



Tripodanthus acutifolius and *Psittacanthus cuneifolius*: Loranthaceae. Sources of natural antimicrobials

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INTRODUCTION

From ancient times humanity knows the benefits of the medicine of natural origin. The knowledge of these sources of medicinal substances is transmitted from one to another generation, thus spreading the uses of diverse extractive forms, obtained from different plant parts. Microbial infections are one of the main causes of mortality and morbidity all over the world according to WHO reports. The development of microbial resistance to conventional therapies has been increased during the last decades. These facts support the importance of antimicrobial activity studies, and the requirement alternative sources for this therapy that could contribute to fight against microbial infections. The purpose of this work is to comparatively study the antibacterial activity of three different extractive forms prepared from *Tripodanthus acutifolius* (Ruiz & Pav.) Blume and *Psittacanthus cuneifolius* (Ruiz & Pav.) Van Tieghem, two members of the Loranthaceae family. Commercial antibiotics were included in order to extent the comparative study.

METHODOLOGY

Plant species were collected in the Tucumán province, in the northwestern Argentina, taxonomically identified, and voucher specimens were deposited in the herbarium. Plant material was dried and ground to a coarse powder. The leaves of each species were subjected to three different extractive forms (according to popular uses): infusions, decoctions and tinctures according to Farmacopea Argentina 6th ed. The extracts were dried and the obtained material represented the extracted material (EM). Phenolic compounds content of each extractive form was determined according to Singleton *et*

al., 1999, using coumarin as standard. Imipenem, oxacillin, cefotaxime, vancomycin and ampicillin were used as commercial reference antibiotics. Antimicrobial tests included eight Gram-negative strains: *Escherichia coli* (301), *Enterobacter cloacae* (302), *Acinetobacter freundii* (303), *Proteus mirabilis* (304), *Pseudomonas aeruginosa* (305), *Klebsiella pneumoniae* (310), *Serratia marcescens* (313), and *Morganella morganii* (320); and four Gram-positive strains: *Staphylococcus aureus* (F7), coagulase-negative and methicillin-sensitive *Staphylococcus aureus* (F20), *Enterococcus faecali* (F208), and *Enterococcus faecium* (F229), isolated from superficial wounds (Hospital 'Nicolás Avellaneda', SIPROSA, Tucumán) and five American Type Culture Collection (ATCC) strains: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterococcus faecali* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213) and *Staphylococcus aureus* (ATCC 25923). The strains were incubated in MH agar (24 h, 37 °C), thereafter suspensions of each strain were prepared in sterile normal saline (SNS) up to the 0.5 value of McFarland scale, which were then diluted with SNS up to 10⁷ CFU/mL, the so called working suspensions (WS), before each assay. The performed tests were:

-Agar macrodilution: Test was performed in Petri plates (90 mm diameter), using MH agar with the addition of serial dilutions of each extractive form. Two µL of each WS were inoculated in fixed points. After incubation at 37°C for 24 h, each plate was examined and the growth of each inoculated point was evaluated in comparison with growth controls (MH agar



plates without extract). Antibacterial activity was determined by the absence of growth. The assays were performed by triplicate for each extractive form at each concentration level.

- Broth microdilution. Test was performed in 96-well plates, using 50 μL of bacterial suspensions ($5 \cdot 10^5$ CFU/mL) in MH supplemented broth (with CaCl_2 and MgCl_2) with the addition of serial dilutions of either each extractive form or commercial antibiotic in a final volume of 100 μL . Growth controls (without extract or commercial antibiotic added) were also included. After incubation (24 h, 37°C) the plates were examined. The absence of growth indicated antibacterial activity. The minimum inhibitory concentration (MIC) was defined as the minimal plant extract or commercial antibiotic concentration that inhibits bacterial growth. A 25 μL aliquot was inoculated in MH agar, incubated at 37°C for 24 h, and the minimum bactericide concentration was determined (minimum plant extract or commercial antibiotic concentration that produced 99.9% reduction in the CFU/mL, in comparison with growth controls). All tests were performed by triplicate for each extractive form or commercial antibiotic.

RESULTS AND DISCUSSION

Macrodilution assays were carried out as screening tests, and these results led to microdilution tests. The latter allowed to obtain precise MIC and MBC values. The results showed that plant tinctures studied with the highest antibacterial activity, regarding the aqueous extractive forms (what is evidenced in inferior MIC/MBC values). The tinctures of both plants inhibited the growth of the 17 strains studied in either the same concentrations or higher than 101 μg EM/mL. *T. acutifolius* aqueous extracts (infusions and decoctions) higher antibacterial activity than those of *P. cuneifolius*, the former were active against the 17 tested strains either at the same concentrations or higher than 126 μg EM/mL. *P. cuneifolius* infusions exhibited neither bacteriostatic nor bactericide activity against 5 Gram-negative and 2 Gram-positive strains, and bactericide on *Proteus mirabilis* (Gram-negative) and three strains of *Enterococcus* sp. (Gram-positive). This extractive form only showed activity either at the

same concentrations or higher than 1595 μg EM/mL. The antibacterial activity profile of *P. cuneifolius* decoction was similar to that of the infusion, being active either at the same concentrations or higher than 607 μg EM/mL. Data obtained by microdilution assays demonstrated that MIC and MBC values of the plant extracts are, in general, higher those values obtained with reference antibiotics. However in some cases were inferior, e.g., MIC values for *T. acutifolius* aqueous extracts against *A. freundi*, inferior than those of cefotaxime (a wide-spectrum *beta*-lactamic antibiotic).

CONCLUSIONS

The results indicate that *T. acutifolius* and *P. cuneifolius* represent interesting sources of natural antibiotics, with an activity comparable to that of commercial antibiotics. Our study also demonstrated the importance of the extractive form used, since the biological activity is strongly affected by solvent and the applied extraction method. The antimicrobial activity of the studied plants could be partially attributed to their relationship, since both belong to the Loranthaceae family.

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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