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ORIGINAL ARTICLE

ANTIFUNGAL POTENTIAL OF PLANT SPECIES FROM BRAZILIAN *CAATINGA* AGAINST DERMATOPHYTES

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SUMMARY

Trichophyton rubrum and *Trichophyton mentagrophytes* complex, or *Trichophyton* spp. are the main etiologic agents of dermatophytosis, whose treatment is limited by the high cost of antifungal treatments, their various side effects, and the emergence of resistance amongst these species. This study evaluated the *in vitro* antidermatophytic activity of 23 crude extracts from nine plant species of semiarid vegetation (*caatinga*) found in Brazil. The extracts were tested at concentrations ranging from 1.95 to 1,000.0 µg/mL by broth microdilution assay against the reference strains *T. rubrum* ATCC 28189 and *T. mentagrophytes* ATCC 11481, and 33 clinical isolates of dermatophytes. All plants showed a fungicidal effect against both fungal species, with MIC/MFC values of the active extracts ranging from 15.6 to 250.0 µg/mL. Selected extracts of *Eugenia uniflora* (AcE), *Libidibia ferrea* (AE), and *Persea americana* (AcE) also exhibited a fungicidal effect against all clinical isolates of *T. rubrum* and *T. mentagrophytes* complex. This is the first report of the antifungal activity of *Schinus terebinthifolius*, *Piptadenia colubrina*, *Parapiptadenia rigida*, *Mimosa ophthalmocentra,* and *Persea americana* against both dermatophyte species.

KEYWORDS: *Trichophyton*; Dermatophytosis; Susceptibility; Plant extracts.

INTRODUCTION

Trichophyton rubrum and *Trichophyton mentagrophytes* complex, or *Trichophyton* spp. are the main etiologic agents of dermatophytosis in many parts of the world, including Brazil[1,2,3]. Overall, this contagious infection, commonly referred to as ringworm or tinea, is restricted to the outermost layers of the epidermis and its appendages, resulting in either a mild or intense inflammatory reaction, and in many cases, it is long lasting and difficult to treat[1]. Topical antifungal agents, mainly azoles or allylamines, are currently used for the treatment of most dermatophytoses. In some cases, such as infections of the nail and hair, a systemic treatment is required[4,5,6]. However, therapeutic efficacy can be limited by antifungal side effects and/or resistance, patient non-adherence or therapy discontinuation, the cost of medication, and drug interactions[7,8]. Antifungal drugs have a limited number of cellular targets such as ergosterol and the enzymes involved in its synthesis, nucleic acids and the cell wall synthesis, and the formation of microtubules[9]. Studies on compounds with potential antifungal action are important, not only for the treatment of dermatophytosis, but also to lead to the discovery of new cellular targets for the treatment of fungal infections.

Natural products have contributed significantly in healthcare since ancient times, when they have been extensively used in folk medicine for the treatment of various diseases[10]. Indeed, the antimicrobial activity of extracts and essential oils from native plants, including their effects on dermatophytes, has been reported worldwide[6,11,12,13,14,15]. Importantly, they exhibit a large chemical diversity and biological activity, representing an alternative easily obtainable for the treatment of various diseases.

In this study, the *in vitro* antifungal activity of different extracts obtained from nine plant species of semiarid vegetation (*caatinga*) of Northeast Brazil were investigated against *T. rubrum* and *T. mentagrophytes* complex.

MATERIAL AND METHODS

Microorganisms: *T. rubrum* ATCC 28189, *T. mentagrophytes* ATCC 11481, and 33 clinical isolates of *T. rubrum* (n = 24) and *T. mentagrophytes* complex (n = 9) were used in this study. The clinical isolates were recovered from nail and skin infections, which were obtained from the fungal collection of the Medical Mycology Laboratory of the *Universidade Estadual de Maringá.*

Plant material: Nine plant species of the semiarid vegetation (*caatinga*) of Northeast Brazil (seven native and two cultivated) were selected for this study. The voucher specimens were deposited and identified at the herbaria of the *Universidade Federal de Pernambuco*

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(UFPE), *Universidade Federal do Rio Grande do Norte* (UFRN), and *Instituto Agronômico de Pernambuco* (IPA) (Table 1).

Preparation of crude extracts: The plant parts were dried at 45 ºC, until a constant weight was achieved, and ground into powder using a Willey mill (Adamo®). The extract solutions were obtained by reflux using water (aqueous extract, AE), ethanol: water (1:1, v/v, ethanolic extract, EE), or acetone: water (1:1, v/v, acetone extract, AcE) as previously described[16] (Table 1). Stock solutions (2,000.0 µg/mL) of crude extracts were prepared in water containing 10% dimethylsulfoxide [DMSO, v/v (Sigma-Aldrich, USA)] and twofold serial dilutions were prepared in growth medium (1,000.0 –1.95 µg/mL), and added once in each assay. The final concentration of DMSO in the assays did not exceed 1% (v/v).

In vitro antifungal assay: The minimum inhibitory concentrations (MIC) of crude extracts against dermatophytes were determined using the standard broth microdilution assay[17], except for the conidia counts that were performed in a Neubauer chamber. The conidial suspensions were adjusted to obtain a final concentration ranging from 2.5×10^3 to 5×10^3 CFU/mL. Terbinafine was used as a quality control. Wells containing 1% DMSO (v/v) and wells without fungal cells in each plate served as growth and sterility controls, respectively. For determination of minimal fungicidal concentrations (MFC), the contents from the wells showing no growth were transferred to Sabouraud dextrose agar plates and incubated at 25 ºC for seven days. Selected extracts (according to MIC values and quantity available for testing) were also assayed against 33 isolates of *T. rubrum* (n = 24) and *T. mentagrophytes* complex (n = 9). All experiments were performed in duplicate on two different occasions.

The antifungal activity of the extracts was ranked according to MIC values using the criteria established by SCORZONI *et al*.[18]: MIC ≤ 75.0 µg/mL, classified as strong activity; 75.0 < MIC ≤ 150.0 µg/mL, moderate activity; 150.0 < MIC ≤ 250.0 µg/mL, weak activity, and MIC > 250.0 µg/mL, inactive.

RESULTS AND DISCUSSION

The Brazilian *caatinga* is one of the richest biomes in terms of biodiversity, harboring several native or cultivated plant species. Many plant species of this region are used in folk medicine or as commercial herbal to treat different conditions. However, few ethnobotanical and ethnopharmacological studies have been conducted on these medicinal plants[19]. Moreover, antifungal activity against dermatophytes has not yet been studied for many of these plant species. Previous results

Table 1
Plant species from Brazilian *caatinga*: tradicional use and *in vitro* antifungal activity of extracts from selected plant parts

Species (family) (voucher number)	Local name	Origin	Part tested	*T. rubrum** ATCC 28189 MIC and MFC (µg/mL)			*T. mentagrophytes** ATCC 11481 MIC and MFC (µg/mL)			Folk medicine use
				AE	EE	AcE	AE	EE	AcE	
Eugenia uniflora Linn (Myrtaceae) (11763 UFRN)	Pitanga	N	Leaves	125.0	125.0	62.5	125.0	125.0	31.3	Throat complaints[11]
Schinus terebinthifolius Raddi (Anacardiaceae) (8758 IPA)	Aroeira	N	Stem bark	1000.0	62.5	NA	1000.0	62.5	NA	Injury, inflamation of internal organs, gastritis, ulcer[19]
Piptadenia colubrina Vell. Benth (Mimosaceae) (38384 IPA)	Angico	N	Stem bark	500.0	125.0	250.0	500.0	125.0	250.0	Bronchitis, gastritis, pneumonia, colds[38]
Parapiptadenia rigida Benth. Brenan (Fabaceae) (83115 UFPE)	Angico vermelho	N	Stem bark	250.0	250.0	15.6	250.0	250.0	15.6	Asthma, bronchitis[19]
Libidibia ferrea Mart. (Caesalpiniaceae) (88145 IPA)	Pau-ferro	N	Stem bark	62.5	62.5	62.5	31.3	31.3	31.3	Blow, throat complaints, bronchitis, anemia, swelling, back pain, injury, labyrinthitis, renal problems, inflammation, stress, fatigue[19]
Psidium guajava Linn. (Myrtaceae) (8214 UFRN)	Goiaba	C	Leaves	125.0	62.5	125.0	125.0	62.5	125.0	Stomach ache, dysentery, digestive problems, headache, inflammation, gingivitis, throat complaints, leukorrhea and skin diseases[11,19]
Mimosa ophthalmocentra Mart. ex Benth (Mimosaceae) (83114 UFPE)	Jurema vermelha	N	Stem bark	125.0	125.0	NA	125.0	125.0	NA	Bronchitis, cough[19]
Mimosa tenuiflora Wild. Poir (Mimosaceae) (83113 UFPE)	Jurema preta	N	Stem bark	62.5	NA	62.5	62.5	NA	62.5	Injury, inflammation, fever[19]
Persea americana Mill. (Lauraceae) (89420 IPA)	Abacate	C	Leaves	NA	31.3	31.3	NA	31.3	31.3	Renal problems[19]

The activity of the extracts was classified as follows: MIC ≤ 75.0 µg/mL, classified as strong activity; 75.0 < MIC ≤ 150.0 µg/mL, moderate activity; 150.0 < MIC ≤ 250.0 µg/mL, weak activity, and MIC > 250.0 µg/mL, inactive [18]. Crude extract: AE - aqueous, EE: ethanol:water and AcE: acetone:water. N: Native; C: Cultivated. *Terbinafine (MIC/MFC) ≤ 0.004 µg/mL. NA: Not analyzed.

Table 2
MIC_{50} and MIC_{90} of extracts of *Libidibia ferrea*, *Persea americana* and *Eugenia uniflora* against 33 clinical isolates of dermatophytes.

Isolates	N	*Libidibia ferrea* (AE)		*Persea americana* (AcE)		*Eugenia uniflora* (AcE)	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
T. rubrum	24	31.3	62.5	31.3	62.5	62.5	125.0
T. mentagrophytes complex	9	31.3	62.5	62.5	62.5	31.3	62.5

N – number of isolates; MIC_{50} and MIC_{90}: concentration (µg/mL) of each extract that inhibited growth by 50 and 90% of all isolates, respectively.

by our research group have shown that the nine plant species (seven native and two cultivated) selected from this biome exhibited growth-inhibitory activity against various *Candida* species[16]. In this study, the antifungal activity of these plants was evaluated against *T. rubrum* and *T. mentagrophytes* complex.

First, *T. rubrum* ATCC 28189 and *T. mentagrophytes* ATCC 11481 reference strains were used for the initial screening of 23 crude extracts obtained from the plant species of the Brazilian *caatinga* and results are reported in Table 1. The presence of 1% DMSO (v/v) did not affect fungal growth. Overall, all plants showed a fungicidal effect (MIC and MFC values of each extract were exactly at the same concentration) against either species, with MIC/MFC values of the active extracts ranging from 15.6 to 250.0 µg/mL. The highest values of MIC/MFC (1,000.0 µg/mL and 500.0 µg/mL, against both species) were observed for AE of *Schinus terebinthifolius* and for AE of *Piptadenia colubrina,* and these extracts were considered inactive according to the criteria of SCORZONI *et al*.[18]. The lowest MIC/MFC values (15.6 µg/mL), against both species, were obtained for AcE of *Parapiptadenia rigida*. No differences in MIC/MFC values were observed between the solvents used for preparing the crude extracts of *Libidibia ferrea*, *Persea americana, Mimosa tenuiflora,* and *Mimosa ophthalmocentra*, and except for the last species, all crude extracts displayed strong antifungal activity. For the other plants, there was a two- to 16-fold decrease in MIC/MFC values depending on the solvent used in extract preparations. Indeed, the type of solvent used during extraction can affect both the yield and the number and type of phytochemicals obtained. The aqueous mixtures of ethanol or acetone have been used to extract mainly soluble polyphenols from plant material[20]. Polyphenols comprise a large family of compounds found in several plant species, which have a chemical structure consisting of at least one phenolic ring and which display various biological properties, including antifungal activity[21]. A high content of condensed tannins was detected in AcE of stem bark of *P. rigida*[22], which may be responsible for the antidermatophytic activity reported previously[21] and in the present study. However, the presence of other phytochemical groups cannot be ruled out.

Currently, there are still few reports describing the antifungal activity of extracts or substances derived from plant species against dermatophytes[6,11,12,13,14,15]. Among the plant species analyzed here, most have shown antifungal activity against *Candida* and *Cryptococcus* species[11,16,23,24,25,26,27,28]. More precisely, most extracts analyzed in this study were active against four *Candida* species (*C. albicans, C. dubliniensis, C. glabrata,* and *C. krusei*) with a MIC range of 15.62 to 500.0 µg/mL. However, in contrast to our results, MFC values were at least four times greater than the MIC values[16]. Regarding dermatophytes, only the antifungal activity of *E. uniflora, L. ferrea, M. tenuiflora,* and *P. guajava* had been previously described. An inhibitory effect on dermatophytes growth was observed for essential oils[29] and EE from leaves of *E. uniflora* cultivated in the Brazilian cerrado[14]. In the latter study, the authors reported MIC values of EE ranging from 500.0 to 1,000.0 µg/mL for *T. rubrum* and *T. mentagrophytes*[14], which differs from our results (125.0 µg/mL for both species). Chemical analyses of *E. uniflora* leaves revealed the presence of terpenes, mainly oxygenated sesquiterpenes, which may be responsible for the antifungal activity[21,25,29]. By using an agar well-diffusion method, LIMA *et al*.[30] reported that 2,500.0 µg/mL of AE and EE of stem bark from *M. tenuiflora* inhibited the growth of clinical isolates of four dermatophyte species (*T. rubrum, T. mentagrophytes, Microscopum canis* and, *Epidermophyton floccosum*). The antidermatophytic activity of *M. tenuiflora* may be attributed to tannins[21], which represent the major class of compounds in stem bark of this plant[31]. In the study conducted by DUTTA *et al*.[32], tinctures from leaves and stem bark of *P. guajava* (which mimics the popular use) at concentrations ranging from 5 to 15% exhibited a fungicidal effect on dermatophytes. SUWANMANEE *et al*.[33] reported the antidermatophytic activity of AE of leaves from *P. guajava* cultivated in Thailand, with MIC of 2,670.0 µg/mL, and 3,330.0 µg/mL for *T. rubrum* and *T. mentagrophytes*, respectively, which also differ from our results (125.0 µg/mL for both species). Several compounds have been detected in the leaves of *P. guajava*[34], and the antibacterial and antifungal activities may be due to the content of polyphenols, such as flavonoids, and hydrolysable and condensed tannins[21,22,35,36,]. Condensed tannins have also been detected in the aqueous extract of *L. ferrea*[22], which in this study showed strong activity against both species of dermatophytes. By using the agar well-diffusion assay, LIMA *et al*.[30] found that 1,250.0 µg/mL AE and EE of *L. ferrea* inhibited the growth of clinical isolates of *T. rubrum, T. mentagrophytes, M. canis,* and *E. floccosum.* Finally, SCHMOURLO *et al.*[28] evaluated the AE obtained by decoction of aerial parts of *S. terebinthifolius* on the growth of *T. rubrum*, by using an agar well-diffusion (1,000.0 µg/mL) and broth microdilution (10^{-6} µg/mL – 1,000.0 µg/mL) methods, and the results showed no inhibitory effect for this extract. Similarly, no antidermatophytic activity was detected for the AE from the stem bark of this plant in the present study. On the other hand, the EE of bark showed strong antifungal activity with MIC/MFC of 62.5 µg/mL for both fungal species. The differences observed between the results described in the literature and the present study may be due to the conditions of plant cultivation, such as soil type and climate, which affect the production of active compounds[37], extraction systems for plant compounds[20], and the antifungal susceptibility testing, which can affect the MIC values of the extracts[18].

According to the MIC/MFC values obtained with dermatophyte reference strains and the availability of plant extracts, *E. uniflora*, *L. ferrea,* and *P. americana* were also tested in clinical isolates, which were

all susceptible to terbinafine (MIC ≤ 0.004 µg/mL, Table 2). Selected extracts showed a fungicidal effect (MIC=MFC) against all clinical isolates of *T. rubrum* and *T. mentagrophytes* complex, corroborating the results obtained with the reference strains. MIC/MFC values of extracts against isolates of *T. rubrum* ranged from 7.8 to 250.0 µg/mL for *E. uniflora* (AcE), 7.8 to 62.5 µg/mL for *L. ferrea* (AE), and 15.6 to 62.5 µg/mL for *P. americana* (AcE). For *T. mentagrophytes* complex, the MIC/MFC values of these extracts ranged from 7.8 to 62.5 µg/mL for *E. uniflora*, 15.6 to 62.5 µg/mL for *L. ferrea*, and 7.8 to 62.5 µg/mL for *P. americana*. Table 2 shows the MIC_{50} and MIC_{90} of extracts of these three plant species, for 50 and 90% growth-inhibition of all isolates, respectively. Overall, a slight increase (twofold) in the MIC_{90} values was observed for all extracts against both fungal species. However, except for AcE of *E. uniflora*, which exhibited moderate activity against most clinical isolates (MIC_{90} of 125.0 µg/mL), the AE of *L. ferrea* (MIC_{90} of 62.5 µg/mL) and AcE of *P. americana* (MIC_{90} of 62.5 µg/mL) showed strong activity against most isolates.

In conclusion, all the *caatinga* plants studied showed a fungicidal effect against *T. rubrum* and *T. mentagrophytes* complex. Except for *E. uniflora, L. ferrea*, *M. tenuiflora,* and *P. guajava,* the antifungal activity of *S. terebinthifolius*, *P. colubrina*, *P. rigida*, *M. ophthalmocentra,* and *P. americana* against both fungal species was described for the first time. These results are useful as a preliminary step towards further antidermatophytic-guided studies of plant species from the Brazilian *caatinga*.

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