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Biofilm Inhibition: The Role of Fixed Oil from *Caryocar coriaceum* in Fighting Resistant Bacterial Communities

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Abstract: Biofilms, formed by microbial communities that increase resistance to antibiotics, are responsible for chronic infections, making their combat a therapeutic priority. Taking this into account, the fruit *Caryocar coriaceum* stands out for its potential in the treatment of infectious diseases. The different parts of this plant can be used, and the fixed oil extracted from its fruit, rich in fatty acids, is indicated as responsible for its biological activities. Thus, the objective of this study was to evaluate the chemical composition of the fixed oil extracted from the fruits of *C. coriaceum* (FOCC), in addition to analyzing its action in the inhibition and pre-formed biofilm disruption of bacteria. The fixed oil was extracted from the internal mesocarp through exhaustive extraction with n-hexane, resulting in a yield of 38.29%. For antibiofilm evaluation, multidrug-resistant bacterial strains were exposed to the oil, and the antibiofilm activity was verified through biofilm formation and pre-formed biofilm disruption assays. The chemical analysis of the fixed oil of *C. coriaceum* (FOCC) identified eight fatty acids, representing 98.2% of the total composition, with a predominance of oleic acid (60.1%) and palmitic acid (33.5%). FOCC demonstrated approximately 70% inhibition of *Streptococcus mutans* biofilm formation at a concentration of 10 mg/mL and approximately 60% inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In pre-formed biofilm disruption, FOCC showed low efficacy against *S. mutans* and *P. aeruginosa* but showed greater activity against *Enterococcus faecalis* and *S. aureus*. These results indicate that FOCC has the potential to prevent biofilms, but its pre-formed biofilm disruption capacity is still limited.

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1. Introduction

Antimicrobial resistance (AMR) poses an increasing threat to global health, characterized by the development of resistance in microorganisms such as bacteria, fungi, and parasites to previously effective treatments. This phenomenon results in the ineffectiveness of antimicrobial therapy and necessitates the adoption of alternatives, such as combination drug therapy, to enhance treatment efficacy [1–3]. The number of deaths caused by multidrug-resistant (MDR) microorganisms has been rising annually, with scientific forecasts indicating that, by 2050, deaths could surpass 10 million, exceeding those caused by cancer, unless effective measures are implemented [4,5].

Bacterial resistance may be associated with various alternative forms of resistance, such as gene transfer responsible for resistance production, overexpression of efflux pumps [6,7], and the creation of mechanisms like biofilms. These are characterized by communities of microorganisms that can behave as organisms of the same or different species, organized and surrounded by a polymeric extracellular matrix, adhering to both biotic and abiotic surfaces. Biofilms are responsible for a large portion of chronic infections, as they increase resistance to antibiotics and allow bacteria to survive in unfavorable conditions, making their control a relevant therapeutic measure [1,8]. Among oral biofilms, *Streptococcus mutans* is well recognized as the major cariogenic species due to its acidogenicity and aciduricity [9].

Given the need for alternative measures, medicinal plants have been widely investigated for their potential in ethnopharmacology, standing out as a rich source of biologically active substances with promising applications as antimicrobial agents, especially essential oils extracted from them. The applicability of these substances has been demonstrated in innovative therapies for treating diseases caused by MDR microorganisms with clinically relevant antimicrobial activities. Furthermore, there is evidence that combining these substances may result in synergistic interactions, increasing their efficacy against bacteria and fungi [10–14].

Various studies have evaluated the anti-biofilm activity of natural products, demonstrating efficacy against different multidrug-resistant bacterial strains, including both Gram-positive and Gram-negative species, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [15–17]. Thus, the search for natural products with antimicrobial activity has increased, and the scientific community's interest can be directed toward highlighting the biodiversity of the Cerrado biome, which has revealed significant properties, underscoring its biological importance [18,19]. This Brazilian biome is home to a large diversity of species used in the treatment of infectious diseases by traditional communities, but many of these plants still remain unexplored from a chemical and pharmacological point of view [18,19].

The genus *Caryocar*, found in the Cerrado, has been the subject of various scientific studies due to its biological actions, which include pharmacological and biological properties such as anti-inflammatory, antioxidant, antimicrobial, and antiparasitic activities. The species *Caryocar coriaceum* Wittm., popularly known as "pequi", stands out for its potential to treat diseases caused by microorganisms. Different parts of this plant can be used, and the fixed oil extracted from its fruit, rich in fatty acids, is considered responsible for its biological activities [20–24].

It is important to highlight that *C. coriaceum* extract is capable of intensifying the action of conventional antibiotics against multiresistant microorganisms [21]. However,

to our knowledge, there are no reports on the antibiofilm potential of the fixed oil from the fruits of this plant. Therefore, the primary objective of the research was to evaluate the phytochemical composition of the fixed oil extracted from the fruits of *C. coriaceum*, as well as to analyze its action in inhibiting and eradicating bacterial biofilms formed by Gram-positive and Gram-negative strains of relevance to public health.

2. Materials and Methods

2.1. Fruit Collection, Exsiccation and Obtaining Licenses

Ripe and healthy fruits of *Caryocar coriaceum* were collected, totaling 300 fruits. The collection took place in Serra do Pequi, located in the Environmental Protection Area (APA) of Chapada do Araripe, in the municipality of Jardim, Ceará, Brazil, at the coordinates 07°29′269″ S and 39°18′050″ W, during the month of February (2021). The species was identified, and a specimen was deposited at the Geraldo Mariz Herbarium (UFP) with the identification number 88,948. Before the collection, the study obtained approval and registration in the Biodiversity Authorization and Information System (SISBio, Brazil) with registration number 77450-1 and in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), with the registrations A4848B1.

2.2. Extraction of Fixed Oil

To extract the fixed oil from *C. coriaceum* (FOCC), the epicarps and external mesocarps were removed. The internal mesocarp of *C. coriaceum*, obtained from 300 fruits, was dehydrated at 40 °C for 7 days, yielding 760 g of dry material. The mesocarp was ground and subjected to exhaustive extraction with n-hexane at room temperature for 72 h. Subsequently, the solvent was removed from the sample using a rotary evaporator. The FOCC was stored in an amber bottle at room temperature until chemical analyses and biological assays were performed, with a total extraction yield of 291.06 g (38.29%).

2.3. Oil Hydrolysis and Identification of Fatty Acids

The fixed oil of *C. coriaceum* (0.2 g) was saponified for 10 min under reflux at 45 °C, using a solution of potassium hydroxide in methanol (1.5 g of KOH in 35 mL of CH_3OH), according to the method described by [25]. The methyl esters of fatty acids were extracted from the reaction medium with dichloromethane. The resulting organic phase was washed with distilled water, dried, and filtered.

The analysis of the chemical components of FOCC was performed by gas chromatography coupled with mass spectrometry (GC-MS). The transesterified FOCC was diluted in dichloromethane to 1%, and 1 µL of this solution was injected into an Agilent 6890 chromatograph (Palo Alto, CA, USA) with a split flow of 1:20, equipped with an Agilent 5973 N mass selective detector. The injector temperature was maintained at 250 °C. The separation of the constituents occurred in an HP-5MS capillary column (5% Phenyl, 95% Dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 µm) with helium gas as the carrier at a flow rate of 1.0 mL/min. The temperature program for the oven started at 60 °C, increasing by 3 °C/min up to 240 °C. The mass detector operated in electron ionization mode (70 eV), with a scanning rate of 3.15 s^{-1} and a mass range between 40 and 450.The temperatures of the transfer line, ion source, and quadrupole analyzer were maintained at 260 °C, 230 °C, and 150 °C, respectively.

The identification of the compounds was made by comparing the mass spectra with the NIST 2016 library (2.2. Mass Spectral Library (NIST/EPA/NIH), National Institute of Standards and Technology, Gaithersburg, MD, USA). For quantification, the diluted samples were injected into an Agilent 7890 chromatograph with a flame ionization detector (FID) operating at 280 °C. The analysis conditions followed the previous procedure, except for

using hydrogen as the carrier gas at a flow rate of 1.5 mL/min. The percentage composition was determined by electronic integration of the FID signal, calculating the area of each peak relative to the total area (area%).

2.4. Evaluation of Bacterial Antibiofilm Potential

2.4.1. Strains, Culture Medium, Inocula and Drugs

The bacterial strains analyzed to assess the antibiofilm potential of FOCC included *Streptococcus mutans* (INCQS 00,446, ATCC 25,175), *Enterococcus faecalis* (INCQS 00,018, ATCC 14,506), *Staphylococcus aureus* (ATCC 25,923), and *Pseudomonas aeruginosa* (ATCC 9027). These microorganisms were provided by the Microbiology and Molecular Biology Laboratory of the Regional University of Cariri (URCA) and the Oswaldo Cruz Foundation (FIOCRUZ). The strains were cultured on BHI agar (Brain Heart Infusion) and incubated in a biological oven for 24 h at 37 °C.

After incubation, the cells were diluted in a 0.85% NaCl solution, and the suspensions were adjusted to a concentration of 5×10^5 CFU/mL, as described by Araújo et al. [26]. FOCC was weighed, dissolved in dimethyl sulfoxide (DMSO), and diluted in sterile water to obtain MIC concentrations (Minimum Inhibitory Concentration, 10 mg/mL) and MIC × 10 (100 mg/mL). The reference drug used was chlorhexidine gluconate (CG) as a standard antibiofilm reference, and DMSO served as a solvent for the substances.

2.4.2. Biofilm Formation Assay

Initially, the minimum inhibitory concentration (MIC) of the fixed oil against planktonic pathogenic bacteria was evaluated using the serial broth microdilution method, as described in the standardized protocols. This test allowed determining the lowest concentration capable of visibly inhibiting bacterial growth, which is a widely recognized approach for studies of antimicrobial activity. The results obtained demonstrated that the MIC of the analyzed samples was higher than 1024 μg/mL. Biofilm formation was evaluated in microtiter plates using the crystal violet method, where 160 μL of culture medium (BHI), 20 μL of distilled water, and 20 μL of bacterial inoculum adjusted to 1.5×10^8 CFU/mL were added to the plates. For sterility control, distilled water replaced the bacterial inoculum. After incubation at 37 °C for 24 h, the plates were washed three times with 0.9% saline solution and incubated at 55 °C. Then, 200 μL of crystal violet was added for 15 min, followed by washing with distilled water, elution with 100% ethanol, and absorbance reading at 492 nm, following the methodology described by [27,28] with adaptations.

2.4.3. Anti-Biofilm Formation Assessment

To evaluate the biofilm formation inhibition capacity of FOCC, 20 μL of FOCC at MIC concentrations (10 mg/mL) and MIC × 10 (100 mg/mL) were added to microtiter plates, along with 20 μL of bacterial inoculum (1.5×10^8 CFU/mL) and 160 μL of culture medium. A 0.85% NaCl solution was used as a control for growth and sterility. The plates were incubated at 37 °C for 24 h. After this period, planktonic cells were removed by three washes with 0.9% saline solution. The biofilm was then fixed by incubating the plates at 55 °C for 1 h and stained with 0.4% crystal violet for 15 min. The plates were washed three more times with saline solution, and the biofilm was eluted with absolute ethanol, followed by an optical density reading at 492 nm. The antibiofilm activity was determined by comparing the results with the growth control.

2.4.4. Pre-Formed Biofilm Disruption Assay

After the 48 h biofilm formation period, the biofilms were treated with 20 μL of different concentrations (MIC and MIC × 10) of FOCC and CG while maintaining controls in the microdilution plates. The plates were incubated at 37 °C for 24 h, followed by the

removal of excess liquid. A triple wash with saline solution was then performed, followed by incubation at 55 °C for 1 h and staining with 0.4% crystal violet for 15 min. The dye was removed with saline solution, and the biofilm was eluted with 100% ethanol. Absorbance was measured at 492 nm, as per [28].

2.5. Statistical Analysis

The GraphPad Prism software (Software Inc. version 6, San Diego, CA, USA) was used for statistical analysis. The arithmetic mean of the triplicates for each concentration tested was calculated, and the data were then subjected to one-way ANOVA analysis ($p < 0.05$; * $p < 0.1$; **** $p < 0.0001$), with the application of the Tukey post hoc test.

3. Results

3.1. Chemical Composition of Fixed Oil

The identification of fatty acids by GC-MS revealed the chemical composition of FOCC, with eight fatty acids identified, representing 97.97% of the total composition of the fixed oil (Table 1 and Figure 1). It was observed that FOCC contains a higher concentration of unsaturated fatty acids, with oleic acid being the predominant compound at 59.78%, followed by palmitic acid, a saturated fatty acid, at 32.45%. Along with compounds in low concentrations, such as linoleic, stearic, and elaidic acids.

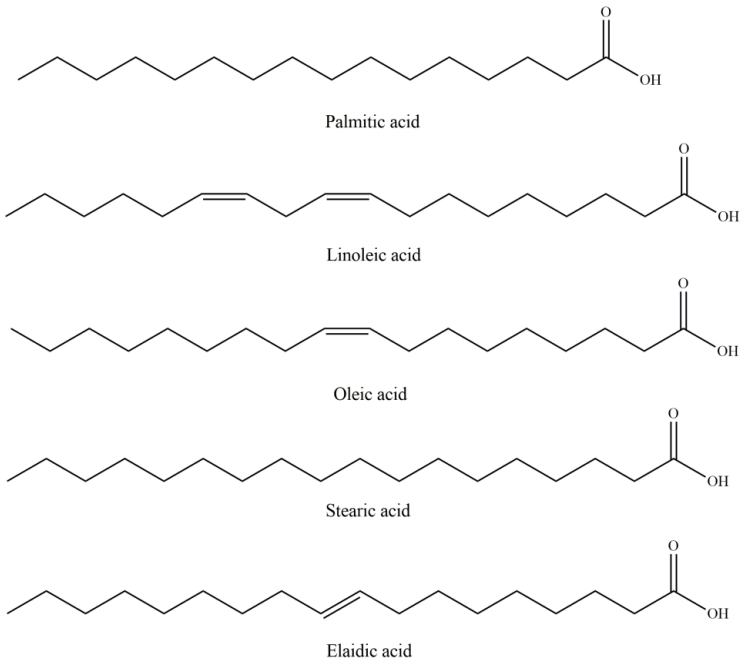

Figure 1. Chemical structure of compounds identified in fixed oil of Caryocar coriaceum (FOCC).

Table 1. Phytochemical profile of fixed oil of *Caryocar coriaceum* (FOCC).

Fatty Acids	Retention Time	[%]
Palmitic acid (C16:0)	24.31	32.45
Linoleic acid (C18:2)	46.19	1.47
Oleic acid (C18:1*cis*)	51.15	59.78
Stearic acid (C18:0)	51.61	2.36
Elaidic acid (C18:1*trans*)	52.2	1.91
Total		97.97

3.2. Anti-Biofilm Formation Activity

The antibiofilm activity of FOCC and chlorhexidine gluconate (CG) is detailed in Figure 2. FOCC showed promising results against both Gram-positive and Gram-negative strains, exhibiting approximately 70% inhibition of *S. mutans* biofilm formation at the lowest concentration tested (MIC 10 mg/mL). Regarding *E. faecalis*, a lesser effect was observed, with significant results only at concentrations of 100 mg/mL (MIC × 10). The MIC concentrations for *S. aureus* and *P. aeruginosa* (Gram-negative) resulted in effective inhibition of approximately 60%, similar to the action of CG at the same concentrations. Notably, against *P. aeruginosa* (Figure 2d), FOCC demonstrated superior inhibition compared to the reference drug.

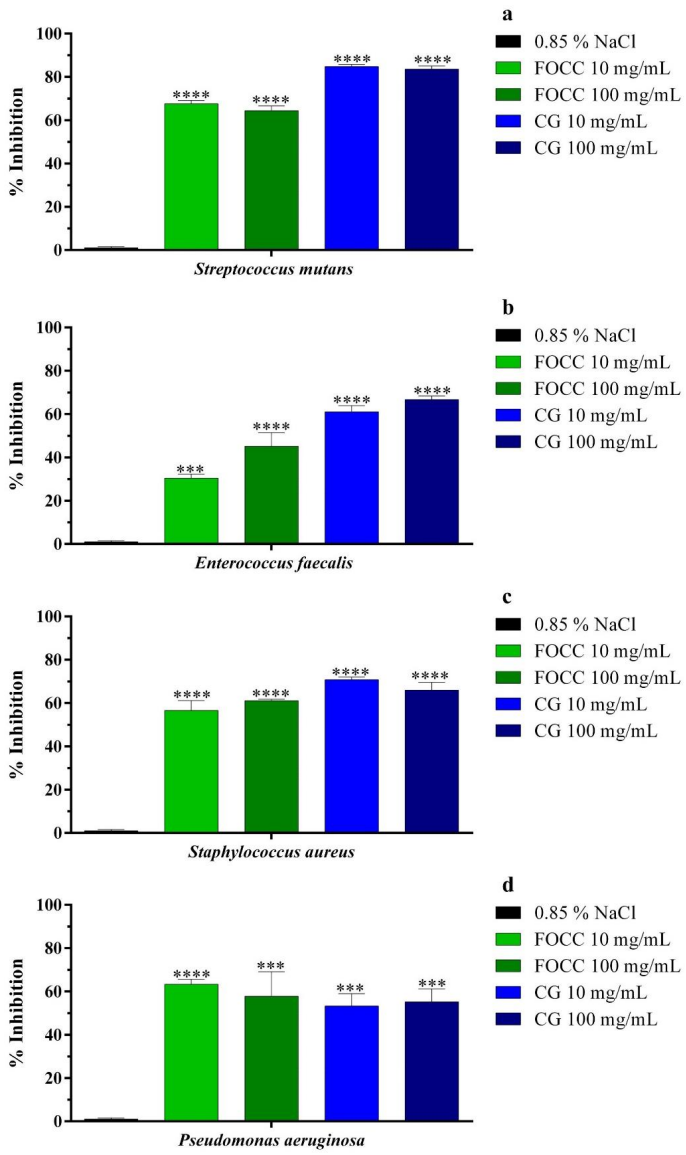

Figure 2. Antibacterial biofilm formation capacity of *Caryocar coriaceum* fixed oil (FOCC) and chlorhexidine gluconate (CG) antibiotic against *Streptococcus mutans* (**a**), *Enterococcus faecalis* (**b**), *Staphylococcus aureus* (**c**), and *Pseudomonas aeruginosa* (**d**); *** = $p < 0.001$, **** = $p < 0.0001$.

3.3. Pre-Formed Biofilm Disruption Capacity

In contrast to the biofilm formation inhibition results (Figure 2), FOCC demonstrated inferior or non-significant results in pre-formed biofilm disruption, as illustrated in Figure 3. FOCC, tested at MIC and MIC × 10 concentrations, did not show clinical relevance against *S. mutans* and *P. aeruginosa*, which may suggest higher resistance, similar to the action of

CG on *P. aeruginosa* (Figure 3d). However, for other Gram-positive strains, such as *E. faecalis* and *S. aureus*, greater pre-formed biofilm disruption efficacy was observed, even surpassing CG's action at 10 mg/mL concentrations.

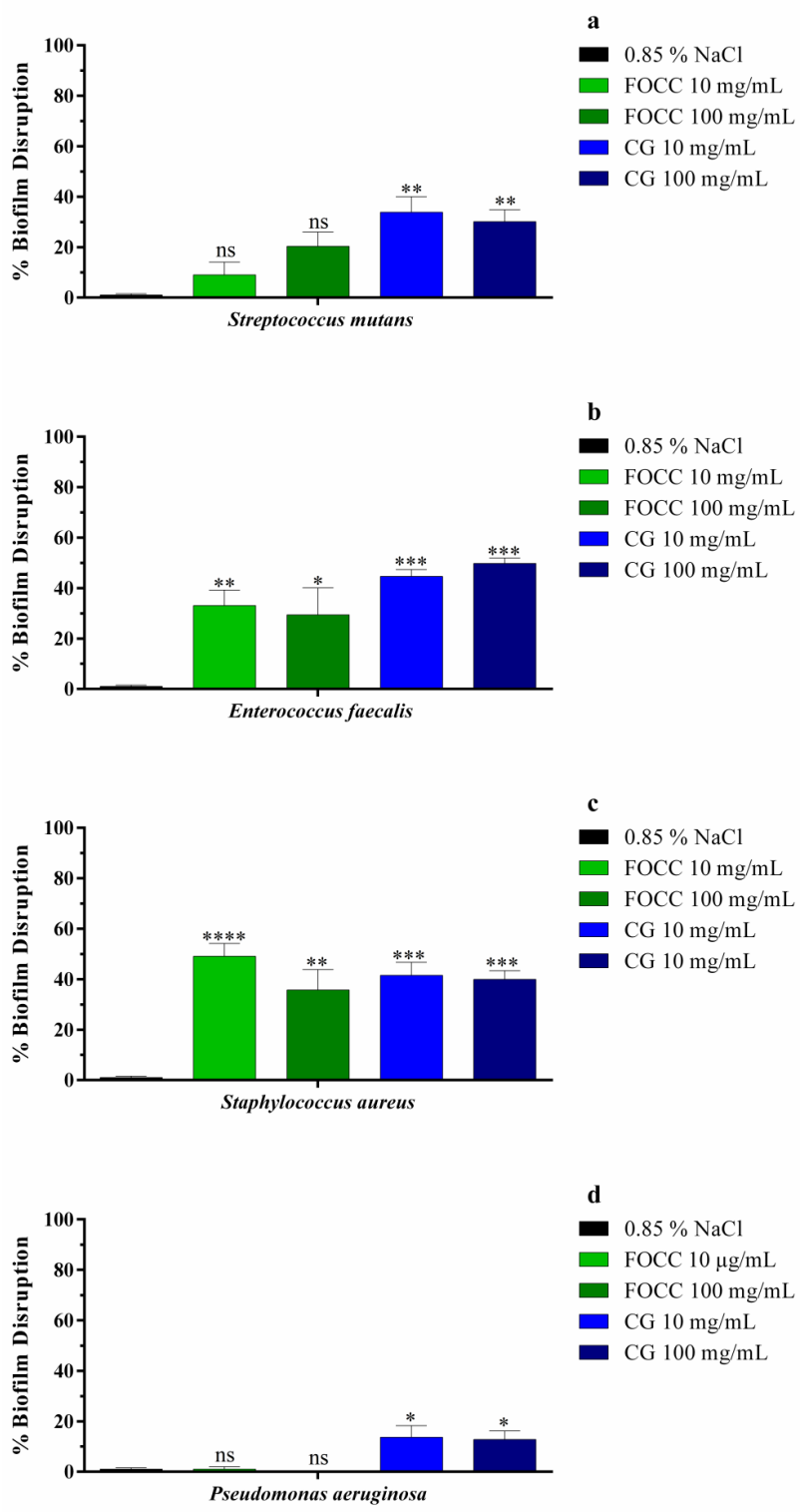

Figure 3. Bacterial pre-formed biofilm disruption capacity of fixed oil of *Caryocar coriaceum* (FOCC) and antibiotic chlorhexidine gluconate (CG) against *Streptococcus mutans* (**a**), *Enterococcus faecalis* (**b**), *Staphylococcus aureus* (**c**), and *Pseudomonas aeruginosa* (**d**); * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, ns: not significant.

4. Discussion

Caryocar coriaceum is a plant species widely used in traditional folk medicine for the treatment of wounds, muscle pain, and chronic arthritis, as well as in the management of respiratory, gastric, and inflammatory diseases [20]. Our study demonstrates that the fixed oil from the mesocarp of *C. coriaceum* mainly contains unsaturated fatty acids in its chemical composition, with a particular predominance of oleic acid (60.1%) and palmitic acid (33.5%), as shown in Table 1. The chemical composition obtained in this study is corroborated by other research conducted previously. In prior studies by our research group, [29] analyzed the fixed oil from the mesocarp of *C. coriaceum* and demonstrated that oleic acid (61%) and palmitic acid (33%) were the most abundant components. Refs. [30,31] also described oleic and palmitic acids as predominant components in their fixed oil samples.

These findings indicate that both fatty acids are potential chemical markers of the fixed oil from *C. coriaceum*. Oleic acid, found as the primary compound in the studies, mainly occurs chemically bound to triglycerides in natural oils (animal and vegetable) and fats. Furthermore, it is described in the literature as possessing antioxidant activities [32], antimicrobial properties [33,34], and anti-inflammatory effects [35–37]. Palmitic acid, in turn, exhibits anti-inflammatory, analgesic [38,39], antitumor [40], and antiviral [41] activities.

This study is the first to report the anti-biofilm potential of the fixed oil from the mesocarp of *C. coriaceum*. FOCC does not present antibacterial activity against planktonic bacteria at clinically relevant concentrations, presenting a MIC > 512 μg/mL [31]. The analyses demonstrate that the FOCC shows promising potential as an anti-biofilm agent against Gram-positive bacteria (*S. mutans* and *S. aureus*) and Gram-negative bacteria (*P. aeruginosa*). The exact mechanism of action of fixed oils against microorganisms is still poorly understood; however, it is suggested that unsaturated fatty acids present in these natural products inhibit bacteria by affecting the synthesis of endogenous fatty acids [42] and reducing extracellular polymeric substances [43]. This antimicrobial activity may also be related to the destruction of the cell membrane and interference in cellular processes (signal transduction and transcription) [44].

The potential of fixed oils in combating pathogenic biofilms is a topic that has been little explored in the literature. Much more attention has been given to the use of unsaturated fatty acids against these microbial communities. Petroselinic acid, for example, significantly inhibited biofilm formation in methicillin-resistant and sensitive strains of *S. aureus*. Furthermore, this acid suppressed the production of virulence factors, such as staphyloxanthin, lipase, and α-hemolysin [45]. Nicol et al. [46] showed that palmitoleic and myristoleic acids reduced biofilm formation in *Acinetobacter baumannii* (an important agent of nosocomial infections), as well as promoting biofilm dispersion and drastically decreasing bacterial motility. Atomic force microscopy experiments also showed that both acids can act against the initial adhesion process. Other unsaturated fatty acids that are part of the major composition of plant or microbial-derived extracts with anti-biofilm activity have also been reported [47–49].

In our study, we also investigated the ability of FOCC to eradicate pre-formed biofilms. FOCC showed significant efficacy in eradicating biofilms, primarily from the Gram-positive bacterium *S. aureus* at a concentration of 10 mg/mL, compared to the control. The anti-biofilm action of FOCC indicates that this oil is ineffective against most pre-formed microbial biofilms, highlighting that bacterial cells in biofilms are more resistant than those in a planktonic state [50]. However, this also underscores the potential of the fixed oil from *C. coriaceum* against the biofilm of *S. aureus*.

The anti-biofilm activity of oleic acid, the major component of the fixed oil from *C. coriaceum*, has been consistently described in the literature. Khadke et al. [51] demonstrated that oleic acid (20 μg/mL) significantly inhibited the biofilm formation of

A. baumannii without affecting the growth of its planktonic cells (CIM > 500 µg/mL), in addition to reducing bacterial motility. Molecular dynamics simulations also showed that this compound binds to acyl-homoserine lactone synthetase (AbaI), which is involved in quorum sensing. Additionally, oleic acid has also been reported as an inhibitor of biofilms of *S. aureus* [52,53] in a dose-dependent manner, potentially due to causing rupture in the cell membrane [54,55]. Thus, these studies corroborate our results and suggest that the anti-biofilm activity of FOCC, especially against *S. aureus*, may be associated with the presence of oleic acid as a major compound without ruling out its synergistic action with other compounds present.

Biofilms represent a significant challenge for public health, as they are complex structures formed by microbial communities adhered to surfaces, making them highly resistant to conventional treatments. This resistance complicates the pre-formed biofilm disruption of infections, especially when biofilms form on medical devices, such as catheters and prostheses, potentially leading to persistent and recurrent infections [56,57]. Given this problem, research into new strategies to combat biofilms is crucial. Innovations in this field can significantly improve clinical outcomes, reduce the use of antibiotics, decrease the risk of developing bacterial resistance, and alleviate the economic burden on healthcare systems.

According to previous studies, it is suggested that the use of fixed oil of *C. coriaceum* can be considered biologically safe [29,58]. Silva et al. [58] reported that after a 9-week period of administration of fixed oil from this plant in Swiss mice (100 mg/kg/day), no significant changes were observed in food intake, body weight, or blood glucose levels in the animals evaluated. Furthermore, Almeida-Bezerra et al. [29] also reported that the fixed oil of *C. coriaceum* did not show toxicity when evaluated in an experimental model using *Drosophila melanogaster*.

5. Conclusions

The fixed oil of *Caryocar coriaceum* exhibited a predominant composition of unsaturated fatty acids, particularly highlighting oleic acid and palmitic acid. Additionally, it demonstrated promising activity in inhibiting bacterial biofilm formation, especially against *S. mutans*, *S. aureus*, and *P. aeruginosa*, although its efficacy in eradicating pre-formed biofilms was limited. The anti-biofilm formation potential, combined with the presence of oleic acid, suggests that this natural product could be an interesting natural alternative for preventing bacterial infections associated with biofilms.

It is suggested that new in vitro and in vivo studies be conducted to investigate the possible mechanisms of action of the fixed oil of *C. coriaceum*, as well as of its major compounds, oleic acid and palmitic acid, against *S. mutans*, *S. aureus*, and *P. aeruginosa*.

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References

1. Kamali, E.; Sharifi, S.; Goudarzi, G.; Hashemi, A.; Goudarzi, H.; Gholami, A.; Najar-Peerayeh, S.; Azizi, O. Evaluation of Antimicrobial Resistance, Biofilm Forming Potential, and the Presence of Biofilm-Related Genes among Clinical Isolates of *Pseudomonas aeruginosa*. *BMC Res. Notes* **2020**, *13*, 27. [CrossRef] [PubMed]
2. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *Lancet* **2022**, *399*, 629–655. [CrossRef] [PubMed]
3. McEwen, S.A.; Collignon, P.J. Antimicrobial Resistance: A One Health Perspective. In *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals*; Wiley: Hoboken, NJ, USA, 2018; pp. 521–547. [CrossRef]
4. World Health Organization (WHO). Antimicrobial Resistance. 2021. Available online: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed on 2 September 2024).
5. O'Neill, J. *Review on Antimicrobial Resistance: Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*; UK Government: London, UK, 2016; pp. 1–80. Available online: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf (accessed on 26 January 2025).
6. Freires, M.S.; Junior, O.M.R. Resistência Bacteriana pelo Uso Indiscriminado da Azitromicina Frente a COVID-19: Uma Revisão Integrativa. *Res. Soc. Dev.* **2022**, *11*, e31611125035. [CrossRef]
7. Huang, L.; Luo, Q.; Li, Y.; Wang, J.; Wu, Z. Bacterial Multidrug Efflux Pumps at the Frontline of Antimicrobial Resistance: An Overview. *Antibiotics* **2022**, *11*, 520. [CrossRef] [PubMed]
8. Rather, M.A.; Gupta, K.; Mandal, M. Microbial Biofilm: Formation, Architecture, Antibiotic Resistance, and Control Strategies. *Braz. J. Microbiol.* **2021**, *52*, 1701–1718. [CrossRef]
9. Yang, S.; Zhang, J.; Yang, R.; Xu, X. Small molecule compounds, a novel strategy against *Streptococcus mutans*. *Pathogens* **2021**, *10*, 1540. [CrossRef] [PubMed]
10. Vaou, N.; Pratsinis, H.; Kletsas, D.; Tsigou, A.; Giamarellos-Bourboulis, E.J.; Eliopoulos, G.M.; Katsambas, A.; Avgerinos, A. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. *Microorganisms* **2021**, *9*, 2041. [CrossRef] [PubMed]
11. Kebede, T.; Gadisa, E.; Tufa, A. Antimicrobial Activities Evaluation and Phytochemical Screening of Some Selected Medicinal Plants: A Possible Alternative in the Treatment of Multidrug-Resistant Microbes. *PLoS ONE* **2021**, *16*, e0249253. [CrossRef] [PubMed]
12. Ugboko, H.U.; Nwinyi, O.C.; Orabueze, I.A.; Ikegbunam, M.N. Antimicrobial Importance of Medicinal Plants in Nigeria. *Sci. World J.* **2020**, *1*, 7059323. [CrossRef] [PubMed]
13. Castronovo, L.M.; Nicoletti, M.; Benedetti, R.; Rossi, M. Medicinal Plants and Their Bacterial Microbiota: A Review on Antimicrobial Compounds Production for Plant and Human Health. *Pathogens* **2021**, *10*, 106. [CrossRef]
14. Silva, J.T.C.; Lima, J.R.; Coelho, S.C.; Albuquerque, R.A. Phytochemical Analysis and Evaluation of the Antibacterial and Antibiotic Potentiation Activities of the Aqueous Extract of *Cordia oncocalyx* Allemão (Boraginaceae). *Pharm. Sci. Adv.* **2024**, *2*, 100042. [CrossRef]
15. Reichling, J. Anti-Biofilm and Virulence Factor-Reducing Activities of Essential Oils and Oil Components as a Possible Option for Bacterial Infection Control. *Planta Med.* **2020**, *86*, 520–537. [CrossRef]
16. Rosca, A.S.; Antoun, M.; Lopez, A.I.; Gutierrez, S.; Didiot, E.; Leblond, P.; Vernis, L.; Gabard, J. Six Bacterial Vaginosis-Associated Species Can Form an in Vitro and Ex Vivo Polymicrobial Biofilm That Is Susceptible to *Thymbra capitata* Essential Oil. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 824860. [CrossRef] [PubMed]
17. Mendes, S.G.; Ribeiro, P.R.; Figueiredo, A.S.; Ferreira, A.L.; Oliveira, S.M. Co-Regulation of Biofilm Formation and Antimicrobial Resistance in Acinetobacter Baumannii: From Mechanisms to Therapeutic Strategies. *Eur. J. Clin. Microbiol. Infect. Dis.* **2023**, *42*, 1405–1423. [CrossRef] [PubMed]
18. Violante, I.M.P.; Silva, R.N.; Carvalho, P.H.B.; Moreira, D.A.; Duarte, D.S.; Pereira, J.A. Antimicrobial Activity of Some Medicinal Plants from the Cerrado of the Central-Western Region of Brazil. *Braz. J. Microbiol.* **2012**, *43*, 1302–1308. [CrossRef] [PubMed]
19. Jesus, G.S.; Silva, C.P.; Almeida, C.A.; Lima, G.P.; Santos, C.C.; Lima, F.P. Antimicrobial Potential of Essential Oils from Cerrado Plants Against Multidrug-Resistant Foodborne Microorganisms. *Molecules* **2020**, *25*, 3296. [CrossRef] [PubMed]
20. Almeida-Bezerra, J.W.; Bezerra, J.J.L.; Silva, V.B.D.; Coutinho, H.D.M.; Costa, J.G.M.D.; Cruz-Martins, N.; Almeida, J.R.G.S. *Caryocar coriaceum* Wittm. (Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs. *Plants* **2022**, *11*, 1685. [CrossRef]
21. Almeida-Bezerra, J.W.; Silva, P.S.T.; Oliveira, F.F.B.; Lima, F.O.G.; Santos, S.C. *Caryocar coriaceum* Fruits as a Potential Alternative to Combat Fungal and Bacterial Infections: In Vitro Evaluation of Methanolic Extracts. *Microb. Pathog.* **2023**, *181*, 106203. [CrossRef] [PubMed]
22. Lemos, I.C.S.; Lima, J.R.F.; Carvalho, K.A.T.; Pereira, A.G. Ethnobiological Survey of Plants and Animals Used for the Treatment of Acute Respiratory Infections in Children of a Traditional Community in the Municipality of Barbalha, Ceará, Brazil. *Afr. J. Tradit. Complement. Altern. Med.* **2016**, *13*, 166–175. [CrossRef]

23. Silva, V.R.P.; Almeida, J.R.G.S.; Guedes, G.M.M. Anti-Inflammatory Activity of Pequi Oil (*Caryocar brasiliense*): A Systematic Review. *Pharmaceutics* **2023**, *17*, 11. [CrossRef] [PubMed]
24. Queiroz, J.C.E.; Leite, J.R.S.A.; Vasconcelos, A.G. Prospecting Plant Extracts and Bioactive Molecules with Antimicrobial Activity in Brazilian Biomes: A Review. *Antibiotics* **2023**, *12*, 427. [CrossRef]
25. Geris, R.; Santos, N.A.C.D.; Amaral, B.A.; Maia, I.D.S.; Castro, V.D.; Carvalho, J.R.M. Biodiesel de Soja: Reação de Transesterificação para Aulas Práticas de Química Orgânica. *Quim. Nova* **2007**, *30*, 1369–1373. [CrossRef]
26. Araújo, N.J.S.; do Socorro Costa, M.; da Silva, A.R.P.; Paulo, C.L.R.; Silva, C.A.P.; Felix, F.J.; de Morais Oliveira-Tintino, C.D.; Barbosa Filho, J.M.; de Freitas, T.S.; Braga, M.F.B.M.; et al. In Vitro Evaluation of the Susceptibility of Bacterial Biofilms to Hecogenin Acetate. *Acta Trop.* **2024**, *253*, 107157. [CrossRef] [PubMed]
27. Stepanovic, S.; Cirkovic, I.; Ruzicka, F. Quantification of Biofilm in Microtiter Plates: Overview of Testing Conditions and Practical Recommendations for Assessment of Biofilm Production by *Staphylococci*. *APMIS* **2007**, *115*, 891–899. [CrossRef] [PubMed]
28. Andrade, J.C.; Santana, L.C.L.; Brito, A.S.; Chaves, T.P.; de Oliveira, J.G.; Quintans-Júnior, L.J.; Melo Coutinho, H.D.; Tintino, S.R.; Galvão Rodrigues, F.F. UPLC-MS-ESI-QTOF Characterization and Evaluation of the Antibacterial and Modulatory Antibiotic Activity of *Ziziphus joazeiro* Mart. Aqueous Extracts. *S. Afr. J. Bot.* **2019**, *123*, 105–112. [CrossRef]
29. Almeida-Bezerra, J.W.; Silva, P.S.T.; Almeida, J.R.G.S.; Cruz-Martins, N.; Coutinho, H.D.M. Exploring the Fluconazole-Resistance Modifying Activity and Potential Mechanism of Action of Fixed Oil from *Caryocar coriaceum* Wittm. (Caryocaraceae) Against *Candida* Species. *Chem. Biodivers.* **2024**, *21*, e202301960. [CrossRef] [PubMed]
30. Figueiredo, P.R.L.; Costa, R.G.; Alves, D.C.; Carvalho, A.F.M.; Costa, J.G.M.; Coutinho, H.D.M.; Menezes, I.R.A. *Caryocar coriaceum* Wittm. (Pequi) Fixed Oil Presents Hypolipemic and Anti-Inflammatory Effects In Vivo and In Vitro. *J. Ethnopharmacol.* **2016**, *191*, 87–94. [CrossRef]
31. Pereira, F.F.G.; Feitosa, M.K.S.B.; Costa, M.S.; Tintino, S.R.; Rodrigues, F.F.G.; Menezes, I.R.A.; Coutinho, H.D.M.; Costa, J.G.M.; Sousa, E.O. Characterization, Antibacterial Activity and Antibiotic Modifying Action of the *Caryocar coriaceum* Wittm. Pulp and Almond Fixed Oil. *Nat. Prod. Res.* **2020**, *34*, 3239–3243. [CrossRef]
32. Guzmán, D.C.; Olguín, H.J.; García, E.H.; Peraza, Á.C.; Mejía, G.B.; Bárcenas, S.P.; García, D.R. Oleic Acid Reduces Oxidative Stress in Rat Brain Induced by Some Anticancer Drugs. *Chem. Biol. Interact.* **2024**, *398*, 111086. [CrossRef]
33. Selvaraj, A.; Syed, A.; Basith, S.; Manikandan, R. *Sapindus mukorossi* Gaertn. and Its Bioactive Metabolite Oleic Acid Impedes Methicillin-Resistant Staphylococcus aureus Biofilm Formation by Down Regulating Adhesion Genes Expression. *Microbiol. Res.* **2021**, *242*, 126601. [CrossRef]
34. Petrović, M.; Petrović, S.; Gligorić, E.; Vučković, I.; Nikolić, N. Poly(Methyl Methacrylate) with Oleic Acid as an Efficient *Candida albicans* Biofilm Repellent. *Materials* **2022**, *15*, 3750. [CrossRef] [PubMed]
35. Nakatsuji, T.; Kao, M.C.; Zhang, L.; Zouboulis, C.C.; Gallo, R.L.; Huang, C.M. Sebum Free Fatty Acids Enhance the Innate Immune Defense of Human Sebocytes by Upregulating Beta-Defensin-2 Expression. *J. Investig. Dermatol.* **2010**, *130*, 985–994. [CrossRef] [PubMed]
36. Dozsa, A.; Dezso, B.; Toth, B.I.; Bacsi, A.; Poliska, S.; Camera, E.; Picardo, M.; Zouboulis, C.C.; Bíró, T.; Schmitz, G. PPARγ-Mediated and Arachidonic Acid-Dependent Signaling Is Involved in Differentiation and Lipid Production of Human Sebocytes. *J. Investig. Dermatol.* **2014**, *134*, 910–920. [CrossRef] [PubMed]
37. Pegoraro, N.S.; Xavier, L.S.; Cardoso, M.L.C.; Zanon, P.E.; Aguiar, C.S.; Figueiredo, B.D.; Cruz, N.C.; Estrela, M.L.; Deffune, E.; Catalani, L.H. The Association of Oleic Acid and Dexamethasone Acetate into Nanocapsules Enables a Reduction in the Effective Corticosteroid Dose in a UVB Radiation-Induced Sunburn Model in Mice. *Pharmaceutics* **2024**, *16*, 176. [CrossRef] [PubMed]
38. Charlet, R.; Pujos-Guillot, E.; Gaudet, F.; Lounes, S.; Gaudin, P.; Dubourdeaux, M.; Fournière, M.; Khoutami, M. Oleic Acid and Palmitic Acid from *Bacteroides thetaiotaomicron* and *Lactobacillus johnsonii* Exhibit Anti-Inflammatory and Antifungal Properties. *Microorganisms* **2022**, *10*, 1803. [CrossRef]
39. Li, S.; Fan, W.; Li, Y.; Liang, J.; Wang, G.; Li, X.; Liu, P. Palmitic Acid and Trans-4-Hydroxy-3-Methoxycinnamate, the Active Ingredients of Yaobishu Formula, Reduce Inflammation and Pain by Regulating Gut Microbiota and Metabolic Changes After Lumbar Disc Herniation to Activate Autophagy and the Wnt/β-Catenin Pathway. *Biochim. Biophys. Acta Mol. Basis Dis.* **2024**, *1870*, 166972. [CrossRef]
40. Zhu, S.; Ren, S.; Jiang, H.; Jiang, J.; Zhao, X. Palmitic Acid Inhibits Prostate Cancer Cell Proliferation and Metastasis by Suppressing the PI3K/Akt Pathway. *Life Sci.* **2021**, *286*, 120046. [CrossRef]
41. Librán-Pérez, M.; Figueira, J.F.; Fernández-Gómez, B.; Antúnez, A.C.; Abreu, H.L.; García-Reyero, N.; Villena, C.R.; Soengas, J.L.; Mallo, J.G. Antiviral Activity of Palmitic Acid via Autophagic Flux Inhibition in Zebrafish (*Danio rerio*). *Fish Shellfish Immunol.* **2019**, *95*, 595–605. [CrossRef] [PubMed]
42. Zheng, C.J.; Yoo, J.S.; Lee, T.G.; Cho, H.Y.; Kim, Y.H.; Lee, H.J. Fatty Acid Synthesis Is a Target for Antibacterial Activity of Unsaturated Fatty Acids. *FEBS Lett.* **2005**, *579*, 5157–5162. [CrossRef]
43. Yuyama, K.T.; Oliveira, A.M.; Oliveira, R.L.; Cavalcanti, T.P.M.; Bezerra, T.K.A. Unsaturated Fatty Acids Control Biofilm Formation of *Staphylococcus aureus* and Other Gram-Positive Bacteria. *Antibiotics* **2020**, *9*, 788. [CrossRef]

44. Dayrit, F.M. The Properties of Lauric Acid and Their Significance in Coconut Oil. *J. Am. Oil Chem. Soc.* **2015**, *92*, 1–15. [CrossRef]
45. Lee, J.H.; Kim, Y.G.; Lee, J. Inhibition of *Staphylococcus aureus* Biofilm Formation and Virulence Factor Production by Petroselinic Acid and Other Unsaturated C18 Fatty Acids. *Microbiol. Spectr.* **2022**, *10*, e0133022. [CrossRef] [PubMed]
46. Nicol, M.; Kwun, M.J.; Ghimire, A.; Thapa, T.J.; Lee, S.J.; Jeong, K.C. Unsaturated Fatty Acids Affect Quorum Sensing Communication System and Inhibit Motility and Biofilm Formation of *Acinetobacter baumannii*. *Int. J. Mol. Sci.* **2018**, *19*, 214. [CrossRef]
47. Supardy, N.A.; Ismail, M.F.; Husain, K.; Leman, M.N. Bioactive Compounds of Sp. IBRL PD4.8 Inhibit Growth of Fouling Bacteria and Attenuate Biofilms of FB3. *Pol. J. Microbiol.* **2019**, *68*, 21–33. [CrossRef]
48. Abdel-Aziz, M.M.; Emam, T.M.; Raafat, M.M. Hindering of Cariogenic *Streptococcus mutans* Biofilm by Fatty Acid Array Derived from an Endophytic *Arthrographis kalrae* Strain. *Biomolecules* **2020**, *10*, 811. [CrossRef] [PubMed]
49. Dou, J.; Ahn, S.; Zeng, C.; Oh, K.; Son, Y.; Xu, H. Willow Bark-Derived Material with Antibacterial and Antibiofilm Properties for Potential Wound Dressing Applications. *J. Agric. Food Chem.* **2023**, *71*, 16554–16567. [CrossRef] [PubMed]
50. Lopes, L.; Figueiredo, D.; Melo, T.; Cardoso, C.; Almeida, A.; Fernandes, R. Chemical Composition, Cytotoxicity, Antimicrobial, Antibiofilm, and Anti-Quorum Sensing Potential of *Mentha piperita* Essential Oil Against the Oral Pathogen *Streptococcus mutans*. *J. Toxicol. Environ. Health Part A* **2024**, *87*, 824–835. [CrossRef] [PubMed]
51. Khadke, S.K.; Shirsat, S.E.; Nadar, S.S.; Rathod, V.K. Assessment of Antibiofilm Potencies of Nervonic and Oleic Acid Against *Acinetobacter baumannii* Using In Vitro and Computational Approaches. *Biomedicines* **2021**, *9*, 1133. [CrossRef] [PubMed]
52. Stenz, L.; François, P.; Fischer, A.; Huyghe, A.; Tangomo, M.; Hernandez, D.; Cassat, J.; Linder, P.; Schrenzel, J. Impact of Oleic Acid (Cis-9-Octadecenoic Acid) on Bacterial Viability and Biofilm Production in St *Staphylococcus aureus*. *FEMS Microbiol. Lett.* **2008**, *287*, 149–155. [CrossRef] [PubMed]
53. Grumezescu, A.M.; Saviuc, C.; Chifiriuc, M.C.; Hristu, R.; Mihaiescu, D.E.; Balaure, P.; Mihaiescu, R.; Usnic, A. Oleic Acid Core-Shell Nanofluid Inhibits *Staphylococcus aureus* Biofilm Development. *IEEE Trans. Nanobiosci.* **2011**, *10*, 269–274. [CrossRef] [PubMed]
54. Kenny, J.G.; Ward, D.; Josefsson, E.; Jonsson, I.M.; Hinds, J.; Rees, H.H.; Lindsay, J.A.; Foster, S.J. The *Staphylococcus aureus* Response to Unsaturated Long Chain Free Fatty Acids: Survival Mechanisms and Virulence Implications. *PLoS ONE* **2009**, *4*, e4344. [CrossRef] [PubMed]
55. Lee, J.H.; Kim, Y.G.; Lee, J. Supercritical Fluid Extracts of Moringa Oleifera and Their Unsaturated Fatty Acid Components Inhibit Biofilm Formation by *Staphylococcus aureus*. *Food Control* **2017**, *80*, 74–82. [CrossRef]
56. Hall-Stoodley, L.; Costerton, J.W.; Stoodley, P. Bacterial Biofilms: From the Natural Environment to Infectious Diseases. *Nat. Rev. Microbiol.* **2004**, *2*, 95–108. [CrossRef] [PubMed]
57. Arciola, C.R.; Campoccia, D.; Speziale, P.; Montanaro, L.; Costerton, J.W. Biofilm Formation in *Staphylococcus* Implant Infections: A Review of Molecular Mechanisms and Implications for Biofilm-Resistant Materials. *Biomaterials* **2012**, *33*, 5967–5982. [CrossRef]
58. Silva, A.A.; Borges, O.M.A.; Alencar, N.M.N.; Vasconcelos, R.P.; Oliveira, A.C.; Gaban, S.V.F. Effect of *Caryocar coriaceum* Wittm. fixed oil on body weight and metabolic parameters of mice fed a high-fat diet. *Food Chem. Adv.* **2024**, *5*, 100855. [CrossRef]