Presence of Antifungal Principles in Ethanol Extracts of *Larrea divaricata* Cav.

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In nature a large number of different types of compounds play an important role in the natural defense of living organisms.

Plants synthesize a vast array of secondary metabolites as defense mechanism for protecting themselves against pathogen infections. These metabolites are therefore gaining importance for their possible biotechnological applications.

Previously we reported a screening of ethanol extracts of some selected plants for their *in vitro* antifungal activities. These plants are used in the popular medicine of the northwest of Argentina because of their vulnerary and antiinflammatory properties. Among them *Larrea divaricata* Cav. inhibited the growth of yeasts and some filamentous fungi.

In the present study we report results on biocompounds with antifungal properties isolated from *L. divaricata* ethanol extracts.

Plant material *of Larrea divaricata* (family Zygophyllaceae) was collected in Amaicha del Valle, Tucumán, Argentina. Fresh plants were air-dried and ground to a coarse powder.

Microorganisms: We used four wood-destroying fungi and four phytopathogenic molds. We also assayed the antifungal activities against yeast.

Ethanol extract: Dry plant material (10 g) was macerated in 100 ml of EtOH 96° for 48 hr at 30°C in a shaker (40 cycles/min).

Fractionation and isolation of active compounds: Chromatographic fractionation of phytochemicals were performed by:

(a.i) Ascendant and bidimensional thin-layer chromatography (TLC) on Kiessel-gel 60 F_{254} plates (Merck). The solvent was toluene–ethyl acetate formic acid (4:5:1).

(a.ii) The best separation was achieved on a column packed with Kiessel-gel (200–400 mesh). Different eluents such as toluene, ethyl acetate, chloroform, and methanol were used.

Antifungal suceptibility tests:

(b.i) Hyphal radial growth rate of filamentous fungi and the percentage of growth inhibition was evaluated.

(b.ii) Broth dilution test in a 96-well microtiter plates.

(b.iii) Bioautography that combines TLC with a bioassay *in situ*.

(b.iii) Zonal hyphal inhibition.

Three different fractions (Fr. A, Fr. B, and Fr. C) were isolated as the main components from *L. divaricata* extracts after TLC analysis. Among them only Fr. B showed a growth inhibitory effect toward various fungi.

With the aim of preparing higher amounts of the bioactive fraction (Fr. B) a column chromatographic fractionation of the extract on Kiessel-gel was performed. The MeOH eluate had the antifungal activity. This fraction was further fractionated by TLC from which we isolated a fraction (Fr. 5) with antifungal activity against *A. niger* Tiegh. and *Trichoderma* spp. These results were obtained by bioautographic analysis.

This report is a first step in a long process toward the examination and elucidation of compounds with fungistatic activity found in ethanol extracts of *L. divaricata.*

Our data suggest that *L. divaricata* contains at least an antifungal agent against yeasts and filamentous fungi. The extract of *L. divaricata* can be recommended as a supplement to casing or substrates for fructification after additional investigation of antifungal activity on Higher Basidiomycetes mushrooms.

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