B197 Screening of antibacterial active extracts obtained from Amazon rain forest plants

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Seven hundred and five organic and aqueous extracts obtained from 429 plants belonging to 70 different families native to the Amazon rain forest were submitted to a screening against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. Plants were collected according to a phytochemical and chemosystematic approach. They were dried and ground and macerated with methanol : dichloromethane (1:1) in order to obtain the organic extract. A following water maceration was done so as to result two extracts from each plant material. Extracts were prepared to 20 times the desired test concentration (2 mg/mL) in water or DMSO 50%. Broth microdilution method was performed to evaluate the antimicrobial activity of the extracts. The bacterial inoculum of each ATCC strain were obtained from fresh colonies in blood agar plates. They were initially prepared to a concentration of 1.5 x 10^8 CFU/mL and were then diluted to 1,5 x 10^2 CFU/mL. From these diluted bacteria suspensions, 190 µL were transferred to each well of the microplates. Ten µL of the extract solutions were added to the wells and the microplates were then incubated at 35º C, for 18 to 20 hours. Results were visually analyzed.
Two out of the 705 extracts showed activity against *E. faecalis*: organic extracts obtained from aerial parts of *Rapanea* sp. and organic extract obtained from aerial parts of *Smilax* sp. One extract showed activity against *S. aureus*: organic extract obtained from stem of *Ruizterania* sp. MICs were determined (<100 µg/mL each extract).

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B198 Alphitolic acid: an unusual triterpenoid from leaves of *Bixa orellana* and evaluation of its antifungal activity

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Bixa orellana L. is a reputed traditional remedy used for several purposes by native people in Latinoamerica. The main of these is the aplication of "annato", a kind of paste obtained with the aril of the seeds, as a dermatological protection and treatment of skin diseases.
In a previous screening (1) antifungal activity was detected on dichloromethane and methanol extracts from leaves of *B. orellana* using agar disk diffusion assay. With the aim of isolating the active compounds, dichloromethane extract was obtained in a Soxhlet apparatus and submitted to a biossay-guided fractionation. It was separated by MPLC on Si60 eluting with a gradient of hexane-AcOEt-MeOH (1:0:0 to 0:1:0 to 0:0:1). Fraction VI inhibited the growth of *Microsporum gypseum* CECT 2908 and *Trichophyton mentagrophytes* CECT 2795 in an agar overlay bioautographic method. HSCCC and CC on Sephadex® LH-20 were used to isolate the active compound from fraction VI. Its structure was elucidated by standard spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, DEPT, H,H-COSY, HSQC, HMBC, EI-MS, CI-MS and IR) and by comparison with our own triterpene database. The final elucidation of the active compound was performed by comparison of its NMR data with those of the related compounds betulinic and goreishic acid and identified as alphitolic acid (2α,3β-dyhidroxy-20(29)-lupen-28-oic acid), a pentaciclic triterpene.
Alphytolic acid occurs rarely in nature. It was previously isolated from *Zizyphus joazeiro* and *Licania heteromorpha* and its antifungal activity was established against *Candida albicans* (2).

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