Chemical Composition, Antibacterial and Larvicidal Activities of Zanthoxylum rhoifolium Lam Fruits Essential Oil

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Abstract: The analysis by GC-MS, of the essential oil of the fruits from this species permitted the identification of nine chemical constituents, representing 86.99 % and having as majorities: trans β-ocimene (33.90 %), linalool (23.27 %) and undecanone (16.19 %). The essential oil was submitted to analysis for the verification of the antibacterial activity, by the method of diffusion in paper discs in Müller Hinton agar medium, where one bacteria were tested Gram positive (Staphylococcus aureus) and four Gram negative (Listeria monocytogenes, Pseudomonas aeruginosa, Shigella flexneri and Salmonella choleraesuis), which demonstrated significant results. Assays for the verification of larvicidal activity were made with Aedes aegypti larvae with the oil being active.

Key Words: Zanthoxylum rhoifolium; essential oil; antibacterial activity; larvicidal activity.

Introduction: The classes of the Rutaceae family are widely distributed in the tropical, subtropical and temperate regions of the world, with approximately 1,600 species, with a great occurrence in Australia and Africa. In Brazil, the family is represented by about 29 classes and 182 species 1. From the representatives of this family it is detached the Zanthoxylum rhoifolium Lam., an arboreal species with a height varying from 6.0 to 12.0 m, pricked of composed leaves, which, because of the shape and density of its treetop and to the fact of its fruits being consumed by some birds, can be successfully used in landscape, in plantings viewing the recovery of vegetation and degraded areas 2.

Previous studies demonstrated that essential oils from the aerial parts (leaves, fruits,
flowers) of the *Zanthoxylum rhoifolium* from the south of Brazil were analyzed by GC, GC-MS and CPGC. The principal chemical constituents are: germacrene D (34 %) and bicyclogermacrene (23 %), from the leaves, from the fruits: menth-2-en-1-ol (46.2 %), β-myrcene (30.2 %), (-)-linalool (15 %) and (-)-α-terpineol (8.45 %), while in the flowers it was detached β-myrcene (65 %) and menth-2-en-1-ol (5.4 %). Reports indicate that the oils from the leaves and fruits were bioactive with antibacterial activity to *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella choleraesuis*, whereas the essential oil from the flowers was inactive 3.

Significant percentage variations in the main compounds of the *Z. rhoifolim* essential oil have been reported. Boehme 4 shows that in species from Costa Rica, the principal constituents were germacrene D (14.6 %), limonene (12.5 %), trans-2-hexenal (11.3 %), β-elemene (9.2 %), 2-undecanone (9.2 %), myrcene (7.9 %), bicyclogermacrene (7.5 %), and germacrene A (5.2 %). In another brazilian report, germacrene D and bicyclogermacrene were found in a concentration of 11.7 and 25.4 %, respectively 5.

*Zanthoxylum armatum* and *Zanthoxylum limonella* essential oils showed larvicidal activities 6,7 and *Z. rhoifolium* is utilized by the inhabitants that live along the Madeira River (Porto Velho Rondônia) for cancer and malaria treatment 8.

The interest for the study of this species is, mainly, because of the fact that it shows great antimicrobial potential, and because it is a largely popular species used as a febrifuge, stomachic medicine and in the treatment of colic.

**Experimental**

**Plant Material:** The fruits were collected in August 2005 in the county of Salitre, Ceará, Brazil, under license granted by the Brazilian Institute of the Environment and Natural Renewable Resources – IBAMA (015/2005 NP). The botanical identification (38231) was granted by the Prisco Bezerra Herbarium of the Biology Department, Federal University of Ceara.

**Oil isolation:** The fruits (380 g) were submitted to hydro-distillation in Clevenger type apparatus for 2 hours, supplying oil with revenue of 0.5 %, based in the fresh weight of the vegetal material. The oil obtained was dried in anhydrous sodium sulfate, filtered, stored in amber glass, sealed and kept under refrigeration before analysis.

**Analytical conditions:** Analysis of the volatile constituents was carried out on a Hewlett-Packard Model 5971 GC-MS using a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness); carrier gas helium, flow rate 1 mL/min. and with split mode. The injector temperature and detector temperature were 250°C and 200°C, respectively. The column temperature was programmed from 35°C to 180°C at 4°C/min. and then 180°C to 250°C at 10°C/min. Mass spectra were recorded from 30-450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library and two other computer libraries. MS searches using retention indices as a pre-selection routine 9,10, as well as by visual comparison of the fragmentation pattern with those reported in the literature 10,11,12.
Antimicrobial assays: The test was carried out with bacteria supplied by the INCQS (National Institute of Control of the Quality in Health) from the Oswaldo Cruz Foundation -FIOCRUZ. They were: two Gram positive (Staphylococcus aureus ATTC 12692 and Listeria monocytogenes ATCC 19117) and three Gram negative (Pseudomonas aeruginosa ATCC 15442, Shigella flexneri ATCC 12022 and Salmonella choleraesuis ATCC 13314). The analysis of the antibacterial activity was performed by the method of diffusion in paper disc in Müller Hinton agar medium. The bacteria were seeded in the medium surface, in Petri plaques. Discs of 6 mm of diameter were used, soaked in 10 μL of test solution (1000 to 100 μg/mL) of the oil. The plaques were incubated at 35°C, and after 24 h the results were read. The test was performed in triplicate and the results expressed in mm by the average around the discs in the 3 repetitions. The assay was monitored with discs soaked with ampicillin (30 μg) and amikacin (30 μg) as positive control and as negative control 0.15 % TWEEN/H₂O.

Larvicidal bioassay: Aliquots of the essential oil of the fruits were tested (0.1 to 1 mg/mL) diluted in water and DMSO. Ten larvae of Aedes aegypti in the third stage of development were added to glass tubes containing test solution and kept at environmental temperature. After 24 hours, it was counted the number of dead larvae and calculated the CL₅₀. The test was performed in triplicate with three repetitions and followed by a positive control (potassium dichromate) and one negative (water/DMSO).

Results and Discussion: The essential oil of the fresh fruits of the Zanthoxylum rhoifolium was analyzed in CG-MS for the determination of the chemical constituents. Nine components were found, representing a total of 86.99 % (Table 1). trans-β-ocimene (33.90 %), linalool (23.27 %) and 2-undecanone (16.19 %) were identified as majority components differing from the results found in the literature. Differences in essential oil composition according to area of collection are well documented in Brazil. One example is the essential oil of Eugenia uniflora, which has a different composition, when collected at Rio Grande do Sul State (Southern Brazil – 30°S) in contrast to Ceará State (Northeastern Brazil – next to equator), this can be due to environmental conditions or may be due to infra-specific genetic variation.

In the antibacterial analysis, it was verified that among the analyzed vines, the zones of Staphylococcus aureus, Salmonella choleraesuis and Shigella flexneri presented to be sensitive to antibacterial action of the essential oil, being the inhibition halos of 15.13 and 10 mm, respectively. Respect to other tested vines, there were no inhibition halos produced (Table 2). Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria, this is ascribed to the presence of an outer membrane, which possessed hydrophilic polysaccharide chains as a barrier to hydrophobic essential oils. Accordingly, the high degree of susceptibility of Shigella flexneri and Salmonella choleraesuis was unexpected, showing an indicative to future tests with other bacterial vines.

It is becoming more challenging to chemically control the mosquito A. aegypti. Besides toxicity, resistance has been reported in many areas, where it is largely used. An
alternative to conventional chemical control is the utilization of natural products from plants and essential oils. The oil demonstrated to be active against *Aedes aegypti* larvae with CL$_{50}$ of 156.7 µg/mL, verifying its larvicidal potential $^{14,15}$.

The antibacterial and larvicidal activities may be attributed to the presence of compounds found in *Zanthoxylum rhoifolium* fruits essential oil, *tans-*β-lactone $^{18}$, linalool $^{19}$ and 2-undecanone $^{20}$. Yet, there isn’t a relation of which chemical component was responsible by the activities evaluated in this study and if occurred synergisms among them. It is possible that the chemical components, which make up the essential oil were complimentary to each other and actually perform better as a whole. In this way, studies with the goal of testing the isolated components are being performed to relate activity/substance.

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**References**


Table 1. Chemical composition percentage of the fruits of *Zanthoxylum rhoifolium*

<table>
<thead>
<tr>
<th>Compound</th>
<th>KI</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>α-Pinene</td>
<td>933</td>
<td>2.52</td>
</tr>
<tr>
<td>β-Mircene</td>
<td>989</td>
<td>0.84</td>
</tr>
<tr>
<td>β-Phellandrene</td>
<td>1027</td>
<td>1.43</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>1521</td>
<td>0.30</td>
</tr>
<tr>
<td><em>trans</em>-β-Ocimene</td>
<td>1047</td>
<td>33.90</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>1090</td>
<td>5.86</td>
</tr>
<tr>
<td>Linalool</td>
<td>1099</td>
<td>23.27</td>
</tr>
<tr>
<td>2-Undecanone</td>
<td>1292</td>
<td>16.19</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>1383</td>
<td>0.53</td>
</tr>
<tr>
<td>Germancrene D</td>
<td>1478</td>
<td>2.45</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>87.29</td>
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Table 2. Evaluation of the antibacterial activity of the fruits of *Zanthoxylum rhoifolium*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition halo diameter (mm)</th>
<th>AMC</th>
<th>AMP</th>
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<tbody>
<tr>
<td></td>
<td>1000</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>15 ± 0.47</td>
<td>10 ± 0.60</td>
<td>7 ± 0.47</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>7 ± 0.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>7 ± 0.47</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>10 ± 0.47</td>
<td>8 ± 0.81</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella choleraesuis</em></td>
<td>13 ± 0.47</td>
<td>9 ± 0.81</td>
<td>-</td>
</tr>
</tbody>
</table>

(AMC) Amikacin 30 µg
(Amp) Ampicillin 30 µg
- insensitive.