Antimicrobial activity of copaiba (Copaifera spp) balsams

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ABSTRACT: Antimicrobial activity of copaiba (Copaifera spp) balsams. Present communication describes the antimicrobial tests realized with 11 oleoresins from different trees collected in Amazonas and Pará States. The activity was tested against Gram-positive (Staphylococcus aureus, and Bacillus subtilis) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. Bacterial cultures was grown in Nutrient Agar, and inocula were prepared by dilution overnight cultures with the Muller-Hinton liquid medium (10^4 u.v./ml). The in vitro evaluation of antimicrobial activity was performed by microdilution method. Results were expressed as minimal inhibitory concentration (MIC). Cloranphenicol was used as positive control. Results revealed inactivity of all the samples against Gram-negative bacteria until 1000 ìg/ml. However, oils 2, 5 and 10 shown significant activity against B. subtilis and S. aureus. Most oils showed different levels of inhibition, confirming the importance of the standardization, once Copaiba Balsam is freely used in folk medicine.

Key words: copaiba oil, Copaifera sp, Leguminosae, antimicrobial activity

INTRODUCTION

Copaiba oils, also described as copaiba balsams or oleoresins, are produced by extraction of the trunk of the trees belonging to the genus Copaifera (Leguminosae). These trees are largely distributed in northern South America, mainly in the Brazilian Amazonas rain forest, in the states of Pará and Amazonas (Veiga Júnior & Pinto, 2002). By the last edition of the Index Kewensis, there are 72 species of the genus Copaifera, being 16 only present in Brazil.

Phytochemical studies carried out on the oleoresin of Copaifera revealed the presence of about 72 sesquiterpene hydrocarbons and 28 diterpenes, but there are significant differences of chemical composition occurring among the species (Cascon & Gilbert, 2000; Pontes et al., 2003).

Copaiba oils have been used for the treatment of cancer, ulcer, syphilis, bronchitis, and diarrhoea, and as bactericidal, anti-helmintic, analgesic, anti-inflammatory, gastro-protective, antitumor, and trypanomidal agents (Ticusi et al., 2002). The oils are used in the flavour and cosmetic industry, and as diesel-like fuels (Monti et al., 1996).

INTRODUCTION

There are many copaiba oleoresins being used and marketed based on popular use, and spite of this, little is known of the relationship between the phytochemical composition and biological and toxicological valuation.

It's necessary that medications of natural origin be standardized chemically and evidence be presented as to efficacy and safety use. The analysis and chemical standardization of the oleoresins of different species of Copaifera is clearly essential if one is to relate chemical composition with biological activity and so to permit validation as a safe and effective phyto-medicine with adequate quality control (Gilbert et al., 1997).

The present study describes the antimicrobial tests realized with 11 oleoresins from different trees collected in Amazonas and Pará States, Brazil.

MATERIAL AND METHOD

Plant material

The 9 oleoresins numbered 1 to 9 were collected in February 2003, in the region of Altamira, Pará State; while the oleoresins numbered 10 and 11 were collected in January 2004, in the region of Eirunepé, Amazonas State. Previous botanical identification of the samples 1 to 9 was made by Prof. Jorge Tamashiro, Institute of Biology, Unicamp, Campinas, SP.

The oleoresins 1, 3, 7, 8 and 9 were previously identified as Copaifera multijuga Hayne. All the samples are deposited in the Museu Paraense Emílio Goeldi, Belém, Pará, for identification.

Cultures and Media

Staphylococcus aureus CCT 2740, Bacillus subtilis CCT 2576, Escherichia coli CCT 0547 and Pseudomonas aeruginosa ATCC 13388 were cultured in Nutrient Agar (Merck) at 37°C for 24h. The suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^6 CFU/ml. This suspension was diluted in sterile Muller-Hinton liquid medium (Merck) to a final concentration of 1.0 x 10^4 CFU/ml. Diluted culture
was then used in the tests.

**Antimicrobial Assay**

A microdilution broth susceptibility assay was used for the evaluation (Elloff, 1998). Stock solutions of oleoresins were prepared in DMSO. Serial dilutions were prepared in sterile Muller-Hinton liquid medium in a 96-well microtiter plate from 1.000 to 5 μg/mL. One hundred microliters of each suspension was then added to each well. The last row containing only the antimicrobial agent without microorganism was used as negative control. Four wells with the medium and the inocula were used to verify the absence of contamination and to check the validity of the inoculum. The plates were incubated for 24h at 37°C. After incubation, fifty microliters of 2,3,5- triphenyltetrazoliun chloride was added to each well and the plates were incubated for more 3h. The first well without color was determined as the minimal inhibitory concentration (MIC). Minimum inhibitor concentrations of cloranphenicol were also determined.

**RESULT AND DISCUSSION**

The antimicrobial evaluation (Table 1) of the oleoresins from *Copaifera* spp resulted in different levels of inhibition against Gram positive bacteria. Oils 2, 5 and 10 showed significant activity against *B. subtilis* and *S. aureus*, with MIC values of 5 μg/mL, comparable with cloranphenicol used as control.

The other oils presented variation in inhibition, with MIC values of 300-550 μg/mL (*B. subtilis*) and 125-550 μg/mL (*S. aureus*). Exception was showed by the oils 1 and 11 against *B. subitilis* and oils 9 and 11 against *S. aureus*, that don’t presented inhibition until 1.000 μg/mL. However, all samples were inactive against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) until 1.000 μg/mL.

For the previous botanical identification may be observed that even in the same specie there are different levels of inhibition, that may be explained for the different chemical composition of the oils depending not only the specie but also the age and collection period.

These results partially highlight the antimicrobial potential of Copaiba oleoresins as traditional remedy, and differences between the levels of inhibition confirm the importance of the standardization.

**TABLE 1.** Minimal inhibitory concentration (MIC, μg/mL) from 11 Copaiba oleoresins.

<table>
<thead>
<tr>
<th>Copaiba oils</th>
<th><em>B. subtilis</em></th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>550</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2</td>
<td>5</td>
<td>5</td>
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<tr>
<td>3</td>
<td>350</td>
<td>150</td>
<td>-</td>
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</tr>
<tr>
<td>4</td>
<td>300</td>
<td>150</td>
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<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>6</td>
<td>450</td>
<td>125</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7</td>
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<td>225</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
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<td>-</td>
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<tr>
<td>10</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>15.6</td>
<td>7.8</td>
<td>1000</td>
<td>15.6</td>
</tr>
</tbody>
</table>

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**REFERENCE**


VEIGA JÚNIOR, V. F., PINTO, A. C. O género *Copaifera* L.