

ANTIFUNGAL PROPERTIES OF BRAZILIAN CERRADO PLANTS

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SHORT COMMUNICATION

ABSTRACT

Ethanollic extracts from leaves of *Hyptis ovalifolia*, *H. suaveolens*, *H. saxatilis*, *Hyptidendrum canum*, *Eugenia uniflora*, *E. dysenterica*, *Caryocar brasiliensis* and *Lafloensia pacari* were investigated for their antifungal activity against dermatophytes. The most effective plants were *H. ovalifolia* and *E. uniflora*, while *Trichophyton rubrum* was the most sensitive among the four dermatophytes species evaluated. This study has demonstrated antifungal properties of Brazilian Cerrado plant extracts in "in vitro" assays.

Key words: Dermatophytes, antifungal activity, plant extracts, susceptibility tests.

The antifungal agents (griseofulvin, azole derivatives, allylamines and morpholines) used in the treatment of dermatophyte infections, can sometimes have adverse side effects such as gastrointestinal disturbances, cutaneous reactions, hepatotoxicity and leukopenia (2,6). Besides these factors, the acquired resistance to certain antifungals (3,5,13) and the high cost of these synthetic medicines limit the treatment of the dermatophytosis. Plants of Brazilian Cerrado have been broadly used in popular medicine (10). The antifungal activity of extracts and bioactive compounds of *Eugenia dysenterica* D.C. and *Annona crassiflora* Mart., against pathogenic fungi *Cryptococcus neoformans* and *Candida* species were reported by Costa *et al.* (4) and Silva *et al.* (11), respectively. The current study was carried out to determine the *in vitro* antifungal activity of Brazilian Cerrado plant extracts against dermatophytes isolates from different clinical specimens.

Leaves of *Hyptis suaveolens* (L.) Poit., *H. ovalifolia* Benth., *H. saxatilis* A. St. Hil. ex Benth., *Hyptidendrum canum* (Pohl ex Benth.) Harley (syn.: *Hyptis cana* Pohl ex Benth.), (Lamiaceae), *Eugenia uniflora* L., *E. dysenterica* (Myrtaceae), *Caryocar*

brasiliensis Camb. (Caryocaraceae) and *Lafloensia pacari* St. Hil. (Lythraceae) were collected in the Brazilian Cerrado, Goiás State, and identified by Prof. Heleno Dias Ferreira (Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Goiás). Air-dried leaves were powdered and exhaustively extracted with ethanol 95% at room temperature for 2 days. Extracts were filtered and the solvent removed under reduced pressure at 40°C. Preliminary antifungal assays were performed using thirty isolates of dermatophytes and leaves extracts at concentration of 1000 µg/mL. Fragments (2 mm) of dermatophytes were inoculated on Mycobiotic agar previously incorporated with extracts, and the antifungal activity was determined by total growth inhibition. *H. ovalifolia* and *E. uniflora*, which showed the best results in the preliminary assays, were chosen for further evaluation of the minimal inhibitory concentration (MIC). The susceptibility test was performed as described by Cáceres *et al.* (1). The extracts were solubilized in 1 mL of dimethyl sulfoxide (DMSO) and serially two-fold diluted in Mycobiotic agar medium to obtain a concentration range of 7.8 to 1000 µg/mL. Mycobiotic agar

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medium containing only DMSO diluted in the same way, which did not influence fungal growth, was included as control. Dermatophytes suspended in 0.85% sterile physiological saline with tween 80 (0.05%) and standardized at OD₉₀ 530 nm (9) were inoculated (10 µL) in holes of 3 mm done in the medium using a Steers inoculator. The inoculated plates were then incubated at 25°C for 5 days and the MIC determined as the lowest concentration of the plant extracts able to inhibit any visible fungal growth. Duplicate plates were used for each assay. The susceptibility standard test using terbinafine (10 µg/mL), as the positive control, were performed applying the same technique.

Antifungal activity of extracts at the concentration of 1000 µg/mL was observed in seven out of eight plants tested. *H. ovalifolia* and *E. uniflora* were the most promising inhibitors, inhibiting completely the growth of the 30 dermatophytes tested. Further evaluation of the minimal inhibitory concentration of these two plants showed that *H. ovalifolia* was the most active, inhibiting 25 out of the 30 dermatophytes at 250 µg/mL. In addition, strains of *Trichophyton rubrum* were the most susceptible as 7 out of the 10 isolates were inhibited in this concentration. *E. uniflora* showed antifungal activity on 19 out of the 30 isolates at the concentration of 500 µg/mL (Table 1).

Antifungal activity of medicinal plants against some dermatophytes has also been reported by other researchers. Cáceres *et al.* (1) showed that from 44 aqueous plant extracts, 50% inhibited the growth of dermatophytes including *Microsporum canis* and *T. mentagrophytes*. In addition, the fungitoxicity had been previously described in *Annona crassiflora* leaf ethanolic extract and essential oil from leaves of *E. dysenterica*, which were found to inhibit the growth of *Candida* species and *Cryptococcus neoformans* (4,11). In the present study, *E. uniflora* and *H. ovalifolia* appeared very promising for antifungal therapy. Their activity were consistent with there of the pharmacological activity shown by other members of genus *Hyptis* (7,8). Iwu *et al.* (7) observed that

essential oil of *H. suaveolens* displayed good antimicrobial activity against yeast and filamentous fungi. Among the four species of dermatophytes tested in the present study, *T. rubrum* was the most sensitive to the plant extracts. This observation has particular interest because *T. rubrum* is the most common specie of dermatophyte, and represents between 80 to 90% of all chronic and recurrent infections (12). This species also shows resistance to the antifungal product used for the dermatophytosis treatment (13). Our findings indicate that traditional medicines remain a valuable resource for the discovery of natural pharmaceutical products.

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RESUMO

Propriedades antifúngicas de plantas do Cerrado brasileiro

A atividade antifúngica de extratos etanólicos de folhas de *Hyptis ovalifolia*, *H. suaveolens*, *H. saxatilis*, *Hyptidendrum canum*, *Eugenia uniflora*, *E. dysenterica*, *Caryocar brasiliensis* e *Lafoensia pacari* sobre isolados de dermatófitos foi verificada. Os extratos mais ativos foram os de *H. ovalifolia* e *E. uniflora*, enquanto que *Trichophyton rubrum* foi o dermatófito mais sensível a ação das plantas. Estes dados demonstram as propriedades antifúngicas de plantas do Cerrado em ensaios *in vitro*.

Palavras-chave: Dermatófitos, atividade antifúngica, extratos de plantas, teste de suscetibilidade.

Table 1. Cumulative percentage inhibition of dermatophyte isolates after treatment with different concentrations of *H. ovalifolia* and *E. uniflora* ethanolic extracts.

Plants/Dermatophytes (Nº of strains)		Concentration (µg/mL)							
		7.8	15.6	31.2	62.5	125	250	500	1000
<i>Hyptis ovalifolia</i>	<i>M. canis</i> (5)		20	20	20	40	100		
	<i>M. gypseum</i> (5)				20	20	60	60	100
	<i>T. rubrum</i> (10)				70	70	100		
	<i>T. mentagrophytes</i> (10)						70	70	100
<i>Eugenia uniflora</i>	<i>M. canis</i> (5)							40	100
	<i>M. gypseum</i> (5)							60	100
	<i>T. rubrum</i> (10)							100	
	<i>T. mentagrophytes</i> (10)							40	100

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