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Research Article

Antioxidant Activity of the Fixed Oil from the Fruits of *Caryocar coriaceum* Wittm. (Caryocaraceae)

José Thyálisson da Costa Silva[1], Francisca de Fátima Silva de Sousa[1], Marcos Aurélio Figueirêdo dos Santos[1], Severino Denicio Gonçalves de Sousa[2], Flávio Silva Tampelini[2], Rebeca Azevedo de Lima Madeira[3], Francisca Sâmara Muniz dos Santos[1], Rafael de Carvalho Mendes[3], Dieferson Leandro de Souza[1], Luis Pereira de Morais[1], Márcia Jordana Ferreira Macêdo[1], Mayumi Kimura[4], João Pereira da Silva Junior[1], Nadghia Figueiredo Leite Sampaio[4], Magaly Lima Mota[1], Andreia Matias Fernandes[1] and José Weverton Almeida-Bezerra [1]*

[1] *Regional University of Cariri, Crato - CE, Brazil.*

[2] *Federal University of Mato Grosso, Cuiabá – MT, Brazil.*

[3] *Faculty of Medicine Estácio IDOMED, Juazeiro do Norte – CE, Brazil.*

[4] *Faculty of Medicine Estácio IDOMED, Iguatu – CE, Brazil.*

***Corresponding Author:** Prof. Dr. José Weverton Almeida-Bezerra, Department of Biological Chemistry, Regional University of Cariri, 63105-000, Crato, CE, Brazil.*

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Abstract

Oxidation is a crucial metabolic process that generates energy in cells but also results in the production of free radicals, leading to oxidative stress and the development of chronic diseases such as cancer and neurodegenerative disorders. Reactive oxygen species (ROS) can cause damage to DNA and cellular molecules, making the production of antioxidants essential to mitigate these effects. Fatty acids, in addition to their structural functions, have antioxidant properties and are recognized for protecting against oxidative stress and inflammation. This study focuses on the fixed oil from the fruits of *Caryocar coriaceum* Wittm., commonly known as pequi, which is rich in fatty acids such as oleic, linoleic, and palmitic acids. The aim was to evaluate the *in vitro* antioxidant activity of this oil using the DPPH free radical method. After oil extraction, assays were performed that demonstrated a high antioxidant capacity of the oil, with an IC_{50} of 75.22 μg/mL, similar to the positive control (ascorbic acid, with an IC_{50} of 9.77 μg/mL). The results indicate that the fixed oil of *C. coriaceum* is effective in inhibiting oxidation at significant concentrations, highlighting its antioxidant properties and potential in preventing degenerative diseases. The study suggests incorporating the oil into the diet as a promising strategy to promote health, reinforcing the importance of natural resources in therapeutic approaches. Additionally, further research is recommended to explore its applications in preventive medicine.

Keywords: *Pequi; Cerrado; Chapada do Araripe; Fatty acid; Free radicals*

1. Introduction

Oxidation is an essential process in cellular metabolism, resulting in the generation of energy needed to maintain vital cellular functions. However, oxygen metabolism in cells also leads to the formation of free radicals. Oxidants, which are compounds naturally generated during metabolism, can cause significant damage when not properly controlled [1]. Oxidative stress is linked to the development of various chronic and degenerative diseases, such as cancer, heart disease, neurodegenerative diseases including Alzheimer's, and also plays a role in the aging process. The balance between oxidative stress and the body's antioxidant defenses appears to influence carcinogenesis [2].

The generated carbon radicals can react with oxygen, forming peroxyl radicals, which in turn attack new polyunsaturated fatty acid chains, propagating the oxidation. The result is the oxidation of multiple fatty acid molecules. Hydroperoxides generated during lipid peroxidation have a short lifespan and, upon reacting with metals, can form compounds like aldehydes (malondialdehyde, acrolein, crotonaldehyde) and epoxides, which are highly reactive and can cause further DNA damage [3].

In living organisms, the production of free radicals is controlled by a series of antioxidant compounds, which can be produced internally (such as superoxide dismutase) or acquired through diet and other sources. Among these compounds are tocopherols (vitamin E), ascorbic acid (vitamin C), polyphenols, selenium, and carotenoids. When there is an antioxidant deficiency, cumulative oxidative damage can occur. Antioxidants have the ability to neutralize or stabilize free radicals before they can damage essential biological structures within cells [4].

An alternative is fatty acids, which, in addition to their structural and metabolic functions, also possess antioxidant properties. They are known for their ability to reduce inflammation and protect against oxidative stress. These fatty acids act as precursors to signaling molecules, such as prostaglandins and leukotrienes, which modulate inflammatory and immune responses in the body. Moreover, the inclusion of fatty acid sources in the diet has been associated with protection against cardiovascular, neurodegenerative diseases, and certain types of cancer, precisely due to their role in controlling oxidative processes [5].

These lipids can help stabilize cell membranes and protect cells from the damage caused by phospholipid oxidation. In combination with antioxidants, fatty acids form a more robust barrier against the harmful effects of free radicals, promoting cellular integrity and contributing to the redox balance in the body [6]. Supplementation with essential fatty acids has been increasingly studied as a strategy to strengthen antioxidant defenses, especially in individuals with diets poor in protective compounds or exposed to high levels of oxidative stress [7].

Among the fatty acids, the fixed oils from the fruits of *Caryocar coriaceum* Wittm. (Caryocaraceae) (Figure 01), popularly known in Brazil as "pequi," stand out. The oil extracted from this species is a rich source of fatty acids, particularly oleic, linoleic, and palmitic acids, which confer significant antioxidant properties to the fruit. According to studies, pequi oil exhibits important biological activities, including anti-inflammatory, antimicrobial, antinociceptive, and gastroprotective actions, and is widely used in traditional medicine to treat inflammations, wounds, rheumatism, and respiratory diseases [8].

Figure 1: *Fruit of Caryocar coriaceum Wittm. (Caryocaraceae) in a Cerrado area in Chapada do Araripe (Crato, Ceará).*

Thus, due to the high content of fatty acids present in the oil, the hypothesis is raised that it has antioxidant properties. Making *C. coriaceum* oil a valuable resource in the prevention of degenerative diseases. Therefore, this study aimed to evaluate the in vitro antioxidant activity of the fixed oil of *C. coriaceum* fruits.

2. Methods

2.1 Botanical Material Collection

A total of 300 mature fruits of *C. coriaceum* were collected from Serra do Pequi, located within the Environmental Protection Area (APA) of Chapada do Araripe, in Jardim, Ceará, Brazil, at coordinates 07°29'269''S and 39°18'050''W. The collected specimens were deposited in the UFP Herbarium – Geraldo Mariz, with voucher number 88,948. The research was approved and registered in the Biodiversity Authorization and Information System (SISBio) under number 77450-1, and in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) with code A4848B1.

2.2 Fixed Oil Extraction

First, the epicarp and outer mesocarp were removed. The inner mesocarp was manually separated, then dehydrated in an oven at 40°C for seven days, resulting in a total of 760 g of material. The inner mesocarps were crushed and subjected to exhaustive extraction with n-hexane at room temperature for 72 hours. After this process, the solvent was evaporated from the sample using a rotary evaporator. The obtained oil was stored in amber bottles until experiments were conducted [9].

2.3 Antioxidant Activity

The antioxidant assay was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical method. Initially, 100 µL of a 0.3 mM DPPH solution was added to 96-well plates along with 50 µL of ethanol and 50 µL of the *Caryocar coriaceum* fixed oil (FOCC), resulting in final concentrations ranging from 0 to 1,024 µg/mL. The plate was incubated at room temperature for 30 minutes, and the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control, and the antioxidant effect was calculated as a percentage, as well as the IC_{50} value [10].

2.4 Statistical Analysis

The antioxidant assays were performed in quadruplicate, and the data were expressed as the mean ± standard error of the mean (± SEM). The data were analyzed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test at 95% confidence. The half-maximal inhibitory concentration (IC_{50}) was calculated using nonlinear regression analysis. All analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

3. Results and Discussions

As shown in Figure 02, the FOCC demonstrates high antioxidant capacity against the DPPH free radical, with results similar to the positive control, ascorbic acid. The IC_{50} values for FOCC and ascorbic acid were 75.22 µg/mL and 9.77 µg/mL, respectively. The lowest evaluated concentration of FOCC (32 µg/mL) was able to inhibit 17.28% of the free radical, compared to 63.67% inhibition by ascorbic acid. The highest concentration evaluated (1,024 µg/mL) of the natural product inhibited 65.05% of DPPH, demonstrating a high antioxidant potential at clinically relevant concentrations.

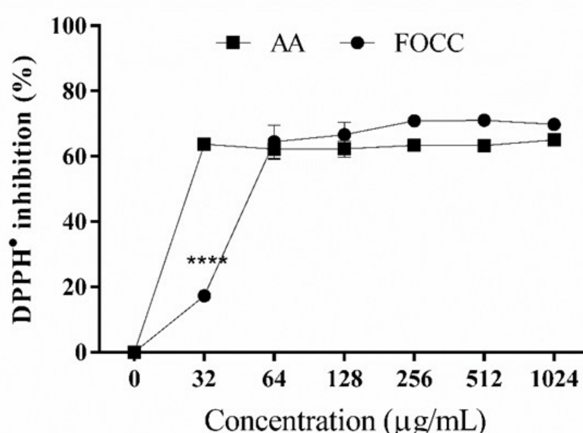

***Figure 2**: Antioxidant activity of fixed oil of Caryocar coriaceum (FOCC) and ascorbic acid (AA).*

Among the various studies conducted on *Caryocar coriaceum*, the investigation of antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical reduction method is the most frequent [8]. In this procedure, 2.5 mL of *C. coriaceum* samples are added to a DPPH solution (60 µM) with a volume of 1 mL, resulting in concentrations that vary according to each study, reaching up to 1000 µg/mL. Blanks are used to measure the absorbance of extraction solvents, such as ethanol, methanol, or water. Known antioxidants, such as vitamin C (ascorbic acid) and BHT (butylated hydroxytoluene), are used as positive controls, while the negative control consists of the DPPH solution alone, used for comparison. After 30 minutes of reaction in a dark environment, the absorbance of the solutions is measured with a spectrophotometer. When a sample exhibits antioxidant activity, the original purple color of DPPH tends to fade to yellow, indicating a higher antioxidant potential the more yellow the solution becomes.

In addition to the fixed oil, the aqueous extract of this species' leaves showed the highest DPPH reduction capacity, with an IC_{50} value 15 times lower than that of ascorbic acid, used as a positive control [11]. The authors observed that at concentrations of 100 and 250 µg/mL, the DPPH scavenging by the leaf and fruit peel extracts of *C. coriaceum* was comparable to that of ascorbic acid. Alves et al. [12] also confirmed that the peels and pulps of this plant's fruits have antioxidant potential, with the pulp being the most active, having an IC_{50} value of 49.4 µg/mL.

In vivo studies on antioxidant activity were conducted by Duavy et al. [13], who found that leaf extracts and pulp oil from the fruits of *C. coriaceum* offered protection to Drosophila melanogaster (fruit flies) against the oxidant agent paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride). In this experiment, the researchers fed the flies a sucrose solution (4%) containing paraquat (1 mM), as well as a group that received the same solution but with aqueous extract of *C. coriaceum* leaves at varying concentrations (1–5 mg/mL), along with a control group (without paraquat and extract). After one week, the live flies were anesthetized on ice and homogenized in a phosphate buffer solution (20 mM), pH 7.4 (50 flies/mL). The homogenate was centrifuged at 3500 ×g for 10 minutes at 4°C, and the supernatant was collected and kept on ice until the tests were performed. These procedures were conducted to measure reactive species levels and evaluate the product's antioxidant efficacy. For this, the levels of reactive species were quantified using the DCFDA assay, which is based on the deacetylation of 2',7'-dichlorofluorescein diacetate (DCF-DA). The reaction medium contained potassium phosphate buffer pH 7.4 (75 mM), DCFH-DA (5 µM final), and the fly supernatant (10 µL). Fluorescence was measured in a Shimadzu spectrophotometer, with excitation at 488 nm and emission at 525 nm, for 20 minutes. Results were expressed in arbitrary fluorescence units (AFU), using a standard curve with DCF. Lipid peroxidation was evaluated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in the samples. In this assay, the authors incubated the supernatant solution in an acidic medium (8.1% SDS (100 µL), 0.8% TBA (500 µL), and 20% acetic acid pH 3.5 (500 µL)) for one hour at 100°C. TBARS levels were determined spectrophotometrically at 532 nm, using malondialdehyde (MDA) as a standard. The natural products demonstrated the ability to reduce ROS levels and lipid peroxidation, as well as decrease the activity of the antioxidant enzymes catalase and glutathione-S-transferase. Moreover, leaf and pulp extracts were able to downregulate mRNA expression of stress-related genes such as catalase, superoxide dismutase, thioredoxin reductase, and Keap-1 (Kelch-like ECH-associated protein 1).

4. Conclusion

The present study demonstrated that the fixed oil from the fruits of *Caryocar coriaceum* Wittm. has significant antioxidant activity, evidenced by the DPPH free radical reduction method. The obtained results indicate that the oil is capable of inhibiting oxidation at relevant concentrations, comparable to the positive control, ascorbic acid. This antioxidant activity is attributed to the high content of fatty acids present in the oil, which not only serve as an energy source but also play a key role in cellular protection against oxidative stress and its harmful consequences. In addition to contributing to the understanding of the bioactive properties of *C. coriaceum* fruits, the findings suggest that incorporating this oil into the diet may have beneficial implications for preventing degenerative diseases related to oxidative stress. The research reinforces the relevance of using natural resources as promising alternatives in the development of therapeutic strategies, highlighting the importance of further studies to explore the oil's bioactivity in broader contexts, including in vivo assays and its potential application in preventive medicine. In summary, the fixed oil of *C. coriaceum* represents a valuable resource in the search for natural methods to promote health and well-being.

Conflict of Interest

The authors declare no conflict of interest.

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