

Antimicrobial Activity of *Hyptis ovalifolia* Towards Dermatophytes

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The essential oil and the aqueous, hexane and methanolic fractions from Hyptis ovalifolia leaves were evaluated for their antifungal activity in vitro against 60 strains of dermatophytes: 10 strains of Microsporum canis, 10 of M. gypseum, 20 of Trichophyton rubrum and 20 of T. mentagrophytes. The extracts inhibited growth of the dermatophytes tested at different concentrations. The most biologically active was the essential oil from the leaves which inhibited 57 isolates (95%) at a concentration of ≤ 500 $\mu\text{g/ml}$.

Key words: dermatophytes - *Hyptis ovalifolia* - antifungal activity

Mycotic infections are probably the most common cause of skin disease in developing countries of tropical regions. Dermatophytosis is the most frequent superficial fungal infection in Brazil. The drugs used against dermatophytosis exhibit several side effects and have limited efficacy (Gupta et al. 1998, Carazo et al. 1999). So that there is a distinct need for the discovery of new safer and more effective antifungal agents. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world (Irobi et al. 1993). This use has been supported by the isolation of active antifungal compounds from plant extracts (Costa et al. 2000, Silva et al. 2001, Souza et al. 2002, Passos et al. 2002).

Previous reports on the activity of ethanolic extract from leaves of *Hyptis ovalifolia* against dermatophytes have been reported by Souza et al. (2002). In this study the antifungal activity of the essential oil and the aqueous, hexane, and methanolic fractions from *H. ovalifolia* leaves against 60 dermatophytes is described.

MATERIALS AND METHODS

Plant material - *Hyptis ovalifolia* Benth. (Lamiaceae) was collected in Goiânia, state of Goiás, Brazil and identified by Prof. Heleno Dias Ferreira, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Goiás (UFG). Voucher samples were preserved at the herbarium of the UFG.

Preparation of plant extract - The essential oil of *H. ovalifolia* leaves was obtained by steam distillation in a Clevenger-type apparatus for 5 h. The ethanolic extract of dried leaves was partitioned between chloroform and water. The chloroform fraction after evaporation was

partitioned between hexane/methanol. The hexane, methanol and aqueous fractions were reduced to dryness and solutions of each as well as of the essential oil were dissolved in dimethyl sulfoxide (DMSO) prepared for subsequent bioassay.

Dermatophyte isolates - The microorganisms used for the biological evaluation were clinical isolates provided by Laboratório de Micologia, Instituto de Patologia Tropical e Saúde Pública, UFG, and identified by standard procedure (Rebell & Taplin 1970). They were: 10 *Microsporum canis*, 10 *M. gypseum*, 20 *Trichophyton rubrum* and 20 *T. mentagrophytes*. Sabouraud's dextrose agar at 25°C was used to maintain isolates. In antifungal assays, the samples were transferred to potato dextrose agar and maintained for 7 days at 25°C.

Antifungal assay - The susceptibility test was performed as described by Cáceres et al. (1993), with the following modifications. The extracts were solubilized in DMSO and a two-fold serial dilution was performed in Sabouraud with chloramphenicol and cycloheximide agar medium, to obtain a concentration range of 3.9 to 1000 $\mu\text{g/ml}$, poured into Petri dishes. Thirty seven wells of 3 mm were opened using a Steers inoculator. To each well 10 μl of a dermatophyte suspension were added. This suspension was prepared using 0.85% sterile physiological saline with Tween 80 (0.05%) and adjusted with a spectrophotometer at 530 nm to obtain 90% transmission (Lima et al. 1993). The inoculated plates were then incubated at 25°C for 5 days and the minimal inhibitory concentration (MIC) was defined as the lowest concentration that substantially inhibited growth of the microorganism detected visually. Duplicate plates were used for each assay. A growth control of the test strains and a susceptibility standard test using terbinafine (10 $\mu\text{g/ml}$) as the reference system were performed applying the same technique.

RESULTS

The results of antifungal assay showed that the essential oil, aqueous, methanolic, and hexane fractions obtained from *H. ovalifolia* leaves possess antifungal activity against the dermatophytes studied (Tables I, II).

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TABLE I
Antifungal activity of different fractions of *Hyptis ovalifolia* leaves against dermatophyte isolates

Fractions	Dermatophyte (n = 60)	Cumulative number of strains inhibited at concentrations (µg/ml)				
		125	250	500	1000	not inhibited
Aqueous	<i>M. canis</i> ^a	0	0	6	10	0
	<i>M. gypseum</i> ^a	0	0	2	4	6
	<i>T. rubrum</i> ^b	0	2	8	10	10
	<i>T. mentagrophytes</i> ^b	0	0	0	0	20
Methanolic	<i>M. canis</i> ^a	1	6	8	8	2
	<i>M. gypseum</i> ^a	0	2	7	10	0
	<i>T. rubrum</i> ^b	3	9	19	19	1
	<i>T. mentagrophytes</i> ^b	0	6	16	17	3
Hexane	<i>M. canis</i> ^a	0	1	2	6	4
	<i>M. gypseum</i> ^a	0	0	2	6	4
	<i>T. rubrum</i> ^b	0	0	3	10	10
	<i>T. mentagrophytes</i> ^b	0	0	3	9	11

M: *Microsporium*; T: *Trichophyton*; a: n = 10; b: n = 20

TABLE II
Antifungal activity of essential oil of *Hyptis ovalifolia* leaves against dermatophyte isolates

Fraction tested	Dermatophyte (n = 60)	Cumulative number of isolates inhibited at concentrations (µg/ml)							
		7.8	15.6	31.2	62.5	125	250	500	1000
Essential oil	<i>M. canis</i> ^a	0	4	0	0	7	9	10	10
	<i>M. gypseum</i> ^a	1	2	4	5	9	9	10	10
	<i>T. rubrum</i> ^b	2	9	10	13	15	20	20	20
	<i>T. mentagrophytes</i> ^b	0	0	0	3	7	19	20	20

M: *Microsporium*; T: *Trichophyton*; a: n = 10; b: n = 20

The antifungal activity of *H. ovalifolia* leaves for all dermatophytes at the concentration ≤ 1000 µg/ml showed that the aqueous fraction inhibited the growth of 40% (24/60), the methanolic fraction of 90% (54/60), the hexane fraction of 51.7% (31/60), while the essential oil inhibited 100% of 60 dermatophytes tested.

The susceptibility of dermatophyte species towards the different fractions differed. *T. rubrum* was the most susceptible species to most fractions tested. Three isolates of this species were inhibited by the methanolic fraction at 125 µg/ml and two isolates were inhibited by the essential oil at 7.8 µg/ml. *T. mentagrophytes* was the least susceptible of the dermatophytes. The aqueous fraction had no activity against this species, while the essential oil, on average, the most active against all dermatophytes tested only showed growth inhibition for this species in high concentration. Terbinafine, used as control inhibited all dermatophytes at 10 µg/ml.

DISCUSSION

The results obtained in our study show wide spectrum antifungal properties for the hexane, methanolic, and aqueous fractions and for the essential oil of *H. ovalifolia* leaves. In the earlier studies Souza et al. (2002) verified that the crude extract of this plant inhibited growth of dermatophyte isolates at concentrations between 15.6 to

1000 µg/ml. Paula et al. (2001) showed that a crude extract of the leaves of this plant has activity against *Cryptococcus neoformans*, one of the most important fungi responsible for meningitis in HIV patients.

The broad spectrum of activity of the methanolic fraction which inhibited 90% of dermatophytes isolated is only surpassed by that of the essential oil which inhibited the growth of all dermatophytes tested at concentration ≤ 1000 µg/ml. The broad activity could be explained by essential oil composition. The leaves of *H. ovalifolia* investigated by GC/MS showed that the main constituent representing 60% of the essential oil isolated by column chromatography was identified by spectral methods as (R)-6-[(Z)-1-heptenyl]-5,6-dihydro-2H-piran-2-one.

The antifungal activity of essential oils from other plants is well known. The essential oil of *Eugenia dysenterica* shows good activity against *C. neoformans* (Costa et al. 2000) and *H. suaveolens* is active against *Candida albicans* (Asekun et al. 1999). Thus the broad spectrum of antifungal activity is consistent with results obtained by several researchers for other essential oils which have shown protection action against both bacterial and fungal pathogens (Janssen et al. 1987).

The variation of susceptibility in vitro from one species to another is evident from the results. *T. rubrum*

the commonest etiological agent of dermatophytosis in Goiânia (Costa et al. 1999, 2002) was the most susceptible dermatophyte. This species is the cause of between 80-90% of all chronic and recurrent infections (Torres-Rodrigues et al. 1998). The fact that current dermatophytosis therapy can cause considerable side-effects and adverse effects in some patients (Gupta et al. 1998), renders plant-derived compounds of special interest because they are generally safer and often more effective substitutes for the synthetically produced antimicrobial agents.

The essential oil of *H. ovalifolia* leaves is thus of particular interest as a source of new anti infective agents for dermatophytic infections in humans. An investigation on a larger scale would allow a better evaluation of the susceptibility phenomena of dermatophytes to essential oil of *H. ovalifolia*.

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