TITLE: SUSCEPTIBILITY OF *Mycobacterium phlei* TO LICHEN EXTRACTS IN DISK DIFFUSION TESTS: INTERFERENCE OF SOLVENTS USED IN SOLUBILIZATION OF EXTRACTS

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ABSTRACT

The disk diffusion test is one of the most used to evaluate the effect of antibiotics on bacteria of clinical interest. There are several factors that can affect the results of this test such as the culture medium, inoculum concentration and incubation conditions. In addition, the search for antimicrobial substances in extracts of plants and other organisms should consider other factors such as the extract concentration and the solvents employed in its solubilization, since the diffusion capacity of the substances contained in extracts depend not only its molecular weight and its appropriate solubilization, but also the diffusion capacity of the solvent. This study aimed to verify the influence of solvents used for solubilization of organic extracts of *Cladina dendroides* in antimicrobial tests disk diffusion. Organic extracts were obtained with ether, chloroform and acetone and tested against *Mycobacterium phlei*. Paper disks of 6mm in diameter were impregnated with 20uL of each extract at a concentration of 2mg/mL solubilized in the respective solvents used to obtain the extract. Simultaneously organic extracts were also solubilized in DMSO at the same concentration and impregnated in paper disks with the same diameter. Thus there was obtained 2 disks for each extract, a solubilized in the extractor solvent and another in DMSO. As a negative control were used disks impregnated with solvents employed in solubilizing the extracts and as positive control commercial disks of amikacin. All discs were deposited on Petri plates containing Mueller Hinton agar medium with inoculum *M. phlei* at a concentration of 107CFU/mL. The plates were incubated at 36oC for 24h and the results evaluated by formation and size of the halos of inhibition around the disks. It was observed that the ether, chloroform and acetone extracts solubilized in DMSO inhibited the growth of *M. phlei* with halos 14mm, 15mm and 12mm, respectively. However, the same extracts solubilized in the solvents extractors were inactive against bacterial strain evaluated. This demonstrates that the solvent used in antimicrobial test disk diffusion to solubilize extracts should be selected carefully because it may lead to wrong results. In this study, the *C. dendroides* extracts produced different results, depending on the solvent used for their solubilization, with positive antibacterial action when solubilized in DMSO and negative when solubilized in other solvents tested.

Keywords: Lichen extracts, Disk diffusion test, *Cladina dendroides*

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