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Anti Dermatophyte Activities of *Eucalyptus camaldulensis* in Comparison with Griseofulvin

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ABSTRACT

Methanolic leaf extracts of Eucalyptus camaldulensis were investigated for in vitro antifungal activities against *Microsporum canis*, *Microsporum gypseum*, *Tricophyton rubrum*, *Tricophyton schoenleinii*, *Tricophyton mentagrophytes* and *Epedermophyton floccosum*. The studies were carried out using broth dilution method, agar dilution method and inhibitory zone estimation. The effects of the plant extract were compared with those of griseofulvin. *Eucalyptus camaldulensis* showed antifungal activity against all the dermatophytes tested with MIC values ranging from 0.4 to 1.6 mg/mL using inhibitory zone estimation, 0.4-1.6 mg/mL using agar dilution method and 0.2 to 1.6 mg/mL using broth dilution method. The minimum fungicidal concentration (MFC) of the extracts ranged from 0.8 to 6.4 mg/mL. The results obtained suggest that *E. Camaldulensis* has anti-dermatophyte activity.

Keywords: *Antifungal activities; Eucalyptus camaldulensis; Griseofulvin; Dermatophytes*

Human infections, particularly those involving the skin and mucosal surface constitute a serious problem, especially in tropical and subtropical developing countries; dermatophytes and Candida spp. being the most frequent pathogen. Herbal medicines have been important sources of products for the developing countries in treating common infections including fungal diseases. Some studies have demonstrated that the oil and leaf extracts of Eucalyptus spp. have antifungal and repellent activity [1-2]. Crude methanolic extract of *E. Camaldulensis* has been reported to inhibit the growth of *Candida albicans* [5]. Also, it has been shown that ethanolic leaf extract of *Eucalyptus camaldulensis* had marked fungicidal effect against clinical dermatophytic fungal isolates; *Microsporium gypseum* and *Trichophyton mentagrophytes* [6].

The phytochemical analysis of the crude extracts of the Eucalyptus spp. revealed the presence of saponin, saponin glycosides, steroid, cardiac glycoside, tannins, volatile oils, phenols and balsam (gum) [5]. Qualitative phytochemical tests, thin layer chromatography (TLC) and TLC-autography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents. Some compounds in the leaves of *E. Camaldulensis* include: essential oil (1 to over 2%), betulinic acid, eucalyptic acid, eucalyptolic acid, oleanolic acid and ursolic acid [7]. In this study, the antifungal effect of *E. Camaldulensis* growing in Iran has been studied against some dermatophyte spp including *Microsporum canis*, *M. gypseum*, *Tricophyton rubrum*, *T. schoenleinii*, *T. mentagrophytes* and *Epedermophyton floccosum*, which have not been studied previously.

MATERIALS AND METHODS

Plant Material

The leaves of *E. Camaldulensis* were collected from national herbarium garden Tehran, Iran in 2001.

Extraction and Isolation

The leaves of *E. Camaldulensis* were dried at room temperature (20-23ºC) and ground into a powder using a blender. The dried leaf powder was (200 g) extracted with 80% methanol (800 mL) by refluxing for 3 hours. The solution was filtered and evaporated to dryness with a rotary evaporator. A 256 mg/mL stock solution of extract was prepared in DMSO and kept at -20ºC for future use.

Microorganisms

Dermatophyte strains included PTCC (Persian Type Culture Collection) (5070) *Microsporum gypseum*,

Table 1. Inhibitory effects of griseofulvin on selected fungal species.

Strain	Griseofulvin concentration (mg/mL)								
	0.064	0.032	0.016	0.008	0.004	0.002	0.001	0.0005	0.00025
Microsporum canis	27	20	20	12	8	2	-	-	-
M. gypseum	30	18	15	2	-	-	-	-	-
Tricophyton rubrum	25	22	10	5	-	-	-	-	-
T. schoenleinii	35	25	25	23	20	11	٥	-	-
T. mentagrophytes	30	27	20	12	5	5	-	-	-
Epedermophyton floccosum	30	20	17	12	11	8	5	-	-

MICs were regarded as the lowest concentration that produced a visible zone of inhibition. Assay was performed using inhibitory zone estimating. The MIC values represent the average of 3 independent experiments.

PTCC (5060) *Microsporum canis*, PTCC (5069) *Trichophyton rubrum* PTCC (5143) and *Trichophyton schoenleinii* were obtained from Iranian scientific and industrial institute. *Trichophyton mentagrophyte* and *Epedermophyton floccosum* were isolated from patients at Mycology department of Iran Medical Science University. Dermatophyte strains identity were confirmed by slide culture and urease test [8]. All strains were maintained in 20% glycerol and 10% lactose at -190ºC in liquid nitrogen.

Inucolum Preparation

All the fungi spp. was maintained on Sabouraud dextrose agar. Sterile distilled water containing 0.05% Tween 80 was added to the surface growth and spores and hypae were scraped off with a sterile wire loop. A spectrophotometer set at 530 nm used to adjust the suspension to 90% transmittance. This resulted in a concentration of about 1×10^6 CFU/mL.

Inhibitory Zone Estimating

Qualitative antifungal screening was carried out using the agar-well diffusion assay [9]. Twenty mL of sterilized sabouraud dextrose agar medium were poured into a 15 cm Petri dish. Twenty µL of inoculums suspension of each test organism was distributed evenly over the surface. A 6mm well was cut in the centre of each plate using the wide-end of a sterilized Pasteur pipette. Fifty µl of serial dilution of methanolic leaf extracts of Eucalyptus camaldulensis or giseofolvin were placed into the wells. The plates were incubated for 5 days at 30ºC. Results of the qualitative screening were recorded as the average diameter of the inhibition zone surrounding the wells containing the test solution. Results were compared with griseofulvin. The MIC was regarded as the lowest concentration that produced a visible zone of inhibition.

Agar Dilution Method

Two mL of melted sabouraud was added to each of 10 sterile universal containers (numbered 1-10). 2 mL of the methnaolic leaf extract solution of *E. Camaldulensis* (12.8 mg/mL) was added to container 1. The contents were mixed and 2 mL transferred to container 2. This serial dilution was repeated through to container 9. 2mL was discarded from container 9. Eighteen mL of melted sabouraud dextrose agar was added to each container; the contents were mixed and poured into a 9 cm diameter Petri dish (numbered 1-10). After solidification of the medium, 20 µl of inoculum were spread onto each plate. The plates were incubated at 30ºC for 5-7 days. The final concentrations of *E. Camaldulensis* extract ranged from 6.4 to 0.025 mg/mL. For griseofulvin, the final concentration ranged from 0.064 to 0.00025 mg/mL. The MIC was the lowest drug concentration at which there was no visible fungal growth after incubation [10].

Broth Dilution Method

One mL of sterile liquid sabouraud medium was added to 11 sterile capped tubes, each. 1mL of *E. Camaldulensis* methanolic leaf extract suspension (12.8 mg/mL in medium) was added to tube 1. The contents were mixed and 1mL was transferred to tube 2. This serial dilution was repeated through to tube 9. 1 mL was discarded from tube 9. Fifty µL of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of *E. Camaldulensis* extract ranged from 6.4 to 0.025 mg/mL. For griseofulvin, the final concentration ranged from 0.064 to 0.00025 mg/mL. The tubes were incubated at 30ºC for 72 h. The fungal growth in each tube was detected turbidometrically at 530 nm. MIC was defined as the drug concentration at which the turbidity of the medium was the same as the medium control.

Ten µl aliquot of cell suspension from the tube without observed growth of fungi was inoculated on to sabouraud dextrose agar, and Minimum fungicidal concentration (MFC) of test compound was determined as

Table 2. Inhibitory effects of methanolic leaf extracts of *E. camaldulensis* on selected fungal species.

Strain	Methanolic leaf extracts of *E. camaldulensis* concentration(mg/mL)								
	6.4	3.2	1.6	0.8	0.4	0.2	0.1	0.05	0.025
Microsporum canis	20	18	10	12	8	-	-	-	-
M. gypseum	23	20	٥	-	-	-	-	-	-
Tricophyton rubrum	35	20	11	7	2	-	-	-	-
T. schoenleinii	15	15	10	5	-	-	-	-	-
T. mentagrophytes	25	20	14	10	4	-	-	-	-
Epedermophyton floccosum	33	21	10	10	5	-	-	-	-

MICs were regarded as the lowest concentration that produced a visible zone of inhibition. Assay was performed using inhibitory zone estimating. The MIC values represent the average of 3 independent experiments.

Table 3. Antifungal effects of methanolic leaf extracts of E. camaldulensis and griseofulvin on selected fungal species.

Strain	MIC (mg/mL)	
	Methanolic leaf extracts of *E. camaldulensis*	Griseofulvin
Microsporum canis	1.6	0.004
M. gypseum	1.6	0.016
Tricophyton rubrum	1.6	0.004
T. schoenleinii	0.4	0.001
T. mentagrophytes	0.4	0.002
Epedermophyton floccosum	0.4	0.002

MICs were determined visually as the concentration that gave 100% inhibition. Assay was performed using agar dilution method. The MIC values represent the average of 3 independent experiments.

Table 4. Inhibitory effects of methanolic leaf extracts of E. camaldulensis and griseofulvin on selected fungal species.

Strain	MIC (mg/mL)	
	Methanolic leaf extracts of *E. camaldulensis*	Griseofulvin
Microsporum canis	0.8	0.001
M. gypseum	1.6	0.008
Tricophyton rubrum	1.6	0.004
T. schoenleinii	0.4	0.001
T. mentagrophytes	0.2	0.002
Epedermophyton floccosum	0.2	0.001

MICs were determined visually as the concentration that gave 100% inhibition. Assay was performed using broth dilution method. The MIC values represent the average of 3 independent experiments.

the lowest concentration of the agent at which no colonies were seen after 4 days at 30ºC [11].

RESULTS

Inhibitory Zone Estimating

All tested fungi were affected by griseofulvin and *E. Camaldulensis* methanolic leaf extract. The greatest inhibitory effect of griseofulvin was recorded with *Tricophyton schoenleinii* (35 mm; inhibition zone). *Tricophyton mentagrophytes* showed the most susceptibility against *E. Camaldulensis* methanolic leaf extract (35 mm). The results are presented in Table 1 and Table 2.

Agar Dilution Method

The MICs of griseofulvin and *E. Camaldulensis* methanolic leaf extract obtained by the agar dilution assay are presented in Table 3. Griseofulvin showed the greatest and the least antifungal activity against *Tricophyton schoenleinii* and *Microsporum gypseum*, respectively. The MICs of *E. Camaldulensis* methanolic leaf extract ranged from 0.4 to 1.6 mg/mL. *Epedermophyton floccosum*, *Tricophyton schoenleinii* and *Tricophyton mentagrophytes* were the most susceptible strains against *E. Camaldulensis* methanolic leaf extract.

Broth Dilution Method

The MICs of griseofulvin and *E. Camaldulensis* methanolic leaf extract obtained by the broth dilution assay are presented in Table 4. The MICs of griseofulvin and *E. Camaldulensis* methanolic leaf extract ranged from 0.001 to 0.008 mg/mL and 0.2 to 1.6 mg/mL, respectively. The least sensitive strain to griseofulvin was *Microsporum gypseum***.** The most sensitive dermatophyte strains to *E. Camaldulensis* methanolic leaf extract were *Tricophyton mentagrophytes* and *Epedermophyton floccosum***.** The MFCs of *E. Camaldulensis* methanolic leaf extract ranged from 0.8 to 6.4 mg/mL (Table 5)**.**

Table 5. Fungicidal effects of methanolic leaf extracts of E. camaldulensis and griseofulvin on selected fungal species.

Strain	MFC (mg/mL)
	Methanolic leaf extracts of *E. camaldulensis*
Microsporum canis	6.4
M. gypseum	3.2
Tricophyton rubrum	1.6
T. schoenleinii	0.8
T. mentagrophytes	0.8
Epedermophyton floccosum	0.8

MFC was determined as the lowest concentration of the agent at which no colonies were seen after 4 days. The MFC values represent the average of 3 independent experiments.

DISCUSSION

The emergence of anti fungal resistant strain of various fungi such as Candida, dermatophyte and cryptococcus neoformans has prompted research into developing new strategies for fighting fungal infections [12] which may be less toxic to man. Some studies have demonstrated the inhibitory effects of essential oil and leaf extract of Eucalyptus spp. against some fungi strains [1-4]. In this study**,** the inhibitory effects of methanolic leaf extracts of *Eucalyptus camaldulensis* were studied against six species of pathogenic dermatophytes including *Microsporum canis*, *Microsporum gypseum*, *Tricophyton rubrum*, *Tricophyton schoenleinii*, *Tricophyton mentagrophytes* and *Epedermophyton floccosum*. Inhibitory zone estimating, agar dilution method and broth dilution method were used in this study, and the results were compared with each other and griseofulvin.

Our results demonstrated that methanolic leaf extract of *E. Camaldulensis* has concentration dependent antifungal activity against all tested strains. All the methods used, showed that *Tricophyton mentagrophytes* was the most susceptible strain to methanolic leaf extracts of *E. Camaldulensis*.

As expected from a crude extract with many components, the methanolic leaf extract of *E. Camaldulensis* had a much larger MICs value than griseofulvin. However as the methanolic leaf extract of *E. Camaldulensis*, is known to have a very low toxicity [13], one might conclude that the mixture would probably produce less side-effects and toxicity compared with conventional chemotherapeutic agents.

The results obtained suggest that *E. Camaldulensis* can be used in treating diseases caused by the test organisms.

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