supercritical fluid extraction. *The Journal of Supercritical Fluids*, 1119 (1): 229-237.

D'Alessandro, A., Hansen, K. C., Eisenmesser, E. Z., & Zimring, J. C. (2019). Protect, repair, destroy or sacrifice: a role of oxidative stress biology in inter-donor variability of blood storage? *Blood Transfus*, 17 (4): 281-288.

Dacie, J. V., & Lewis, S. M. (2001) *Practical Haematology*. In: Lewis, S.M., Bain, B.J. and Bates, I. Eds., Practical Heamatology, 9th Edition, Churchill Livingstone, Harcourt Publishers Limited, London, 444-451.

Dutra, J. P., Santos, A. A. M., Barbieri, R. L., & March, M. M. (2021). *Butiá para todos os gostos*. Brasília: Ed: Embrapa.

Dzierzak, E., & Philipsen, S. (2013). Erythropoiesis: development and differentiation. *Cold Spring Harb. Perspect Med*, 3 (4): 1-16.

Farag, M. R., & Alagawany, M. (2018). Erythrocytes as a biological model for screening of xenobiotics toxicity. *Chem-Biol Interact*, 279 (1): 73-83.

Farag, M. R., Alagawany, M., & Tufarelli, V. (2017). *In vitro* antioxidant activities of resveratrol, cinnamaldehyde and their synergistic effect against cyadox-induced cytotoxicity in rabbit erythrocytes. *Drug Chem Toxicol*, 40 (1): 196-205.

Filho, V. M. B., Waczuk, E. P., Kamdem, J. P., Abolaji, A. O., Lacerda, S. R., Costa, J. G. M., Menezes, I. R. A., Boligon, A. A., Athayde, M. L., Rocha, J. B. T., & Posser, T. (2014). Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). *Industrial Crops and Products*, 55 (1): 280-288.

Hemthanon, T., & Ungcharoenwiwat, P. (2022). Antibacterial activity, stability, and hemolytic activity of heartwood extract from Caesalpinia sappan for application on nonwoven fabric. *Electronic Journal of Biotechnology*, 55 (1): 9-17.

Khoshbin, A. R., Mohamadabadi, F., Vafaeian, F., Babania, A., Akbarian, S., Khandozi, R., Sadrebazaz, M. A., Hatami, E., & Joshaghani, H. R. (2015). The effect of radiotherapy and chemotherapy on osmotic fragility of red blood cells and plasma levels of malondialdehyde in patients with breast cancer. *Reports of Practical Oncology & Radiotherapy*, 20 (4): 305-308.

Kuhn, V., Diederich, L., KellerIV, T. C. S., Kramer, C. M., Lückstädt, W., Panknin, C., Suvorava, T., Isakson, B. E., Kelm, M., & Cortese-Krott, M. M. (2017). Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, anemia. *Antioxidants & Redox Signaling*, 26 (13): 718-742.

Kumagai, L., & Hanazaki, N. (2013). Ethnobotanical and ethnoecological study of *Butia catarinensis* Noblick & Lorenzi: contributions to the conservation of an endangered area in southern Brazil. *Acta Botanica Brasilica*, 27 (1): 13-20.

Moody, J. O., Adebiyi, O. A., & Adeniyi B. A.; (2004). Do *Aloe vera* and Ageratum conyzoides enhance the anti-microbial activity of traditional medicinal soft soaps (Osedudu)? *Journal of Ethnopharmacology*, 92 (1): 57-60.

Morio, F., Loge, C., Besse, B., Hennequin, C., & Le, P. P. (2010). Screening for amino acid substitutions in the Candida albicans Erg11 protein of azolesusceptible and azole-resistant clinical isolates: new substitutions and a review of the literature. *Diagnostic Microbiology and Infectious Disease*, 66 (4): 373-384.

Oteiza, P. (1994). A mechanism for the stimulatory effect of aluminum on iron-induced lipid peroxidation. *Archives of Biochemistry and Biophysics*, 308 (2): 374-379.

Rangel, M., Malpezzi, E. L., Susini, S. M., & Freitas, J. C. (1997). Hemolytic activity in extracts of the diatom Nitzschia. *Toxicon*, 35 (2): 305-309.

Reddy, C. S. S. S., Subramanyam, M. V. V., Vani, R., & Devi, S. A. (2007). *In vitro* models of oxidative stress in rat erythrocytes: Effect of antioxidant supplements. *Toxicology in Vitro*, 21 (8): 1355-1364.

Rockett, F., Schmidt, H., Rodrigues, E., Flôres, S., & Rios, A. (2021). Application of refrigeration and packing can extend Butiá fruit shelf life. *Food Bioscience*, 42 (1): 1-9.

Rodrigues, C. E., Schäfer, L., Gregolon, J. G. N., Oliveira, J. F., Baez, O. P., Deolindo, C. T. P., Melo, A. P. Z., Singer, R. B., Kist, T. B. L., & Hoff, R. (2022). Determination of amino acid content, fatty acid profiles, and phenolic compounds in non-conventional edible fruits of seven species of palm trees (Arecaceae) native to the southern half of South America. *Food Research International*, 162 (1): 1-10.

Roggia, I., Dalcin, A. J. F., Ourique, A. F., Cruz, I. B. M., Ribeiro, E. E., Mitjans, M., Vinardell, M. P., & Gomes, P. (2020). Protective effect of guarana-loaded liposomes on hemolytic activity. *Colloids and Surfaces B: Biointerfaces*, 187 (1): 1-10, 110636.

Schön, W., Ziegler, C., Gärtner, H., & Kraft, G. (1994). Heavy ion induced membrane damage: hemolysis of erythrocytes and changes in erythrocyte membrane fluidity. *Radiat Environ Biophys* 33 (3): 233-241.

Woźniak, M., Mrówczyńska, L., Waśkiewicz, A., Rogoziński, T., & Ratajczak, I. (2019). The role of seasonality on the chemical composition, antioxidant activity and cytotoxicity of Polish propolis in human erythrocytes. *Revista Brasileira de Farmacognosia*, 29 (3): 301-308.