Marlena Dudek-Makuch [1], Krzysztof Makuch[2], Ryszard Koczorowski[2], Anna Kędzia[3], Anna Michalak[4]

The effect of Dentofresh® on yeast-like fungi

Działanie preparatu Dentofresh® na grzyby drożdżopodobne

[1] Department of Pharmacognosy, Poznan University of Medical Sciences, Poland
[2] Clinic of Geriatric Dentistry, Poznan University of Medical Sciences, Poland
[3] Department of Oral Microbiology, Medical University of Gdansk, Poland
[4] Medical Affairs Department, Phytopharm Kleka SA, Nowe Miasto nad Wartą, Poland

Abstract

Introduction. The mouthwash Dentofresh® produced by Phytopharm Kleka SA, intended for adults and children over the age of 6, is a prophylactic preparation. Using the mouthwash after having brushed the teeth has a preventive and healing influence on the hard tissues of teeth and gingiva. The mouthwash contains: eucalyptus, mint, thyme and sage oils, which show anti-inflammatory and antimicrobial activity.
Aim. The aim of the study was to evaluate the effectiveness of Dentofresh® against strains of yeast-like fungi from the genus *Candida*.
Material and methods. The study was conducted on 15 strains of yeast-like fungi from the genus *Candida* isolated from infections, and five reference strains. The fungicidal activity of the preparation (MFC – Minimal Fungicidal Concentration) against the strains of yeast-like fungi at the concentration recommended by its producer was evaluated.
Results. Dentofresh® exhibited great fungicidal activity at the concentration recommended by the producer as quickly as 15 minutes after it was applied (against 87% of the strains), while after 30 minutes, all the tested strains of yeast-like fungi were destroyed.
Conclusions. The preparation was effective against the genera of yeast-like fungi which are usually resistant to common *antifungal* medicines.

Keywords: *Candida* sp., essential oils, Dentofresh®.

Streszczenie

Wstęp. Płyn do płukania jamy ustnej Dentofresh® firmy Phytopharm Klęka SA, przeznaczony dla dorosłych oraz dzieci powyżej 6. roku życia, jest preparatem, który ma zastosowanie w profilaktyce. Stosowanie płukanki, co najmniej dwa razy dziennie po szczotkowaniu zębów, wywiera działanie profilaktyczno-lecznicze na twarde tkanki zębów i dziąsła. Płukanka w swoim składzie zawiera kompozycję naturalnych olejków ziołowych: eukaliptusowego, miętowego, tymiankowego i szałwiowego, mających działanie przeciwzapalne i przeciwbakteryjnie.
Cel. Celem pracy była ocena skuteczności preparatu Dentofresh® wobec szczepów grzybów drożdżopodobnych z rodzaju *Candida*.
Materiał i metody. Badania objęły 15 szczepów grzybów drożdżopodobnych z rodzaju *Candida* wyizolowanych z zakażeń oraz pięć szczepów wzorcowych. Oceniono grzybobójczą aktywność preparatu (MFC – Minimal Fungicidal Concentration, minimalne stężenie grzybobójcze) w stężeniach użytkowych wobec szczepów grzybów drożdżopodobnych.
Wyniki. Dentofresh® wykazał wysoką aktywność grzybobójczą w stężeniach użytkowych już po 15 minutach działania (wobec 87% szczepów), po 30 minutach działania ginęły wszystkie testowane szczepy grzybów drożdżopodobnych. Preparat działał skutecznie wobec gatunków grzybów drożdżopodobnych, które zazwyczaj wykazują oporność na częściej stosowane leki przciwgrzybicze.
Wnioski. Preparat był skuteczny wobec gatunków drożdży, zazwyczaj odpornych na typowe leki przeciwgrzybiczne.

Słowa kluczowe: *Candida* sp., olejki eteryczne, Dentofresh®.

Introduction

Systematic daily hygienic procedures are a basic means of preventing diseases of the oral cavity. In addition to cleaning teeth with a brush and toothpaste, emphasis placed on the importance of mouthwashes with comprehensive or specific anti-caries, anti-inflammatory, antibacterial and antifungal activity (inhibiting the multiplication of pathogenic bacteria and fungi), which maintain the cleansing effect and refresh the mouth.

Correct oral cavity care not only make it possible to prevent caries as well as inflammation of gingiva and periodontium, but also shortens an

ongoing disease, alleviates its symptoms and decreases chances of complications.

It is very important to perform complementary hygienic procedures, especially if there are additional factors predisposing to infections, such as the use of dentures or orthodontic appliances, long-term antibiotic therapy, reduced immunity often resulting from every day fatigue or stress [1].

In such cases, fungal infections within the oral cavity may develop. Most frequently the pathogenic fungi participating in such infections, include those from the genus *Candida*, particularly from the species *C. albicans* [2].

Recently there has been an increase in the number of patients suffering from oral candidiasis caused by strains of fungi resistant to the frequently applied antimycotics [3, 4].

Natural substances, including plant materials rich in essential oils and active compounds obtained from them, are gaining importance in the prevention and treatment of fungal infections of the oral cavity, gingival inflammation, periodontal diseases and in inhibiting the formation of supragingival plaque [5]. They not only show a wide range of biological activity, including antibacterial and antifungal activity, but are also characterised by therapeutic safety and good tolerance.

The following plants from the family *Lamiaceae* with confirmed pharmacological activity are the most widely used species: peppermint leaves (*Menthae piperitae folium*), thyme leaves (*Thymi folium*), leaves of various species of sage, including lavender leaf sage (*Salviae lavadulaefolia folium*), and eucalyptus leaves (*Eucalypti folium*) from the Myrtle family (*Myrtaceae*). Essential oils from the abovementioned plants are ingredients of the mouthwash Dentofresh®, intended for adults and children over the age of 6, whose antifungal activity was the subject of this study.

Aim

The aim of this study was to evaluate the effectiveness of Dentofresh® produced by Phytopharm against strains of yeast-like fungi from the genus *Candida,* isolated from material obtained from patients with oral infections, and against reference strains.

Material and methods

The study was carried out at the Laboratory of Oral Microbiology, Medical University of Gdansk, Poland.

The non-alcohol mouthrinses – Dentofresh® at concentrations recommended by the producer (undiluted preparation) were tested. Dentofresh®. It is composed of: Aqua, Xylitol, Glycerin, PEG-35 Castor Oil, Poloxamer 407, Mentha Piperita Oil, Thymus Vulgaris Flower/Leaf Oil, Eucalyptus Globulus Leaf Oil, Salvia Lavendulaefolia Leaf Oil, Eucalyptol, Menthol, Thymol, Sodium Fluoride, Aroma, Citric Acid, Methylparaben, Propylparaben, CI 42051, CI 47005.

Fungicidal activity test

The strains of yeast-like fungi used in the study were isolated from material obtained from patients with oral infections. The material was inoculated into Sabouraud medium and incubated at 37^0 C for 24 hours. The cultured strains of yeast-like fungi were identified on the basis of cell morphology in preparations stained by the Gram method, appearance of the colony on Sabouraud medium and CHROMagar Candida medium (BioRad), the germ tube test, an ability to produce chlamydospores, and their biochemical features (20C AUX bioMerieux) [6, 7]. The study involved 15 strains of yeast-like fungi from the genus *Candida* isolated from infections, belonging to the following species: *Candida albicans* (five strains), *Candida glabrata* (two strains), *Candida guilliermondii* (one strain), *Candida kefyr* (one strain), *Candida krusei* (two strains), *Candida lusitaniae* (one strain), *Candida parapsilosis* (one strain), *Candida tropicalis* (two strains), and five reference strains from: *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 66032, *Candida krusei* ATCC 14234, *Candida parapsilosis* ATCC 22019 and *Candida tropicalis* ATCC 750.

The fungicidal (MFC – Minimal Fungicidal Concentration) activity of Dentofresh® at concentrations recommended by the producer (undiluted preparation) against the abovementioned strains of yeast-like fungi was evaluated. In order to do this, 0.1 ml of suspension of the tested strain containing 10^6 CFU (Colony Forming Units) in 1 ml was added to 1 ml of the preparation. Next, after 15 and 30 minutes, 0.1 ml samples were taken and inoculated into 2 ml of BHI broth (Brain Heart Infusion Broth, Merck). The BHI broth inoculated with 0.1 ml of the fungal culture constituted a growth control of the tested strain. The inoculations and control media were cultured at 37^0 C for 24 hours in aerobic conditions. A lack of any growth of the yeast-like fungi in the medium suggested fungicidal activity of the preparation.

Results

Dentofresh®, evaluated in this study, showed considerable activity against the tested yeast-like fungi.

Table 1 presents the results of the fungicidal activity (MFC) of Dentofresh® at the concentration recommended by its producer (undiluted preparation) against 15 strains of yeast-like fungi from the genus *Candida* isolated from infections and against five reference strains.

The study demonstrated that 15 minutes after application of Dentofresh® (MFC), 87% of the strains were killed, and after 30 minutes, all the tested strains of yeast-like fungi were destroyed.

Table 1. Fungicidal activity (MFC) of Dentofresh® against yeast-like fungi at concentration recommended by producer

Tabela 1. *Aktywność grzybobójcza (MFC) Dentofreshu® przeciwko grzybom drożdżopodobnym w stężeniach zalecanych przez producenta*

Yeast-like fungi *Rodzaj szczepów*		Number of strains *Liczba szczepów*	Concentration recommended by producer (MFC) *Stężenie zalecane przez producenta*	
			Activity after 15 min *Aktywność po 15 min*	Activity after 30 min *Aktywność po 30 min*
Candida albicans		5	4	5
Candida glabrata		2	2	2
Candida guilliermondii		1	1	1
Candida kefyr		1	0	1
Candida krusei		2	2	2
Candida lusitaniae		1	1	1
Candida parapsilosis		1	1	1
Candida tropicalis		2	2	2
Total number yeast-like fungi		15	13	15
Reference strains:				
Candida albicans	ATCC 10231	1	1	1
Candida glabrata	ATCC 66032	1	1	1
Candida krusei	ATCC 14234	1	1	1
Candida parapsilosis	ATCC 22019	1	1	1
Candida tropicalis	ATCC 750	1	1	1

0 – indicates lack of activity;
0 – wskazuje na brak aktywności

Discussion

In recent years, the interest in using natural plant substances has increased, which is connected with their effectiveness and a high safety level confirmed by many years of application in medicine. Essential oils have been used in folk medicine and as culinary and aromatic ingredients for ages [8]. They show antibacterial, antifungal, antiviral and antiprotozoal activity. To date, there have been no reports of increasing resistance of bacteria or fungi to the activity of essential oils.

The mouthwash Dentofresh® produced by Phytopharm, intended for adults and children over the age of 6, is a prophylactic preparation. It contains a mixture of natural essential oils – eucalyptus, mint, thyme and sage oils – and, the main active compounds found in oils, such as: eucalyptol, menthol, thymol and sodium fluoride. The antibacterial and antifungal activity of the aforementioned components has been widely studied.

Gram-positive bacteria are more sensitive to the activity of essential oils than Gram-negative ones. The lower sensitivity of Gram-negative bacteria to essential oils may be explained by the fact that diffusion of the components of essential oils through the outer cell membrane is limited because of the presence of a hydrophilic barrier, which hinders transport of macroparticles and hydrophobic components [8]. In the case of Gram-positive bacteria, direct contact of the components of essential oil with the lipophilic cell wall is possible, which leads to damage or complete rupture of the cell membrane. It has been stated that essential oils containing significant numbers of monoterpenes cause increased cytoplasm liquidity, disrupt the spatial organisation of the proteins found in cytoplasm, inhibit cellular respiration and affect ion transport processes [8–10].

Eucalyptus oil and its main component, 1.8-cyneol (eucalyptol), show wide antimicrobial potency, including antibacterial action (also against methicyllin-resistant strains of *Staphylococcus aureus* (MRSA)), antiviral and antifungal activity against strains from the genus *Candida* and the species *Aspergillus fumigatus* (MIC 0.6 mg/ml), *Aspergillus niger* (0.6 mg/ml) [11]. Moreover, its immunostimulant, anti-inflammatory, antioxidant and analgesic activity has also been proven [12].

Research suggests that the oil from peppermint leaves (*Menthae piperitae*) shows activity against numerous microorganisms, including bacteria from the following species: *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumannii, Serratia marcescens Micrococcus luteus, Haemophilus influenzae, Streptococcus pneumoniae* and *Bacillus subtilis* as well as yeast-like and mould fungi [8, 13–19].

Many of studies have also proven antifungal activity of mint oil for 48.75% of the strains, which was strongest against *Candida albicans, C. tropicalis* and *C. parapsilosis*. It exhibits fungicidal activity at concentrations from 2.8 to 25.0 µg/ml. Such a broad range of effective concentrations

may be connected with the place of origin of the plants (climatic differences) and may result from differences in cultivation conditions or methods of extraction. Menthol, the main component of mint oil (over 50%), is regarded to be responsible for the antimicrobial activity of the oil [1, 20]. However, some studies have demonstrated that even mint oils with low menthol content show strong antimicrobial activity, which suggests that this activity is due to a group of compounds [21].

It has also been shown that decoction from peppermint leaves inhibits adhesion of many microorganisms to oral cavity tissues (including *C. albicans, C. glabrata, C. tropicalis* and *C. krusei*), but inhibition of fungi adhesion to acrylic material (dentures) has not been confirmed. The details of this phenomenon are not known, but it may be caused by the influence on adhesin synthesis or be connected with mechanical damage of adhesins present within the cell wall of the yeast-like fungi from the genus *Candida* [1].

Great antimicrobial effectiveness has also been shown by thyme oil obtained from the leaves of common thyme (*Thymus vulgaris*) or Spanish thyme (*Thymus zygis*). The main components of thyme oil are thymol its isomer, carvacrol, and *p*-cymene [22]. At low concentration (MIC ranging from 20–250 μg/ml), it inhibits growth of bacterial strains such as *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Proteus vulgaris* and fungi from the species *Aspergillus flavus*, whereas at higher concentration (MIC ranging from 330–1330 μg/ml), it also inhibits growth of strains of *Escherichia coli*. Experiments have confirmed great effectiveness of the antimicrobial activity of some components of the oil, including thymol. It is active against bacteria from the species *Streptococcus pyogenes*, S*treptococcus pneumoniae*, *Staphylococcus aureus, Haemophilus influenzae*, *Escherichia coli* and *Pseudomonas aeruginosa* [23].

Thyme oil and its components show strong activity against fungi from the genus *Candida*, including the following species: *C. albicans, C. krusei, C. tropicalis, C. guillermondii, C. glabrata, C. tropicalis, C. parapsilosis* and the species *Aspergillus fumigatus* [10, 22]. The mechanism of the activity consists in reducing the content of ergosterol in the cell membrane, which, as a result, is damaged, potassium ion leakage, spheroplast disintegration and, in consequence, death of the cell [22].

Sage oil, obtained from leaves of lavender leaf sage (*Salvia lavanduleafolia*) mainly contains: α-, β-thujone (30–35%), camphor (20–30%), 1.8-cyneol (10–30%), borneol (3–15%) and β-pinene (5–12%), and, in smaller quantities: α-pinene (4–7%), sabinyl and borneol acetate (1–5%), limonene (2%), carvacrol, rosmarinic acid, luteinol, linalool, geraniol, α-terpineol and γ-terpinene (⩽ 1%). Unlike sage oil obtained from garden sage leaves (*Salvia officinalis*), it contains a smaller amount of thujone, which shows toxic activity at high concentration [9].

It exhibits a wide range of antimicrobial activity towards Gram-positive bacteria, such as *Staphyloccocus aureus, Streptococcus mutans, Bacillus subtilis* and *Micrococcus luteus* (MIC 2.31–3.42 mg/ml), and Gram-negative bacteria, including *Escherichia coli* (MIC 3.42 mg/ml) [9].

Dentofresh®, evaluated in this study, showed great activity against the tested yeast-like fungi, which is connected with strong antimicrobial activity of the components of essential oils (**Table 1**). 15 minutes after application of Dentofresh® (MFC), 87% of the strains were killed, and after 30 minutes, all the tested strains of yeast-like fungi were destroyed. It is noteworthy that some of the fungal species under study, including *Candida glabrata, Candida krusei* and *Candida tropicalis*, often show resistance to antifungal medicines usually applied locally (**Table 1**). These fungi frequently participate in oral candidiasis and prosthetic stomatopathy (connected with the use of dentures).

Previous research also confirmed that Dentofresh® strongly inhibited formation of bacterial dental plaque, particularly in vestibular and lingual surfaces of teeth (measurement of PCR and API rates). It was shown, that the mouthwash inhibited inflammation of oral mucosa (measurement of BoP and PBI rates), which proved its anti-inflammatory properties. In addition, the majority of the subjects stated that it resulted in a feeling of cleanness and freshness in the oral cavity after rinsing [5].

The experiments, conducted in this study, confirm that the mouthwash Dentofresh® is an effective agent complementing oral hygienic procedures, and that its antifungal, antibacterial, anti-inflammatory and plaque-inhibiting properties allow prevention of caries as well as gingival and periodontal inflammation. In the case of advanced disease, the use of Dentofresh® shortens its duration, alleviates the symptoms and reduces chances of complications. Moreover, many years of using essential oils in medicine, dentistry, food and cosmetic industries prove that preparations containing essential oils can be safely applied to adults and children.

Conclusions

1. Dentofresh® showed great fungicidal activity (MFC) at the concentration recommended by the producer as soon as 15 minutes after application (87% of strains), whereas after 30 minutes, all the tested strains of yeast-like fungi were killed.
2. The mouthwash was effective against the species of yeast-like fungi that are usually resistant to frequently applied antifungal medicines and often participate in oral cavity infections.

Acknowledgments

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

[1] Carretto C, Almeida R, Furlan M, Jorge A, Junqueira J. Antimicrobial activity of Mentha piperita L. against Candida spp. Braz Dent Sci. 2010;13:4–9.
[2] Szponar E, Bobowicz Z, Orkiszewska M. The occurrence and sensitivity to antibiotics Candida strains isolated from the oral mucosa. Dent Forum. 2005;32:25–29.
[3] Scully C, El-Kabir M. Candida and oral candidosis: a review. Crit Rev Oral Biol Med. 1994;5:125–157.
[4] Ellpoial AN.B, Samaranayakel LP. Oral candidal infections and antimycotics. Crit Rev Oral Biol Med. 2000;1l:172–198.
[5] Chrzęszczyk D, Konopka T, Zawada Ł. Comparative analysis of mouthrinses efficacy when used as plaque inhibitory agents. J Stoma. 2013;66:37–53.
[6] Adam HJ, Richardson SE, Roscoe M, Boroumandi S, Gris M, Yau YC.W. An implementation strategy for the use of chromogenic media in the rapid, presumptive identification of Candida species. Open Mycol J. 2010;4:33–38.
[7] Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium in the isolation of yeast from clinical specimens. J Med Microbiol. 2005;54:981–985.
[8] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem. 2003;10:813–829.
[9] Pierozan MK, Pauletti GF, Rota L, Santos AC.A, Lerin LA, Di Luccio M, Mossi AJ, Atti-Serafini L, Cansian RL, Vladimiroliveira J. Chemical characterization and antimicrobial activity of essential oils of Salvia L. species. Ciênc Tecnol Aliment, Campinas. 2009;29:764–770.
[10] Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – an overview. Forsch Komplementmed. 2009;16:79–90.
[11] Bansod S, Rai M. Antifungal activity of essential oils from indian medicinal plants against human pathogenic aspergillus fumigatus and A. niger. World J Med Sci. 2008;3:81–88.
[12] Sadlon AE, Lamson DW. Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices. Altern Med Rev. 2010;15:33–47.
[13] Pattnaik S, Subramanyam VR, Kole C. Antibacterial and antifungal activity of ten essential oils in vitro. Microbios. 1996;86:237–246.
[14] Hammer KA, Carson CE, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86:985–990.
[15] Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006;6:39.
[16] Megalla SE, El-Katlawi NE.M, Ross SA. A study of antimicrobial action of some essential oils constituents. Herba Pol. 1980;26:181–186.
[17] Griffin SG, Wyllie SG, Markham JL, Leach DN. The role of structure and molecular properties of terpenoidsin determining their antimicrobial activity. Flavour Fragr J. 1999;14:322–232.
[18] Chao S, Young G, Oberg C, Nakaoka K. Inhibition of methicillin-resistant Staphylococcus aureus (MRSA) by essential oil. Flavour Fragr J. 2008;23:444–449.
[19] Saharkhiz MJ, Motamedi M, Zomorodian K, Pakshir K, Miri R, Hemyari K. Chemical composition, antifungal and antibiofilm activities of the essential oil of Mentha piperita L. ISRN Pharmaceutics. 2012;2012:718645.
[20] Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. FEMS Yeast Res. 2005;5:867–873.
[21] 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;67:1249–1255.
[22] Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Cavaleiro C, Gonçalves MJ, Martinez-de-Oliveira J. Antifungal activity of Thymus oils and their major compounds. JEADV. 2004;18:73–78.
[23] Sienkiewicz M, Denys P, Kowalczyk E. Antibacterial and immunostimulatory effect of essential oils. Int Rev Allergol Clin Immunol. 2011;17:1–2.

Correspondence address:
Collegium Stomatologicum UM
4 Święcickiego Str., 60-781 Poznan, Poland
e-mail: dudum@poczta.onet.pl
phone: +48 61 8546 708