Evaluation of Antimicrobial and Modulatory activity of the extract of
Richardia brasiliensis Gomes

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The emergence of resistant microorganisms and also the toxicity associated with antimicrobial drugs increase the need of research for new active principles. Richardia brasiliensis, a weed used popularly as an expectorant, antiemetic, and diaphoretic. The extracts have coumarins, flavonoids, steroids, triterpenoids, alkaloids and resin, as secondary metabolites. The present study aimed to test the potential antimicrobial and modulator of the ethanolic and hexanic extracts of R. brasiliensis. The ethanolic and hexanic extracts were tested for their antimicrobial effect and in combination with aminoglycosides and antifungal against standard and multi-resistant microorganisms by the broth microdilution method with culture medium Brain Heart Infusion (BHI). It was observed that the association between antibiotics and ethanolic and hexanic extracts showed clinically relevant results on the tests with multi-resistant bacteria. The natural products from R. brasiliensis demonstrated a modulating action against the microorganisms used. These results can represent a new effort to combat antibiotic resistant bacteria.

Keywords: Richardia brasiliensis, Antimicrobial activity, Modulatory effect, Multi-resistant microorganisms.

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Medicinal plants have been used by man since antiquity as a means to alleviate or even cure diseases, this knowledge was passed from generation to generation, especially after the creation of writing. With the development of new technologies could further study of these plants, isolating substances responsible for its pharmacological effect and thus creating drugs with the active principle purified and improved⁴. Richardia brasiliensis is a plant belonging to the Rubiaceae family which includes about 637 genera and nearly 10,700 species. It is one of the largest families between the dicotyledons, being abundant in subtropical regions worldwide⁶. Popularly is known as the white poaia, poaia-of-the-field or poaia. It is an annual plant, herbaceous, prostate, branched, stem densely hirsute, measuring 20-50 cm in length. It has marked presence in the agricultural regions of the Midwest, South and Southeast of Brazil³-⁵. R. brasiliensis is popularly used in Brazil as infusion or decoction of the root as an expectorant, antiemetic, and diaphoretic while plants of the same family are also used as anti-inflammatory and to treat hemorrhoids, coughs, bronchitis, and headache⁶.

In the phytochemical prospection of the aerial parts (stalk fragments, petiole and leaf), was observed the presence of coumarins, flavonoids, steroids, terpenoids, alkaloids and resins. In the underground part were found the same substances from the top except for flavonoids and alkaloids⁷. Pinto et al.⁸ isolated phytocompounds from R. brasiliensis as Isorhamnetin-3-O-rutinoside, Oleanolic acid, acid p-Hydroxy-benzoic, m-Methoxy-p-hydroxy-benzoic acid and Scopoletin. All classes of phytocompounds indicated by the literature presented several biological activities, demonstrating the necessity of more pharmacognostic and ethnobiological studies.

The aim of this study was to evaluate the potential antimicrobial and modulator of ethanolic and hexanic extracts of the leaves of R. brasiliensis against pathogenic microorganisms.

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Methodology

Microorganisms

The microorganisms used in testing were obtained from National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation, Ministry of Health, were used three standard strains of bacteria, *Escherichia coli* ATCC10536; *Staphylococcus aureus* ATCC25923; *Pseudomonas aeruginosa* ATCC15442. To evaluate the modulatory activity of the natural products were used the following bacterial isolates multi-resistant: *Pseudomonas aeruginosa* 22, *Escherichia coli* 27 and *Staphylococcus aureus* 358 with resistance profiles tested. The fungal strains used were: the standard strain *Candida tropicalis* ATCC 40042 and *Candida krusei* ATCC 2538. Both for antimicrobial evaluation is to evaluate the modulator effect. All strains were maintained in culture medium Heart Infusion Agar – HIA (Difco Ltda) and before testing the cells were cultured for 24 hrs at 35°C using media Brain Heart Infusion Agar – BHI (Difco laboratories Ltda).

Plant material

Leaves of *R. brasiliensis* were collected in the city of Joao Pessoa, Paraiba, Brazil. The plant material was identified by Dr. Maria de Fátima Agra and one voucher specimen was deposited in the herbarium Prof. Lauro Pires Xavier with a voucher number #Agra et al 3195#.

Preparation of ethanol extract and fractions of the Richardia brasiliensis leaves

Fresh leaves of *R. brasiliensis* (275 gm) were ground and macerated and the en gave into extraction with ethanol and hexane solvents for a period 72 hrs. The solvent was distilled in a rotary evaporator at 80°C under reduced pressure. The extracts obtained were weighed (10.2 and 11.4 gm, respectively) and stored at room temperature.

Drugs utilized

The antibiotics used in the tests were the aminoglycosides: amikacin, gentamicin and neomycin (Sigma Co., St. Louis, USA). Antifungal drugs used were: Amphotericin B, Nystatin, Mebendazole and Benzoylmethronidazole. All solutions were prepared using sterile water following the manufacturer's recommendations.

Minimal Inhibitory Concentration (MIC)

The method used was the broth microdilution. The ethanolic and hexanic extracts of *R. brasiliensis* were dissolved using Dimethylsulfoxide (DMSO) and diluted to a concentration of 1024 µg/mL using sterile water. The bacteria inoculates were diluted using BHI 10% to a final concentration of 10^5 UFC/mL. It was used 100 µL of the inoculum into each well of a 96-well microdilution plate and, after that, submitted to a dilution of 1:2 in series using 100 µL of the extract, varying concentrations between 512 a 8 µg/mL. The microdilution plates were incubated for 24 hrs at 35°C. The reading of MIC was made using the resazurin indicator, while the fungal tests reading was verified absence or presence of turbidity. The MIC was determined as the lowest concentration where no growth of the microorganism was observed, according to NCCLS.

Modulation tests with antimicrobials

To verify if the natural product could alter the action of antimicrobial drugs against the strains tested, was used the method proposed by Coutinho et al. The ethanolic and hexanic extracts of *R. brasiliensis* were tested in sub inhibitory concentration (MIC/8=128µg/mL). Were distributed 100 µL of the solution containing BHI with the inoculum and the extract in each well. After this, 100 µL of the antimicrobial was mixed with the solution on the first well, after dilution of 1:2, varying the concentrations of the aminoglycosides and antifungal between 2500 to 2.44 µg/mL and 512 to 2 µg/mL, respectively.

Results

After testing the minimum inhibitory concentration with bacteria and with fungi were found results ≥ 1024 µg/mL for both ethanol extract and hexane extract. However, both ethanol extract and hexane extract showed modulation levels clinically relevant against multi-resistant bacterial strains *S. aureus* 358 and *E. coli* 27. In the study of the modulatory activity, both the ethanolic extract and the hexane extract against multi-resistant bacterial strains was observed a 9.8 µg/mL of MIC when the natural product was associated with Neomycin and 2.44 µg/mL associated with Gentamicin against *E. coli* 27. Against *S. aureus* 358 was observed 19.53 µg/mL of MIC in combination with Amikacin and 2.44 µg/mL with Gentamicin. It was not observed modulating effect clinically relevant against *P. aeruginosa* (Table 1).
The modulation tests with fungi *Candida tropicalis* and *Candida krusei* showed MIC ≥ 1024 µg/mL in all of the tests (Table 2).

### Discussion

In the phytochemical prospection of the aerial parts of *R. brasiliensis* were found flavonoids, steroids, triterpenoids, alkaloids and resin. Studies show that secondary metabolites like flavonoids and terpenes have an antimicrobial in species, *Coussarea platyphylla* Mull. Arg also shows the presence of triterpenes and Phytosterol in *Guettarda grazielae*. Steroids have also been isolated from the specie *R. grandiflora*.

The ethanol and hexanic extracts showed no antimicrobial activity clinically relevant before the tested strains of fungi and bacteria according to Houghton et al. (MIC ≥ 1024 µg/mL). Results are divergent from Figueiredo et al. These divergent results probably occur due the fact of plants were been reaped in different regions and also the use of different methodologies, as disk diffusion. The maximum concentration of the antimicrobial agent used in this study was 512 µg/mL, this concentration was a bit more higher of the lowest MIC that was found by Figueiredo et al. According to Greger & Hadacek the methodology used in the study cited above is not well accepted because it’s not possible to precise the quantity of natural product that spreads through the agar.

The resistance of the microorganisms occurs by several mechanisms like efflux pump, production of enzymes that inactivate the drug and change the target of action of the antibiotic. The resistance mechanisms are transmitted through exchange of genetic material between microorganisms of the same species.

Many studies have shown that extracts and essential oils from plants can modify the activity of antimicrobial and thereby improve its performance by decreasing the concentration necessary for there to be growth inhibition. Fact demonstrated with the plants *Turnera ulmifolia*, *Momordica charantia*, *Mentha arvensis*, *Cordia verbenacea*. The results indicate the potential of this and other natural products for future clinical and *in-vivo* assays. These assays must be directed using information from the traditional knowledge and after an appropriate study about the toxicity and chemical prospection.

The tests of modulation showed with the bacteria modifying effect clinically relevant against *S. aureus* (associated with gentamicin) and *E. coli* (associated with neomycin and gentamicin). This effect is probably coming from the secondary metabolites present in the plant under study. The capacity that natural products have to modify the action of antimicrobials can be seen from studies that show that association of synthetic drugs and plant extracts can act reversing microbial resistance eliminating plasmids and inhibiting the efflux pump.

Bioprospecting, a vital step in the pharmaceutical production process, is also one of the most controversial and socially complex aspects in the pharmaceutical industry. The use of the traditional knowledge for this process have the aim to alleviate social inequalities by providing access to end products, such as new drugs or biotechnologies. So, countries rich in biodiversity can develop these

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**Table 1**—Test result for modulation of bacterial resistance (µg/mL).

<table>
<thead>
<tr>
<th></th>
<th>EERB + Antibiotic</th>
<th>HERB + Antibiotic</th>
<th>Antibiotic alone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>39.1</td>
<td>39.1</td>
<td>156.2</td>
</tr>
<tr>
<td>Neomycin</td>
<td>9.8</td>
<td>9.8</td>
<td>78.1</td>
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<tr>
<td>Gentamicin</td>
<td>2.4</td>
<td>2.4</td>
<td>9.8</td>
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<tr>
<td><em>Staphylococcus aureus</em> 358</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>19.5</td>
<td>19.5</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em> 22</td>
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<tr>
<td>Amikacin</td>
<td>156.2</td>
<td>321.5</td>
<td>312.5</td>
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<tr>
<td>Neomycin</td>
<td>156.2</td>
<td>156.2</td>
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<tr>
<td>Gentamicin</td>
<td>19.5</td>
<td>19.5</td>
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</tr>
</tbody>
</table>

EERB = Ethanol extract of *Richardia brasiliensis*. HERB = Hexane extract of *Richardia brasiliensis*.

**Table 2**—Test result for modulation of fungal resistance (µg/mL).

<table>
<thead>
<tr>
<th>Candida tropicalis ATCC40042</th>
<th>EERB + Antibiotic</th>
<th>HERB + Antibiotic</th>
<th>Antibiotic alone</th>
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<tbody>
<tr>
<td>Mebendazole</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
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<tr>
<td>Amphotericin B</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
</tr>
<tr>
<td>Nystatin</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
</tr>
<tr>
<td>Benzoilmetronidazol</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Candida krusei ATCC2538</th>
<th>EERB + Antibiotic</th>
<th>HERB + Antibiotic</th>
<th>Antibiotic alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mebendazole</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
<td>≥ 1024</td>
</tr>
<tr>
<td>Amphotericin B</td>
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<td>Nystatin</td>
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<td>Benzoilmetronidazol</td>
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EERB = Ethanol extract of *Richardia brasiliensis*. HERB = Hexane extract of *Richardia brasiliensis*.
resources to combat social and environmental problems. This is one of the most important role of the studies of the folk medicine and the traditional knowledge.

**Conclusion**

The extracts of *R. brasiliensis* demonstrated results with clinical relevance showing moderate modulation effect against *S. aureus* 358 and *E. coli* 27. Being this a pioneering study on the modulating effect of *R. brasiliensis*. Deepest research about its secondary metabolites could help to elucidate which one of these metabolites is responsible for such effects.

**Conflict of Interest**

There is no conflict of interest

**Acknowledgment**

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**References**