SVOA Microbiology

ISSN: 2634-534X

ScienceVolks Open Access

Original Research

Fixed Oils of Medicinal Palms of Arecaceae from Chapada do Araripe: Chemical Composition and Antibacterial Potential

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Abstract

This study aimed to analyze the chemical composition of fixed oils extracted from the fruits of *Acrocomia aculeata, Syagrus cearensis* and *Attalea speciosa*, in addition to evaluating their efficacy in combating resistant microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*. The ripe fruits were collected in the region of Barbalha, Ceará, and the extracted oils were analyzed by gas chromatography and mass spectrometry (GC/MS) to identify the compounds present. The antibacterial activity was tested using the microdilution method in 96-well plates, evaluating the inhibition of bacterial growth at different concentrations of the oils. The chromatographic analysis of the fixed oils of *Acrocomia aculeata*, *Attalea speciosa* and *Syagrus cearensis* revealed the predominance of saturated fatty acids, with lauric acid being the most abundant (41.71% to 47.21%). Oleic and myristic acids were also significant, while stearic and linoleic acids appeared in smaller amounts. *Attalea speciosa* showed inhibition of 40.17% against *Escherichia coli* and 40.77% against *Staphylococcus aureus* (1000 µg/mL). *Acrocomia aculeata* inhibited 44.76% of *S. aureus* (1000 µg/mL), and *Syagrus cearensis* had moderate activity against *E. coli.*

Keywords: *Antimicrobial, Phytotherapy, Health, Medicinal plants.*

1. Introduction

Microbial resistance, exacerbated by the overuse of antimicrobials, facilitates the emergence of multidrug-resistant microorganisms [1]. Treatable infections become more difficult to cure, increasing morbidity, mortality, and costs to the health system. The spread of resistance, at the community and international level, is a serious threat to global public health [2]. Antibiotic consumption grew by 39% between 2000 and 2015, with projections of an increase of up to 200% in the coming years. It is estimated that deaths from untreatable infections could reach 10 million annually after 2050 [3].

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https://doi.org/10.58624/SVOAMB.2025.06.003

Received: December 10, 2024

Published: January 30, 2025

Citation: Moreira FC, Rolón M, Coronel C, Gómez CV, Alfonso JJ, Gómez A, Gonçalo MABF, Maia Filho A, Silva JTC, Silva RR, Sousa JD, Sousa JD, Albuquerque ES, Felício MF, Santos MAF, Melo JA, Morais-Braga MFB, Coutinho HDM, Silva VB, Almeida-Bezerra JW, Costa AR. Fixed Oils of Medicinal Palms of Arecaceae from Chapada do Araripe: Chemical Composition and Antibacterial Potential. *SVOA Microbiology 2025,* 6:1, 17-24. doi: 10.58624/SVOAMB.2025.06.003

1. Introduction

Microbial resistance, exacerbated by the overuse of antimicrobials, facilitates the emergence of multidrug-resistant microorganisms [1]. Treatable infections become more difficult to cure, increasing morbidity, mortality, and costs to the health system. The spread of resistance, at the community and international level, is a serious threat to global public health [2]. Antibiotic consumption grew by 39% between 2000 and 2015, with projections of an increase of up to 200% in the coming years. It is estimated that deaths from untreatable infections could reach 10 million annually after 2050 [3].

Among the most relevant bacterial species in this context, *Escherichia coli* and *Staphylococcus aureus stand out*. These bacteria are known for their ability to develop resistance to multiple antibiotics, which poses a significant challenge for the treatment of hospital-acquired infections [4]. Antimicrobial resistance compromises the effectiveness of control measures and is compounded by the difficulty of access to medicines in developing countries. The absence of effective antibiotics makes their proper use even more difficult [5].

Brazil has a rich biodiversity and occupies a prominent position in the production of herbal medicines, offering significant therapeutic potential through the extraction of medicinal plants, including essential oils, resins, tinctures, and extracts [6]. The effectiveness of herbal treatments depends on the correct identification and proper use of plants, which are exploited for medicinal purposes in the Caatinga and are fundamental for the health of local communities, especially in Chapada do Araripe, in Cariri Cearense, where the diversity of plant species is significant [7].

These species of medicinal plants from Chapada do Araripe highlight the relevance of some specific botanical families, such as Fabaceae, Asteraceae, Malvaceae and Rubiaceae species of the Arecaceae family, they are also widely used in the popular pharmacopoeia. The order Arecales, to which the Palm Trees belong, has 181 genera and about 2,600 species, and Brazil is home to 37 genera and 299 species. In Chapada do Araripe, environmental conditions favor its diversity [8, 9].

In this family, it is noted that species such as *Acrocomia aculeata* (Jacq.) Lodd. ex Mart*., Syagrus cearensis Noblick and Attalea speciosa* Mart. are notoriously investigated for the treatment of infections and parasites widely recognized for their therapeutic properties, highlighting their importance in folk medicine [10, 11]. Several applications can be demonstrated in the literature, such as use for the treatment of urinary tract infections, inflammation, antimicrobial and antiparasitic [12-15].

These properties may reflect the composition of the oils extracted from these palm trees, which have a high concentration of fatty acids with potential to fight pathogens. These fatty acids demonstrate antibacterial properties that can be exploited in medical treatments [16, 17]. In addition, the acids of interest are analyzed in the pulps and almonds of different species, evidencing their pharmacological potential [18].

Based on the pharmacological and biological properties of the species *Acrocomia aculeata, Syagrus cearensi*s and *Attalea speciosa*, it is noted that these species have the potential to combat and control multidrug-resistant pathogenic bacteria, a promising alternative to the exhaustion of effectively active antibiotics. Thus, to investigate the chemical composition of fixed oils extracted from ripe fruits, in addition to evaluating the efficacy of these oils in inhibiting the growth of resistant pathogenic microorganisms, such as *Staphylococcus aureus* and *Escherichia coli.*

2. Materials and Methods

2.1 Obtaining a license and collecting samples

The ripe fruits of the species: *Acrocomia aculeata* (Jacq.) Lodd. ex Mart*., Syagrus cearensi*s Noblick and *Attalea speciosa* Mart. were collected in the APA (Área de Proteção Ambiental) of Chapada do Araripe (39°24'28" W, 07º20'51" S), in the municipality of Barbalha, Ceará. To carry out the collection, authorization was obtained from the platforms of the SISBIO (Sistema de Autorização e Informação em Biodiversidade) and the SISGEN (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) under registration numbers 80987-1 and A23DEE0, respectively. The species were identified in the field by botanist Dr. José Weverton Almeida-Bezerra.

2.2 Fixed oil extraction

After collecting the ripe fruits, the almonds were separated and thoroughly cleaned. Then, the material was crushed and dehydrated at 40°C for 72 h in a drying oven. The seeds then went through a cold extraction process using n-hexane for 92 hours, in a 1:2 ratio, to release lipophilic compounds. After filtration, the liquid fraction obtained was concentrated in a rotary evaporator with a water bath, resulting in the extraction of the fixed oils, which were stored in amber containers at room temperature (28°C) to be used in biological assays [19].

2.3 Chromatographic Analysis and Mass Spectrometry (GC/MS)

For the determination of fatty acids, an indirect approach was adopted using methyl esters. A total of 0.2 g of the oil was subjected to reflux saponification using a solution of potassium hydroxide in methanol for 2 h [20]. After saponification, the pH was adjusted, and the free acids were converted to methyl esters in an acidic environment.

The analysis of the fixed constituents of the oil was performed by Gas Chromatography coupled to Mass Spectrometry (GC/MS) with a Hewlett-Packard spectrometer model 5971. Separation was performed in a column of fused non-polar DB-1 capillary silica 30 m x 0.25 mm id 0.25 µm Helium gas was used as a carrier gas, with a flow rate of 0.8 mL/min and in the split mode configuration.

The injector and detector temperatures were set at 250°C and 200°C, respectively. The thermal profile of the column started at 35°C, increasing to 180°C at a rate of 4°C/min, and then reaching 250°C at a rate of 10°C/min

Mass spectra were acquired in a range of 30 to 450 m/z, with a volume of 1 µL of the 5 µg/mL dichloromethane solution being injected. The identification of the compounds was performed by comparing the obtained spectra (70 eV) with data from a database embedded in the spectrometer and in two other computers, using retention indices determined in relation to the series of n-alkane homologues from C7 to C30 [21]. A visual comparison was also performed with data from the mass spectra library (NIST and Wiley) [22, 23] to confirm the results.

2.4 In vitro antibacterial activity

2.4.1 Strains, culture medium and inoculum

The antibacterial activity of the fixed oils was evaluated using bacterial strains: *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The culture medium used was Brain Heart Infusion (BHI, Merck KGaA, Darmstadt, Germany) for 24 h at 37 °C The screening test followed the methodology recommended by CLSI M7-A113 [24], with specific adaptations for the experimental conditions.

After culture and growth, the bacteria were diluted in test tubes containing 3mL of sterile saline solution (0.9% NaCl) for inoculum preparation, which were shaken in a vortex device and the turbidity was compared with the McFarland standard scale adjusted to 0.5 (equivalent to 1.5 x 10^8 colony-forming units CFU/mL).

2.4.2 Minimum Inhibitory Concentration (MIC)

The antibacterial properties of the fixed oils were evaluated by the Minimum Inhibitory Concentration (MIC) to verify the ability to inhibit bacterial growth. A matrix solution was prepared with 100 µL of inoculum and 900 µL of culture medium (BHI) in 96-well microtiter plates, followed by the addition of fixed oils at concentrations of 500 and 1000 µg/mL. As a positive control, 1350 µL of BHI with 150 µL of bacterial suspension were used. The plates were incubated in a bacteriological incubator at 37 ºC for 24 h [25]. After incubation, the plates were read using liquid resazurin as a developer, waiting 1 hour for the oxidation-reduction reaction to occur, indicating the presence or absence of bacterial growth.

2.5 Statistical analysis

All trials were conducted in triplicate, and the results were analyzed using the GraphPad Prism version 6 program (Graph Pad Software Inc., San Diego, CA, USA), The data were analyzed using two-way ANOVA with Post Hoc Bonferroni test.

3. Results

Chemical composition

According to the chromatographic analysis of the fixed oils of the fruits of *A. aculeata, A. speciosa* and *S. cearensis*, it was possible to identify 8 chemical compounds that represented 96.50%, 97.42% and 94.13%, respectively, of the total chemical composition present in the fixed oils. The compounds belong to the classes of saturated, monounsaturated and polyunsaturated fatty acids. Lauric acid, known for its antimicrobial properties, was the predominant saturated fatty acid in the fixed oils of *A. aculeata, A. speciosa* and *S. cearensis* with 41.71%, 47.21% and 43.73%, respectively. The fixed oils of *A. aculeata* showed a relevant concentration of oleic acid of 24.36%. In *S. cearensis*, myristic acid stood out with a significant proportion of 18.29%, followed by *A. speciosa* with 15.77%. Regarding the phytochemicals present in lower concentrations (<3%), both stearic acid and linoleic acid were identified in all species (Table 1).

Table 1. Percentage composition of fixed oils extracted from *Acrocomia aculeata*, *Attalea speciosa* and *Syagrus cearenses.*

Fatty acids	Base structure	Fixed Oil (%)		
		Acrocomia aculeata	*Attalea speciosa*	*Syagrus cearensis*
Caprylic acid	C8:0	7.14	5.03	6.14
Capric acid	C10:0	3.16	4.25	4.85
Lauric acid	C12:0	41.71	47.21	43.73
Myristic acid	C14:0	7.92	15.77	18.29
Palmitic Acid	C16:0	6.77	6.18	6.43
Linoleic acid	C18:2	2.85	1.98	1.12
Oleic Acid	C18:1	24.36	14.85	10.91
Stearic Acid	C18:0	2.59	2.15	2.66
Saturated		69.29	80.59	82.10
Unsaturated		27.21	16.83	12.03
Total identified		96.50	97.42	94.13

3.2 Antibacterial activity

The analyses revealed that the fixed oil *A. speciosa* showed a significant inhibition of *E. coli*, with percentages of 40.17 ± 2.52% at the concentration of 1000 µg/mL and 24.77 ± 5.83% at the concentration of 500 µg/mL. The S. *cearensis* plant showed an inhibition of 32.86 ± 5.16% at 1000 µg/mL and 15.30 ± 2.78% at 500 µg/mL. In turn, *A. aculeata* showed an inhibition of 29.84 ± 0.88% at a concentration of 1000 µg/mL, with undetected activity (ND) at 500 µg/mL Figure 1. In relation to *S. aureus* to *A. speciosa* also showed good antibacterial activity, with 40.77 ± 2.15% inhibition at a concentration of 1000 µg/mL and 31.96 ± 4.60% at 500 µg/mL. For *S. cearensis*, inhibition was greatly reduced, reaching only 5.95 ± 1.14% per 1000 µg/mL, with undetected activity (ND) at 500 µg/mL. On the other hand, *A. aculeata* stood out with an inhibition of 44.76 ± 1.50% at 1000 µg/mL and 33.59 ± 0.81% at 500 µg/mL Figure 2.

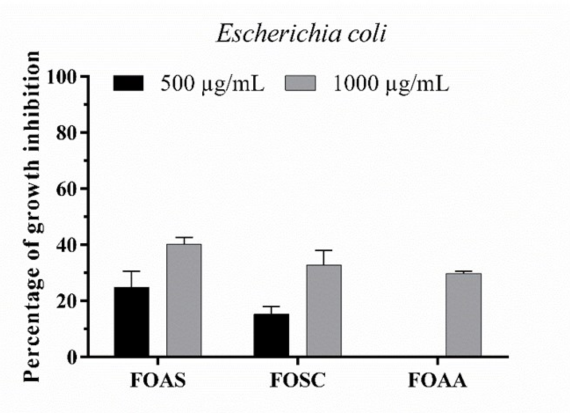

***Figure 1.** Antibacterial activity of fixed oils A, speciosa (FOAS), S. cearensis (FOSC) and A. aculeata (FOAA) against Escherichia coli, expressed as percentage of inhibition of bacterial growth.*

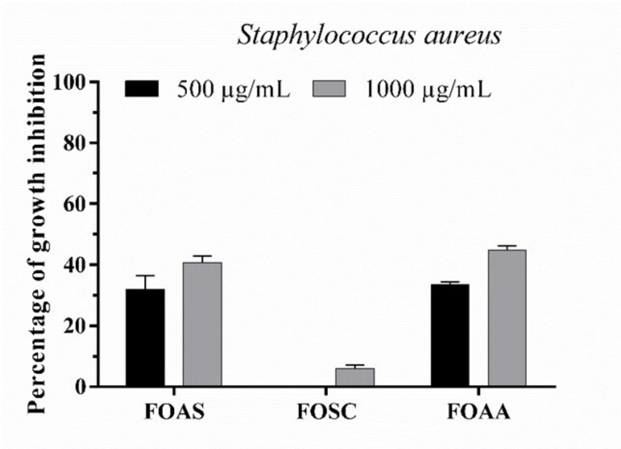

Figure 2. *Antibacterial activity of fixed oils A, speciosa (FOAS), S. cearensis (FOSC) and A. aculeata (FOAA) against Staphylococcus aureus, expressed as percentage of inhibition of bacterial growth.*

4. Discussion

According to the findings of this study, they identified high concentrations of lauric acid in the phytochemical composition of *A. aculeata*, *S. cearensis* and *A. speciosa*. Lauric acid, one of the main compounds in the Arecaceae family, is recognized for its biological properties, including antioxidant and antimicrobial action, positioning it as a promising therapeutic compound [26]. *A. aculeata* fixed oil is a rich source of oleic acid, which contributes significantly to its beneficial properties [25]. On the other hand, the fixed oil of *S. cearensis* stands out for its high content of myristic acid, which is relevant for its nutritional and functional properties [27].

In addition, the chemical composition of fixed oils extracted from *A. aculeata* fruit can vary considerably depending on factors such as geographic origin and agricultural management practices. The analyses carried out indicated that the lipid fraction of "macaúba" oil has a fatty acid profile susceptible to environmental influences, resulting in variations in its chemical composition [28]. These variations can impact both the chemical composition and nutritional properties of the oil, affecting its potential for biotechnological applications [29].

Lauric acid has antimicrobial action against *S. aureus*, interfering in the biosynthesis of fatty acids, essential for the growth and survival of this bacterium. This interference can inhibit its development, making lauric acid a promising antimicrobial agent [30]. In addition, arginate laurate, derived from lauric acid, is effective against *Escherichia coli*, acting by damaging the bacterial cell membrane. This action quickly inactivates cells, without destroying them, and its effectiveness is enhanced by gentle physical methods, which increase their performance [31].

The antibacterial action of lauric acid and its derivatives occurs through three main mechanisms: first, it destroys the cell membranes of gram-positive bacteria and enveloped viruses; second, it interferes with cellular processes such as signal transduction and transcription; and, finally, it stabilizes human cell membranes, with positive implications for health [32]. In addition, lauric acid inhibits the growth of pathogenic microorganisms and modulates immune responses, contributing to homeostasis and protection against infections [30]. In this way, it is not only an antibacterial agent, but also a health promoter.

Oleic acid, present in *A. aculeata*, specifically in mesocarp and almond oils, is found in concentrations higher than those of commercial lauric oils, such as coconut, palm kernel and babassu oils, evidencing the potential of *A. aculeata* oil for therapeutic applications [33]. Oleic acid has a significant antibacterial potential, interacting with the cell wall of bacteria, increasing its permeability and causing the release of intracellular content, which results in cell death. In addition, it inhibits essential metabolic processes in bacteria, reinforcing their antibacterial action [34].

S. cearensis stands out for its high content of myristic acid. This saturated fatty acid is valued for its properties in various industrial applications, including the production of cosmetics and personal care products [35]. Myristic acid (C14) has antibacterial properties, with a minimum inhibitory concentration (MIC) of 1600 µg/mL against MSSA (methicillin-sensitive *Staphylococcus aureus*). However, its antibacterial efficacy may be lower than that of other medium -chain fatty acids, such as lauric acid (C12), which has shown better results in some studies [36].

This comparison underscores the importance of considering the selection of compounds based on antimicrobial efficacy and their applications in different contexts.

Such property may be a reflection of the composition of the oils extracted from these palm trees, containing a high concentration of fatty acids, which can be used to fight pathogens. These components have antibacterial properties that can be exploited in medical treatments [16, 17]. In addition, the compound acids of interest are analysed in the pulps and almonds of different species, highlighting both their pharmacological potential [18].

The evaluation of the acute and subacute toxicity of the oil extracted from the pulp of *Acrocomia aculeata* in rats did not reveal significant toxicity in in vitro and in vivo tests, such as the Trypan Blue exclusion test and the *Galleria mellonella* model, suggesting safety for consumption. The oil did not affect blood clotting times, indicating a potential beneficial effect on decreasing platelet aggregation [37]. In contrast, *Syagrus cearensis* showed moderate toxicity in analyses with *Artemia salina*, with mortality at high concentrations [14]. *Attalea speciosa* has toxic potential in some parts, especially in the shell, while its almonds are generally safe, but excessive consumption can cause digestive discomfort [38].

5. Conclusion

The fixed oils of the fruits of *Acrocomia aculeata*, *Attalea speciosa* and *Syagrus cearensis* showed rich compositions, with saturated fatty acids corresponding to 69.29% in *A. aculeata*, 80.59% in *A. speciosa* and 82.10% in *S. cearensis*. In these same species, respectively, lauric acid stands out, present in the highest concentrations. Regarding antibacterial activity, *Attalea speciosa* showed the most significant inhibition against *Escherichia coli* (40.17% at 1000 μg/mL) and *Staphylococcus aureus* (40.77% at 1000 μg/mL). *Acrocomia aculeata* also stood out, with an inhibition of 44.76% at 1000 μg/mL against *S. aureus*. In contrast, *Syagrus cearensis* had less pronounced antibacterial activity. These results indicate the antimicrobial potential of the plants studied, suggesting that their fixed oils could be explored as therapeutic alternatives to fight bacterial infections. The available resources are limited, which prevents wider exploration. However, the materials demonstrate strong potential to facilitate the creation of cost-effective treatments against these bacteria by increasing their accessibility.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors thank Universidade Regional do Cariri (URCA, Brazil).

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