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Original Article

# Fatty Acid Profile and Antimicrobial Activity of Oils Extracted from Seeds of Oleaginous Plant Species

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**ABSTRACT**

**Background:** The *in vitro* antimicrobial effect of lipids has been studied and antimicrobial action of these lipids has been observed in various microbial groups. **Objectives:** The research studied the fatty acid profile and antimicrobial action of seed oil *Acrocomia emensis*, *Attalea burretiana* and *Carpotroche brasiliensis* against strains of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. **Materials and Methods:** The analyzes were extracted and then hydrolysis and methylation were performed for later by Gas Chromatography coupled to a Mass Spectrometer. The determination of the antimicrobial action was determined by the agar diffusion method. **Results:** In species *Acrocomia emensis* and *Attalea burretiana* lauric acid was the most abundant fatty followed by myristic acid. In the oil of *Carpotroche brasiliensis* the hidnocarpic, chaumoogric and gorlic acids were in a greater concentration. The antimicrobial activity was observed in inhibition zones between 8-14 mm, so these oils can be made possible used as pharmaceutical adjuvants in antibiotic preparations.

**Keywords:** Oil plants, Antimicrobial activity, Fatty acids.

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## INTRODUCTION

The *in vitro* antimicrobial effect of lipids has been studied for over a century[1] and its ability to destabilize living cell membranes causing an imbalance of their selective permeability properties and resulting in dissipation of solute gradients. Another explanation is that lipids interact with sites within the microorganism cell, influencing biochemical functions and its viability loss.[2]

Some studies have evaluated antimicrobial lipid activity in various microbic groups. In the group of Gram-positive bacteria Bergsson *et al.* (2001) observed antimicrobial action of lauric, palmtoleic and capric acids against *S. aureus* and group streptococci A and B.[3]

In the group of gram-negative bacteria, Bergsson, Steingrimsson and Thermar (1999) identified that monocaprine eliminated all the strains of *Neisseria gonorrheae* tested, and that lauric acid and palmitoleic acid had action against *Helicobacter pylori* after ten minutes of incubation.[4]

Excessive use of synthetic drugs culminated in the growing problem of bacterial resistance, but these drugs did not follow the growth of this resistance, with regard to the improvement in their action against them, which led researchers and pharmaceutical industries to develop medicines having as a base product that contain simple natural compounds.[2-5]

Health researchers work with the evidence of simple and less specific antimicrobial compounds, acting in conjunction with more specific synthetic antibiotics, to launch a two-front attack on microorganism and ensure a more promising effect.[5-6]

This research aimed to investigate the profile of fatty acids and the antimicrobial action of the fixed oils of *Carpotroche brasiliensis*, *Attalea burretiana* and *Acrocomia emensis*, which are oilseed species. The advantage of studying antimicrobial actions in these oils lies in the fact that they are abundant natural compounds in nature and a renewable source of raw material to be explored. It is noteworthy that this exploitation must be done sustainably and, in a way, and rhythm that do not, in the long run, to reduce biological diversity, thus maintaining its potential to meet the needs of present and future generations.

## MATERIALS AND METHODS

### Plant Material

The fruits of *Acrocomia emensis* were collected in Bonito de Minas, MG, Brazil in June 2013. The fruits of *Attalea burretiana*, known as Coco Palmeirinha, and *Carpotroche brasiliensis*, popularly called Sapucainha, were collected in Joaíma, MG, Brazil in February and July 2013. The fruits of *Acrocomia emensis* and *Carpotroche brasiliensis* were collected

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directly from the plant, while the fruits of *Attalea burretiana* were collected in the soil. The identification of species was made by description of Lorenzi (1992; 2010).[7-8] An exsicata of each species was deposited in the Herbarium of the State University of Montes Claros under numbers 4,362 (*A. emensis*), 4,361 (*A. burretiana*) and 4,360 (*C. brasiliensis*).

### Fruit Processing

The fruits of the three species were washed with water and neutral detergent, were drying in the sun and the separation of the shell, pulp and seeds was done manually. The seeds were used for oil extraction, but before they were drying at 40°C.

### Oil Extraction

*A. emensis*, A. *burretiana* and *C. brasiliensis* fruits oil extraction was made by pressing in the brand type *Expeller* "Oekotec" model CA59G of laboratory scale. Subsequent to extraction, gross oils were subjected to decantation for 24 hr and the supernatant was used in the analysis.

### Fatty Acid Profile

### Sample preparation: Hydrolysis and Oil Methylation

In a 2 mL cryogenic tube, 10 mg of the oil was dissolved in 100 mL of a solution of ethanol (95%)/potassium hydroxide 1 mol/L (5%). After vortexing for ten seconds, the oil was hydrolyzed in a domestic microwave oven (Panasonic Piccolo), at a power of 80 W (Power 2), for five minutes. After cooling, 400 µL of 20% hydrochloric acid, 20 mg of sodium chloride - NaCl and 600 µL of ethyl acetate P.A. were added. After vortexing for ten seconds and resting for five minutes, an aliquot of 300 µL of the organic layer was removed, placed in microcentrifuge tubes and dried by evaporation, thus obtaining the free fatty acids.[9]

Free fatty acids were metilated with 100 mL of boro trifluoride ($BF_3$) in 14% methanol and heated for ten minutes in water bath at 60°C. After dilution with 400 mL of methanol, they were analyzed by Gas Chromatography (GC).

### Analysis Method: Gas Chromatography coupled to Mass Spectrometer

The analyzes were performed on Gas Chromatographer HP7820A (Agilent) equipped with flame ionization detector. The column used was the Innowax (HP) 15m x 0.25mm x 0.20µ with temperature gradient was used: 70°C (0 min.), 7°C/min. up to 240°C; Injector (Split of 1/30) at 250°C and detector at 260°C. Hydrogen was used as drag gas (3 mL/min.). The sample injection volume was 1 mL. The data acquisition program applied in the analysis was EZChrom Elite Compact (Agilent). The quantitative analysis was done by standardization of area by GC-DIC. The identification of peaks was made by comparing retention times with pure patterns of meatilated fatty acids SUPELCO37 and by Gas Chromatography coupled to Mass Spectrometer (GC-MS).

The chromatographer used was GCMS-QP 2010 Ultra (Shimadzu). The column used was the RXI-1MS 30 m x 0.25 mm x 0.25 µm (Restek) with a temperature of 70°C (2min), 5°C/min, up to 250°C. The injector temperature was 250°C, split (1:20), the GC-MS interface temperature and the MS detector (with 70 eV electronic impact) were 250°C. The drag gas was the helium at 1.5 mL/min. The sample injection volume was 1 µL. The data acquisition software used was the GC-MS Solution (Shimadzu). The mass spectra generated were compared to those of the spectral library: NIST11 - *National Institute of Standards and Technology - Mass Spectral Library*.

### Biological Activity

To determine the antibacterial activity the seeds of *A. emensis*, *A. burretiana* and *C. brasiliensis*, the agar diffusion method was used, described by Clinical and Laboratory Standards Institute (CLSI) and adapted, seeking to find some Inhibitory activity of the development of *Pseudomonas aeruginosa* (ATCC 25619), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538). ATCC strains were provided by the Oswaldo Cruz Foundation's reference microorganisms laboratory. The medium of culture used was Mueller Hinton Agar (MHA). The inoculation of the plates was performed following the standardized rules by CLSI (2012).[10]

Sterile discs of 6 mm in diameter, were previously impregnated with different concentrations (100%; 50%; 25%; 12.5%; 6.25%; 3.12%; 1.56%; 0.78%) of seed oil of the *A. emensis*, A. *burretiana* and *C. brasiliensis* diluted in hexane P.A., as oil did not dilute in tween 80 and dimethylsulfoxide (DMSO).

The impregnated discs were placed in a greenhouse (at 35°C for five minutes for evaporation of the hexane used in the dilution of the sample) and later each disc was softly pressed against the previously inoculated Petri plate.

As a negative control, a disk impregnated with P.A. was used, the disk was dried in an oven at 35°C for five minutes before being fixed on the plate. As positive controls, different LB Laboclin® antibiotic discs were used, commonly used in antimicrobial sensitivity tests, as recommended by CLSI (2012). Then the plates were placed inverted in a bacteriological greenhouse at 35°C for 24 hr.[10]

After the incubation period, the inhibition halos diameters were measured, including the disc diameter, as guided by CLSI (2012). All tests were done in triplicate, and the value of halo was determined by the average measurements in millimeters obtained.[10]

## RESULTS AND DISCUSSION

### Identification and Quantification of Fatty Acids

The chromatographic profile of the *Acrocomia emensis* seed oil (Tucum coconut) was very similar to the profile of *Attalea burretiana* (Palmeirinha coconut). In both eleven different fatty acids were identified, represented by the peaks in the chromatogram, of which four were unsaturated and the rest had saturated chain.

There was a variation in fatty acid concentrations between the two species (Table 1). Noteworthy for caprylic acids (C8: 0), capric (C10: 0) and palmitoleic (C16: 1) in which double concentrations were observed in the oil of Palmeirinha coconut. Miristical acid (C14: 0) and alpha linolenic (C18: 3) were the only fatty acids of the Tucum coconut that surpassed, in concentration, those of Palmeirinha coconut with content, on average, 27% and 33% higher. Palmeirinha coconut oil, as well as Tucum coconut oil, were rich in lauric acid, with a concentration of 55.5% and 44.6%, respectively (Table 1).

Fatty acid acid profiles of the seed oil, the two coconut species studied, are expected for coconut oils (Table 2).

Similar profiles were observed by Kumar (2011), Faria *et al.* (2008), Fortes and Baugh (1999) and Ferreira, Faza and Hiarryc (2012) varying only the concentration of fatty acids.[11-14]

The study by Kumar (2011) evaluated the variation between the fatty acid profile of *Cocos nucifera* from 60 tall, 14 dwarf and 34 hybrid palms.[11] Faria *et al.* (2008) performed the chemical characterization of sour coconut almonds.[12] Fortes and Baugh (1999) investigated the fatty acid profile of *Acrocomia sclerocarpa* (coconut Macaúba)[13] and Ferreira, Faza and Hiaryc (2012) by *Attalea dubia* (Indaiá) and *Orbignya phalerata* (Babassu).[14]

In all coconuts, high levels of lauric acid were observed, as found in the Tucum and Palmeirinha coconuts. Kumar (2011) presented concentrations of this acid between 42 - 52% in coconuts of Bahia, Faria *et al.* (2008) 42.1% in Sour coconut, Fortes and Baugh (1999) 38.89% in

**Table 1: Identification and quantification of *A. emensis* and *A. burretiana* seed oil fatty acids analyzed by GC-DIC and confirmed by GC-MS.**

| Peak | Fatty acids | Nomenclature IUPAC | Conventional Nomenclature | RT | *A.emensis* % | *A.burretiana* % |
|---|---|---|---|---|---|---|
| 1 | C8:0 | octanoic acid | caprylic acid | 1.74 | 4.17 | 9.72 |
| 2 | C10:0 | decanoic acid | capric acid | 3.71 | 4.03 | 8.82 |
| 3 | C12:0 | dodecanoic acid | lauric acid | 6.42 | 55,50 | 44.61 |
| 4 | C14:0 | tetradecanoic acid | myristic acid | 9.06 | 15.80 | 11.68 |
| 5 | C16:0 | hexadecanoic acid | palmitic acid | 11.62 | 5.55 | 7.05 |
| 6 | C16:1 | hexadecenoic acid | palmitoleic acid | 11.88 | 0.13 | 0.26 |
| 7 | C18:0 | octadecanoic acid | stearic acid | 14.04 | 2.25 | 3.08 |
| 8 | C18:1 | octadecenoic acid | oleic acid | 14.24 | 8.62 | 8.94 |
| 9 | C18:2 | octadecadienoic acid | linoleic acid | 14.75 | 2.86 | 3.40 |
| 10 | C18:3 | 9,12,15-octadecatrienoic acid | α-linolenic acid | 16.28 | 0.12 | 0.08 |
| Others | | | | | 0,78 | 2,13 |
| % total | | | | | 100 | 100 |

IUPAC: International Union of Pure and Applied Chemistry. RT: Retention time

**Table 2: Fatty acid composition (%) in coconuts almonds fats Indaiá (*Attalea dubia*), Babassu (*Orbignya phalerata*), Macaúba (*Acrocomia sclerocarpa*), Sour coconut (*Butia capitata*), Coconut of Bahia (*Coconut nucifera*),[11-14] Tucum (*Acrocomia emensis*) and Palmeirinha (*Attalea burretiana*).**

| Fatty acids | Indaiá[14] | Babaçu[14] | Macaúba[13] | Coquinho azedo[12] | Coco da Bahia[11] | Tucum | Palmeirinha |
|---|---|---|---|---|---|---|---|
| C8:0 | 10.9 | 9.2 | 2.10 | 7.8 | 2.77-7.21 | 4.17 | 9.72 |
| C10:0 | 8.3 | 9.6 | 3.72 | 8.0 | 3.46-5.94 | 4.03 | 8.82 |
| C12:0 | 55.8 | 54.7 | 38.89 | 42.1 | 42.42-52.52 | 55.50 | 44.61 |
| C14:0 | 11.6 | 11.8 | 11.00 | 10.5 | 18.12-23.05 | 15.80 | 11.68 |
| C16:0 | 3.8 | 4.8 | 17.35 | 6.0 | 7.59-12.99 | 5.55 | 7.05 |
| C16:1 | - | - | - | - | - | 0.13 | 0.26 |
| C18:0 | - | 2.05 | 4.34 | 4.0 | 2.45-4.07 | 2.25 | 3.08 |
| C18:1 | 5.8 | 6.5 | 22.40 | 16.9 | - | 8.62 | 8.94 |
| C18:2 | - | 0.9 | - | 4.2 | - | 2.86 | 3.40 |
| C18:3 | - | - | - | - | - | 0.12 | 0.08 |
| C20:0 | - | - | - | 0.1 | 0.02-0.31 | 0.20 | 0.25 |

(-) There was no presence

Macaúba and Ferreira, Faza and Haryc (2012) 55.8% in Indaiá and 54.75% in Babaçu. Miristical acid (C14:0) was the second largest concentration for Indaiá, Babaçu, coconuts of Bahia, Tucum and Palmeirinha and oleic acid (C18:1) for Macaúba and Sour coconut.[11-14]

Nine different fatty acids were identified, of which six were unsaturated and the rest showed saturated chain. In Sapucainha, as well as in the coconuts of Tucum and Palmeirinha, the lauric acids (C12:0), palmitic (C16:0), palmitoleic (C16:1), steric (C18:0), oleic (C18:1) and linoleic (C18:2). Among these, palmitic acid stands out for displaying higher concentration (7%) (Table 3).

The presence of chaulmoogric cyclpentine acids (C18:1), hydnocarp (C16:1) and gorlic (C18:2) was identified, according to the chemical composition already described for brazilian chaulmoogra oil.[15-16]

The concentration of chaulmoogric acids (C18:1) and gorlic (C18:2) was 20.8 and 12.4%, respectively. Hydnocarpic acid content (C16:1) stands out, since its concentration is 47% of total fatty acids identified in Sapucaininha oil.

Compatible profile was found by Lima *et al*. (2005) when they researched the fatty acids of *C. brasiliensis* and obtained hydnocarpic acids (40.5%), chaulmoogric (14.0%) and gorlic (16.1%) of seeds. The author also identified palmitic acid (C16:0) and estearic acid (C18:0), but only isolated and emphasized the cyclopentines, as the intention was to test the anti-inflammatory and analgesic activity of these acids in rats. Acids had oral anti-inflammatory effect and showed peripheral analgesic effect.[17]

In the study with the same species Oliveira *et al*. (2009) found the same profile as majority acids, adding only myristic and oleic acid, which were not identified by Lima *et al*. (2005) but are in accordance with the profile found in this research.[16-17]

The *Hidnocarpus wightiana* had a fatty acid profile similar to that of sapucainha. palmitic acid (C16:0), estearic (C18:0), oleic (18:1), hydnocarpic (16:1), chalmoogric (C18:1) and gorlic (18:2) were found in the respective concentrations of 7.4%, 1.6%, 5.2%, 36%, 31% and 18%.[18]

Cyclpentic acids are typical of the old Flacourtiaceae family. The genres of this family known as chaulmoogras are: *Hydnocarpus*, *Carpotroche*, *Caloncoba*, *Oncoba*, *Lindackeria* e *Mayna*.[19]

The species *C. brasiliensis* belongs to the genus *Carpotroche* that belonged to the Flacourtiaceae family. However, phylogenies taken from data from deoxiribonucéic acid sequences (DNA) isolated from chloroplasts

**Table 3: Identification and quantification of fatty acids in the seed oil of *Carpotroche brasiliensis* by GC-DIC and confirmed by GC-MS.**

| Peak | Fatty acids | Nomenclatura IUPAC | Nomenclatura Convencional | RT | *C.braziliensis* (%) |
|---|---|---|---|---|---|
| 1 | C12:0 | Ácido dodecanóico | Ácido laurico | 6.29 | 0.2 |
| 2 | C16:0 | Ácido hexadecanóico | Àcido palmitico | 11.62 | 7.0 |
| 3 | C16:1 | Ácido hexadecenóico | Ácido palmitoleico | 11.88 | 1.8 |
| 4 | C16:1 | Ácido 11(2-ciclopentenil) undecanóico | Ácido hidnocarpico | 13.68 | 47 |
| 5 | C18:0 | Ácido octadecanóico | Ácido esteárico | 14.03 | 1.1 |
| 6 | C18:1 | Ácido octadecenóico | Ácido oleico | 14.22 | 3.7 |
| 7 | C18:2 | Ácido octadecadienóico | Ácido linoleico | 14.74 | 1.1 |
| 8 | C18:1 | Ácido 13(2-ciclopentenil tridecanóico | Ácido chaulmoogrico | 16.02 | 20.8 |
| 9 | C18:2 | Ácido 13(2-ciclopentenil tridecenóico | Ácido górlico | 16.22 | 12.4 |
| | Others | | | | 4.9 |
| | % total | | | | 100 |

IUPAC: International Union of Pure and Applied Chemistry. RT: Retention time

**Table 4: Reference values of antibiotic discs used as positive controls in sensitivity tests.**

| | | Inhibition halo measurements (mm) Reference values[10] | |
|---|---|---|---|
| Antibiotics | Micro-organism | Resistant | Sensitive |
| Tetracycline 30 µg | *E. coli* | ≤ 11 | ≥ 15 |
| | *S. aureus* | ≤ 14 | ≥ 19 |
| Chloramphenicol 30 µg | *E. coli* | ≤ 12 | ≥ 18 |
| | *S. aureus* | | |
| Imipenem 10 µg | *E. coli* | ≥19 | ≥ 23 |
| | *S. aureus* | ≥13 | ≥ 16 |
| | *P. aeruginosa* | ≥ 15 | ≥ 19 |

**Table 5: Medium values of the inhibition halls of gross oils *Acrocomia emensis*, *Attalea burretiana* e *Carpotroche brasiliensis* in different concentrations (%).**

| | | Measurements (mm) of inhibition halos in concentrations (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Òil Plant species | Microorganism | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 |
| | *S. aureus* | 12 | 10 | 9 | 8 | 8 | 8 | 8 | 8 |
| *A. emensis* | *E. coli* | 11 | 11 | 11 | 11 | 9 | 8 | - | - |
| | *P. aeruginosa* | 12 | 12 | 10 | + | 9 | 8 | 8 | - |
| | *S. aureus* | 10 | 10 | 8 | 8 | 8 | - | - | - |
| *A.burretiana* | *E. coli* | 13 | 13 | 11 | 11 | 11 | 10 | 10 | 9 |
| | *P. aeruginosa* | 11 | 10 | 10 | 10 | 10 | 8 | 8 | 8 |
| | *S. aureus* | - | - | - | - | - | - | - | - |
| *C. brasiliensis* | *E. coli* | - | - | - | - | - | - | - | - |
| | *P. aeruginosa* | 14 | 11 | 10 | 9 | 8 | - | - | - |

(-) There was no presence

proposed a taxonomic rearrangement and divided the Flacourtiaceae family into two main ones, Achariaceae and Salicaceae. *Carpotroche* became a genre of the Achariaceae family.[20]

## Antimicrobial Analysis

The criteria for determination and interpretation of halos measures were established according to CLSI (2012). The selection of tetracycline (30 µg), chloramphenicol (30 µg) and imipenem (10 µg) antibiotics used as positive control (Table 4) was made by inhibiting the growth of gram-positive and gram-negative bacteria. The measurement of the inhibition halos, found for all antibiotic discs, were within the standards established by CLSI (2012).[10]

For natural products, there is no consensus on the acceptable level of inhibition. Some authors consider only similar results to those of antibiotics, while others consider good potential even those with higher or lower inhibition levels.[21] Thus, the results found were interpreted according to CLSI standards, 2012 and all inhibition halos values were considered important.[10-21]

In the gross oil of the seeds of *A. emensis* and *A. burretiana* was observed antibacterial action against *Staphylococcus aureus* (Table 5). In species *A. emensis*, activity was found in all tested concentrations, while *A. burretiana* was observed minimum inhibitory concentration (MIC) of 6.25%. In both species, it was noted that the size of the halos was proportional to increased concentration. However, when they were compared to the halos standard of positive controls, which were in accordance with the halo standards adopted by CLSI (2012),[10] it was noted that the gram-positive bacterium was resistant to the antibacterial action of Tucum coconut oil and Palmeirinha coconut oil. No antimicrobial activity of *C. brasilensis* oil against *S. aureus* was not found in any of the concentrations tested.

Similar result was found by Kabara *et al.* (2011). The authors tested 30 fatty acids, their derivatives and their bactericidal properties compared to twelve positive microorganisms. Among the tested microorganisms, it was observed that *S. aureus* was resistant in all tests. However, the authors interpreted that *Pneumococci* and group A *Streptococci* were sensitive, especially to lauric acid, which is the acid observed in greater concentration in *A. emensis* and *A. burretiana*.[22]

The result of Heczko *et al.* (2011) diverges from Kabara *et al.* (2011) because it found that lauric acid (C12:0) was the most active, among 4 different fatty acids tested, eliminating 95% of 242 *S. aureus* strains in a low concentration (0.39 mol. $L^{-1}$).[22-23]

Despite displaying conflicting results regarding *S. aureus*, studies highlighted lauric acid as a good antimicrobial agent against gram-positive strains. It was observed that this acid predominates in species *A. emensis* (55.50%) and *A. burretiana* (44.61%), so it is interesting that these oils are tested in other gram-positive strains such as *Pneumococci* and group A *Streptococci*. Regarding *S. aureus*, it is believed that if fatty acids and/or their monoglycerides were separated and isolated from *A. emensis* and *A. burretiana* oil and applied directly to the microorganism, antimicrobial activity would be more effective.

In fact, Conley and Kabara (2011) tested the antimicrobial activities of mono and fatty acids against a variety of bacteria that included *S. aureus*. The spectrum of monoglyceride activity was narrower compared to that of fatty acids. However, monoglycerides were more active in MICs against *S. aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. Monolaurine (lauric acid glyceride) has been more active than other glycerides and more active than free fatty acids against most bacteria. Monolaurin and monocaprine (glyceride capric acid) were the only ones where activity was observed against *S. aureus* and were more active than their respective fatty acids.[24]

Another study showing greater effectiveness in monoglycerides was Lee, Kim and Shin (2002) who tested the activity of linolenic acid (C18:3), with and without monoglycerides, against *S. aureus* and allowed monolaurin and monomyristin (glyceride myristic acid) increase the antimicrobial activity of this acid.[25] Bergsson *et al*. (2001) found great results when they tested monocaprine against *S. aureus*, with 99% elimination in a 1.56 mol/L concentration.[3]

In the oils of *A. emensis* e *A. burretiana* was found antimicrobial activity against *Escherichia coli*. The MIC of *A. emensis* for this microorganism was 3.12%. *C. brasiliensis* did not show halo of inhibition against *E. coli.*

There are different studies on the action of lipids against *E. coli.*, Marounek *et al*. (2003) determined the sensitivity of two *E. coli* strains against fatty acids. This study found that caprylic and capric acid has antimicrobial activity against *E. coli*.[26] *A. emensis* and *A. burretiana* have these two acids in their profile and it is possible to observe, on average, twice the concentration of these two acids in *A. burretiana*. This can justify your best activity in the disk diffusion test against *E. coli*.

By interpreting MIC, Skrivanova *et al*. (2006) observed inhibitory effect of caprylic and capric acid, in their respective concentrations of 21 mM and 29 mM, against *E. coli* and *Salmonella sp*.[27] Nair (2005) contained that monocaprine, glyceride of capric acid, effectively eliminated *E. coli* when added at high concentrations (50 mM) in milk and apple juices.[28-29] Unlike these studies, Preuss *et al*. (2005) in a study on the bactericidal effect of monolaurine in bacteria, it did not find antimicrobial activity of this glyceride in *E. coli* and *Klebsiella pneumoniae*, while *Helicobacter pylori* was sensitive.[30]

Studies allow us to find that pH can influence antimicrobial activity of lipids. Marounek *et al*. (2003) evaluated the effect of pH on *E. coli* growth and noted that the fall of it positively influences the effect of caprylic and capric acids against this microorganism. Bergsson, Steingrimsson and Thorm (2002) share this theory because they observed resistance from *E. coli* and *Salmonella sp*. to a diversity of monoglycerides tested at neutral pH.[26,31]

The decrease of pH (<5) in the medium treated with lipids is highly effective in the elimination of *Samonella sp*. and *E.coli* with a reduction in colony formative units by over one million times in ten minutes.[32] In this regard, *A. emensis*, *A. burretiana* and *C. brasiliensis* can be tested in order to evaluate this procedure as a power of the antimicrobial activity of these oils.

*A. emensis* oil, *A. burretiana* and *C. brasiliensis* inhibited the growth of *P. aeruginosa*. The largest sizes of halos found in Tucum (12 mm) and Palmeirinha coconut (11 mm), both in the 100% concentration, allow you to infer that *P. aeruginosa* is resistant to the antimicrobial action of these oils, according to halo patterns of inhibition established by CLSI (2012).

The MIC of oil against *P. aeruginosa* was 6.25%. In the 100% concentration, the largest inhibition halo of all oils tested (14 mm) was observed in this oil. The pattern of this halo compared to the Imipenem antibiotic allowed *P. aeruginosa* exhibited intermediate growth in the antimicrobial action of Sapucaininha oil. This indicates that this microorganism can only be inhibited if the large amounts of this raw material is administered.

The study by Agoramoorthy *et al*. (2007) expressed a similar result when testing the antimicrobial activity of fatty acids from *Excoecaria agallocha* in *P. aeruginosa*. The authors tested an extract of this plant with a mixture of all the fatty acids identified in it and obtained inhibition halos between 11-7.6 mm for *P. aeruginosa*. The halo decreased in the same proportion of the extract concentration.[33] The same pattern was found for *A. emensis*, *A. burretiana* and *C. brasiliensis*, whose inhibition halos were between 14-8 mm between the three species.

Equivalent result was found by Suliman (2013) that investigated the profile of fatty acids of *Swietenia macrophyllaking* and analyzed the antimicrobial activity of the oil from its seed against *S. aureus*, *P. aeruginosa* and *E. coli*. Linoleic acids (39.21%), oleic (18.82%) are found (17.65%) palmtoleic (0.59%) palmitic (15.47%) in oil composition. The largest inhibition halo for *S. aureus* and *P. aeruginosa* was 11 mm (1000 µg/mL). Although they inhibited growth, according to positive control, these microorganisms were resistant to oil activity. In tests with *E. coli* was not observed inhibition halo.[34]

The study of Ashala *et al*. (2010) tested, in disk essay, the *Balanites aegyptiaca* gross oil against the same microorganisms tested by Suliman (2013), but observed better activity against *S. aureus* (20 mm) and *E. coli* (17 mm) in concentration 10 µL/disc.[34-35]

In tests with *A. emensis*, *A. burretiana* and *C. brasiliensis*, the halo that characterizes antimicrobial activity was not observed in the disc containing only negative control, i.e the hexan solvent P.A. The absence of halo shows that a choice of the same, for the dilution of the oil of the three species, was adequate, as there was no interference in the results of the tests.

In the present work it was noted that species *A. emensis* e *A. burretiana* had a better activity than *C. brasiliensis*, in general, with regard to this group of bacteria. The antimicrobial activity of *E. coli* and *S. aureus* may be associated with caprylic acids (C8:0), capric (C10:0), myristic (C14:0), alpha-linolenic (C18:0) and araquidic (C20:0), as these were not identified in the profile of *C. brasiliensis*, whose oil did not present activity against these microorganisms.

Antimicrobial activity against *P. aeruginosa* may be linked to fatty acids that are common the three species. They are the lauric acids (C12:0), palmitic (C16:0), palmtoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2). Cyclpentic acids are described in the literature as widely used acids before the advent of sulfas, to combat *Mycobacterium leprae*.[16] These acids, represented by the hydnocarp (C16:1), chaulmoogric (C18:1) and gorlic (C18:2), are present only in *C. brasiliensis* and may also be associated with antimcrobial activity against *P. aeruginosa*, either isolatedly or synergically to the other compounds.

According to Desbois and Smith (2010), unsaturated fatty acids usually tend to be more active against gram-positive than gram-negative. When fatty acids have the same carbon chain length, there is a higher power of unsaturated microorganisms and the more double bonds the better the activity of fatty acid.[36] This theory is in accordance with the result obtained for *P. aeruginosa* with the oils of *A. emensis, A. burretiana* and

*C. brasiliensis*, but conflict with the result obtained for *S. aureus* and *E. coli*, where acids associated with their antimicrobial activity are mostly saturated.

Microorganisms when *in vitro* tested vary in their sensitivity to antimicrobial activity of lipids in general. The type of lipid that is most active also varies according to the type of microorganism it will fight. The structure of the lipid often seems to influence its activity, and insaturated fatty acids in general, and saturated fatty acid monoglycerides are considered generally more active. PH is another factor that affects inhibitory and microbicidal action of lipids. In general, acid pH enhances this action.[36]

Fatty and monoglyceride acids have stood out as the best lipid forms capable of eliminating pathogens known to infect humans and domestic animals.[37] Therefore, it is believed that in the oils of *A. emensis*, *A. burretiana* and *C. brasiliensi*, will be observed better activity with the isolation of its fatty acids and/or synthesis of their respective monoglycerides.

The antimicrobial activity demonstrated with *A. emensis*, *A burretiana* and *C. brasiliensis* shows that these raw materials can be exploited by interacting them with conventional antibiotics or other agents, aiming to identify synergistic combinations. The activity presented in this work can be explored to enable its applications as pharmaceutical adjuvants in antibiotic preparations.

The adjuvant is a substance added to the medicine to prevent changes, correct or improve the organoleptic, biopharmacotechnical and technological characteristics of the drug.[38] Thus, combinations could be produced from these oils to improve power and expand the spectrum of pharmaceutical products used for antimicrobial purposes. They can also be useful as a food additive, in the form of a natural preservative in industrial canned with low toxicity related to natural products.

## CONCLUSION

The fatty acid profile of the three species has been drawn and their respective fatty acids identified and quantified. In species *Acrocomia emensis* and *Attalea burretiana*, lauric acid was the most abundant fatty acid followed by the miristic. In the species *Carpotroche brasiliensis* the majority fatty acid was the hydnocarpic, followed by chaumoogric and gorlic. Antimicrobial activity was observed in the oils of the three species studied. The antimicrobial potential observed in the oils of *A. emensis* seeds, *A. burretiana* and *C. brasiliensis* can be explored to enable their applications as pharmaceutical adjuvants in antibiotic preparations and also natural preservatives for food.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ATCC:** American Type Culture Collection; **$BF_3$:** Boron trifluoride; **CLSI:** Clinical and Laboratory Standards Institute; **DMSO:** Dimethylsulfoxide; **DNA:** Deoxyribonucleic acid; **GC:** Gas Chromatography; **CG/DIC:** Gas Chromatograph Coupled to Flame Ionization; **GC-MS:** Gas Chromatography coupled to Mass Spectrometer; **GCMS-QP:** Gas Chromatography coupled to Mass Spectrometer - Quadrupole; **MHA:** Mueller Hinton Agar; **MIC:** Minimal Inhibitory Concentration; **MS:** Mass Spectrometer; **NaCl:** Sodium chloride; **NIST11:** National Institute of Standards and Technology - Mass Spectral Library; **P.A:** Pure for Analysis.

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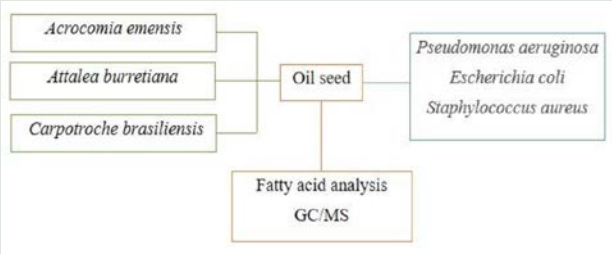
## GRAPHICAL ABSTRACT




## SUMMARY

- This research work evaluated the composition of fatty acids and the antimicrobial action of oils from seeds of oleaginous species.
- Based on this study, it can be determined that in the species *Acrocomia emensis* and *Attalea burretiana*, lauric acid was the most abundant fatty acid followed by myristic acid. In *Carpotroche brasiliensis* oil, hydnocarpic, chaumogric and goric acids were in higher concentration.
- Antimicrobial activity was observed in zones of inhibition between 8-14 mm.

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