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SHORT REPORT

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In vitro antifungal activity of extracts and alkaloid compounds from *Piper arboreum* against dermatophytes

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ABSTRACT

Piper is widely distributed in subtropical regions and species of this genus are known for their potent pharmacological activities. *Piper arboreum* Aubl. is a traditional medicinal plant popularly known as "pau-de-angola", "jaborandi", and chili pepper, demonstrating antifungal, trypanocidal, antibacterial, and antioxidant activities. The leaves of *P. arboreum* were extracted using Soxhlet and dichloromethane to obtain the extract, which was then fractionated using solvents of different polarities. Samples were analyzed using ultra-high-performance liquid chromatography coupled with mass spectrometry equipped with an electrospray ionization source. Antifungal microdilution assays were performed, and scanning and transmission electron microscopy were used to assess the invasion of the pretreated nail. The minimum inhibitory concentration (MIC) values of the extract and a dichloromethane fraction were, respectively, 62.5 µg/ml and 16.0 µg/ml against *Trichophyton rubrum*, and 125 µg/ml and 62.5 µg/ml, and 500 µg/ml and 500 µg/ml against *T. mentagrophytes*, and *Microsporum gypseum*, respectively. No growth was observed on nail fragments exposed to the extract (at concentrations > 64 µg/ml and then inoculated with spore suspension. Transmission electron microscopy revealed strong inhibition of hyphal growth and an irregular growth pattern following treatment with the extract and the dichloromethane fraction. Results demonstrated the antifungal properties of the *P. arboreum* extract and its dichloromethane fraction against dermatophytes, with the identification of three different alkaloid compounds. The cytotoxicity was specific towards the fungal cells, and morphological and ultrastructural analyses indicated damage to the cell wall and cytoplasmic membrane as the potential mechanism of action. The leaf material used to generate the extract can be taken from the plant without any detrimental effect thus enabling strategies to be implemented for the exploitation of this species.

1. Introduction

Piper is one of the most diverse genera among the basal clades of angiosperms and is widespread in tropical wet forests around the world (Dyer & Palmer, 2004), with about 2,000 species (Quijano-Abril et al., 2006). The genus *Piper* is widely distributed in tropical and subtropical regions and is known for its aromatic herbs (Biswas et al., 2022; Guerrini et al., 2009). Some species of *Piper* have already been demonstrated to have potent pharmacological activities, as well as exhibit great chemical diversity of their secondary metabolites (Tran et al., 2024; Yadav et al., 2020). Essential oils produced by *Piper* species have been found to comprise monoterpenes (germacrene A, α-pinene), sesquiterpenes (germacrene B, germacrene D, α-humulene, β-copaen-4-α-ol), phenylpropanoids (humulene epoxide II, muurola-4,10(14)-dien-1-β-ol), aldehy-

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des (cinnamaldehyde), ketones, and long chain alcohols (Cysne et al., 2005).

P. arboreum Aubl., popularly known as "pau-de-angola", "jaborandi", and chili pepper, has been shown to demonstrate antifungal, trypanocidal, antibacterial, and antioxidant activities (Bernuci et al., 2016; Regasini et al., 2009; Regasini et al., 2008). Furthermore, phytochemical studies have revealed that leaves of this species contain several amides, such as arboreumine, N-[10-(13,14-methylenedioxyphenyl)-7*(E)*-pentaenoyl]-pyrrolidine, and N-[10-(13,14-methylenedioxyphenyl)-7*(E)* (da Silva et al., 2002). Regasini et al. (2009) showed that the essential oil of *P. arboreum* is active against *Trypanosoma cruzi* protozoa and the fungal species, *Cladosporium sphaerospermum*, *C. cladosporioides*, *Candida krusei*, *C. parapsilosis*, and *Cryptococcus neoformans*.

Dermatophytosis represents the fourth cause of disease with global incidence estimated at 20 to 25% within the healthy population (Hay et al., 2014; Rouzaud et al., 2018; White et al., 2014; Yousefian et al., 2024). The high prevalence of dermatophytosis is related to the fact that the fungi are more resistant to the host's innate defense and thus can remain as a residual infection that can be lifelong if not treated correctly (Lahmer et al., 2024; Worek et al., 2014). The fungal species that cause dermatophytosis belong to three genera: *Epidermophyton*, *Trichophyton* and *Microsoprum* (Rinaldi, 2000). *T. rubrum* is the most common dermatophyte and causes dermatophytoses, such as a tine pedis and tinea corporis (Dalla Lana et al., 2016). Imidazoles, butenafine, and terbinafine are among the antifungal drugs used for the topical treatment of dermatophytosis (Baghi et al., 2016; Watanabe, 1999), while triazoles, griseofulvin, and terbinafine are used as oral systemic therapies for severe dermatophytosis (Lesher Jr, 1999; Rani et al., 2013). However, the toxicity and interactions associated with these drugs, the need for long treatment regimens, the rise of fungal resistance, and high treatment costs highlight the need for new, more efficient, and safe antifungal drugs (Bennett et al., 2000; de Pauw, 2000; Olson & Troxell, 2023).

The present study evaluated the antidermatophytic effect and cytotoxicity of *P. arboreum* leaf extract and fractions and investigated the possible mechanisms of action.

2. Materials and methods

2.1. Plant material

Leaves from *P. arboreum* were collected from 01/2016 to 06/2017 in Diamantina do Norte, Paraná, Brazil (latitude 18°14'17" S and longitude 43°36'40" W). The plant was identified by botanical professor José Tadeu Weidlich Motta and his exsiccata were deposited in the herbarium of the Botanical Museum of Curitiba. The aerial parts of *P. arboreum* were collected at the Caiuá Ecological Station, Diamond of the North-PR and identified by professor Mariza Barion Romagnolo of the Biology Department/Riparian Vegetation Laboratory/Nupelia and its exsiccata deposited at the State University of Maringá Herbarium (HUEM 15942). After collection, *Piper* leaves were weighed and dried in an air oven circulating (QUIMIS®, model Q-31), at a temperature of 36 °C. After drying, the plant samples were crushed in a knife mill (Tecnal Marconi® model TE 048), packaged, and stored in a dry place and protected from light.

2.2. Preparation and fractionation of P. arboreum extract

The leaves of *P. arboreum* were dried in a circulating air oven and ground in a knife mill, after which they were extracted using Soxhlet and dichloromethane as a solvent over 8 h to obtain the extract. A part of the extract was lyophilized and stored at –10 °C until use, while the other part was submitted to column chromatography on silica gel 60 (0.063-0.2 mm, Macherey-Nagel) and submitted to fractionation with solvents with different degrees of polarity to give six different fractions: hexane, hexane/dichloromethane 80:20, hexane/dichloromethane 50:50, dichloromethane, dichloromethane/ethyl acetate 50:50 and methanol (Achenbach et al., 1987; Chauret et al., 1996; Obici et al., 2008).

2.3. Fungal strains and growth conditions

T. rubrum ATCC 28189, *T. mentagrophytes* ATCC 11480, and *Microsporum gypseum* ATCC 14683 were cultured at 28 °C in Sabouraud dextrose agar tubes for around 25 days. *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 28707 were grown in Sabouraud dextrose broth at 37 °C and maintained on Sabouraud dextrose agar at 4 °C. Before the assays, spores were collected in sterile saline and suspensions were adjusted to 1.0×10^5 spores/ml.

2.4. Antifungal assays

The minimal inhibitory concentration (MIC) of the extract and its fractions against fungal strains was determined according to the M38-A2 broth microdilution reference procedure of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute-CLSI, 2008a, 2008b). Fungal inocula of 0.4×10^4 to 5×10^4 conidia/ml were prepared in RPMI 1640 medium with L-glutamine and without bicarbonate and buffered with 0.165 M 3-(morpholino) propanesulfonic acid (MOPS). Serial two-fold dilutions of the extract and fractions were performed in 96-well microdilution plates containing 100 µl of RPMI medium. After this, inoculum (5 µl) was added to each well and the plates were incubated at 28 °C for 72 h. The MIC was defined as the lowest concentration that resulted in inhibition of visual growth.

2.5. Checkerboard assay

The checkerboard test, using the microdilution method, was performed to evaluate the in vitro interaction of the antifungal fluconazole (FLU) or nystatin (NYS) with *P. arboreum* extract against *T. rubrum*. The fractional inhibitory concentration (FIC) index was determined, which is defined as the sum of the MIC of each drug in combination, divided by the MIC of the drug used alone. An FIC index of ≤ 0.5 is considered synergism, > 4 is antagonism, and > 0.5 but ≤ 4 is indifferent (Odds, 2003).

2.6. Effect on the invasion of nails

Distal fragments of normal human fingernails were collected from a healthy volunteer who was not receiving antifungal therapy (Macura et al., 2003). Nail fragments were cut into pieces approximately 2 mm × 2 mm in diameter and autoclaved at 121 °C for 15 min. The nail pieces were then saturated with the extract and fractions at different concentrations for 1 h in test tubes. After this, the surface of the nail pieces was inoculated with 50 µl of spore suspension, which were then incubated in a humidified atmosphere at 28 °C for 7–14 days.

Scanning and transmission electron microscopy (SEM and TEM) were used to assess nail invasion. For SEM analysis, the nail pieces were prefixed in a solution of 2.5% sodium glutaraldehyde and 0.1 M sodium cacodylate buffer, dehydrated in increasing ethanol concentrations, and critical point dried. The samples were then coated

with gold and analyzed on a Quanta™ 250 scanning electron microscope (FEI Company, Hillsboro, OR, USA).

For TEM analysis, the nail pieces were washed with PBS and fixed in a solution of 2.5% sodium glutaraldehyde and 0.1% sodium cacodylate buffer then postfixed in 1% osmium tetroxide (OsO_4), 0.8% potassium ferrocyanide, and 10 mM calcium chloride ($CaCl_2$) in 0.1 M cacodylate buffer. After this, the samples were dehydrated in increasing acetone concentrations and soaked in EPON resin at 60 °C for 72 h. Ultrafine sections were obtained, stained with uranyl acetate and lead citrate, and then examined on a Quanta ™ 250 transmission electron microscope.

2.7. Cytotoxicity assay

The cytotoxic effect of the *P. arboreum* extract and the dichloromethane fraction was analyzed as described in Benassi-Zanqueta et al. (2018). The tests were performed in triplicate using VERO epithelial cells (ATCC CCL81). In 96-well plates, 5×10^4 cells/well in DMEM supplemented with 10% fetal bovine serum (FBS) were added and the plates were incubated for 24 h at 37 °C and 5% CO_2. Thereafter, the well culture medium was aspirated and different concentrations of extract and fraction were added (1000 to 1.95 µg/ml). After 24 h of incubation at 37 °C and 5% CO_2, the culture medium was removed and the wells were washed twice with PBS. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Ambresco®, USA) was dissolved in 2 ml PBS and then 50 µl was added to all wells except the blank. Plates were incubated at 37 °C and 5% CO_2, protected from light for 4 h, after which the MTT was removed and 150 µl dimethylsulfoxide (DMSO; Ambresco®, USA) was added to each well. Plates were shaken and the reading was performed on an Asys Expert Plus Plate Reader (Biochrom, Cambridge, UK) at a wavelength of 550 nm. DMSO was used as a negative control. Data were calculated as the percentage of inhibition, and the concentration for 50% cellular toxicity (CC_{50}) was determined as the concentration that reduces the optical density of the treated cells by 50% relative to the untreated cells.

2.8. High-resolution mass spectrometry (HRMS) analysis

Samples were analyzed by ultra-high-performance liquid chromatography (UHPLC; Nexera X2, Shimadzu, Kyoto, Japan) coupled with HRMS (QTOF Impact II, Bruker, Billerica, MA, USA) equipped with an electrospray ionization source. The capillary voltage was operated in positive ionization mode, set at 4500 V, and with an endplate offset potential of –500 V. The dry gas parameters were set to 8 l/min at 200 °C with a nebulization gas pressure of 4 bar. Data were collected from m/z 50 to 1300 with an acquisition rate of 5 spectra/s, and the ions of interest were selected by auto MS/MS scan fragmentation. C18 column (75 × 2.0 mm i.d.; 1.6 µm Shim-pack XR-ODS III) was used for chromatographic separation. Gradient mixture of solvents A (H_2O) and B (acetonitrile with 0.1% formic acid; v:v) was as follows: 5% B for 0–1 min, 30% B for 1–3 min, 95% B for 3–12 min, maintained at 95% B for 12–15 min, and 5% B 15–17 min, at 40 °C (Chauret et al., 1996; Freixa et al., 2001; Holetz et al., 2002).

2.9. Statistical analysis

All tests were carried out in triplicate, and the data were analyzed through Analysis of Variance (ANOVA). Tukey's test was conducted, and a *p*-value of ≤ 0,05 was considered significant compared with the control group. The statistical analysis was performed with the program Graph-Pad Prism 4, USA.

3. Results and discussion

3.1. MIC Results

The MIC of the *P. arboreum* extract was first determined against various fungal species, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *T. rubrum*, *T. mentagrophytes*, and *M. gypseum* (Table 1). The best effect was observed against *T. rubrum*, and thus, fractions of the extract were subsequently tested against this species and the other dermatophytes.

Table 1. Minimum inhibitory concentration (µg/ml) of *P. arboreum* extract and fluconazole against dermatophytes and yeasts

Yeasts	*Piper arboreum* extract	Fluconazole
C. albicans ATCC 10231	> 1000	7.8
C. tropicalis ATCC 28707	> 1000	250
C. parapsilosis ATCC 22019	500	1.9
Dermatophytes		
T. rubrum ATCC 28189	62.5	125
T. mentagrophytes ATCC 11480	125	31.25
M. gypseum ATCC 14683	500	1000

The results represent mean values for at least three separate experiments. Standard errors were less than 10%.

3.2. Fractionation and antifungal composition

The *P. arboreum* extract was submitted to fractionation with solvents with different degrees of polarity to give six different fractions: hexane (HE), hexane/dichloromethane 80:20 (HD 80:20), hexane/dichloromethane 50:50 (HD 50:50), dichloromethane (DI), dichloromethane/ethyl acetate 50:50 (DA), and methanol (ME). The extract and fractions showed activity against *T. rubrum*, in particular (Table 2). The DI fraction showed the most effective fungicidal activity (16 µg/ml) against *T. rubrum*, followed by the extract itself, both of which were more effective when compared with those of FLU (MIC of 125 µg/ml) and the other fractions, which had MICs that ranged from 125 to 500 µg/ml.

Table 2. Evaluation of the *P. arboreum* extract and its fractions against dermatophytes

Dermatophytes	Extract	HE	HD (80:20)	HD (50:50)	DI	DA	ME	FLU
T. rubrum ATCC 28189	62.5	250	125	125	16.0	125	500	125
T. mentagrophytes ATCC 11480	125	500	250	250	62.5	500	1000	31.25
M. gypseum ATCC 14683	500	> 1000	> 1000	> 1000	500	62.5	250	1000

HE: hexane, HD: hexane/dichloromethane (80:20), HD: hexane/dichloromethane (50:50), DI: dichloromethane, DA: dichloromethane/ethyl acetate (50:50), ME: methanol, FLU: fluconazole. The results represent mean values for at least three separate experiments. Standard errors were less than 10% of means.

Silva (2004) showed that *P. arboreum* presents pyrrolidine amides, which are considered markers of the species. Monoterpenes and sesquiterpenes were also identified in the essential oils of this species (Durant-Archibold et al., 2018; Navickiene et al., 2006). The pyrrolidine amides present several biological activities, particularly antifungal activity against opportunistic fungi, such as *C. sphaerospermum* (da Silva et al., 2002), *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. neoformans* (Regasini et al., 2009).

The checkerboard method was used to evaluate the association of the antifungal drugs, FLU and NYS, with the *P. arboreum* extract and the DI fraction against dermatophyte species (Table 3). The FIC indices indicated a synergic interaction between the DI fraction and both antifungal drugs against both *T. rubrum* and *T. mentagrophytes* (values ranging from 0.1 to 0.4). The extract, on the other hand, showed a synergic effect when associated with FLU or NYS against *T. rubrum*, and a indifferent effect against *T. mentagrophytes* (FIC index > 0.5). Previous reports of synergistic combinations of antifungal compounds have not been extensive or encouraging. In a review of combination therapy in systemic mycosis, Polak (1990) discussed the combination of amphotericin B with flucytosine as one of the better-established synergistic combinations of antifungal agents that have been used clinically to treat candidiasis. The combination of an azole with a polyene resulted in conflicting outcomes, depending on the species and the strain tested, and specific antagonism was observed with *C. albicans*. Since the ID fraction concentration effective in vitro is achievable in vivo, the combination of this fraction with FLU or NYS represents an attractive perspective for the development of new management strategies for dermatophytosis.

Table 3. Fractional inhibitory concentration (FIC) indices for the associate of *P. arboreum* extract or dichloromethane (DI) fractionwith fluconazole (FLU) or nystatin (NYS) against *T.rubrum* and *T. mentagrophytes*

Dermatophytes	Extract × FLU	Extract × NYS	DI × FLU	DI × NYS
T. rubrum ATCC 28189	0.270	0.344	0.155	0.209
T. mentagrophytes ATCC 11480	0.625	0.500	0.376	0.326

The results represent mean values for at least three separate experiments. Standard errors were less than 10% of means.

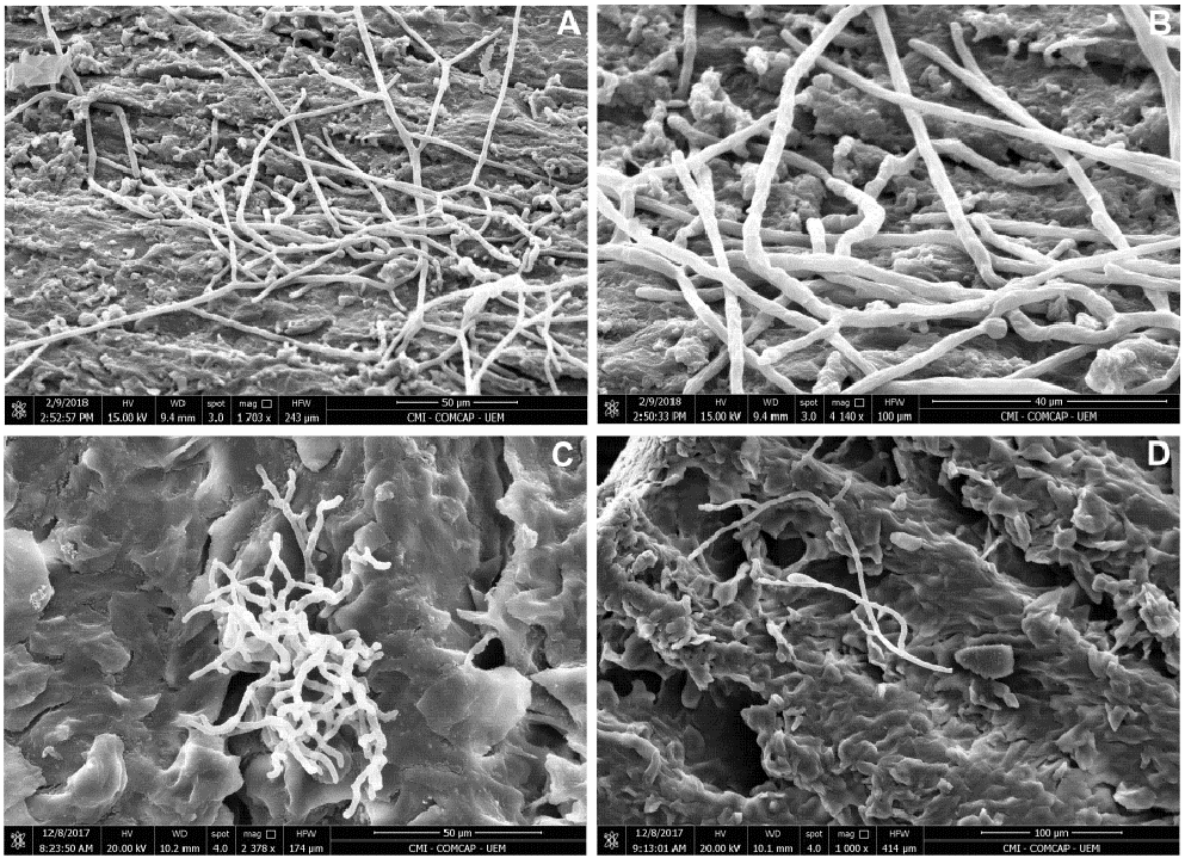

Figure 1. Scanning electron microscopy
A) and B) control cells of T. rubrum. C) *T. rubrum* treated with *P. arboreum* extract at 64 µg/ml. D) *T. rubrum* treated with the dichloromethane fraction at 32 µg/ml.

For topical treatment of dermatophytosis to be successful, it must be able to penetrate the nail plate, which is a thick barrier with a compact structure (Andrade et al., 1996; Butler et al., 2024; Walters et al., 1981). The nail pieces were saturated with *P. arboreum* extract and dichloromethane fraction and then inoculated with a *T. rubrum* spore suspension. Strong inhibition of hyphal growth was observed for cells pretreated with both the extract and the DI fraction (Figures 1C and 1D, respectively).

T. rubrum treated with 64 and 32 µg/ml of the extract and 16 µg/ml of the DI fraction presented short hyphae and irregular growth. In TEM, control cells had intact membranes and cell walls, with dense cytoplasm (Figure 2A). After treatment with 64 and 32 µg/ml of extract and 16 µg/ml of DI fraction (Figure 2B, 2C and 2D), structural changes were visible including alterations in the space between the cell wall and membrane, damage to the cell wall and cytoplasmic membrane, changes to the cytoplasm, and the destruction of organelles. The changes could lead to fungal growth inhibition. Similar structural changes were observed on nails that had not been exposed to extract or fractions that had intact membranes and cell walls, with dense cytoplasm (Figure 2A). Structural changes were visible for cells on pretreated nails (64 and 32 µg/ml of the extract and 16 µg/ml of the DI fraction (Figures 2B, 2C, and 2D, respectively), which included alterations in the space between the cell wall

and membrane, damages to the cell wall and cytoplasmic membrane, changes to the cytoplasm, and the destruction of organelles. These changes could lead to fungal growth inhibition. Similar structural changes were observed by Ridzuan et al. (2018) for *T. rubrum* treated with hydroxychavicol, a phenolic compound of betel leaf (*Piper betle*), and miconazole. Growth inhibition and short hyphae were also observed in *T. rubrum* after treatment with natural compounds, such as copaiba oil and copalic acid (Nakamura et al., 2017).

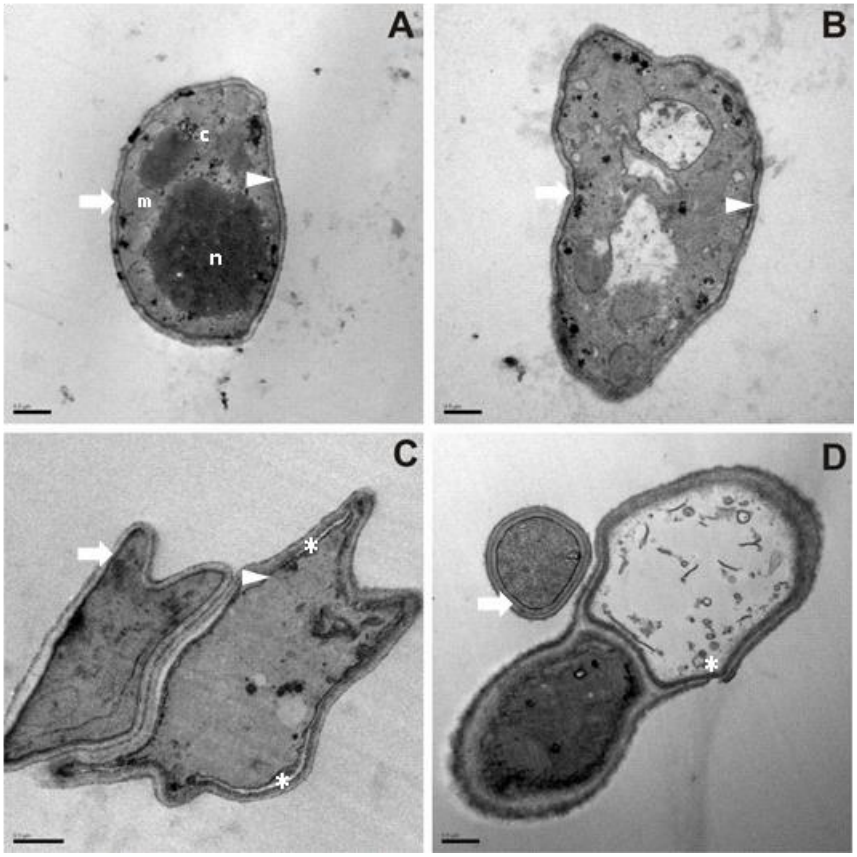

Figure 2. Transmission electron microscopy

A) Control cells of *T. rubrum*. B) *T. rubrum* treated with *P. arboreum* extract at 64 µg/mL. C) *T. rubrum* treated with *P. arboreum* extract at 32 µg/mL. D) *T. rubrum* treated with the dichloromethane fraction at 16µg/mL. In the micrographs, the following structures are indicated: nucleus (n); mitochondria (m); cytoplasm (c); white arrow: cell wall; white arrowhead: cytoplasmic membrane; membrane and cell wall damage (*).

Dermatophytosis is a superficial fungal infection with a worldwide morbidity rate of approximately 20%, which is caused by more than 30 species belonging to three main genera (*Epidermophyton*, *Microsporum*, and *Trichophyton*) (Gupta et al., 2024; Jones et al., 1973; Seebacher et al., 2008). According to a study carried out at the Gregorio Marañón hospital in Madrid, Spain, the most common species that caused dermatophytosis were *T. rubrum* (60%), *T. mentagrophytes* (21%), and *M. canis* (10%) (Rupérez et al., 2013), which emphasizes the importance of new treatments against these fungi (Mayorga et al., 2016).

Plants have a significant role in the development of new drugs to combat microbial infections. The hydroethanolic extract of the leaves of *P. regnellii* was previously shown to have antimicrobial activity against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the yeasts *C. krusei* and *C. tropicalis* (Holetz et al., 2002). Recently, Nascimento et al. (2015) isolated amide piperetine from the roots of *P. arboreum* and evaluated the activity of extracts, fractions, and essential oils against *S. aureus*, *M. gypseum*, and *Epidermophyton floccosum*, showing potent antibacterial and antifungal activity.

The potential cytotoxic effects of the *P. arboreum* extract and the DI fraction were tested on VERO cells; both of which showed CC_{50} values higher than the values of the MIC values, indicating that the extract and DI fraction were more toxic to the fungal cells than to the VERO cells (Table 4).

Table 4. Cytotoxicity test of the *P. arboreum* extract and the dichloromethane (DI) fraction in VERO cells, as assessed by the MTT method

Dermatophytes	CC_{50} (µg/ml)
P. arboreum extract	148.33 ± 20.21
DI fraction	91.67 ± 2.89

The results represent mean values for at least three separate experiments. Standard errors were less than 10% of means.

3.3. Chemical composition

iHRMS ionization processes are more robust and allow the construction of hypotheses based on literature data, having a remarkable level of confidence since the purification of chemical compounds are high-cost process often not feasible to Three compounds were identified in the crude *P. arboreum* extract and the DI fraction analyzed by UHPLC-HRMS/MS (positive ion mode). The identification of these compounds was proposed from a review of the genus *Piper*, in addition to the mass error value. Only molecular formulas with ≤ 5 ppm of error were considered in this study (Brenton & Godfrey,

2010).These compounds were putatively identified as the alkaloids, *n*-[10-(13,14-methylenedioxyphenyl)-7*(E)*-pentanoyl]-pyrrolidine ($C_{16}H_{17}NO_3$[M+H]), 4-butoxy-N-(furan-2-ylmethyl)benzamide ($C_{16}H_{19}NO_3$ [M+H]), and arboreumine ($C_{25}H_{40}N_2O_5$ [M+K]). The identification was established based on the fragmentation pattern of the main ion by MS/MS analysis, and compared to those already described in the literature (Table 5). In a previous paper, Navickiene et al. (2000) described various amides bearing isobutyl, pyrrolidine, dihydropyridone, and piperidine moieties that have been isolated from Piperaceae species. The amides isolated from stems of *P. hispidum* and from seeds of *P. tuberculatum* were active against the fungus *Cladosporium sphaerospermun* as evaluated by direct bioautography.

Table 5. Constituents of the *P. arboreum* extract and the dichloromethane fraction (Data of the compounds identified by UHPLC-HRMS/MS)

Molecular formula	Exact mass (*m/z*)	Precursor ion (*m/z*)	Mass error (ppm)	t_R/min
$C_{16}H_{17}NO_3$ (M+H)	272.1281	272.1277	1.47	4.76
$C_{16}H_{19}NO_3$ (M+H)	274.1437	274.1431	2.19	4.78
$C_{25}H_{40}N_2O_5$ (M+K)	487.2568	487.2575	1.44	5.50

t_R: Retention time. The results represent mean values for at least three separate experiments. Standard errors were less than 10% of means.

4. Conclusions

In the present study, the *P. arboreum* extract and its dichloromethane fraction demonstrated antifungal activity against dermatophytes. Furthermore, three different alkaloid compounds were identified. The cytotoxicity was specific towards the fungal cells, and morphological and ultrastructural analyses indicated damage to the cell wall and cytoplasmic membrane as the potential mechanism of action. For topical treatment of dermatophytosis to be successful, it must be able to penetrate the nail plate. The nail pieces saturated with the *P. arboreum* extract and dichloromethane fraction and then inoculated with a *T. rubrum* spore suspension show a strong inhibition of hyphal growth was strongly inhibited on nails pretreated with both the extract and DI fraction. In terms of conservation, the results showed that leaf material could be useful for antifungal uses, and it could be used without any detrimental effect on the plant. However, extracts may be derived from whole plants or specific parts of plants such as leaves, stems, barks, roots, flowers, and/or fruits, and may be either total extracts or selective extracts. Each extract may contain hundreds of different compounds with complexity providing its challenges in terms of both batch variability and defining mechanisms of action. We suggest more conclusive studies to evaluate these biological components.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. On request, the associated author can provide more information.

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None.

CRediT authorship contribution statement

Fabiana Brusco Lorenzetti: Conceptualization, Data curation, Formal analysis, Investigation
Carla Maria Mariano Fernandez: Methodology
Eliana Harue Endo: Methodology, Writing
Regina Yasuko Makimori: Methodology
Mariza Barion Romagnolo: Methodology
César Armando Contreras Lancheros: Methodology
Marcia Regina Pereira Cabral: Methodology
Maria Helena Sarragiotto: Methodology
Celso Vataru Nakamura: Methodology
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Supplementary File

None.

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