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Research Article

Does the Fixed Oil from the Pequi Fruit Extracted by Extractivists from Chapada of Araripe have an Antibacterial Effect?

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Abstract

Popularly, the fixed oil from the fruits of *Caryocar coriaceum* Wittm. extracted by extractivists from Chapada of Araripe, it is used for the treatment of various diseases, especially for the treatment of infectious and parasitic diseases. Due to this popular use, it was hypothesized that this product had an effect against pathogenic microorganisms. Therefore, this work aimed to determine the chemical composition of this oil, as well as to evaluate its antibacterial and modulatory effect against pathogenic bacteria. The oil was extracted according to the artisanal techniques of extractivists from Chapada of Araripe. For chemical analysis, initially the oil was subjected to transesterification reactions using methanol and KOH as catalyst. Subsequently, the methyl esters were analyzed using a gas chromatograph coupled to mass spectrometry. As for the antibacterial assays, the methodology of serial microdilution in broth against standard and multi-drug-resistant bacteria was followed (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). In addition, the modulatory effect of drugs (erythromycin, gentamicin and norfloxacin) at sub-inhibitory concentrations (MIC/8) was evaluated. The Gas Chromatography results showed a total of four constituents in the oil, with oleic acid (59.46%) and palmitic acid (33.58%) being the major compounds. Regarding the antibacterial activity, the oil did not present an intrinsic effect, however, when associated with antibiotics, it was able to increase its antibacterial activities, such as for erythromycin against *P. aeruginosa* and *E. coli*, and for this strain there was a reduction of antibiotic MIC (32 µg/mL) to 2 µg/mL.

***Keywords:** Caryocar coriaceum*; Caryocaraceae; Antibiotic; Fatty acid, Ethnopharmacology.

1. Introduction

Currently, the resistance acquired by bacteria has drawn the attention of researchers around the globe, as it puts the effectiveness of antibiotics at risk (Jain, et al., 2021). Although bacterial resistance is something normally acquired over the years, there are other factors that can contribute to the development of this resistance, the main one being the inappropriate and excessive use of antibiotics (Abadi et al., 2019; Ventola, 2015). Among the microorganisms that have a high probability of acquiring resistance are nosocomial bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus* (Mitik-Dineva et al., 2009).

In view of this problem, research in search of new therapeutic resources has intensified in recent decades (Gupta et al., 2017). As alternatives, there are products from medicinal plants, such as fixed oils, essential oils, extracts, tinctures and resins (Bezerra et al., 2019; Santos et al., 2019, Nascimento et al., 2021). Since such products have a variety of chemical compounds with biological activities, they are also used in folk medicine (Gupta et al., 2017).

An oilseed species widely used for the treatment of infectious and parasitic diseases (IPD) in the Chapada of Araripe area (Ceará - Brazil) is *Caryocar coriaceum* Wittm. (Caryocaraceae). This is a producer of fruits known in Brazil as "pequi", which are used by extractivists in the region for the extraction of oil, which is used in cooking and medicinally.

Its popular uses are recorded for the treatment of inflammation, muscle pain, fever, wound healing, sore throat, flu, expectorant, cough, bronchitis, eczema, scalp conditions, lung pain, asthma, burns, rickets, indigestion, heart murmur, fatigue and erectile dysfunction (Cartaxo et al., 2010; Macêdo et al., 2016; Lemos et al., 2016).

These ethnopharmacological uses can be justified by the richness of compounds that the oil presents. Research indicates that the fixed oil of the fruits of *C. coriaceum* has palmitic acid, palmitic acid, palmitic acid, myristic acid, myristic acid, palmitoleic acid, linoleic acid, linoleic acid, eicosenoic acid and eicosenoic acid (Costa et al., 2019; Figueiredo et al., 2016; Oliveira et al., 2010; Pereira et al., 2019).

Due to the medicinal use of *C. coriaceum* oil for the treatment of IPD, as well as its vast chemical composition, this work aims to investigate its chemical composition and its antibacterial and modulatory effect.

2. Materials and Methods

2.1 License and Collection of Botanical Material

The project was registered in the Biodiversity Authorization and Information System (SISBio) under registration 77450-1 and the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration A4848B1. A total of 1,000 ripe and healthy fruits of *C. coriaceum* were collected in February 2021 at 15:00 ±00:30 h in an Environmental Protection Area (APA) of Chapada of Araripe, belonging to the municipality of Jardim - CE, Brazil under coordinates 07°29'269''S and 39°18'050''W at 925 m altitude.

2.2 Fixed Oil Obtaining

To obtain *C. coriaceum* oil, the same method used by extractivists from Chapada of Araripe was followed, as detailed in the work by Cavalcanti et al. (2015). For that, 1,000 fruits were submitted to manual extraction of the putamen (internal mesocarp + endocarp + seed) by a technique called "rolling", which consists of moving a sharp object (knife) against the fruit to remove and discard the barks. Subsequently, the putamens were placed in boilers containing 200 L of drinking water and subjected to constant boiling for five hours, in order to fully cook the fruits. These were rubbed on an artisanal metal grater to separate the pulp from the other parts of the fruit. At the end of this step, the fruits were washed with potable water to remove excess pulp, and the liquid solution returned to the boiler, while the remaining parts of the fruits were discarded. The boilers remained for another five hours in boiling and constant manual agitation to obtain the oil through its agglutination on the surface. This was collected and taken to a pan to be boiled for two hours, then filtered and packed in an amber container at a temperature of 10 °C.

2.3 Chemical profile of *C. coriaceum* oil

Initially, the oil from the fruits of *C. coriaceum* was subjected to transesterification reactions using methanol and KOH as a catalyst. Subsequently, the methyl esters were analyzed using a gas chromatograph coupled to mass spectrometry (Oliveira et al., 2010).

The gas chromatography (GC) analysis was performed with Agilent Technologies 6890N GC-FID system, equipped with DB-5 capillary column (30 m × 0.32 mm; 0.50 μm) and connected to FID detector. The thermal programmer was 60 °C (1 min) to 180 °C at 3 °C/min; injector temperature 220 °C; detector temperature 220 °C; split ratio 1:10; carrier gas Helium; flow rate: 1.0 mL/min. The injected volume of methyl esters of *C. coriaceum* fixed oil was 1 μL diluted in chloroform (1:10). Two replicates of samples were processed in the same way. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Identification of the constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of *n*-alkanes, C_7–C_{30}, under identical experimental conditions, compared with the mass spectra library search (NIST and Wiley), and with the mass spectra literature date Adams (2007). The relative amounts of individual components were calculated based on the CG peak area (FID response).

2.4 Antibacterial Activity

2.4.1 Preparation of Substances

The fixed oil of *C. coriaceum* and the antibiotics erythromycin, gentamicin and norfloxacin (Sigma Aldrich Co., St. Louis, USA), in a total of 10 mg, were diluted in 1 mL of dimethyl sulfoxide (DMSO) and later in 8,765 μL of sterile distilled water so that the respective solutions reached a concentration of 1,024 μg/mL (GOMES et al., 2018).

2.4.2 Strains Used and Inoculum Preparation

The standard strains *American Type Culture Collection* (ATCC): *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923, and the multiresistant: *E. coli* 06, *P. aeruginosa* 03 e *S. aureus* 10 (Table 1), stored at -20 °C in the Laboratory of Microbiology and Molecular Biology of the Universidade Regional do Cariri, were subcultured in Brain Heart Infusion Agar and kept in an oven at 37 °C for 24 hours.

Table 1 - Origin and resistance profile of the multidrug-resistant strains used.

Strains	Source	Resistance profile
E. coli 06	Urine culture	Asb, Ca, Cef, Cfo, Cpm, Cro
P. aeruginosa 03	Urine culture	Ami, Cip, Cpm, Ctz, Imi, Lev, Mer, Ptz
S. aureus 10	Swab retal	Amc, Amox, Amp, Asb, Azi, Ca, Cef, Cfo, Cip, Cla, Clin, Eri, Lev, Mox, Oxa, Pen

Legenda: *Amc - Amoxicillin + Clavulanic Acid, Ami - Amikacin, Amox - Amoxicillin, Amp - Ampicillin, Asb - Ampicillin + Sulbactam, Azi - Azithromycin, Ca - Cefadroxil, Cef - Cephalexin, Cfo - Cefoxitin, Cip - Ciprofloxacin, Cla - Clarithromycin, Clin - Clindamycin, Com - Cefepime, Cro - Ceftriaxone, Ctz - Ceftazidime, Eri - Erythromycin, Imi - Imipenem, Lev - Levofloxacin, Mer - Meropenem, Mox - Moxifloxacin, Oxa - Oxacillin, Pen - Penicillin, Ptz - Piperacillin + Tazobactam.*

After the growth period, samples from the respective colonies were diluted in test tubes containing 3 mL of sterile saline solution (NaCl 0.9%). The suspensions were shaken in a Vortex device and their turbidity was compared and adjusted to the 0.5 McFarland scale (1.5 x 10^8 Colony Forming Units/mL) (CLSI, 2018).

2.4.3 Evaluation of antibacterial activity

The Minimum Inhibitory Concentration (MIC) of the fixed oil was determined using the methodology of Javadpour et al. (1996). Eppendorfs were filled with 100 µL of the inoculum and 900 µL of 10% Brain Heart Infusion Broth (BHI), and 100 µL aliquots of each solution were distributed into 96-well microdilution plates (alphabetical direction). Then, serial microdilution (1:1) was carried out with 100 µL of the natural product until the penultimate well, the last one being used to control bacterial growth. The oil concentration on the plates ranged from 512 to 8 µg/mL. The tests were performed in triplicate and the plates were taken to an oven at 37 °C, where they remained for 24 hours. After incubation, 20 µL of sodium resazurin (0.4 mg/mL) was added to the wells of the plates and left for 1 hour at room temperature (25 °C). The permanence of the blue color of resazurin indicated inhibition of bacterial growth, while the change to pink indicated the occurrence of growth.

2.4.4 Antibiotic Action Modifying Activity

Following the methodology proposed by Coutinho et al. (2008), the eppendorfs were filled with 150 µL of inoculum, a volume of OFCc corresponding to its subinhibitory concentration (MIC/8) and sufficient 10% BHI to complete its total volume (1.5 mL). The eppendorfs referring to the modulation controls were filled with 1,350 µL of 10% BHI and 150 µL of inoculum. The solutions were distributed in 96-well microdilution plates (100 µL/well), numerically. Serial microdilution (1:1) was performed up to the penultimate well with 100 µL of antibiotics, whose concentrations on the plates ranged from 512 to 0.5 µg/mL. The tests were performed in triplicate and the plates were incubated for 24 hours in an oven at 37 °C. The reading of the results occurred in the same way as for the CIM.

2.5 Statistical analysis

The antibacterial assays were performed in triplicate, and their means and their respective standard deviations were calculated. Subsequently, they were submitted to a one-way analysis of variance (One-way ANOVA) using the Tukey test at 95% reliability. Such analyzes were performed using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, United States).

3. Results & Discussions

Chemically, the fixed oil manually extracted from the fruits of *C. coriaceum* by extractivists from Chapada of Araripe, has saturated and unsaturated fatty acids (Table 2). Gas Chromatography showed a total of four constituents in the oil, with oleic acid (59.46%) and palmitic acid (33.58%) being the major compounds. Corroborating, therefore, with the results of the study by Oliveira et al. (2010) and Figueiredo et al. (2016), since both studies point out that such acids are the majority. However, it is noteworthy that Figueiredo et al. (2016), found three more fatty acids in the constitution, namely palmitoleic, heptadecenoic and licosenoic acid. This difference may be related to differences in oil preparation, as the preparation changes according to the extractivist group that prepares it (Cavalcanti et al., 2015).

Table 2 - Chemical composition of the fixed oil of *Caryocar coriaceum* Wittm.

Fatty acid	Yield (%)
Palmitic acid (C16:0)	33.58
Stearic acid (C18:0)	2.96
Oleic acid (C18:1)	59.46
Linoleic acid (C18:2)	2.33

Ethnobotanical studies point to the use of fixed oil from the fruits of *C. coriaceum* by residents of Chapada of Araripe for the treatment of infectious and parasitic diseases (Cartaxo et al., 2010; Macêdo et al., 2016; Lemos et al., 2016). However, it was possible to notice that the oil did not show antibacterial activity against standard and multidrug-resistant strains at relevant concentrations (Table 3). Since concentrations greater than 1,000 µg/mL have no clinical relevance (Houghton et al., 2007). Thus, despite the ethnomedicinal use, *in vitro* studies have not demonstrated antibacterial potential.

Table 3 – Intrinsic antibacterial activity of the fixed oil of *Caryocar coriaceum* (FOCC) Wittm.

	SA ATCC	SA 10	EC ATCC	EC 06	PA ATCC	PA 24
FOCC	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024

Although palmitic acid has an antibacterial effect against *Escherichia coli* (Padmini et al., 2020), it was not able to produce the expected antibacterial effect. This effect can be explained by the fact that its presence is only 33.58%, and the other constituents act as antagonists. However, Costa et al. (2011), when evaluating the antibacterial effect of oil from the pulp (external mesocarp) of *C. coriaceum* through the inhibition halo methodology, demonstrated that the ethnomedicinal product showed biological properties against strains of *P. aeruginosa*, *S. aureus*, *Streptococcus pneumoniae* and *Salmonella choleraesuis* at a concentration of 10%. This divergent fact can be justified by the methodology used, as well as the origin of the oil.

Although *C. coriaceum* fixed oil did not have a direct antibacterial effect, it was able to modify the action of antibiotics (Figure 1). This is because natural plant products, such as fixed oils, have the ability to improve or decrease the activity of standard drugs, so these natural products can be a viable alternative to the issue of bacterial resistance (Machado et al., 2019). The modulatory effect can be observed for gentamicin against the three multidrug-resistant bacterial strains. In addition, the oil was responsible for intensifying the antibiotic effect of erythromycin against *P. aeruginosa* and *E. coli*, and for this strain there was a reduction in the MIC of the antibiotic (32 µg/mL) to 2 µg/mL. Finally, in addition to the modulating effect, the fixed oil from the fruits of *C. coriaceum* showed an antagonistic effect for norfloxacin against *P. aeruginosa* and *E. coli*.

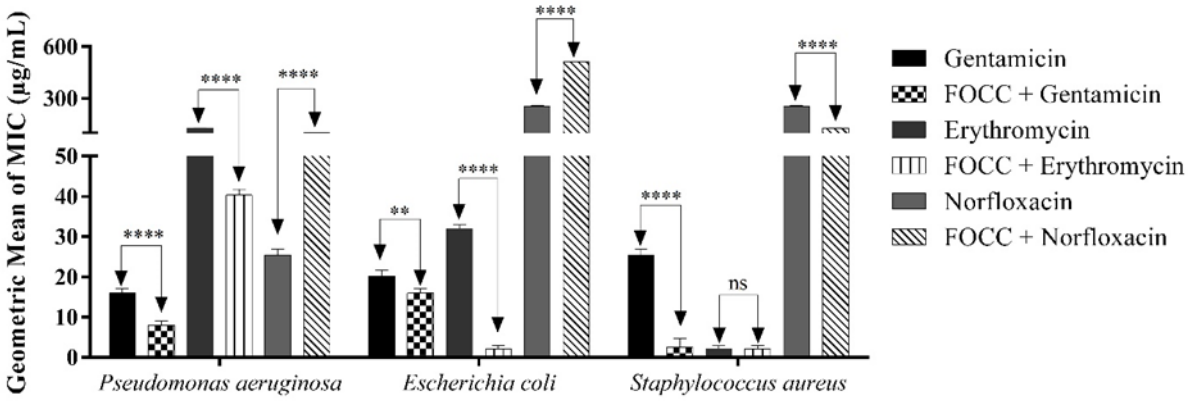

Figure 1: *Antibiotic modulating activity of the fixed oil from the fruits of Caryocar coriaceum (FOCC) Wittm. against multidrug-resistant bacteria. **** = p <0.0001, ** = p <0.01, ns = no significance.*

This modulating effect can be explained by the presence of hydrophobic compounds in the fixed oil (saturated and unsaturated fatty acids), which make the bacterial cell more permeable to the entry of antibiotics, thus increasing its efficiency and reducing the minimum concentration necessary to affect the bacterial cell. (Chan et al., 2015). Furthermore, due to the detergent properties of fatty acids, they may have altered the amphipathic combinations of the bacterial cell membrane. As a result, it may have solubilized membrane components such as lipids and proteins, causing a breakdown in structures and affecting metabolic processes essential for obtaining energy for the bacterial cell, such as the electron transport chain and oxidative phosphorylation. Such membrane damage can also lead to difficulties in nutrient absorption, inhibition of enzyme activity, and generation of toxic peroxidation (Chan et al., 2015; Desbois et al., 2010).

Vegetable oilseed species present compounds capable of intensifying the action of standard drugs, as is the case of fixed oils from the fruits of *Mauritia flexuosa* L.f. (buriti) and *Orbignya speciosa* (Mart.) Barb.Rodr. (babaçu), palm trees that are also used medicinally in the Chapada of Araripe region for the treatment of infectious and parasitic diseases (Pereira et al., 2018; Machado et al., 2019).

4. Conclusion

Although there are reports of the ethnopharmacological use of *C. coriaceum* fixed oil against infectious and parasitic diseases, our *in vitro* study indicated that the oil does not have direct antibacterial activity against pathogenic strains. However, the natural product, which is rich in saturated and unsaturated fatty acids, was able to modulate standard drugs against bacteria that show a certain degree of resistance.

Conflict of Interest

The authors declare no conflict of interest.

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