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Antibacterial activity of lecanoric acid isolated from *Parmelia flaventior*

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INTRODUCTION

Many lichens families date from de mid-nineteenth century, when grow form was considerate an important character. Those lichens produce a large number of secondary aliphatic and phenolic metabolites[1]. Lichen substances haven proven antibiotic which may account for their resistance to attacks by bacterial or fungal pathogens in nature[2,3].

Parmelia flaventior is an interesting lichen specie, greenish to greyish in colour which grow commonly in the central of Mexico. During a study on the antimicrobial activity of Mexican lichen species, the acetone extracts of the lichen *Parmelia flaventior* showed a broad spectrum of antibacterial activity. By column chromatography of the acetone extract an active constituent, lecanoric acid, was found to be present.

The activity of Lecanoric acid was tested against 5 Gramm-positive bacteria, *Staphylococcus aureus* ATCC25923 and ATCC 6538, *Streptecoccus pyrogenes* B hemolytic (type B), *Streptococcus faecalis* and *Bacillus subtilis*; and 3 Gramm-negative bacteria, *Escherichia coli* ATCC 25922 and *Vibrio cholerae* 01 Ogawa and Inaba serotypes. Among the Gramm-positive bacteria, lecanoric acid was found to be very effective (12-27 mm) against all bacteria and minimum inhibitory concentration (MIC) of 128 mcg/ml.

As it was well know the compounds isolated from lichen species are ineffective against Gramm-negative bacteria, however, lecanoric acid was found very effective against *E. coli* (12 mm) and *Vibrio cholerae* 01 Inaba serotype (15 mm) at a MIC of 128 mcg/ml

MATERIALS AND METHODS

Parmelia flaventior was collected at Sierra de Guadalupe, Las Cabañas, Estado de México, México on september of 2008. The plant was authenticated and a voucher specimen (MEXU-EA1) was deposited at the Instituto de Biologia, Universidad Nacional Autónoma de México, México. 0.5 kg of the lichen was exhaustively extracted with acetone and the solvent removed under reduced pressure to afford an extract (20.0 g) which was chromatographed on 200g silica gel using Hexane/ ethyl acetate mixtures as eluting solvents and monitoring by TLC. Hexane: ethyl acetate (70:30) eluted band A which, on crystallization in hexane/ ethyl acetate, gave the white crystals of lecanoric acid (5.0 g, 1 %).pf 180-181 °C[4].

Identification of the compound was made in comparation with the physical and spectroscopic constants which are in agreement with those as lecanoric acid was based on melting point and IR, ^{1}H-NMR,^{13}C-NMR, MS spectral data. The antimicrobial activity of lecanoric acid was tested in triplicate by the disc diffusion technique and the dilution method[5], using Muller-Hinton agar, sterile disc (6 mm diameter) impregnated with 25 mcl (1.78 mg) of lecanoric acid which were

prepared from stock solution of 71.4 mg/ml on acetone/ destilled water 50:50, and oven dried at 35 °C during 30 min. Bacterial strains were incubated for 2-3 h. at 37 °C to obtain 1 X 10^8 UFC/ml for plate inoculation. Plates were incubated at 37 °C for 24 h. for after which inhibition zones were measured.

Different dilution (0.1, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 mcg/ml) were used for calculating the minimum inhibitory concentration (MIC). Inoculum was incubated for 12 h. and spot inoculated with 2 l of culture (10^4 UFC)

RESULTS

Bacteries	Inibition (cm)
Bacillus subtilis	1.2
S. aureus ATCC 25923	1.8
S. aureus ATCC 6538	2.7
E. coli	1.2
Vibrio ch. Inaba	1.5
Vibrio ch. Ogawa	1.3
St. pyogenesβ-hemolítico(B)	2.6
St. faecalis	1.5
Salmonella typhi	0.0

CONCLUSIONS

The obtained results are encouraging to be used as reference in the employment of the dose with antimicrobial action of this product, the minimun inhibition concentration is 128 mcg/ml for V. cholerae and E. coli, and is between 256 and 512 mg/ml for the rest of microbial agents tested.

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