Antimicrobial activity of Mentha piperita L. against Candida spp.

Atividade antimicrobiana de Mentha piperita L. sobre Candida spp.

Claunencil de Fátima Pires CARRETTO
Postgraduate student, Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos, UNESP – Univ Estadual Paulista - SP - Brazil.

Rosilene Batista de Aguiar ALMEIDA
Postgraduate student - Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos - UNESP – Univ Estadual Paulista - São José dos Campos - SP - Brazil.

Marcos Roberto FURLAN
Assistant Professor - Department of Agronomy - Taubaté University – UNITAU - Taubaté - SP - Brazil.

Antonio Olavo Cardoso JORGE
Full Professor - Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos - UNESP – Univ Estadual Paulista - SP - Brazil.

Juliana Campos JUNQUEIRA
Assistant Professor - Department of Biosciences and Oral Diagnosis - School of Dentistry of São José dos Campos - UNESP – Univ Estadual Paulista - SP - Brazil.

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 colected 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 ºC 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 ºC 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 ºC. 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		Extract Concentration (mg/mL)						
strains		125	62.5	31.25	15.62	7.81	3.90	
250								
C. albicans	E	100	100	100	82	0	0	0
(n = 50)	C	100	100	96	50	0	0	0
C. tropicalis	E	100	100	100	90	20	0	0
(n = 10)	C	100	100	100	70	20	0	0
C. glabrata	E	100	100	100	20	0	0	0
(n = 10)	C	100	100	100	0	0	0	0
C. parapsilosis	E	100	100	100	0	0	0	0
(n = 8)	C	100	100	100	0	0	0	0
C. krusei	E	100	100	100	0	0	0	0
(n = 2)	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	%
C. albicans (n=50)	34	68
C. tropicalis (n=10)	4	40
C. parapsilosis (n=8)	1	12.5
C. glabrata (n=10)	0	0
C. krusei (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*
C. albicans	Infusion	4.41	0.21	0.088
	Control	4.24	0.28	
C. glabrata	Infusion	4.42	0.18	0.450
	Control	4.36	0.22	
C. tropicalis	Infusion	4.30	0.21	0.390
	Control	4.22	0.24	
C. krusei	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 gratest 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 μL/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, Carretto 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.

References

1. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of the essential oil of Melaleuca alternifólia (tea tree oil). J Appl Microbiol. 2000;88(1):170-5.
2. Bonjar GHS. Inhibition of Clotrimazole-resistant Candida albicans by plants used in Iranian folkloric. Fitoterapia. 2004;75(1):74-6.
3. Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Falcioni L, Cioni PL et al. The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia. 2005;159(3):339-45.
4. Dulger B, Gonuz A, Aysel V. Inhibition of clotrimazole-resistant Candida albicans by some endemic Sideritis species from Turkey. Fitoterapia. 2006;77(5):404-5.
5. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem. 2001;49(9):4168-70.
6. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol. 2005;97(2):305-11.
7. Loguercio AP, Battistin A, De Vargas AC, Henzel A, Witt NM. Atividade antibacteriana de extrato hidro-alcoólico de folhas de jambolão (Syzygium cumini (L.) Skells). Ciência Rural. 2005;35(2):371-6.
8. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol. 2004;35(4):275-80.
9. Suffredini IB, Paciencia MLB, Nepomuceno DC, Younes RN, Varella AD. Antibacterial and cytotoxic activity of Brazilian plant extracts: Clusiaceae. Mem Inst Oswaldo Cruz. 2006;101(3):287-90.
10. Rodrigues CR, Faquin V, Trevisan D, Pinto JEBP, Bertolucci SKV, Rodrigues TM. Nutrição mineral, crescimento e teor de óleo essencial da menta em solução nutritiva sob diferentes concentrações de fósforo e épocas de coleta. Hortic Bras. 2004;22(3):573-8.
11. Martins ER, Castro DM, Castellani DC, Dias JE. Plantas medicinais. Viçosa: UFV; 1998. 220p.
12. Pereira RS, Sumita TC, Furlan MR, Jorge AOC, Ueno M. Atividade antibacteriana de óleos essenciais em cepas isoladas de infecção urinária. Rev Saúde Pub. 2004;38(2):326-8.
13. Ertürk O. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia. 2006;61(3):275-8.
14. Mardegan RC, Furletti VF, Duarte MCT, Foglio MA, Gonçalves RB, Höfling JF. Extratos de plantas medicinais com atividade contra Candida spp. Braz Oral Res. 2005;19(sp.iss.):124.
15. Silva MV, Costa TR, Costa MR, Ferreira EC, Fernandes OFL, Santos SC et al. Growth inhibition effect of Brazilian Cerrado plant extracts on Candida species. Pharmaceutical Biology. 2001;39(2):138-41.
16. Ezzat SM. In vitro inhibition of Candida albicans growth by plant extracts and essential oils. World J Microbiol Biotechnol. 2001;17(7):757-9.
17. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86(6):985-90.
18. Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Med. 2003;69(5):413-9.
19. Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Biochemical activities of Iranian Mentha piperita L. and Myrtus communis L. essential oils. Phytochemistry. 2006;67(12):1249-55.
20. Iscan G, Kirimer N, Kürkcüoglu M, Baser HC, Demirci F. Antimicrobial screening of Mentha piperita essential oils. J Agric Food Chem. 2002;50(14):3943-6.
21. Carretto CFP, Navas EAFA, Cachuté T, Oliveira LD, Junqueira JC, Jorge AOC. Efeitos do chá de tomilho sobre a aderência in vitro de Streptococcus mutans ao esmalte dentário e Candida albicans à resina acrílica. Rev Odontol UNESP. 2007;36(3):281-6.
22. Taweechaisupapong S, Choopan T, Singhara S, Chatrchaiwiwatana S, Wongkham S. In vitro inhibitory effect of Streblus asper leaf-extract on adhesion of Candida albicans to human buccal epithelial cells. J Ethnopharmacol. 2005;96(1-2):221-6.

Recebido em: 04/08/2010
Aprovado em: 07/01/2011

Corresponding author:
Juliana Campos Junqueira
Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos, UNESP – Univ Estadual Paulista,
Francisco José Longo 777, São Dimas, São José dos Campos
CEP: 12245-000, SP, Brazil
E-mail: juliana@fosjc.unesp.br