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Research

Does the Ethnomedicinal Use of Pequi Oil for the Treatment of Infections Reveal its Antifungal Potential?

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Abstract

In Brazil, the use of medicinal plants has been on the rise over the years, especially as an alternative for the treatment of diseases caused by fungal infections. Fungal infections have become a major public health problem, primarily due to the indiscriminate and prolonged use of antibiotics. In this context, among the various species of the Brazilian flora, an endemic species stands out, *Caryocar coriaceum*, popularly known as "pequi," is widely used in nutrition and is also employed by the population for medicinal purposes to treat infectious diseases. Thus, this study aims to evaluate the chemical composition, antifungal action of the medicinal oil from *Caryocar coriaceum*, test the combined effect with the drug Fluconazole, and finally determine the chemical constituents present in the oil of pequi fruits. Initially, the fruits of this species were subjected to boiling in broth microdilution, and the combined effect with Fluconazole was assessed at sub-inhibitory concentrations (1/8 MIC), followed by spectrophotometric readings used to determine the IC_{50}. The *C. coriaceum* species is composed of four fatty acids, of which two were more prevalent, oleic acid and palmitic acid. The fixed oil showed low antifungal activity when evaluated individually for *Candida albicans* and *Candida tropicalis* strains, with IC50 values of 593.8 µg/mL, exhibiting greater modification for the standard drug with IC50 values for C. albicans at 16.07 µg/mL and *C. tropicalis* at 4.77 µg/mL. However, for the Candida krusei strain, the fixed oil exhibited more potent antifungal activity than Fluconazole at concentrations of 32 µg/mL and 64 µg/mL, while Fluconazole had intensified activity at concentrations from 2 µg/mL up to 128 µg/mL. Regarding the potentiating action for *Candida albicans*, *Candida tropicalis* and *Candida krusei* strains, the oil associated with Fluconazole enhanced the antifungal effect with IC_{50} values of 0.02792 µg/mL for *C. albicans*, 0.09903 µg/mL for *C. tropicalis*, and 15.15 µg/mL for *C. krusei*. Thus, the oil contains promising compounds in its composition for the development of medications to treat infectious diseases.

***Keywords:** Candidiasis; Chapada do Araripe; Azoles; Caryocaraceae.*

1. Introduction

Fungal infections caused by species of the *Candida* genus represent a high rate of morbidity and mortality. They are the leading causes of systemic and superficial candidiasis (Navarro-Arias et al., 2019). *Candida* yeasts inhabit as commensals in the normal microbiota of humans and other animals without harming the host. However, when factors disrupt this balance, they trigger virulence factors in the yeasts, rendering them pathogenic. This opportunistic behavior in the host classifies them as opportunistic fungi, responsible for fungal infections referred to as candidiasis (Jovito, 2016; Shiozawa et al., 2018).

Candida albicans is the most frequently observed species in patients diagnosed with candidiasis. However, there has been an increase in the incidence of candidiasis cases caused by other *Candida* non-*albicans* species, such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*. These species are of significant concern, as their incidence is associated with a rising number of fatalities (Guinea, 2014; Sorendino et al., 2018).

In recent decades, infectious and parasitic diseases have been on the rise, posing a significant health problem. This gradual increase is primarily attributed to the extensive and prolonged use of antibiotics (Abadi et al., 2019; Santos et al., 2019). Indiscriminate use of these drugs leads to microbial resistance, driven by selection mechanisms acting on microorganisms, including bacteria, protozoa, and fungi. As a result, the development of new substances has not kept pace with the rapid spread of these microorganisms (Oliveira-Tintino et al., 2018).

As a therapeutic alternative for infectious diseases, underserved communities utilize medicinal plants for treatment due to their low cost, ready availability, and the presence of popular knowledge (Albuquerque et al., 2014). Consequently, research focused on the biological exploration of plants has been carried out both in Brazil and worldwide, increasing interest and understanding of their bioactivities and chemical composition (Goes et al., 2016).

Brazil boasts a vast diversity of plant species, and among the Brazilian flora, a native species in the states of Ceará, Pernambuco, and Piauí stands out. It belongs to the genus *Caryocar* and the family Caryocaraceae. *Caryocar coriaceum* Wittm (Caryocaraceae) is commonly known as "pequi" or "pequizeiro." This species is highly appreciated in culinary applications and, in addition to its use in food, it is employed for various medicinal purposes. In this context, the fixed oil from its fruit is particularly noteworthy for the treatment of infectious diseases, parasitic diseases, as well as skin and subcutaneous tissue conditions (Magalhães et al., 2019).

The medicinal oil derived from the fruit of *Caryocar coriaceum* contains a chemical composition that includes both saturated (such as palmitic, stearic, eicosanoic, benzoic, and lignoceric acids) and unsaturated (like linoleic and oleic acids) fatty acids. These components provide important bioactive compounds effective against pathogenic strains, such as *Candida* spp. yeasts (Alves et al., 2017; Pereira et al., 2019; Serra et al., 2020).

Therefore, one way to combat resistance in the treatment of infectious and parasitic diseases (DIPs) is to investigate substances derived from the fixed oil of pequi fruits, coupled with scientific validation of the species' biological potential and bioprospecting capabilities against *Candida* yeast strains. This research has the potential to contribute to the treatment of fungal diseases and aid in the preservation of the pequi species. This is justified by the increasing number of cases of infectious and parasitic diseases (DIPs), which have been expanding over the years due to the excessive use of antibiotics. Hence, studies focused on the synergistic action of pequi fruit fixed oil are of significant importance, given its rich chemical components in its composition.

Considering these aspects, the research had the general objective of evaluating the chemical composition, antifungal action, and potentiating potential of the medicinal oil of *C. coriaceum* Wittm. (Caryocaraceae). The specific objectives of this research were as follows: to analyze the antifungal activity of the fixed oil from *C. coriaceum* against strains of the *Candida* spp lineage, to determine whether the medicinal oil of *C. coriaceum* is effective in enhancing the action of fluconazole against *Candida* yeasts, and to identify the chemical compounds present in the medicinal oil of pequi fruits.

2. Materials and methods

2.1 License and collection of botanical material

The collection of *C. coriaceum* fruits was carried out in an area of Chapada do Araripe, belonging to the municipality of Jardim – CE. From the material used, branches with fertile parts were collected to make an exsiccate that was deposited in the Herbarium UFP - Geraldo Mariz of the Federal University of Pernambuco, under number 88.948.

This work is registered in the Biodiversity Authorization and Information System (SISBio) under number 77450-1 and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration A4848B1.

2.2 Extraction of fixed oil from *C. coriaceum*

A total of 1,000 fruits of *C. coriaceum* underwent a manual extraction process, removing the putamen (inner mesocarp + endocarp + seeds) using the same method employed by extractors in the Chapada do Araripe region. This technique, known as "rolling," involves running a pointed object (knife) over the fruit to remove and discard the peel. After extraction, the *C. coriaceum* fruits were placed in a boiler with 200 liters of potable water and continuously boiled for five hours to fully cook the fruit. Subsequently, the fruits went through a friction process using a manual metal grater to separate the pulp from the rest of the fruit. They were then washed with potable water to remove excess pulp, while the liquid was reused, and the remaining fruits were not used. Next, the boiler continued to heat for five hours with constant stirring until the oil condensed on the surface. Finally, the condensed oil was collected, boiled in a metal container for two hours, filtered, and stored in an amber container at 10°C.

2.3 Phytochemical analysis

The fixed oil from *C. coriaceum* underwent a transesterification reaction using methanol and KOH as a catalyst. Subsequently, the methyl esters were evaluated using gas chromatography coupled with mass spectrometry (Oliveira et al., 2017).

In the gas chromatography (GC) analysis, an Agilent Technologies 6890N GC-FID system was utilized. It was equipped with a DB-5 capillary column (30 m × 0.32 mm; 0.50 µm) and connected to a Flame Ionization Detector (FID). The heating program started at 60°C for 1 minute, then increased to 180°C at a rate of 3°C per minute. The injector temperature was set at 220°C, the detector temperature at 220°C, and the split ratio was 1:10. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. A 1 µl volume of methyl esters of fatty acids from *C. coriaceum*, diluted in chloroform at a ratio of 1:10, was injected. Two parallel samples were processed in the same way. The relative concentrations of the components were calculated based on the peak areas in the GC chromatogram without any correction factors (Oliveira et al., 2017).

The identification of components was based on the retention index (RI), determined from a homologous series of n-alkanes C_7-C_{30} under identical experimental conditions. This RI was then compared with mass spectral library searches (NIST and Wiley) and mass spectral data from the literature. The relative proportions of the constituents were determined by measuring the peak areas in the GC chromatogram (FID response).

2.4 Antifungal activity

2.4.1 Strains, culture medium, drugs, reagents and solution preparation

The standard strains used were obtained from the Microorganism Collection and Reference in Sanitary Surveillance (CMRVS) at the National Institute of Quality Control in Health (FIOCRUZ-INCQS), namely *Candida albicans* 40006, *Candida tropicalis* 40042, and *Candida krusei* 40095.

The fungal strains were inoculated in Petri dishes containing Sabourand Dextrose Agar (SDA, Kasvi) and incubated for 24 hours at 37°C. Small aliquots were then transferred to test tubes, each containing 3 mL of sterile saline solution (0.9%) adjusted to a turbidity of 0.5 on the scale (1×10^8 CFU/mL). In the determination of the Average Inhibitory Concentration (IC50) and microdilution tests, Eppendorf tubes were used to distribute the Sabourand Dextrose Broth (CSD, Himedia) medium.

Subsequently, Dimethyl Sulfoxide (DMSO 0.5%, Merck, Darmstadt, Germany) was used for diluting the fixed oil. It was then further diluted in sterile distilled water to achieve a concentration of 4,096 µg/mL. These methods were employed to reduce the condensation of DMSO. Fluconazole (Capsule - FLUCOMED, São Paulo, Brazil), from the class of azoles, was used as a positive control (Bezerra et al., 2019).

2.4.2 Determination of Mean Inhibitory Concentration (IC_{50})

In this test, we used the microdilution method in 96-well plates. Each well was filled with 100 µL of Sabourand Dextrose Broth (CSD) containing a 10% fungal inoculum. Then, 100 µL of the natural product or Fluconazole at the same concentration was added to the first well, followed by serial dilution, ranging from concentrations of 1,024 µg/mL to 2 µg/mL. The last well was designated as the growth control. The plates were incubated at 37°C for 24 hours, and subsequently, the results were read using a spectrophotometer at a wavelength of 630 nm (Thermoplate®).

The obtained results were used to construct the cell viability curve and determine the IC_{50} (Bezerra et al., 2019).

2.4.3 Determination of Minimum Fungicide Concentration (MFC)

After the test, the tip of a sterile rod was inserted into each well of the plates to mix the solutions, then it was taken for cultivation in Petri dishes containing solid SDA medium (*Sabourand Dextrose Agar*). After 24 h of incubation at a temperature of 37º C, the plates were inspected observing the fungicidal effect of the oil on the formation of colonies by *Candida* yeasts (Bezerra et al., 2019).

2.4.4 Assessment of the potentiating effect of Fluconazole

Modulation tests were carried out, using a sub-inhibitory concentration (CM/8), where CM represents the matrix concentration of 1024 µg/mL. Sabourand Dextrose Broth medium (CSD, Himedia), with 10% inoculum and oil, was added to 96-well plates. The mixture was added to the first well of each column, Fluconazole (1:1 v/v) and continued dilution until the last well, at a concentration of 1,024 to 2 µg/mL. The plates were incubated at 37º C for 24 h, reading was performed using a spectrophotometer device with an ELISA reader (λ: 630 nm) (Bezerra et al., 2019).

2.5 Statistical analysis

The results of the tests were analyzed using the GraphPad Prism version 6 software, in which the data were analyzed by (One-way ANOVA) using the Tukey test at 95% reliability. And the median inhibitory concentrations (IC_{50}) were calculated by nonlinear regression analysis.

3. Results

3.1 Phytochemical analysis

According to Table 1, which shows the results of the chromatographic analysis of the medicinal oil of *C. coriaceum*, it was possible to identify four fatty acids, corresponding to 98.33% of the total composition. The majority constituent was oleic acid with 59.46% being an unsaturated fatty acid, the second most present fatty acid in the oil was palmitic acid with 33.58% which is part of the saturated fatty acids.

Table 1 - Chemical composition of medicinal *Caryocar coriaceum* oil.

Compounds	Yield (%)
Palmitic Acid	33.58
Stearic acid	2.96
Oleic acid	59.46
Linoleic acid	2.33
Total identified	98.33

3.2 Antifungal activity

In trials evaluating fungal growth, the medicinal oil showed a low antifungal effect, while Fluconazole showed better antifungal activity than the natural product. The evaluation of the potential modulating activity of the fixed oil on the antifungal effect of Fluconazole against the strains *C. albicans*, *C. tropicalis* and *C. krusei* revealed that the combination of this drug associated with the natural product potentiated the antifungal effect, compared to when it was tested in isolation.

The IC_{50} values (Table 2) obtained in the evaluation showed that for *C. albicans* (Figure 1) fixed oil presented higher concentrations than the drug alone and combined. In relation to *C. tropicalis* (Figure 2) Fluconazole combined with the medicinal oil showed a significant change in concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL while from 16 µg/mL to 1024 µg/mL did not modification occurred, as they are similar. For the *C. krusei* strains (Figure 3), there was an intensification of Fluconazole at concentrations from 2 µg/mL to 128 µg/mL, significantly reducing the IC_{50}. It is possible to observe that in *C. krusei* strains, pequi oil showed more potent antifungal activity than Fluconazole at concentrations of 32 µg/mL and 64 µg/mL.

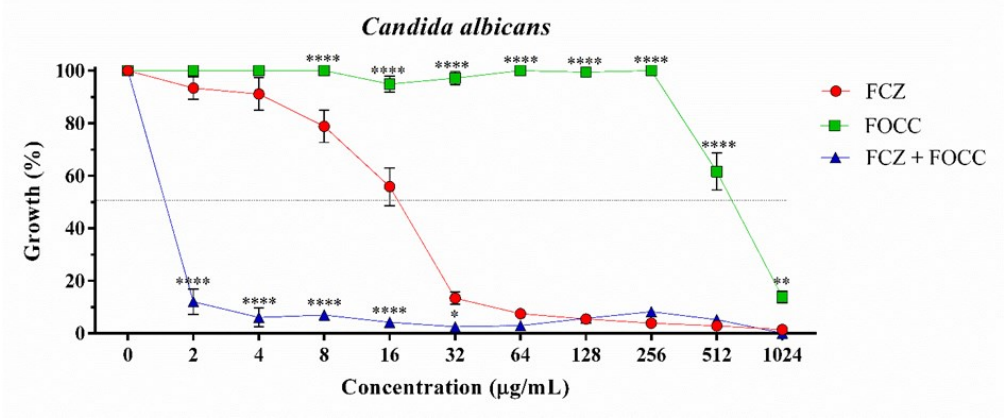

Figure 1 - *Fungal growth curve of Caryocar coriaceum fixed oil (FOCC) and Fluconazole (FCZ) in µg/mL against Candida albicans 40006 INCQS. Statistically significant value with * p < 0.05, ** p < 0.01 and **** p < 0.0001.*

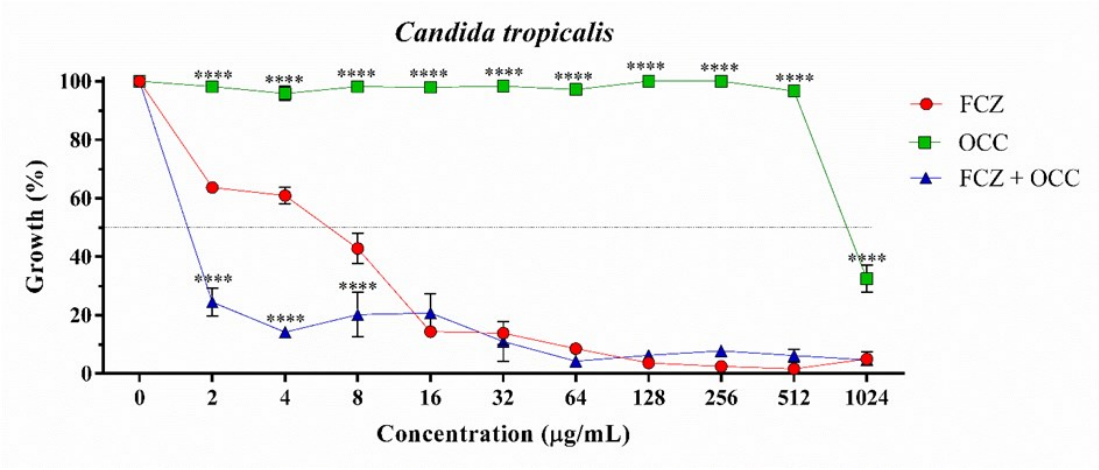

Figure 2 - *Fungal growth curve of Caryocar coriaceum fixed oil (FOCC) and Fluconazole (FCZ) in µg/mL against Candida tropicalis 40042 INCQS. Statistically significant value with * p < 0.05, ** p < 0.01 and **** p < 0.0001.*

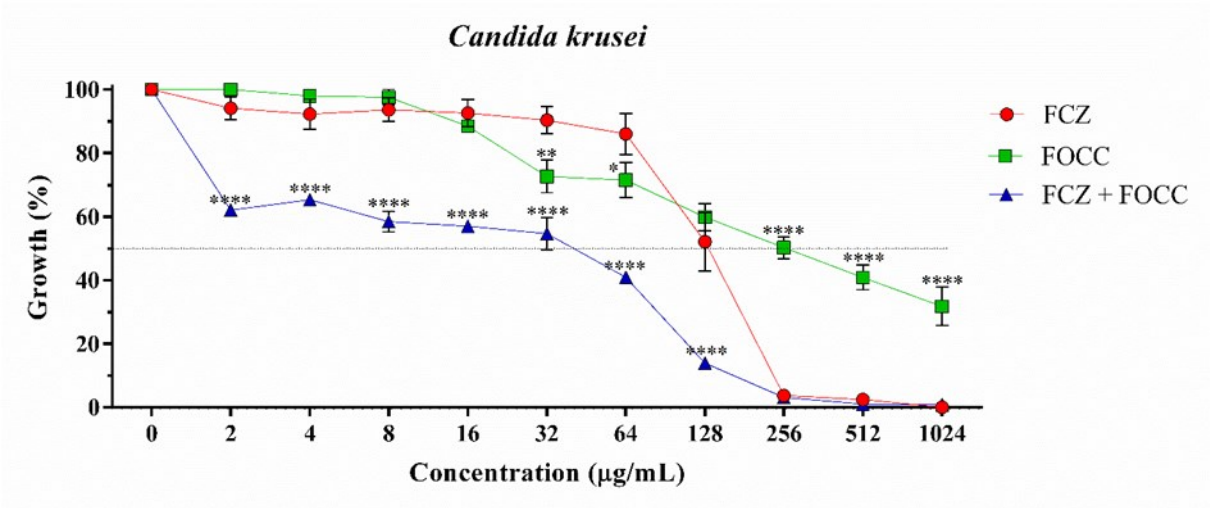

Figure 3 - *Fungal growth curve of Caryocar coriaceum fixed oil (FOCC) and Fluconazole (FCZ) in µg/mL against Candida krusei 40095 INCQS. Statistically significant value with * p < 0.05, ** p < 0.01 and **** p < 0.0001.*

Table 2 - IC_{50} of *Caryocar coriaceum* fixed oil (FOCC) and Fluconazole (FCZ) against *Candida albicans*, *Candida tropicalis* and *Candida krusei*.

Products tested	*Candida albicans*	*Candida tropicalis*	*Candida krusei*
FCZ	16.07 µg/mL	4.775 µg/mL	125.5 µg/mL
FOCC	593.8 µg/mL	593.8 µg/mL	268.8 µg/mL
FCZ + FOCC	0.02792 µg/mL	0.09903 µg/mL	15.15 µg/mL

The antifungal activity of *C. coriaceum* oil was determined by the Minimum Fungicidal Concentration, and samples capable of inhibiting the growth of fungal colonies were considered. It was observed that *C. coriaceum* oil alone, after 24 hours of incubation for the strains, was not able to completely inhibit the growth of colonies, as the MFC was 1024 µg/mL. However, for *C. albicans* INCQS 40006 and *C. tropicalis* INCQS 40042 strains, both Fluconazole alone and in combination with *C. coriaceum* oil showed a much lower MFC, which was 32 µg/mL. In this case, the oil did not modulate the fungicidal effect of Fluconazole for these strains. On the other hand, when tested on the *C. krusei* ATCC 40095 strain, Fluconazole alone and in combination with the oil was able to modulate the fungicidal effect with a MFC of 128 µg/mL.

4. Discussion

In ethnopharmacological studies, the fixed oil of *C. coriaceum* is commonly used in folk medicine due to its anti-inflammatory properties for healing skin lesions and treating respiratory system diseases, including inflammations, asthma, colds, and flu (Almeida-Bezerra et al., 2022). The literature also discusses other benefits of *C. coriaceum*, such as its anticonvulsant properties (Oliveira et al., 2017; Saraiva et al., 2011), antibacterial activity, antibiotic-modifying effects (Pereira et al., 2019), and potential cardioprotective effects (Kerntopf et al., 2013).

In the present study, among the identified fatty acids, the main ones were oleic acid (59.46%) and palmitic acid (33.58%), which were found in significant concentrations in the pequi fruit oil. According to Saraiva et al. (2011) and Costa et al. (2011), there is a prevalence of unsaturated fatty acids (<50%) compared to saturated ones in fixed oil, consistent with other studies in the literature that mention the predominance of unsaturated fatty acids.

In the study by Lima et al. (2007), it is noted that the fatty acids found in the pequi pulp belong to the group of unsaturated fatty acids (61.35%), with oleic acid constituting 55.87%. In experiments conducted by Silva (2015), oleic acid exhibited antifungal activity against strains of *Candida albicans* and *Candida tropicalis*, making it effective in the treatment of vaginal candidiasis. Therefore, the presence of oleic acid in the oil contributed to its antifungal activity.

In the study by Martins et al. (2015), the species *Caryocar brasiliense* was evaluated against the fungus *Candida albicans*, showing that *C. brasiliense* stimulated the growth of the *C. albicans* strain when compared to their respective negative controls. Additionally, in the work of Marques et al. (2002), a similar result was obtained, as *C. brasiliense* also had a stimulating effect on phytopathogenic fungi.

Infections caused by non-*albicans Candida* species are increasingly being described in bloodstream infections. Regarding the epidemiology of candidemia, infections by *C. albicans* are more common in European countries (Poikonen et al., 2010; Hesstvedt et al., 2015), while in the United States and India, there is a higher prevalence of infections caused by non-*albicans Candida* than by *C. albicans* (Nucci et al., 2013; Chakrabarti et al., 2015).

Mayer et al. (2013) emphasizes that the pathogenicity of *Candida* species involves morphological changes, biofilm development, cell surface adhesion, and secretion of hydrolytic enzymes. Consequently, Upadhyay et al. (2014) highlights the importance of using plant derivatives in the treatment of *Candida* species infections as therapeutic agents with fewer adverse effects for disease treatment. In our study, we observed that the fixed oil of C. coriaceum fruit, when combined with the drug, was able to significantly enhance the effect against strains of *C. albicans*, *C. tropicalis*, and *C. krusei*, indicating that the species contains compounds with antifungal properties. Thus, the oil exhibited a potentiating effect for Fluconazole against *Candida* spp. strains.

5. Conclusion

The medicinal oil of *Caryocar coriaceum*, although it demonstrated low antifungal activity when evaluated on its own against *Candida albicans* and *Candida tropicalis* strains, revealed a significant modification in the growth of *Candida krusei* when combined with the drug Fluconazole. Furthermore, the presence of saturated and unsaturated fatty acids in its composition, with the unsaturated oleic acid as the major component, highlights the therapeutic potential of this oil in the formulation of medications to treat infectious diseases. These results suggest the effectiveness of the medicinal use of this oil in overcoming resistance in *Candida* yeast, underscoring the importance of future studies on new antifungals derived from natural products for the prevention and treatment of fungal infections.

Conflict of Interest

The authors declare no conflict of interest.

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