Comparative Study of the Antifungal Potential of the Lichen Extracts of Stereocaulon sp. and Cladonia sp.

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ABSTRACT: Lichens produce compounds with inhibitory effects on some pathogenic microorganisms. In this survey, we evaluate the inhibitory effects of seven different lichen extracts of the genera Stereocaulon and Cladonia on the growth of yeast Saccharomyces cerevisiae and green mold Penicillium digitatum. The extracts that produced the greatest inhibition were those of ethanol, isopropanol, chloroform, ethyl acetate, and acetone while the aqueous and hexane extracts were less effective. Extracts of Stereocaulon sp. had greater inhibitory effect on fungal growth compared to Cladonia sp. Both species of lichens present secondary metabolites with antifungal activity.

KEYWORDS: Antimycotic activity; Growth inhibition; Lichen; Mold; Secondary metabolites.

Introduction. Microorganisms such as fungi and bacteria develop antimicrobial resistance[1,2]. This creates the need to search new compounds to control the pathogens of animals and plants. Additionally, the use of synthetic pesticides in agriculture is being restricted due to their harmful effects on the environment and human health[1,2,3,4]. New compounds have been created from natural sources such as plants, algae, fungi and lichens; the latter are especially interesting from a biochemical viewpoint due to their particular adaptations to hostile environments[5,6].

Lichens are fungi living in close symbiosis with a photosynthetic organism, such as microalgae or cyanobacterium. The fungus component of this association is the mycobiont and the photosynthetic organism is the photobiont[7,8]. This symbiosis has allowed lichens to colonize all over the planet, even extreme habitats like Antarctica, deserts, and high mountains[13,14].

The lichen body is the talus. There three main forms are as follows: crustose lichens form crusts on the substrate, foliose lichens resemble leaves, and fruticose lichens have erect or decumbent growth[9,10,11]. Most lichens form an internally stratified talus consisting of several ordered layers including the upper cortex, the layer of the photobiont, the medulla, and the lower cortex. The photobiont is protected by a fungal layer. Most of their secondary metabolites, collectively known as lichen compounds, accumulate in the medulla[11]. Lichens, along with bryophytes such as mosses and liverworts, play an important role in ecosystems as they capture nitrogen and carbon and create microhabitats favorable to organisms like amphibians and insects[12]. Lichens are excellent indicators of an ecosystem's health due to their high sensitivity to air pollution and habitat destruction[9,15,16].

Lichens produce compounds that protect them from adverse physical and biological factors such as herbivorous animals and pathogens[10,11]. Studies have demonstrated these metabolites have antiviral properties and contain antibiotics, enzyme inhibitors, sunscreens, and growth inhibitors of plants and microorganisms[17,18,19,20,21,22]. Some of these substances correspond to the lichenic acids, the organic acids that allow lichens to degrade rock and make perforations for adherence while contributing to soil formation and the colonization of new areas. Some of these acids, like usnic acid and rhizocarpic acid, also act as photoprotective compounds[23,24]. Usnic acid is present in species of genera such as Usnea and Cladonia and has recognized antibacterial and antifungal properties[25,26,27].

To date, 1,383 species of lichen entailing 304 genera have been identified in Chile[28,29] but it is still necessary to increase our taxonomic knowledge of these lichen and to further characterize their biochemical properties to aid researchers obtain novel medicines or pesticides. The central-southern zone of Chile is especially diverse, but its lichen flora is only partially known and even less is known about their potential biochemical properties. In Chile, there are no bibliographic data on the use of lichen extracts in traditional medicine and the existing information in relation to metabolites with biological activity is extremely limited[30].

The species of two edaphic lichen genera of the order Lecanorales, Stereocaulon and Cladonia have been studied for the biological properties of their secondary metabolites. The genus Stereocaulon has a cosmopolitan distribution and includes approximately 125 species[31] and Cladonia includes over 450 species worldwide[32,33]. In Chile, the diversity of both genera is not fully known; only 7 species of Stereocaulon and 38 species of Cladonia have been documented in Aysén[34] and only two species of Cladonia have been reported in La Campana National Park[35]. The lichens of these genera have been used in folk medicine to treat fever, diarrhea, pains and wounds in other parts of the world[36]. Therefore, it is of interest to study the biological properties of these species against pathogenic microorganisms. The antimicrobial effect of the extracts of several species of both Stereocaulon and Cladonia have been tested on bacteria and fungi[36,37,38,39,40,41,42,43,44,45,46,47,48]. In Chile, the effect of Cladonia aff. rappii on yeasts has been evaluated[49].

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In this study, the biological activity of different lichen extracts of Stereocaulon sp. (Figure 1) and Cladonia sp. (Figure 2) were tested on the growth of baker's yeast (Saccharomyces cerevisiae) and green mold (Penicillium digitatum).

Figure 1. Stereocaulon sp. collected in Manquemapu locality.

Figure 2.. Cladonia sp. collected in El Sauce locality.

Results and Discussion. In the graphs, the bars followed by the same letters do not show significant statistical difference (p = 0.05).

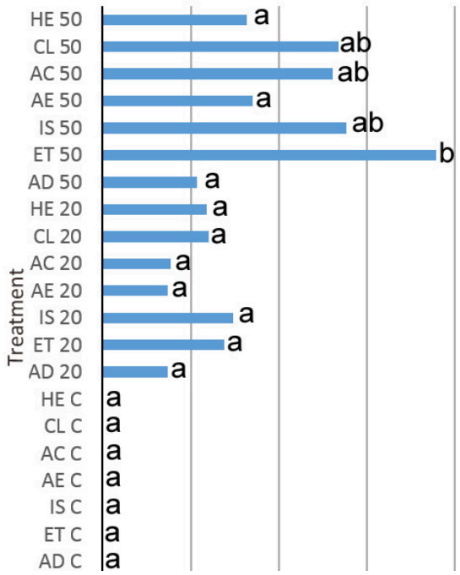

Figure 3. Inhibitory effect of Stereocaulon sp. extracts on S. cerevisiae. Solvents: distilled water (AD), absolute ethyl alcohol (ET), isopropyl alcohol (IS), ethyl acetate (AE), acetone (AC), chloroform (CL), and hexane (HE).

In the trials testing Stereocaulon sp. against S. cerevisiae, as shown in Figure 3, 50 µL ethanol extract (ET 50) is shown to be the best inhibitor since its growth inhibition halo has the largest diameter. ET 50 is followed by chloroform (CL 50), acetone (AC 50), and isopropanol (IS 50). In the experiment with Cladonia sp. extracts, as shown in Figure 4, the best performance is using chloroform (CL 50) and ethyl acetate (AE 50).

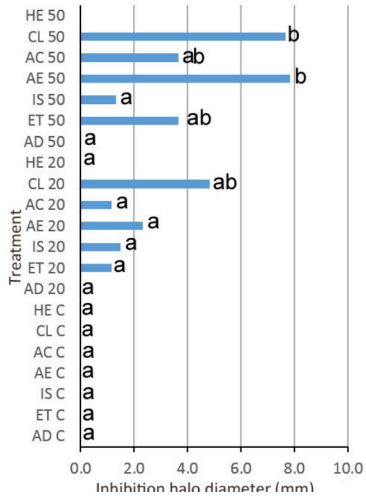

Figure 4. Inhibitory effect of Cladonia sp. extracts on S. cerevisiae.

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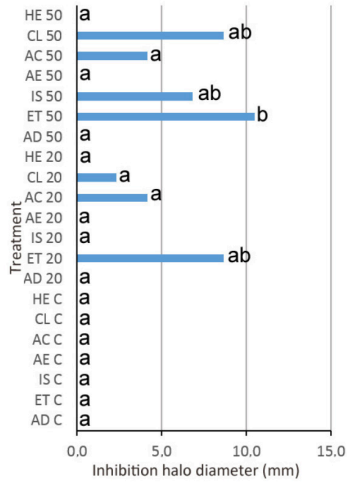

Figure 5. Inhibitory effect of Stereocaulon sp. extracts on P. digitatum.

For P. digitatum treated with Stereocaulon sp., the best performance was obtained with the ethanol extract (ET 50), followed by chloroform (CL 50) and isopropanol (IS 50). The 20 µL ethanol (ET 20) treatment was also very effective as it had a 9.0 mm halo diameter. This shows that P. digitatum is especially sensitive to lichen compounds dissolved in ethanol.

In the experiment with Cladonia sp. extracts, the best performance was observed with the isopropanol extract (IS 50), followed by ethanol (ET 50), chloroform (CL 50) and ethyl acetate (AE 50), as shown in Figure 5.

Aqueous extracts (AD 20 and 50) and hexanes (HE 20 and 50) had low performances in all experiments.

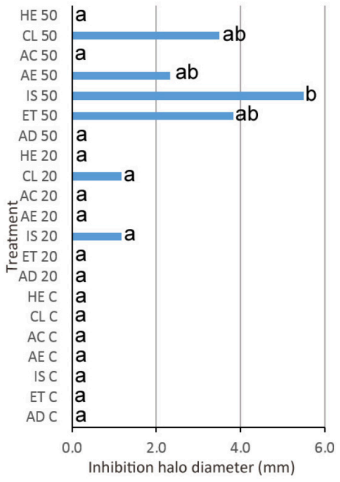

Figure 6. Inhibitory effect of Cladonia sp. extracts on P. digitatum.

Conclusion. The lichens of the genera Stereocaulon and Cladonia used in this project present substances that inhibiting the growth of S. cerevisiae and P. digitatum.

Extracts of Stereocaulon sp. had a better performance than the Cladonia sp. Extracts because they generated larger-diameter halos under the same cultivation conditions for both fungi.

Greater inhibition was observed in the treatments using a higher extract dose (50 µL) which shows the inhibitory effect is dependent on the extract concentration.

The solvents used have no inhibitory effect on fungal growth as demonstrated in the control treatments.

In all the experiments that utilized solid culture medium, the extracts with the greatest inhibition of fungal growth were those of ethanol, chloroform, and isopropanol while the hexane and aqueous extracts had the lowest performance. The ethyl acetate and acetone extracts had varied effects.

The lichen extracts of Stereocaulon sp. and Cladonia sp. have the potential to formulate phytosanitary products or drugs for controlling pathogenic fungi. For this, it will be necessary to determine the components of each extract.

Further research must be conducted to test these extracts on other fungal and bacteria species of agricultural interest as well as to determine the minimum dose necessary to inhibit microorganism growth and study the biochemical profile of each extract.

Methods. The research was carried from March to August 2018 in the teaching laboratory at the Complejo Educacional Chimbarongo in Chimbarongo, Chile.

Stereocaulon sp. was collected in the Manquemapu locality of Purranque, Chile and the Cladonia sp. was collected in a sclerophyllous forest located in El Sauce. The lichen material was dried for two weeks then crushed in a food processor.

The solvents used to make the extracts were distilled water, absolute ethyl alcohol, isopropyl alcohol, ethyl acetate, acetone, chloroform, and hexane. To make the extracts, 80 ml of each solvent was mixed with 20 g of lichen material and macerated for a week at 6 ˚C in hermetic glass jars then sieved and filtered.

The fungi were cultivated on Potato-Dextrose-Agar medium in 90 mm plastic Petri discs. Inoculation was carried out by applying 1 mL of physiological saline solution containing approximately 5,000 colony-forming units (CFU) of Saccharomyces cerevisiae and 3,800 CFU of P. digitatum onto the agar medium.

Mycological susceptibility tests were performed using the disk diffusion method in solid media using 6 mm diameter filter paper discs. In each Petri dish, 3 discs with 20 µL and 3 discs with 50 µL were placed. The Petri dishes were placed in a digital incubator at 25 ˚C for 72 hours.

Four experiments were carried out with 28 treatments. Doses of 0 (C = control), 20 and 50 µL of each of the seven types of extract were evaluated. The control treatment consisted of disks with 50 µL of each solvent. The growth inhibition halos were measured with a ruler for the data analysis.

A unifactorial analysis of variance with a level of significance of p = 0.05 was performed. The means were separated by the Tukey test when there was a significant difference at p = 0.05.

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