



Antifungal Action of the Dillapiole-rich Oil of *Piper aduncum* against Dermatmycoses Caused by Filamentous Fungi

**Roseane G. Ferreira¹, Marta C. Monteiro¹, Joyce Kelly R. da Silva²
and José Guilherme S. Maia^{1,3*}**

¹Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Pará, 66075-110 Belém, PA, Brazil.

²Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Pará, 66075-110 Belém, PA, Brazil.

³Programa de Pós-Graduação em Recursos Naturais da Amazônia, Universidade Federal do Oeste do Pará, 68035-110 Santarém, PA, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JGSM and RGF designed the study, wrote the protocol and wrote the first and final draft of the manuscript. Authors RGF, MCM, JKRDS and JGSM managed the literature searches, analyses of the study, performed the spectroscopy analysis and managed the biological experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims and Study Design: *Piper aduncum* L. is a Brazilian plant with many biological properties attributed to its dillapiole-rich essential oil. Despite the development of antibiotics, bacterial and fungal infections are still a public health issue in the medical field. This study measured the antimicrobial activity of the dillapiole-rich essential oil of *P. aduncum* against pathogenic skin microorganisms.

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*Corresponding author: E-mail: gmaia@ufpa.br;

University of Pará, Brazil. This work was performed in 2014.

Methodology: Gas Chromatography (GC) and Gas Chromatography-Mass spectrometry (GC-MS) have analyzed the oil and its dillapiole-rich fraction. The determination of Minimum Inhibitory Concentration (MIC) and Minimum fungicidal concentration (MFC) values was carried out by microdilution method and counting of formed colonies.

Results: For the strains of *Trichophyton mentagrophytes* (ATCC 9533 and clinical isolate), the oil and its dillapiole-rich fraction exhibited MIC values of 500 µg/ml while the MFC values were 1,500 µg/ml for the oil and 1,000 µg/ml for the fraction rich in dillapiole. For clinical isolates of *T. rubrum* and *Epidermophyton floccosum*, MIC values of 500 µg/ml and MFC 1,500 µg/ml were equal for the oil and the dillapiole-rich fraction, respectively. For clinical isolates of *Microsporum canis* and *M. gypseum*, the MIC and MFC values were 250 µg/ml and 500 µg/ml, respectively. For strains of *Aspergillus fumigatus* (ATCC 40152 and clinical isolate), the oil and its dillapiole-rich fraction have shown the same MIC value of 3.9 µg/ml while the MFC values were 7.8 µg/ml for the strain ATCC 40152, and 15.6 µg/ml for the clinical isolate. The oil and dillapiole-rich fraction did not show antibacterial activity against the strain of *Staphylococcus aureus* ATCC 6538 and its clinical isolate.

Conclusion: The dillapiole-rich essential oil of *P. aduncum* and its dillapiole-rich fraction demonstrates significant antifungal activity against dermatophytes, filamentous fungi and potent antifungal activity against non-dermatophyte filamentous fungi.

Keywords: *Piper aduncum*; *Piperaceae*; dillapiole; *in vitro* antimicrobial activity; dermatophytes.

1. INTRODUCTION

Despite the development of antibiotics, bacterial and fungal infections are still a public health issue in the medical field. The presence of numerous strains resistant to current drugs continues to represent a significant challenge. Medicinal Herbals have been used for this purpose, for many centuries. More recently, there has been an increased interest in natural products due to their availability, reduced side effects, toxicity, and greater biodegradability, if compared with available antibiotics [1]. In this regard, the essential oils of plants can offer a significant therapeutic potential when used alone or in combination with traditional antibiotics.

Piper aduncum L. is a widespread shrub growing wild in the Amazon, known as “pimenta-de-macaco” [syn. *Artanthe adunca* (L.) Miq., *A. elongata* (Vahl) Miq., *Piper aduncifolium* Trel., *P. angustifolium* Ruiz & Pav., *P. elongatum* Vahl, *Steffensia adunca* (L.) Kunth, *S. elongata* (Vahl) Kunth, among many other] [2]. With respect the traditional use, tea leaves of *P. aduncum* occurring in the Amazon region are used against gynecological inflammation, intestinal disorders, as diuretic, to treat pyelitis, cystitis, erysipelas and wound healing [3]. The main constituent found in the leaf essential oil of *P. aduncum* is dillapiole, a phenylpropanoid derivative that ranges from 31% to 97% for plants sampled in tropical areas of North Brazil, Equador, Costa Rica and Cuba [3-6].

The leaf extract and essential oil of *P. aduncum* were active against protozoa parasites and diverse microorganisms, including pathogenic bacteria and fungi, showing properties as antimicrobial, molluscicidal and cytotoxic [7]. Also, the oil of *P. aduncum* showed to be toxic and insecticide against *Ostrinia nubilalis* (a lepidopteran corn borer), *Anopheles marajoara* and *Aedes aegypti* (the mosquitoes that transmit malaria and dengue), and *Solenopsis saevissima* (a painful fire ant). These biological actions were attributed to the dillapiole, its main constituent [8-10]. Dillapiole was also reported acting as a synergist of natural insecticides, including carbamates, organochlorines, pyrethrum, tenulin, and azadirachtin [11,12]. The oil of *P. aduncum* showed antifungal activity against *Crinipellis pernicioso* and *Fusarium solani* f. sp. *Piperis*, two phytopathogenic fungi, which infects the cocoa and pepper crops in the Brazilian Amazon [13,14]. The toxicological evaluation of the oil of *P. aduncum* had low toxicity in mice (LD₅₀ 2,400±191.7 mg/kg) [15].

Diseases caused by dermatophytes generate a large public health problem, leading to reduced quality of life of people affected and the high cost of treatment with synthetic antimicrobials that, in turn, have some drawbacks such as high toxicity, side effects and the emergence of resistant strains [7,16]. Dermatomycosis is a common injury that affects humans and animals. It is often caused by dermatophytes of the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Usually, it infects skin, nails and

scalp, causing lesions characterized by skin irritation, peeling, redness, swelling and inflammation [17,18].

Thus, in view of the significant biological action demonstrated by *P. aduncum* oil, in the present work we decided to evaluate this oil and dillapiole, its main constituent, as antimicrobial agents with therapeutic alternative of low cost and low toxicity, against the microorganisms *Candida albicans*, *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. gypseum*, *Epidermophyton floccosum*, and *Aspergillus fumigatus*. Also, the oil composition of *P. aduncum* and its dillapiole-rich fraction were analyzed by GC and GC-MS.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts (leaves and thin stems) of *P. aduncum* were collected in the city of Santo Antonio do Tauá, the state of Pará, Brazil, in a particular agronomic experiment with plant matrices. Specimens were deposited in the Herbarium of the Emilio Goeldi Museum, the city of Belém, the state of Pará, Brazil, under the number MG150678.

2.2 Plant Processing and Extraction of the Essential Oil

The plant material was air dried for 48 h, ground and submitted to hydrodistillation using a Clevenger-type apparatus (100 g, 3 h). The oil was dried over anhydrous sodium sulfate, and its percentage content was calculated based on the plant dry weight. The moisture content of the plant was calculated using a balance with moisture measurement by infra-red. The oil was kept in an amber vial and stored at 5°C before Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis and the bioassays. The procedure was performed in triplicate.

2.3 Oil Fractionation (Purification of Dillapiole)

The oil (10 g) was submitted to fractionation on silica (230-400 mesh, Merck, 200 g) chromatographic column (80 x 3 cm) using n-hexane and n-hexane-ethyl acetate (10%) as elution solvent and the separated fractions were monitored by thin-layer chromatography

(revealed by vanillin/H₂SO₄, 110°C). Gas Chromatography-Flame Ionization Detection (GC-FID) obtained the content of dillapiole in the oil and the purified fractions. Also, the minor compounds found with the dillapiole in the purified fraction were identified by GC-MS.

2.4 Analysis of the Oil Composition and Dillapiole

The analyses were performed on a GC-MS Thermo Focus DSQ II, under the following conditions: DB-5ms (30 m x 0.25 mm; 0.25 mm film thickness) fused-silica capillary column; programmed temperature: 60–240°C (3°C/min); injector temperature: 250°C; carrier gas: Helium, adjusted to a linear velocity of 32 cm/s (measured at 100°C); injection type: split (2 µl of a 1:1000 hexane solution); split flow was adjusted to yield a 20:1 ratio; septum sweep was a constant 10 ml/min; Electronic Impact-Mass Spectrometry (EI-MS): electron energy, 70 eV; temperature of ion source and connection parts: 200°C. The quantitative data regarding the volatile constituents were obtained by peak area normalization using a GC-FID Thermo Focus operated under similar conditions of the GC-MS, except for the carrier gas, which was nitrogen. The retention index was calculated for all the volatiles constituents using an *n*-alkane (C8-C40, Sigma/Aldrich) homologous series. Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds which were previously analyzed and stored in a private library, as well as with the aid of commercial libraries containing retention indices and mass spectra of volatile compounds commonly found in the essential oils [19,20].

2.5 Antimicrobial Assay

2.5.1 Culture media

The culture media used for the antibacterial activity were Mueller-Hinton broth, Mueller-Hinton agar (Merck, Germany), and mannitol agar (HiMEDIA, India); and for the antifungal activity were RPMI 1640 with glutamine without bicarbonate, phenol red indicator, MOPS pH 7 buffer, Sabouraud dextrose agar (SDA), and Potato agar (HiMEDIA, India).

2.5.2 Strains of microorganisms tested

The strains were from American Type Culture Collection (ATCC), provided by the Instituto

Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz (INCQS/FIOCRUZ, Rio de Janeiro) and their clinical isolates, which were furnished by the Instituto Evandro Chagas (IEC, Belém). The bacterium was *Staphylococcus aureus* ATCC 6538 and its clinical isolate. The fungi were *Trichophyton mentagrophytes* ATCC 9533, *Candida albicans* ATCC 10231 and *Aspergillus fumigatus* ATCC 40152, and its clinical isolate, *C. albicans*, *A. fumigatus*, *T. mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. gypseum*, and *Epidermophyton floccosum*.

2.5.3 Antibacterial assay

The bacterium was cultured on Mueller-Hinton agar and mannitol agar (on Petri dishes). Then the dishes were incubated at 35°C (24 h) for growth analysis. The inoculum was prepared using 3-4 colonies of bacteria transferred to a tube containing 1 ml of Mueller-Hinton broth and making some adjustments to achieve an approximate concentration of 1×10^8 CFU/ml, consistent with the 0.5 McFarland scale (from 0.09 to 0.11). For this step, a turbidimeter apparatus (Densimat-Biomerieux) was used. The suspension was incubated for 1 h to reach an exponential bacterial growth and, then, a serial dilution was made to obtain the inoculum 1×10^3 CFU/ml, according to Clinical and Laboratory Standards Institute [21].

2.5.4 Antifungal assay

For filamentous fungi, the inoculum was prepared based on the M38-A2 standard of Clinical and Laboratory Standards Institute [22]. The fungi were cultured in Petri dishes containing potato dextrose agar and kept in an oven at room temperature: *T. mentagrophytes* (21 days), *T. rubrum* and *E. floccosum* (30 days), and *M. canis* and *M. gypseum* (14 days). Intending to the exponential growth of conidia of *A. fumigatus* was used agar saboraund dextrose, at 37°C, for seven days. The inoculum was prepared by washing the conidia with RPMI medium (5 ml), and the obtained suspension was transferred to a test tube for the settling of larger particles, for 5 min. Then, the suspension was vortexed (Phenix, AT 56 model) for 15 min. The turbidity of the suspension was adjusted to McFarland scale (turbidimeter Densimat, Biomerieux), 0.4 to 5×10^6 CFU/ml. For *A. fumigatus* was made a dilution (1:50) in RPMI medium, resulting in 0.4 to 5×10^4 CFU/ml.

For yeast, the inoculum preparation was based on the M27-A3 standard of Clinical and

Laboratory Standards Institute [23]. *Candida albicans* was cultured on Petri plates containing Sabouraud dextrose agar and kept in an oven at 35°C (24 h). About five colonies of *C. albicans* were suspended in RPMI medium (5 ml), and the suspension was vortexed (15 min). The density of cells was adjusted to McFarland standard scale to 1×10^6 to 5×10^6 CFU/ml. The inoculum was incubated (1 h) to achieve the exponential growth of fungi and, then, dilutions 1:50 and 1:20 were made with RPMI 1640 liquid medium, resulting in a concentration of 1×10^3 to 5×10^3 CFU/ml.

2.5.5 Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The broth microdilution method was used [21]. The dilution of oil and concentrate of dillapiole was done in solution of Tween 20 (0.5%) to obtain the test concentration (20 µl/ml) and, then, with serial dilution using MHA and SDA for the preparation of other concentrations: Bacterial strains ranging from 125 to 2,000 µg/ml; and fungal strains ranging from 1.9 to 2,000 µg/ml [7]. The test was performed in 96 well plates, where each well received 90 µl of the particular concentration of the oil and concentrate of dillapiole, 90 µl of the MHA or SDA media, and 20 µl of the inoculum. The control of microbial growth, control of sterility of the medium and the solvent control were done simultaneously. Each well kept a final volume of 200 µl. The inhibition of growth of bacteria and fungi was revealed by addition of sterile resazurin solution (20 ul, 0.02%, w/v) (Sigma), after incubation (24h, $36 \pm 1^\circ\text{C}$). Then the plates were incubated for another 3 h. The MIC, which is defined as the lowest concentration of oil capable of inhibiting the growth of microorganisms, was determined by the permanence of blue coloration (resazurin) in the wells. The wells that showed no apparent growth were selected to evaluate the MBC and MFC, which were determined by the absence of microbial growth on plates containing MHA and SDA media, respectively [21,22]. The halo formed around the disk was measured using a digital caliper and compared to standards antimicrobial discs: Chloramphenicol (250 µg/ml) for bacteria and amphotericin B (100 µg/ml) for fungi. The MIC is the lowest concentration of antimicrobial agent that prevents the visible growth of a microorganism in a sensibility test of broth microdilution. The MBC was obtained by

seeding method, by removing an aliquot of 10 μ l of the contents of the wells, followed by spreading on Petri plates previously prepared with Mueller-Hinton agar and incubated at 35°C (24 h).

2.6 Statistical Analysis

Data were statistically analyzed by ANOVA (One-way ANOVA), with correction using Tukey's test. The MIC, MBC, and MFC data were expressed as mean \pm standard error of the mean (SEM). The probability accepted as indicative of the existence of significant differences was $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Oil and Purification of Dillapiole

The oil showed a yield of 3.0% and it was analyzed by GC and GC-MS. Twenty-four constituents were identified, and they are listed in Table 1. Dillapiole was the main component in the oil, with 85.9%. The oil was submitted to

purification process using column chromatography, and the content of dillapiole reached 98.4%.

3.2 Antibacterial Activity

The oil and dillapiole-rich fraction did not show antibacterial activity against the strain of *S. aureus* ATCC 6538 and its clinical isolate, at the concentration range used (125-2,000 μ g/ml). Confirming our data, another dillapiole-rich oil of *P. aduncum* from Ecuador did not show antibacterial activity against *S. aureus* ATCC 29213 [4]. But, another sample of *P. aduncum* oil, containing dillapiole as the principal constituent, showed antibacterial activity against *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228, with MIC and MBC values between 250 and 1000 μ g/ml [7]. On the other hand, antibacterial properties against various microorganisms have been attributed to the ethanol extract of *P. aduncum*, due the presence of p-hydroxybenzoic acid, chalcones and chromenes [24,25].

Table 1. Composition of the oil of *P. aduncum* and its dillapiole-rich fraction

Constituents	RI _{Calc.}	RI _{Lit.}	Oil (%)	Dillapiolle-rich fraction (%)
α -Pinene	928	932	0.5	
<i>p</i> -Cymene	1018	1020	0.2	
(<i>E</i>)- β -Ocimene	1038	1044	0.2	
Cyclosativene	1368	1369	0.1	
α -Copaene	1369	1374	0.4	
β -Elemene	1383	1389	0.2	
(<i>E</i>)-Caryophyllene	1411	1417	3.8	0.8
α -Humulene	1447	1452	0.5	
<i>allo</i> -Aromadendrene	1448	1458	0.2	
Germacrene D	1473	1484	0.4	
β -Selinene	1487	1489	0.4	
γ -Cadinene	1507	1513	0.4	
Myristicin	1512	1517	1.5	0.2
α -Calacorene	1536	1544	0.1	
Elemicin	1542	1555	0.2	
(<i>E</i>)-Nerolidol	1555	1562	0.3	
Spathulenol	1567	1577	0.3	
Caryophyllene oxide	1572	1582	0.8	
Globulol	1584	1590	0.5	
Humulene epoxide II	1599	1608	0.1	
Dilapiolle	1615	1620	85.9	98.4
epi- α -Muurolol	1626	1640	0.2	
α -Muurolol	1634	1644	0.2	
Apiole	1665	1677	0.4	0.2
Total			97.0	99.6

RI_{Calc.} = Calculated retention index; RI_{Lit.} = Retention index of literature (Adams, 2007)

Studies of antimicrobial activity of plants have shown controversies. Certain factors may explain these variations, such as extracts of different compositions, different techniques used, the culture media used, changes in pH, the growth conditions (aerobic and anaerobic), the incubation time of the cultured microorganisms, as well as the inoculum size. Also, different strains tested show variation in susceptibility to the various extracts as the origin, seasonality and the plant constituents [26]. Studies have reported that essential oils are more active against fungi than against gram-positive bacteria, estimating that 60% of them have antifungal effects while 35% exhibit antibacterial properties. This information corroborates our findings [27].

3.3 Antifungal Activity

The results of the antifungal assay against the dermatophytes filamentous fungi are shown in Table 2 and Fig. 1. The MIC values for the oil and dillapiole-rich fraction were lower compared to MFC values, indicating fungicidal action against the tested microorganisms, in both products.

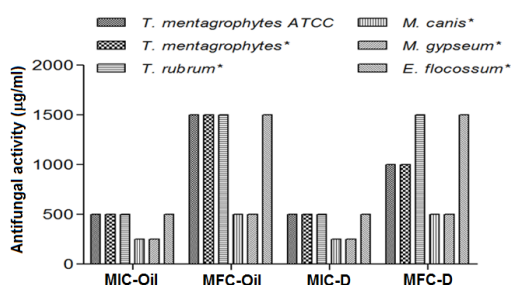


Fig. 1. Antifungal activity of the oil and concentrate of dillapiole against dermatophytic filamentous fungi

The antifungal activity has demonstrated similar MIC and MFC values for *T. mentagrophytes*, *T. rubrum*, and *E. floccosum*, as well as between *M. canis* and *M. gypseum*. However, the strains of the genera *Trichophyton* and *Epidermophyton* showed higher MIC and MFC values, when compared to the strains of the genus *Microsporum*. The explanation for these results would be the hypothesis that anthropophilic species, such as *T. mentagrophytes*, *T. rubrum*, and *E. floccosum*, are more resistant to the antifungal agents. They are better adapted to man and, therefore, exhibit higher values of MIC and MFC than the zoophylic (*M. canis*) and geophilic (*M. gypseum*) species. Also, the results suggest that dillapiole would be responsible for

the antifungal activity of *P. aduncum* oil, since their content is quite high (85.9%), reflecting on the similarity of MIC and MFC values found in oil and dillapiole-rich fraction (99.48%), in almost all tests. This fact is confirmed by the activity observed in the fungus *T. mentagrophytes* ATCC and its clinical isolate, whose MFC values for the dillapiole-rich fraction are smaller (1,000 µg/ml) than in the oil *P. aduncum* (1,500 µg/ml).

The oil of *P. aduncum* was previously submitted to antifungal action against the strains of *T. mentagrophytes* ATCC 16066, showing an MIC value of 500 µg/ml [4], therefore, comparable to our results. A dillapiole-rich fraction (95-98.5%) of *P. aduncum* oil presented fungicidal action on basidiospores and germination of *Clinipellis perniciosa*, a fungus that attacks cacao plantations, with MIC value of 0.6 mg/ml [9,13]. An extract of *P. aduncum*, harvested in Minas Gerais, Brazil, was active against strains of *T. rubrum*, with MIC and MFC values of 0.31 µg/ml and 0.62 µg/ml, respectively. But, the main constituents of the extract were sesquiterpenes, such as germacrene D, (*E*)-nerolidol and δ -cadineno [28].

The greater effectiveness of the *P. aduncum* oil against the filamentous fungi, compared to bacteria and yeasts, can be attributed to its volatility and lipophilic character, which will permeate the membrane of filamentous fungi, allowing its absorption and diffusion [29]. A probable mechanism of action of essential oils occurs in the cell walls of fungi, which is mainly composed of chitin, glucans, and glycopeptides. The oil act in the destruction of components of the cell wall and cell membrane and it causes leakage of their constituents into the cytoplasm that, in turn, suffer a consequent coagulation [1].

The results of antifungal activity for the oil and the dillapiole-rich fraction against *A. fumigatus*, a non-dermatophyte filamentous fungus, are in Table 3 and Fig. 2. Again, it was demonstrated the fungicidal action of these products, presenting MIC values lower than that of MFC values.

The results of the tests against non-dermatophyte filamentous fungi (e.g. *A. fumigatus*), as well as against dermatophyte filamentous fungi (e.g. *T. rubrum*), showed similar values of MIC and MFC for the oil and the dillapiole-rich fraction, suggesting once again that dillapiole is responsible for the antifungal activity of the *P. aduncum* oil.

Table 2. Antifungal activity of the oil of *P. aduncum* and dillapiole-rich fraction against dermatophyte filamentous fungi

Strains	Oil (µg/ml)		Dillapiole-rich fraction (µg/ml)	
	MIC	MFC	MIC	MFC
<i>T. mentagrophytes</i> ATCC 9533	500	1,500	500	1,000
<i>T. mentagrophytes</i> *	500	1,500	500	1,000
<i>T. rubrum</i> *	500	1,500	500	1,500
<i>M. canis</i> *	250	500	250	500
<i>M. gypseum</i> *	250	500	250	500
<i>E. floccosum</i> *	500	1,500	500	1,500

Clinical isolates

Table 3. Antifungal activity for the oil of *P. aduncum* and dillapiole-rich fraction against *A. fumigatus*, a non-dermatophyte filamentous fungi

Strains	Oil (µg/ml)		Dilapiolle-rich fraction (µg/ml)	
	MIC-Oil	MFC-Oil	MIC-D	MFC-D
<i>A. fumigatus</i> ATCC 40152	3.9	7.8	3.9	7.8
<i>A. fumigatus</i> *	3.9	15.6	3.9	15.6

Clinical isolate

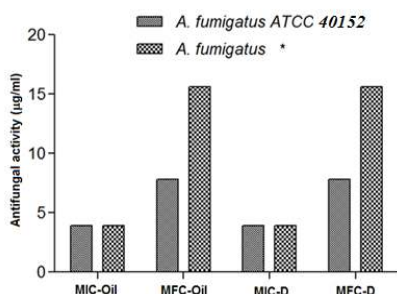


Fig. 2. Antifungal activity for the oil and dillapiole-rich fraction against *A. fumigatus* ATCC 40152 and its clinical isolate*

An alcoholic extract of *P. aduncum* was tested against *A. fumigatus*, with MIC value of 1,000 µg/ml, but without information regarding its chemical composition [30]. Traditional plant essential oils have antifungal activity against *A. fumigatus*, as the *Origanum vulgare* oil, with MIC in the range 0.32 to 0.64 µg/ml [31]. Also, in the oil of *Thymus caespitius* and α-terpineol, its primary constituent, with MIC ranged from 1.25 to 2.5 µg/ml and 0.32 to 0.64 µg/ml, respectively [32].

Furthermore, it has been observed a difference in MIC and MFC values, among dermatophyte filamentous fungi (e.g., *T. rubrum*), and non-dermatophyte filamentous fungi (e.g., *A. fumigatus*). This difference is attributed to the higher pathogenicity of virulence factors of the dermatophytes, which it is related to the

accession process and invasion of host tissue, making that the fungi can be more specialized and, therefore, more pathogenics. The fungi *T. mentagrophytes* and *T. rubrum* can express specific fibrillar adhesins that recognize and bind to mannose and galactose residues of the cell surface. These structures are responsible for making the correct anchoring of these fungi in the host cells and prevent them from being disconnected by external aggressions, such as the act of scratching [33].

Regarding the invasion process, dermatophytes stand out for their lipolytic capacity, with the release of extracellular enzymes when there is contact with the host tissue lipids, facilitating the invasion process. The mannans, a particular component of the glycoprotein of the cell wall of fungi, promote the suppression of the inflammatory response and cell proliferation of the host organism [34].

The oil and concentrate of dilapiolle showed no antifungal activity against *C. albicans*, with MIC and MFC values above 2,000 µg/ml. Previously the oil and the ethanol extract of *P. aduncum* were tested against *C. albicans*, but only the oil showed antifungal activity in concentrations above 2,000 µg/ml [35]. Therefore, with a similar result to that presented in this work.

The high effectiveness of essential oils against filamentous fungi, when compared to yeasts and bacteria can be attributed to the lipophilic character and the volatility of these products, as

well as the nature of the lipophilic and aerobic membranes of filamentous fungi, allowing the oil to be absorbed and diffused across the membrane [29]. According to established criteria, it is possible to evaluate the effectiveness of compounds tested in antifungal studies. Holetz et al. [36] consider that the MIC value less than 100 µg/ml indicates good antifungal activity, MIC value 100-500 µg/ml has moderate activity, MIC value 500-1,000 µg/ml has weak activity and MIC value above 1,000 µg/ml is considered inactive. Aligiannis et al. [37] and Duarte et al. [34] adopted the following values: strong inhibitor, MIC up to 500 µg/ml; moderate inhibitor, MIC from 600 to 1,500 µg/ml; and weak inhibitor, MIC above 1,500 µg/ml.

4. CONCLUSION

Thus, considering the results and the criteria described in the mentioned literature, the oil of *P. aduncum* and the dillapiole-rich fraction showed good antifungal activity for dermatophyte filamentous fungi and potential antifungal activity for non-dermatophyte filamentous fungi. This significant activity can be explained by the high lipophilicity of the oil, which preferentially promotes the passage of dillapiole and other liposoluble substances through the cell walls of fungi over the water-soluble substances.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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