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Original Research Article

Antifungal Activity of Phytochemicals against Samples of *Cladosporium*

ABSTRACT

Cladosporium species are ubiquitous, saprobic dematiaceous fungi, associated with human and animal opportunistic infections. *Cladosporium* has been known to be one of the most airborne fungi causing respiratory allergies diseases, particulary asthma and rhinitis. Antifungal compounds of natural origin, such as terpenes, have received much attention in recente times. They are a promising therapeutic tool for treating fungal infections, and are known for their antimicrobial properties. **Aims:** In this context, the present study aims to evaluate the in vitro antifungal activity of eight phytochemicals commonly found in *Melissa officinalis* L. essential oil (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) against ten samples of *Cladosporium*. **Methodology:** Microbiological screening was performed with the phytochemicals at a concentration of 1.024 μg/mL. Microbiological screening was performed based on the broth microdilution technique. Laboratory tests were carried out at the Mycology Laboratory Department of Pharmaceutical Sciences, located in the Health Sciences Center (CCS) of the Federal University of Paraíba (UFPB). **Results:** Through analysis of results, it is observed that citral showed the best activities of the as samples of *Cladosporium* studied. **Conclusion:** citral representing a new possibility in the arsenal of products for treatment of fungal infections caused by these fungi.

Keywords: **Keywords:** *Phytochemicals; Citral; Antifungal; Cladosporium carrionii, Cladosporium oxysporum, Cladosporium sphaerospermum.*

1. INTRODUCTION

Fungal infections are becoming more frequent because of expansion of at-risk populations and use of treatment modalities that permit longer survival of these patients [1].

Cladosporium species are among the most common fungal inhabitants worldwide, being isolated from almost any environmental source and geographic location [2].

The most common *Cladosporium* species are primarily isolated from soil and plant material, where they are frequently encountered as saprobes or secondary invaders on follicular lesions, concomitant with other plant pathogenic fungi [2-4]. However, several species are important pathogens of plants and some are also able to affect animals including humans [5-7].

Cladosporium is usually associated with allergic rhinitis and asthma [8,9] or localized superficial or deep lesions [10-13], but rarely can cause disseminated infections [14,15]. They are difficult to treat due to long treatment periods, limited treatment options, resistance to common antifungal agents, and their greater prevalence among immunocompromised patients. All of these characteristics invite recurrences [16,17].

There exists a clear need for more, therapeutically effective antifungals. Actually, plants have been an interesting alternative to source of new biologically active compounds [18-20]. The plants produce numerous and varied organic compounds including monoterpenes and sesquiterpenes

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compounds present in essential oils, of which the majority does not directly participate in the plant's growth and development and are generally called secondary metabolites [21,22]

Melissa officinalis L., member of Lamiaceae family, is one of the well known aromatic medicinal plant species. The essential oil is a well-known antibacterial and antifungal agent [23-25]. There have been some previous reports on the chemical constitutions of *M. officinalis.* According to these studies, the major components of the essential oil of *M. officinalis* were citral (geranial and neral), and citronellal [25,26], (*B*)-caryophyllene [27], caryophyllene oxide [27-28], linalool [29], geraniol [30], thymol [31], α-pinene [27], β-pinene [28,32], carvacrol and *iso*-menthone [33].

Previous studies in our laboratory with *M. officinalis* L. essential oil showed strong antifungal activity of this oil against *Cladosporium carrionii* strains (Menezes *et al*., 2015). Therefore, the aim of the present work was to investigate the antifungal activity of eight phytochemicals commonly found in *M. officinalis* L. essential oil against strains *of Cladosporium.*

2. MATERIAL AND METHODS

2.1 Phytochemicals and Synthetic Antifungal

The phytochemicals (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) and amphotericin B (standard drug) were acquired from Sigma-Aldrich®. All them were dissolved in 2% Tween 80 (INLAB®) and up to 0.5 % dimethyl sulfoxide – DMSO (MERCK®) in sterile distilled water to obtain 1.024 µg/mL solutions.

2.2 Cladosporium Samples

For testing of antifungal activity were selected and used ten samples of *Cladosporium. Cladosporium carrionii* strains (URM 2871, 0212, CQ 02), *Cladosporium oxysporum* strains (URM 5234, URM 6056, URM 5412) and *Cladosporium spherospermum* strains (URM 5962, URM 5455, URM 5350, URM 6120) were taken from the Microorganisms Collection of the Mycology Laboratory, at the Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Paraíba, Brazil and from the Pernambuco (Brazil) Federal University, Biological Sciences Center – Mycology Department fungal collection (URM).

The samples were maintained on Sabouraud Dextrose Agar - SDA (DIFCO®) at room temperature (28° to 30℃) and under refrigeration (4℃).

2.3 Inoculum

Stock inoculate suspensions of the *Cladosporium* strains were prepared from 10-days old sabouraud dextrose agar (Difco Lab., USA) cultures grown at 28 ºC. Fungal colonies were covered with 5 mL of sterile saline solution (0.9 %), the surface was gently scraped with a sterile loop, and the saline solution with the fungal elements was transferred to a sterile tube. These suspensions were shaken for 2 min using a vortexer, and allowed to stand for 5 min to allow hyphal fragments to fall out of the suspensions so that the supernatant containing the conidia could be collected. Tubes containing the inocula were standardized to 0.5 McFarland scale (10^6 CFU/mL). Mold conidia were counted using a hemocytometer. The inocula of the conidial suspensions were adjusted using sterile NaCl 0.9 % to contain approximately 10^6 CFU/mL [34,35].

2.4 Antifungal Activity Screening

Microbiological screening was performed with the phytochemicals (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) at a concentration of 1.024 µg/mL. Microbiological screening was performed based on the broth microdilution technique [34,36]. Sterile 96-U-shaped-well microplates were used and each well of the plates contained 100 µL of Sabouraud dextrose broth - SDB (DIFCO®). Then, 100 µL of the products (2.048 µg/mL) were individually added to each line of wells, so that each line of wells corresponded to a phytochemical tested. Finally, 10 µL of fungal inoculum of each strain of *Cladosporium* were added to wells, so that each column corresponded to a strain. The

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microplates were incubated at 28 ℃ being selected those phytochemicals who showed better inhibition profile visual growth of microorganisms after seven days incubation. The standard antifungal was amphotericin B (1.024 µg/mL). Negative control (without drugs) was performed to confirm the viability of the sporangiospores. Sensitivity control for Tween 80 and DMSO was also performed.

3. RESULTS AND DISCUSSION

The results of the microbiological screening of phytochemicals against *Cladosporium* strains are summarized in Table 1.

In 1.024 µg/mL, the concentration of phytochemicals used, it was found that citral showed better antifungal activity, inhibiting the growth of 90 % of the *Cladosporium* strains tested. Resistant strains with only the *C. sphaerospermum* URM 5962. The phytochemicals (+) citronellal, linalool and α-pinene were able to inhibit the growth of at most 3 strains. The (-) citronellal inhibited the growth of 2 strains (*C. carrionii* URM 2871 and *C. carrionii* CQ 02), the β-caryophyllene and geraniol, inhibited only strains *C. sphaerospermum* URM 5962 and *C. carrionii* URM 2871, respectively and β-cymene was not able to inhibit the growth of any of the strains tested, at this concentration.

The term phytochemical relates to chemical compounds, non-nutritive, which naturally occur in plants and exhibit biological activity [37]. Studies involving phytochemicals are of great importance, because they facilitate the utilization of individual components, instead of a mixture like in essential oils, giving more predictability and probably less collateral effects. Several studies point to the various activities of phytochemicals, which are: antimicrobial, antioxidant, anti-inflammatory, analgesic, cardioprotective, anti-hemorrhagic, hepatoprotective, antitussive, antitumor, immunostimulating, anticancer, antiviral, among other [38-44].

Citral (3,7-dimethyl-2-6-octadienal) is the name given to a mixture of two geometric isomers: (2E)-3,7-dimetilocta-2,6-dienal (geranial, *trans*-citral, citral A) and (2Z)- 3,7-dimetilocta-2,6-dienal (neral, *cis*-citral, citral B), which are acyclic α, β-unsaturated monoterpene aldehydes that occur naturally in many essential citrus fruit oils and in other herbs or spices [45].

The citral aroma is stronger and sweeter than that of lemon [46]. Geranial has a strong lemon odor while neral has a sweeter, yet less intense lemon odor. Due to its characteristic lemon aroma, citral has become a flavoring substance of great importance, a heavily used rawmaterial for the pharmaceutical, food, perfume, and cosmetics industries [47,48]. Also it has emerged as the active component of citrus essential oils against pathogens [49].

Citral was reported by presenting antifungal activity [50-52], antibacterial [53,48], anti- *Leshimania* [54] anti-*Trypanosoma cruzi* [55] and insecticide [56].

In the present study, citral showed activity against *Cladosporium* strains, confirming the results obtained in previous studies. Zheng *et al.* [57] demonstrated the antimicrobial activity of citral front of fungal strains of *Penicillium digitatum*. Such phytochemical has brought an action against strains of methicillin-resistant *Staphylococcus aureus*, *Penicillium italicum* and *Rhizopus atolonifer* [58]. In recent studies, citral showed in vitro antifungal potential against strains of *Candida albicans* [59].

Knowing that there are few studies on the activity of essential oils and their phytochemicals in demáceos fungi, particularly on fungi of the Cladosporium genus, and that caused them infections are increasingly common around the world, this work will enable a contribution to scientific research, in respect to the pharmacological research of new antifungal products derived from natural products against these fungi.

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Table 1. Antifungal activity of phytochemicals against samples of Cladosporium- microdilution technique.

Microorganisms	Phytochemicals (1.024 µg/mL)									
	citral	(-) citronellal	(+) citronellal	β-caryophyllene	geraniol	linalool	β-cymene	α-pinene	**Amphotericin B**	**Strain control**
C. carrionii URM 2871	-	-	+	+	-	+	+	-	-	+
C. carrionii 0212	-	+	+	+	+	+	+	+	+	+
C. carrionii CQ 02	-	-	+	+	+	+	+	+	-	+
C. oxysporum URM 5234	-	+	+	+	+	+	+	+	-	+
C. oxysporum URM 6056	-	+	+	+	+	-	+	+	+	+
C. oxysporum URM 5412	-	+	+	+	+	-	+	+	-	+
C. sphaerospermum URM 5962	+	+	+	-	+	-	+	-	-	+
C. sphaerospermum URM 5455	-	+	-	+	+	+	+	+	-	+
C. sphaerospermum URM 5350	-	+	-	+	+	+	+	-	-	+
C. sphaerospermum URM 6120	-	+	-	+	+	+	+	+	+	+

(+): Microbial growth in culture medium (-): Absence of microbial growth

4. CONCLUSION

The results obtained in this study show the pharmacological potential of plant products, particularly, the antifungal potential of citral against *Cladosporium*. The Citral could appear as promising compound to be inserted in pharmaceutical formulations applied to control the survival and dissemination of etiological agents of superficial or systemic opportunistic mycoses. Moreover, the results of this study show the necessity of accomplishment of researches addressed to the evaluation of antimicrobial properties of this phytochemical in different pathogenic microorganisms.

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