

# Chemical Profile and Antimicrobial Activity of *Syagrus oleracea* (Mart.) Becc. Oils and Oils Extracted from *Speciomerus revoili* (Pic.) Larvae

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## ABSTRACT

**Background:** The antimicrobial effect of oils extracted from *Syagrus oleracea* (Mart.) to prove the action and obtain data for the future development of therapeutic products. **Objectives:** The present study was to determine the chemical profile and the antimicrobial potential of oils extracted from *Syagrus oleracea* (Mart.) Becc. almonds and *Speciomerus revoili* (Pic.) larvae to obtain scientific information to prove the action and obtain data for the future development of therapeutic products. **Materials and Methods:** The separation, identification and quantification of fatty acids in oils extracted from the larvae of *S. revoili* (Coleoptera, Chrysomelidae) and almonds of *S. oleracea* (Arecaceae) were performed by gas chromatography coupled with mass spectrometry. The determination of antibacterial activity was performed using microdilution in plates. **Results:** In the oils extracted from *S. revoili* and *S. oleracea*, saturated chain fatty acids, the presence of hexanoic acid (only in almonds), and caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and those of unsaturated chain, oleic acid (omega-9) and linoleic acid (omega-6). *S. revoili* oil showed satisfactory results against *Escherichia coli* and *Staphylococcus aureus*.

**Keywords:** *Syagrus oleracea*, *Speciomerus revoili*, Fatty acids, Antimicrobial activity.

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## INTRODUCTION

*Syagrus oleracea* Mart. Becc., has traditionally been used in the form of root extracts for back pain and flower syrups for bronchitis.<sup>[1]</sup> In the pulp of the fruits are present phenolic compounds that have antioxidant and antimicrobial activities and in the oils of the almonds, saturated and monounsaturated fatty acids.<sup>[2,3]</sup>

Members of the Bruchida family and mainly attack almonds from plants of the Arecaceae family, such as the species *Pachymerus nucelorum* and *Speciomerus revoili*.<sup>[4]</sup> In Brazil, traditional communities use *Pachymerus nucleorum* larva oil, popularly known as coró, to treat cracks in the heel, swelling, wounds,

seborrheic dermatitis, inflammation, thrombosis, newborn's navel and earache.<sup>[5]</sup> Thus, the objective of the work was to determine the chemical profile and the antimicrobial potential of oils extracted from almonds of *S. oleracea* and larvae of *S. revoili*.

## MATERIALS AND METHODS

### Plant material

Fruits of *S. oleracea*, were collected in the city of Josenópolis-MG, Brazil (Location 1: W -16 32' 27.40561" S -42 32' 05.28360"; Location 2: W -16 32' 27.47761" S -42 32' 05.51760"; Location 3: W -16 32' 28.05000" S -42 32' 05.70479"), in the months of February and August 2018.

The plant was identified as *Syagrus oleracea* (Mart.) Becc. and the excicata deposited in the herbarium of the State University of Montes Claros voucher 504. The larvae were identified at the Coleoptera Systematics and Biotechnology Laboratory at the Federal University of Paraná and identified under n° 99, as of the species *Speciomerus revoili*.



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## Fruit Processing

The fruits were washed, dried for 24 hr, separated from the almond shells. The obtained larvae were washed with distilled water and stored (-20°C) for later identification and analysis.

## Extraction of oil from fruits and larvae

The almonds of *S. oleracea* from locations 1 (92.03g), 2 (50.66g) and 3 (56.83g) were crushed, added hexane (P.A.) (3 x 300mL). Larvae of *S. revoili* from localities 1 (11.70g), 2 (1.91g) and 3 (4.62g), were macerated with hexane (P.A.) (3 x 110mL). The materials were vacuum filtered, the filtrates were collected and the solvents evaporated (25 to 36.8°C). Extraction was carried out for each location.

## Fatty acid profile

### Sample preparation: hydrolysis and oil methylation

The analyzes of the three extractions and the hydrolysis and methylation of the oils were analyzed followed by the method described Sande *et al.*, 2018.<sup>[6]</sup>

## 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 0.20 $\mu$  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 (3mL/min.). The sample injection volume was 1L. The data acquisition program applied in the analysis was EZChrom Elite Compact (Agilent). The quantitative analysis was done by standardization of area by CG-FID. 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 (CG-MS).

## Biological Activity

### Tested micro-organisms

Reference strains gram negative of *E. coli* (ATCC 8739) and gram positive of *S. aureus* (ATCC 25923) were used. Sterility tests were performed for the culture medium and oils.

## Microdilution in plate

### Standardization of the inoculum

The microdilution plate test<sup>[7]</sup> started by standardizing the inoculum on a McFarland scale at 0.5 (1.5x10<sup>8</sup> CFU/mL). The inoculum was inserted in Tryptic Soy Brothe nutrient broth and then transferred to Elisa plate wells at a concentration of 5x10<sup>5</sup> CFU/mL.

## Standardization of oils

Oils at concentrations of 240 $\mu$ L, 120 $\mu$ L, 60 $\mu$ L/mL and crude oil (without dilution, as used popularly) were used to determine the minimum inhibitory concentration, as positive controls amoxicillin (*S. aureus*) and tetracycline (*E. coli*) (16 $\mu$ /mL, 8 $\mu$ /mL and 4 $\mu$ /mL) and negative control TBS + Tween 80. The standardization of the oils was carried out in TSB nutrient broth together with Tween 80 and the oils<sup>[7]</sup> (NCCLS, 2003). 20 $\mu$ L were transferred to sterile microplate wells with a final volume of 180 $\mu$ L and incubated for 24 hr. Then it was verified whether there was growth or inhibition of micro-organisms with the addition of 20 $\mu$ L of 1% triphenyl tetrazolium chloride. The assays were performed in three replications.

## Statistical analysis

ANOVA and Tukey's test were performed and  $p < 0.05$  were considered significant results.

## RESULTS AND DISCUSSION

### Oils from *S. oleracea* almonds and *S. revoili* larvae

The yields found were location 1: 5.3g (45.30%) of larva oil and 20.29g (22.05%) of almond oil; location 2: 0.8g (41.89%) of larva oil and 9.98g (19.70%) of almond oil and location 3: 1.5g (32.47%) of larva oil and 9.63g (16.95%) of almond oil. Two unsaturated fatty acids and the other saturated ones were identified. Variations in fatty acid concentrations were observed and hexanoic acid was found only in almond oils. The main fatty acids observed were saturated chains: hexanoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and those of the unsaturated chain: oleic acid (omega-9) and linoleic acid (omega-6) (Table 1).

Higher concentrations of caprylic acids, capric acid and lauric acid were observed in the almond oil of *S. oleraceae* of myristic, palmitic, oleic and linoleic acids in the oil of *S. revoili*. Saturated chain fatty acids were found in high concentration in almond oil (86.0%) (Table 1).

Lauric acid was observed as the main component in *Syagrus romanzoffiana*, *Acrocomia aculeata* and *Syagrus coronata* almonds. Lauric acid has antimicrobial properties and improves the immune system.<sup>[8]</sup> Lauric acid present in *Cocos nucifera* oils showed antimicrobial activity superior to other saturated fatty acids against gram-positive bacteria, some fungi and viruses.<sup>[9]</sup>

Unsaturated fatty acids were found in greater amounts in *S. revoili* larvae, 27.33% oleic acid and 4.63% linoleic acid. Lauric acid has the highest concentrations in both almond oil and larva oil. In *S. oleracea* almonds, fatty acids were found by Nozaki *et al.*, (2012):<sup>[10]</sup> hexanoic acid (0.48 $\pm$ 0.01%), caprylic acid (30 $\pm$ 0.12%), capric acid (6.65 $\pm$ 0.06%), lauric acid (48.34 $\pm$ 0.18%), myristic acid (14.34 $\pm$ 0.07%), palmitic acid (5.14 $\pm$ 0.05%), stearic acid (3,72 $\pm$ 0.07%), oleic acid (7.98 $\pm$ 0.13%) and linoleic acid

**Table 1: Fatty acids obtained from *S. oleracea* and *S. revoili* oils.**

Fatty Acids	Mean ± Standard Deviation (%)	Mean ± Standard Deviation (%)
	<i>S. oleracea</i>	<i>S. revoili</i>
Hexanoic (C6:0)	0.63 ± 0.40	ND
Caprylic (C8:0)	11.97 ± 1.59	0.63 ± 0.40
Cáprico (C10:0)	7.2 ± 0.87	1.37 ± 0.32
Laurelic (C12:0)	44.40 ± 1.48	29.10 ± 3.07
Miristic (C14:0)	13.23 ± 1.28	15.83 ± 0.57
Palmitic (C16:0)	5.4 ± 0.52	14.30 ± 0.52
Stearic (C18:0)	2.30 ± 0.49	3.50 ± 0.52
Oleic (C18:1)	9.87 ± 1.20	27.33 ± 1.06
Linoleic (C18:2)	2.0 ± 0.14	4.63 ± 0.07

The results show the mean ± standard deviation of the analysis performed in triplicate. Statistical differences were observed by the tukey test at 5% probability between *S. oleracea* and *S. revoili* for all fatty acids studied. ND = Not detected.

(63.0±0.03%). In *Attalea speciosa*, the main fatty acids found were lauric acid (42.2%), oleic acid (18.5%), caprylic acid (3.0%), capric acid (2.8%), myristic acid (14.9%), palmitic acid (9.3%), stearic acid (2.9%), and linoleic acid (3.9%).<sup>[11]</sup>

In studies carried out on the oils of the larvae of the beetle *Pacymerus nucleorum*, bruquid that belongs to the same family of the beetle *S. revoili*, found in almonds of the macauba coconut (*Acrocomia aculeata*)<sup>[4]</sup> presented profiles similar to the results of fatty acids present in the larvae *S. revoili* found inside *S. oleracea* cocoa almonds, with variations only in the concentrations of fatty acids.

### Antimicrobial Activity

In the microdilution plate test, using crude oil of *S. revoili*, a bacteriostatic effect was observed, since after transfer to plates with culture medium, a small growth of bacteria was observed. In the other dilutions (240µL, 120µL and 60µL) no inhibition was observed. The positive controls (antibiotics) amoxicillin and tetracycline inhibited micro-organisms at a concentration of 4µg/mL and in the negative control there was bacterial growth.

Medium-chain fatty acids, such as lauric and long-chain oils, are responsible for the inactivation of gram positive and gram negative bacteria.<sup>[12]</sup> The antimicrobial effects occur due to interferences in the cell wall of micro-organisms and in the mechanisms of bacterial virulence.<sup>[13]</sup>

## CONCLUSION

Hexanoic, caprylic, capric, lauric, myristic, palmitic, stearic and unsaturated oleic and linoleic fatty acids were identified. Only the crude oil of the larva of the *S. revoili* beetle showed bacteriostatic activity against gram-negative strains of *E. coli* and *S. aureus*. Larva oil as it is popularly used can have an effect because it is used directly in the ear without any dilution, as observed in the experiments, however, further studies must be performed.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ATCC:** American Type Culture Collection; **CFU:** Colony Forming Unit; **GC:** Gas Chromatography; **GC/FID:** Gas Chromatograph Coupled to Flame Ionization; **GC-MS:** Gas Chromatography coupled to Mass Spectrometer; **P.A:** Pure for Analysis, **TBS:** Fetal Bovine Serum. <https://www.sigmaaldrich.com/IN/en/product/sigma/f9665>

## SUMMARY

- This research work evaluated the fatty acid composition and antimicrobial action of *Syagrus oleracea* (Mart.) Becc. Oils and Oils Extracted from Larvae of *Speciomerus revoili* (Pic.)
- Based on this study, it is possible to determine fatty acids with a saturated chain, the presence of hexanoic acid (only in almonds), and caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and those with an unsaturated chain, oleic acid (omega-9) and linoleic acid (omega-6).
- Antimicrobial activity was observed against *Escherichia coli* and *Staphylococcus aureus*, for *S. revoili* oil

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