

Antimicrobial activity of *Mentha piperita* L. against *Candida* spp.

Atividade antimicrobiana de Mentha piperita L. sobre Candida spp.

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ABSTRACT

The aim of this study was to investigate the in vitro effects of a hydroalcoholic extract, an infusion and the essential oil from *Mentha piperita* L. on the growth of *Candida* spp. The hydroalcoholic extract and essential oil were evaluated for antimicrobial activity against 50 strains of *Candida albicans*, 10 strains of *Candida glabrata*, 10 strains of *Candida tropicalis*, 8 strains of *Candida parapsilosis* and 2 strains of *Candida krusei*. The minimum inhibitory concentration (MIC) of the hydroalcoholic extract was determined by the broth dilution method, and the antimicrobial activity of the essential oil was determined by the agar diffusion test. The effect of an infusion of *Mentha piperita* L. on the adherence of standard *Candida* strains to acrylic resin was also investigated. The results demonstrated that the hydroalcoholic extract of *Mentha piperita* L. showed fungistatic activity against the strains *C. albicans*, *C. tropicalis* and *C. glabrata*. The essential oil showed the strongest inhibitory activity against the strains *C. albicans*, *C. tropicalis* and *C. parapsilosis*. Despite this inhibitory activity, an infusion of *Mentha piperita* L. did not inhibit the adherence of *Candida* to acrylic resin. In conclusion, *Mentha piperita* L. presented significant antimicrobial activity against the strains *C. albicans* and *C. tropicalis* but showed no effect on the adherence of these microorganisms to acrylic resin.

UNITERMS

Mentha piperita; *Candida*; medicinal plants.

INTRODUCTION

Bacteria and fungi are capable of developing resistance to drugs used as therapeutic agents. Microbial resistance is a growing problem, and the future of antimicrobial treatments remains uncertain. Numerous research institutions in Australia [1], Iran

[2], Italy[3], Turkey [4], and elsewhere around the world have conducted studies on the antimicrobial properties of medical plants. Important research concerning the antimicrobial activity of plants has also been performed in Brazil [5-9]. However, laboratory and clinical evidence is still lacking regarding the efficacy and safety of using antimicrobials from plants

in animals and humans. The estimated therapeutic benefits of medicinal plants are principally derived from subjective empirical evidence gained from the practice of traditional medicine [7].

Candida species are opportunistic fungal pathogens that can cause local or systemic infections in humans with predisposing factors, such as the use of dental prostheses, prolonged treatment with antibiotics, or a compromised immune system. Current antifungal drugs have demonstrated limitations such as low potency, low solubility, toxicity and the development of resistance by fungal strains. Thus, the investigation of natural products against *Candida* spp. has increased significantly over the last decade, and these studies have involved more than 250 plant species [6].

Peppermint (*Mentha piperita* L.), an aromatic plant of the Labiatae family, produces an essential oil rich in menthone (14 - 32%) and menthol (30 - 50%). Menthol is widely used in the food, pharmaceutical, hygiene and tobacco industries [10]. The objective of this work was to study the in vitro effects of a hydroalcoholic extract, an infusion and the essential oil from *Mentha piperita* L. on variety of *Candida* species.

MATERIALS AND METHODS

This work was reviewed and approved by the Research Ethics Committee of the School of Dentistry of São Paulo State University/UNESP (08/2007-PH/CEP)

Mentha piperita L. collection

During the morning, *Mentha piperita* L. was collected from the medicinal plant garden of the Agronomy Department of University of Taubaté/UNITAU.

Preparation of a hydroalcoholic extract of *Mentha piperita* L.

The preparation of a hydroalcoholic extract of *Mentha piperita* L. was based on the method of Martins et al. [11]. Fresh leaves were weighed (500 g) and transferred into an amber glass flask containing 1 L of 99.3 °GL ethanol (Merck, Rio de Janeiro, RJ, Brazil). The flask was stored at room temperature, macerated for 15 days, and agitated twice a day for 60 seconds.

Next, the extract was filtered through a 90 µg filter

paper (Framex, Blumenau, SC, Brazil) and analyzed to measure the alcohol content using an alcoholmeter, with a calculated result of 79 °GL ethanol. Then, a 79 °GL ethanol solution was immediately prepared and used as a control substance. Both the hydroalcoholic extract from *Mentha piperita* L. and the control were filter-sterilized through a 0.22 µm ester cellulose membrane (Millipore, São Paulo, SP, Brazil).

Extraction of the essential oil from *Mentha piperita* L.

Essential oil was extracted from *Mentha piperita* L. by hydrodistillation, using a method proposed by Pereira et al. [12]. Fresh *Mentha piperita* L. leaves (100 g) and 250 mL of water were mixed in a glass volumetric flask. The mixture was heated over a Bunsen burner flame and the generated water vapor was collected using a 75 cm serpentine condenser. The essential oil was then separated from the water using a separatory funnel.

Preparation and infusion of *Mentha piperita* L.

An infusion of *Mentha piperita* L. was prepared by combining 10 g of fresh leaves and 100 ml of distilled water in a sealed flask, stirred and left covered for 10 min. Next, the infusion was filter-sterilized through a 90 µg filter paper (Framex, Blumenau, SC, Brazil) and sterilized through a 0.22 µm ester cellulose membrane (Millipore, São Paulo, SP, Brazil).

Microorganisms

The antimicrobial activities of the hydroalcoholic extract and the essential oil from *Mentha piperita* L. were evaluated on 80 *Candida* strains: 50 *C. albicans*, 10 *C. glabrata*, 10 *C. tropicalis*, 8 *C. parapsilosis* and 2 *C. krusei*. These strains were supplied by the Microbiology and Immunology Laboratory of the School of Dentistry of São Paulo State University and were obtained from the human buccal cavity.

The effect of the infusion of *Mentha piperita* L. on the adherence of *Candida* to acrylic resin was tested using standard strains of *C. albicans* (ATCC 18804), *C. glabrata* (ATCC 90030), *C. tropicalis* (ATCC 13803) and *C. krusei* (ATCC 6258).

A *Candida* suspension containing 10⁶ cells/mL was prepared for each *Candida* strain; suspensions were standardized using a spectrophotometer (Micronal, B-582, São Paulo, SP, Brazil) set at a wavelength of 530 nm and an optical density of 0.284.

Determination of the minimum inhibitory concentration (MIC) of the hydroalcoholic extract from *Mentha piperita* L.

The minimum inhibitory concentration (MIC) of the hydroalcoholic extract of *Mentha piperita* L. was determined by the broth dilution method. The hydroalcoholic extract was diluted in Sabouraud broth medium (Difco, Detroit, MI, USA) to the following concentrations: 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.9 mg/mL of the extract. Then, 100 µL of the standardized suspension of each *Candida* strain was added to the medium. The tests were performed in duplicate. The control group consisted of cultures containing Sabouraud broth (Difco, Detroit, MI, USA) and 79° GL ethanol submitted to the same dilution procedure as the hydroalcoholic extract from *Mentha piperita* L. After incubation at 37 °C for 24 hours, the turbidity reading of the medium was taken by visual observation. The lowest concentration of hydroalcoholic extract that inhibited microbial growth in the broth was considered the MIC.

Antimicrobial activity of the essential oil from *Mentha piperita* L.

The antimicrobial activity of the *Mentha piperita* L. essential oil was determined by the agar diffusion test. The standardized *Candida* suspension was seeded by pour plate in Sabouraud dextrose agar (Difco, Detroit, MI, USA) using a proportion of 1:200. Both the essential oil from *Mentha piperita* L. and the control substances were applied with a Steers replicator.

The positive control for growth was performed using 0.1 mg/mL of chloramphenicol (União Química Farmacêutica Nacional S/A, São Paulo, SP, Brazil), and the negative control for growth was performed using 100,000 UI/mL of nystatin (EMS S/A, Hortolândia, SP, Brazil). After incubation at 37 °C for 24 hours, antimicrobial activity was determined by measuring the area of growth inhibition where the essential oil of *Mentha piperita* L. was applied.

Evaluation of the effects of an infusion of *Mentha piperita* L. on the adherence of *Candida* to acrylic resin

Standardized samples of chemically activated acrylic resin (Clássico, Produtos Odontológicos Ind. Ltda, São Paulo, SP, Brazil), measuring 5 mm in diameter and 2.5 mm in thickness, were obtained. Thirty samples were used for each *Candida* strain; 15

were used for the infusion of *Mentha piperita* L. group and 15 for the control group.

After sterilization in an autoclave, the samples were placed in 24-well cell culture plates (Costar Corning, New York, NY USA), containing both 1 ml of Sabouraud broth medium (Difco, Detroit, MI, USA) and either 1 mL of the *Mentha piperita* L. infusion or 1 mL of distilled water (control group). The standardized *Candida* suspension (1 µl) was then added to each well.

After incubation at 37 °C for 24 hours, the samples were removed and washed with 1 ml of 0.85% physiological solution (Labsynth, São Paulo, SP, Brazil). Then, the samples were mixed for 60 seconds in tubes containing 2 ml of 0.85% physiological solution and glass beads. Yeast that adhered to the samples were dispersed, diluted and cultivated in Sabouraud dextrose agar (Difco, Detroit, MI, USA) for 48 hours at 37 °C. The number of colony forming units (CFU/ml) was counted, and the results were analyzed by Student's t test using a 5% level of significance ($p < 0.05$).

RESULTS

Minimum inhibitory concentration (MIC) of a hydroalcoholic extract of *Mentha piperita* L.

The results for the MIC assay are shown in Table 1. The MIC of the hydroalcoholic extract of *Mentha piperita* L. was lower than the control substance for the strains *C. albicans*, *C. tropicalis* and *C. glabrata*. (Table 1).

Antimicrobial activity of the essential oil from *Mentha piperita* L.

Among all the *Candida* strains analyzed ($n = 80$), the essential oil of *Mentha piperita* L. showed an inhibitory activity against 39 strains, exhibiting inhibition halos between 5 and 10 mm. *C. albicans* was the species that was most sensitive to the essential oil of *Mentha piperita* L. (Table 2).

Evaluation of the effects of an infusion of *Mentha piperita* L. on the adherence of *Candida* to acrylic resin

The mean CFU/mL (Log) values for *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* were similar between the infusion and control groups. There were no statistically significant differences between the groups (Table 3).

Table 1 - Percent of inhibition of Candida strains by the hydroalcoholic extract of Mentha piperita L. and control substances

Candida strains		Extract Concentration (mg/mL)						
250		125	62.5	31.25	15.62	7.81	3.90	
<i>C. albicans</i> (n = 50)	E	100	100	100	82	0	0	0
	C	100	100	96	50	0	0	0
<i>C. tropicalis</i> (n = 10)	E	100	100	100	90	20	0	0
	C	100	100	100	70	20	0	0
<i>C. glabrata</i> (n = 10)	E	100	100	100	20	0	0	0
	C	100	100	100	0	0	0	0
<i>C. parapsilosis</i> (n = 8)	E	100	100	100	0	0	0	0
	C	100	100	100	0	0	0	0
<i>C. krusei</i> (n = 2)	E	100	100	100	0	0	0	0
	C	100	100	100	0	0	0	0

E: hydroalcoholic extract of Mentha piperita L.; C: control

Table 2 - Sensitivity of Candida strains to essential oil of Mentha piperita L.

Species	Number of strains inhibited	%
<i>C. albicans</i> (n=50)	34	68
<i>C. tropicalis</i> (n=10)	4	40
<i>C. parapsilosis</i> (n=8)	1	12.5
<i>C. glabrata</i> (n=10)	0	0
<i>C. krusei</i> (n=2)	0	0
Total (n=80)	39	48.75

Table 3 - Mean and standard deviation of Candida CFU/mL (Log)

Species	Groups	Mean	Standard deviation	p-value*
<i>C. albicans</i>	Infusion	4.41	0.21	0.088
	Control	4.24	0.28	
<i>C. glabrata</i>	Infusion	4.42	0.18	0.450
	Control	4.36	0.22	
<i>C. tropicalis</i>	Infusion	4.30	0.21	0.390
	Control	4.22	0.24	
<i>C. krusei</i>	Infusion	4.38	0.18	0.067
	Control	4.24	0.22	

*Student's t test showed no statistically significant difference between the infusion and control groups for the Candida species studied.

DISCUSSION

In the present study, the hydroalcoholic extract of Mentha piperita L. showed MIC values ranging from 15.62 to 62.0 mg/mL for the Candida strains tested. In a study by Ertürk [13], the hydroalcoholic extract of Mentha piperita L. showed strong antifungal activity against a standard strain of *C. albicans*, exhibiting an MIC value of 5 mg/mL. Mardegan et al. [14] also evaluated an extract of dried Mentha piperita L. and observed MIC values between 0.25 and 1.75 mg/

mL for Candida species isolated from patients with periodontal disease.

In this study, 80 clinical strains of Candida isolated from the human oral cavity, representing five different species, were analyzed. The hydroalcoholic extract of Mentha piperita L. showed the greatest fungistatic activity for *C. albicans*, followed by *C. tropicalis* and *C. glabrata*. However, no fungistatic activity was observed for *C. parapsilosis* or *C. krusei*. Silva et al. [15] demonstrated that an ethanol extract of *Annona crassiflora* showed fungistatic activity on strains of

C. albicans, *C. tropicalis* and *C. krusei*. Strains of *C. tropicalis* were more sensitive to the ethanol extract of *Annona crassiflora* than the *C. krusei* or *C. albicans* strains. These results demonstrate that the sensitivity of *Candida* to medicinal plant extracts varies according to the different species of the genus *Candida*.

The essential oil of *Mentha piperita* L. analyzed in this study inhibited 48.75% of the strains tested and *C. albicans* was the species most sensitive to the oil, followed by *C. tropicalis*. Several studies in the literature also observed antimicrobial activity against *C. albicans* for the essential oil of *Mentha piperita* L. [16-19]. The essential oil of *Mentha piperita* L. displayed fungicidal activity at concentrations of 2, 8 and 25 $\mu\text{L}/\text{mL}$ in studies by Yadegarinia et al. [19], Mímica-Dukic et al. [18] and Hammer et al. [17], respectively.

The different results observed in the literature can probably be attributed to natural variations in the composition of the essential oils tested, even among those extracted from the same species, as a result of different collection times, climatic variations and extraction methods, among other factors. Moreover, both differences between the microbiological tests used and different strain sensitivities are important factors for the observed results [3].

Menthol has been considered the chemical component of the essential oil of *Mentha piperita* L. responsible for the antimicrobial activity [20]. However, Yadegarinia et al. [19] suggested that menthol should not be considered the only component responsible for the antimicrobial activity of *Mentha piperita* L. These authors observed strong antimicrobial properties for the essential oil even when it contains low concentrations of menthol, showing that other chemical compounds present also contribute to the

antimicrobial activity of the essential oil from *Mentha piperita* L.

Although the antimicrobial activity of *Mentha piperita* L. has been tested on numerous pathogenic microorganisms, few studies have evaluated its effect on the adherence to the tissues and other structures of the buccal cavity. In the present study, an infusion of *Mentha piperita* L. was evaluated for its ability to inhibit the adherence of *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* to acrylic resin. The results verified that the infusion was unable to inhibit the adherence of the strains tested to acrylic resin. However, using the same methodology described in this study, Carreto et al. [21] verified that an infusion of *Thymus vulgaris* (thyme) inhibited the adherence of *C. albicans* to acrylic resin. Taweechaisupapong et al. [22] also verified that an ethanol extract of *Streblus asper* significantly reduced the adherence of *C. albicans* to human buccal epithelial cells after a one minute exposure to the extract at a concentration of 125 mg/mL. According to the authors, this extract can interfere in the synthesis of adhesins or cause mechanical alterations within the adhesins present in the *Candida* cell wall.

CONCLUSIONS

A hydroalcoholic extract of *Mentha piperita* L. showed fungistatic activity against strains of *C. albicans*, *C. tropicalis* and *C. glabrata*. The essential oil from *Mentha piperita* L. exhibited the greatest inhibitory activity against strains of *C. albicans*, followed by *C. tropicalis* and *C. parapsilosis*. An infusion of *Mentha piperita* L. showed no effect on the adherence of *Candida* to acrylic resin.

RESUMO

O objetivo desse trabalho foi estudar in vitro o efeito do extrato hidroalcoólico, da infusão e do óleo essencial de *Mentha piperita* L. sobre *Candida* spp. O extrato hidroalcoólico e o óleo essencial de *Mentha piperita* L. foram avaliados quanto à atividade antimicrobiana em 50 cepas de *Candida albicans*, 10 *Candida glabrata*, 10 *Candida tropicalis*, 8 *Candida parapsilosis* e 2 *Candida krusei*. A concentração inibitória mínima (CIM) do extrato hidroalcoólico foi determinada pelo método da diluição em caldo e a atividade antimicrobiana do óleo essencial foi realizada por meio do teste de difusão em ágar. Foi observado também o efeito da infusão de *Mentha piperita* L. sobre a aderência de cepas padrão de *Candida* à resina acrílica. Os resultados demonstraram que o extrato hidroalcoólico de *Mentha piperita* L. apresentou atividade fungistática para cepas de *C. albicans*, *C. tropicalis* e *C. glabrata*. O óleo essencial mostrou maior atividade inibitória para cepas de *C. albicans*, seguida por *C. tropicalis* e *C. parapsilosis*. A infusão de *Mentha piperita* L. não inibiu a aderência de *Candida* à resina acrílica. Concluiu-se que *Mentha piperita* L. apresentou maior atividade antimicrobiana sobre cepas de *C. albicans* e *C. tropicalis*, mas não teve efeito sobre a aderência desses microrganismos à resina acrílica.

UNITERMS:

Mentha piperita; *Candida*; óleos voláteis; plantas medicinais.

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