



Evaluation of the antibacterial activity of different extractive forms of Guayacán ritidome

M. A. Sgariglia, J.R. Soberón, E. N. Quiroga, D. A. Sampietro and M. A. Vattuone

Instituto de Estudios Vegetales "Dr. Antonio R. Sampietro", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, (4000) San Miguel de Tucumán, Tucumán, Argentina.
e-mail: instveg@unt.edu.ar

INTRODUCTION

The phytochemical research based on ethnopharmacologic data is an effective approach for the discovery of new metabolites with possible application as antimicrobials. Popular wisdom attributes to *Caesalpinia paraguariensis*, also called 'Guayacán', vulnerary properties, among others. This species is exploited because of its wood properties. It is not reforested, and if felling continues less specimens will remain. The use of its ritidome with medicinal purposes is not destructive, and transforms it in an available natural resource for long time, since this tree reaches very advanced ages.

On the other hand, the microorganisms tested, are pathogen that very frequently develop resistance to the new available synthetic antibiotics, resulting in severe complications, mainly in immunosuppressed patients.

The aim of this study is to evaluate the antibacterial activity of *Caesalpinia paraguariensis* extracts and validate the medicinal use of this argentinian native tree.

MATERIALS AND METHODS

Plant material. Ritidome of *Caesalpinia paraguariensis* (D. Parodi) Burk. (Caesalpiniaceae, Fabaceae) was collected during the period Fall-Winter 2005, in the mount Chaqueño, where this species is representative.

Plant extraction. Aqueous extracts, infusion and decoction were prepared (10 %, w/v) from powered ritidome according to the Pharmacopeia Argentina 6th Ed. Briefly: water extracted

material was filtered through Whatman 1 paper and centrifuged at 13.300 g, at 4 °C for 20 min. Solutions were lyophilized, dissolved in a minimum volume distilled water in order to achieve a solution, and were kept at -20° C.

Solutions were sterilized through a filter membrane (0.22 µm) before use.

The alcoholic extract was prepared at 20 % (w/v) in 96° ethanol according to the technique M of Pharmacopeia Argentina 6th Ed., concentrated in a rotatory evaporator until dryness, and dissolved as for aqueous extracts. The solvent was eliminated by vacuum evaporation and the residue was resuspended in an equal volume of 0.2 % DMSO. The preparation was shaken for 20 min at 40° C, and clarified by centrifugation at 2.310 g. The supernatant was sterilized through a filter membrane (0.22 µm) before use.

Total phenolic compounds (TPC) were determined with the Folin-Ciocalteu reagent, using coumarin as standard.

Microorganisms and Culture Media.

ATCC strains. Gram-positive: *Staphylococcus aureus* (29213), *S. aureus* (25923), *Enterococcus faecalis* (29212). Gram-negative: *Pseudomonas aeruginosa* (27853), *Escherichia coli* (25922).

Isolates. Strains isolated in the Hospital 'N. Avellaneda' SOPROSA, San Miguel de Tucumán. Gram-positive: *S. aureus* CNMS (F20), *S. aureus* (F7), *E. faecalis* (F208), *Enterococcus faecium* (F229). Gram-negative: *Morganella morganii* (F320), *E. coli* (F301), *Enterobacter cloacae* (F302), *Acinetobacter freundii* (F303), *Proteus mirabilis* (F304), *P.*

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aeruginosa (F305), *Klebsiella pneumoniae* (F310), *Serratia marcescens* (F313).

Strain preservation. In semisolid BHI, (Britania®) added with 1% glycerol, at -20°C. *Ps. aeruginosa*, was kept in sterile water with 1% glycerol at room temperature.

Positive controls. Amoxiciline and Ciprofloxacin (Amoxidal® inj. and Ciriax® i.v., Roemmers). Susceptibility test according to NCCLS (1997).

Agar dilution method (Qualitative). Constant volumes of successive dilutions of plant extracts were mixed with the culture medium (MH-agar) at 45° C, poured in Petri dishes (10 cm in diameter) and left to stand at room temperature. Then, 2 µl bacterial suspensions were seeded as points (1 x 10⁵ CFU/ml) on the medium surface. They were incubated at 37° C for 16-20 h. Results were read with respect to a growth control. The test was carried out in duplicate.

Microdilution test.

MIC determination. Sterile polystyrene 96 well microplates were prepared by dispensing serial dilutions of plant extracts or synthetic antibiotics, 50 µl of bacterial suspension (5 · 10⁵ CFU · ml⁻¹) in MH broth medium (Britania®) supplemented with CaCl₂ and MgCl₂ sterile solutions in order to reach 25 mg · l⁻¹ and 12.5 mg · l⁻¹ of Ca⁺² and Mg⁺² concentrations, respectively, as recommended by the NCCLS 1997, in a final volume of 100 µl. Microplates were covered with a sterile plate sealer, carefully mixed and incubated for 16-20 h at 37°C. Results were read with respect to the growth control. The test was carried out in quadruplicate.

MBC determination. 25 µl aliquots, taken from the MIC and neighbor wells, were streaked on Petri dishes containing MH agar medium, and incubated for 16-20 h at 37°C. Then, colonies were counted. Bacterial viability controls were made. The MBC was defined as the lowest concentration of assayed samples which produced 99.9% reduction in CFU · ml⁻¹ when compared with the control. This test was carried out in duplicate.

RESULTS AND DISCUSSION

MIC values were expressed in TPC (µg/ml). Within this great group of compounds are included secondary metabolites, such as phenolic

acids, quinones, catechins, flavones, flavonoids and flavonols, tannins, anthocyanins, etc., which are known by their interesting biological activities (Cowan, 1999). According to the extractive form, and to the plant part used, there are an increasing feasibility to find such compounds in these extracts. The antibacterial activity of organic extracts of 'guayacán' aerial parts has been once reported (Woldemichael *et al.*, 2003).

The macrodilution test showed that the three extracts of *C. paraguariensis* were active on all

tested bacterial species, and provided orientative data concerning effective extract concentrations for each bacterial species. The results of the microdilution test indicated how potent the extracts were against bacteria. The ranges of MIC and MBC values were: infusion 39.76-634.25 and 79.53-1,272; decoction 78.28-1,252 and 78.28-1,252; tincture 30.7-982.5 and 61.4-491.25; respectively. Positive controls: amoxiciline 0.3 ≥ 640 and 1.2-640, ciprofloxacin 0.015 ≥ 128 and 0.12-128.

Antibacterial power varied according to the considered species. Aqueous extracts showed similar MIC values, which suggested a similar composition. When evaluating the MIC values of the positive controls, amoxiciline and ciprofloxacin (wide-spectrum therapeutic agents of different ways and action mechanisms), most Gram negative tested species, were resistant to amoxiciline. *P. mirabilis* (F304) and *Ps. aeruginosa* (F305) were resistant to ciprofloxacin while plant extracts showed an overwhelming activity on this latter group. Gram-positive species were more susceptible to the extracts than Gram-negative species, likewise against amoxiciline, and in general were very sensitive to ciprofloxacin.

CONCLUSIONS

The extracts of *C. paraguariensis* showed bacteriostatic and bactericidal *in vitro* activity on the tested bacterial species.

The antibacterial properties of the extracts suggested potential uses as anti-infectious agents and as preservatives of medicines and food.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y

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REFERENCES

Woldemichael G. M., Singh M. P., Maiese W. M. and Timmermann B. N. (2003) Constituents of antibacterial extract of *Caesalpinia paraguariensis* Burk. *Z. Naturforsch.* **58c**, 70-75.

Cowan M. M. (1999) Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews* **12**: 564-582.

NCCLS (National Committee for Clinical Laboratory Standards) (1997) *Specialty Collection: Susceptibility Testing*. SC21-L. M7-A4. NCCLS, Wayne, PA.

British Society for Antimicrobial Chemotherapy (1991) A guide to sensitivity testing. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy* **27**, Suppl. D: 1-50.