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# Antibacterial and inhibitory activity of biofilm for major phenolic compounds of *Jodina rhombifolia* Hook et Arn (Santalaceae)

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## INTRODUCTION

Rural populations of northwestern Argentina (NWA) used and use leaves and bark of *Jodina rhombifolia*, a tree of Santalaceae family, for sore throats, whooping cough (pertussis), kidney stones or pain, stomach indisposition, articular and muscular pains (Ferraro and Martino, 1990). The most frequently used forms are infusion, and bath preparations. Flavonoids are among biologically active plant chemical compounds, and there is a lot of literature about their properties, *e.g.*, antibacterial.

Bacteria have developed biofilm, mechanism that allows them to secrete substances to adhere to each other and to solid surfaces, thus making very difficult their eradication because of becoming impenetrable to antibiotics. The formation of this film is considered an important virulence factor of negative-coagulase *Staphylococcus* (NCS).

Taking into account the ethnobotanic and phytochemical antecedents of the plant, the aim of this work was to evaluate the antibacterial activity of *Jodina rhombifolia* against Gram-positive and Gram-negative bacteria of collection, and isolates from clinical samples, as well as its action on biofilm formation.

## MATERIALS AND METHODS

**Preparation of the extract.**

Most phenolic compounds were extracted from 15g of dried aerial parts of *J. rhombifolia* until exhaustion with 80% and 50% ethanol. The obtained extract was concentrated *in vacuo* under low-pressure (Mabry *et al.*, 1970; Harbone, 1998), and the residue was used for preparing a mother solution at a concentration of 200 mg/ml in distilled water.

**Bacterial strains.**

ATCC (American Type Culture Collection): *Enterococcus faecalis* 29212, *Staphylococcus aureus* 25923 and 29213, *Escherichia coli* 35218, *Pseudomonas aeruginosa* 27853. Bacteria isolated from clinical samples: *Shigella sonnei*, *Shigella Flexneri, Salmonella enteriditis, Salmonella thiphymurium, Listeria monocytogenes, Enterococcus faecalis, Staphylococcus Haemolyticus, Staphylococcus epidermidis, Staphylococcus cohnni, Staphylococcus hominis*.

**Preparation of the inoculum.**

Strains were activated in BHI broth, subcultured in blood agar or McConkey-agar, and then a suspension was prepared in 2 ml BHI until a final concentration of $10^8$ CFU/ml.

**Microbiologic assays.**

The method of radial diffusion was carried out, using MH agar with 4 mm thickness, which was inoculated with bacterial suspensions. Wells of 1 cm diameter were punched in the agar, and 100 µl of dilute plant extract was applied reaching final concentrations of 20, 10, 5 and 2.5 mg/ml.

To determine the minimum inhibitory concentration (MIC) of the plant extract, plates were prepared by addition of 5 ml MH, and increasing quantities of plant extract until final concentrations of 20, 10, 5 and 2 mg/ml. Two µl of $10^7$ CFU/ml

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were inoculated, a final inoculum of $10^4$ CFU of each bacterial strain being
Obtained. It was incubated at 37°C for 24 h.

The above-mentioned techniques were carried out according to CLSI/NCCLS (Clinical and Laboratory Standards Institute/National Committe for Clinical Standard Laboratory) specifications.

For biofilm production clinical isolates of *S. epidermidis* were selected. In a carbonate multitray 100 µl of a bacterial suspension of *S. epidermidis* were deposited, and the plant extract was added at a final concentration of 50, 25 and 12.5 mg/ml, respectively. The multitray was subjected to incubation, fixation, and stain of the biofilm in order to read in the spectrophotometer. Determinations were carried out in quadruplicate.

## RESULTS

**Table 1.** Results of the inhibition halos by the method of radial diffusion.

| Microorganisms / Extract in mg/ml | Inhibition halos in mm | | | |
|---|---|---|---|---|
| | 20 | 10 | 5 | 2.5 |
| *S. aureus* 29213 | 20 | 18 | 12 | 10 |
| *S. aureus* 25923 | - | - | - | - |
| *E.faecalis* 29212 | 15 | 12 | 9 | 8 |
| *E.coli* 35218 | - | - | - | - |
| *P. aeruginosa* 27853 | 15 | 14 | - | - |
| *S. enteriditis* | - | - | - | - |
| *S. thiphymurium* | - | - | - | - |
| *Shigella flexneri* | - | - | - | - |
| *L. monocytogenes* | 16 | 14 | 12 | 8 |

**Table 2.** MIC values of ATCC strains and isolates of clinic samples.

| Microorganism | MIC mg/ml |
|---|---|
| *Staphylococcus aureus* (ATCC 29213) | 2.5 |
| *S. aureus* (ATCC 25923) | >10 |
| *Enterococcus faecalis* (ATCC 29212) | 10 |
| *Escherichia coli* (ATCC 35218) | >10 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 10 |
| *Salmonella enteriditis* | >10 |
| *Salmonella thiphymurium* | 10 |
| *Shigella flexneri* | >10 |
| *Listeria monocytogenes* | 2.5 |

Plant extract against *S. aureus* 29213 showed a good activity with a MIC value of 2.5 mg/ml (Tables 1 and 2). MIC values of 21 SCN isolates of clinical samples were determined (urine, catheter and conjunctival exudate). Results of MIC values of SCN are shown in Table 3. *S. epidermidis* was the species that showed the highest sensitivity to plant extract (Table 3).

With regard to the inhibition of the biofilm of *S. epidermidis*, extract concentration of 20 mg/ml inhibited its formation, while concentrations of 10 and 5 mg/ml showed a decrease, but not absence, of its production.

**Table 3.** MIC values of 21 SCN strains.

| Microorganisms | Total extract mg/ml | | | | |
|---|---|---|---|---|---|
| | >20 | 20 | 10 | 5 | 2.5 |
| *S. haemolyticus* (n=13) | 46% | 8% | 46% | - | - |
| *S. epidermidis* (n=4) | - - | - - | 100% | - | - |
| *S. cohnni* (n=2) | 50% | 50% | - | - | - |
| *S. hominis* (n=2) | 100% | - | - | - | - |

## DISCUSSION AND CONCLUSIONS

The plant extract showed antibacterial effectiveness against Gram-positive bacteria, and inhibitory activity of the biofilm formation in SCN.

The extract of *Jodina rhombifolia* inhibited biofilm production of *S. epidermidis*, only at the

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concentration of 20 mg/ml. Probably inhibitory effect is due to the fact that the plant extract maintains bacterial population at a lower density than the necessary for achieving biofilm formation.

This property findings of this plant of important use in NWA popular medicine justifies the realization of further studies about the antimicrobial activity of its flavonoids, and to incorporate it in the treatment of diseases of bacterial origin previous innocuousness studies.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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