Informative article

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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST ORAL PATHOGENES – INFORMATIVE ARTICLE

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Periodontal disease and Dental caries associated with dental plaque are the most common bacterial diseases, but also, significant oral health problem is *Candidiasis. Candida albicans,* is an opportunistic pathogen that can, under certain conditions proliferate and cause infections. The need for prevention and alternative forms of treatment and products for oral diseases comes from the rise in disease incidence, increased resistance by pathogenic bacteria to currently used chemotherapeutics. The products derived from medicinal plants have proven to be a source of biologically active substances, and thanks to their active principles, products based on medical herbs are more prevalent in modern phytotherapy. Essential oils are complex natural mixtures of volatile secondary metabolites – aliphatic and aromatic, terpinen and phenyl-propane compounds isolated from plants. The main constituents of essential oils are terpenes and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones, which are responsible for the fragrant and biological properties of plants. Different oils produce various pharmacological effects such as anti-inflammatory, antioxidant and anticancerogenic properties, but also oils are biocides. There are numerous *in vitro* studies that dealt with the research activities of natural herbal substances against oral bacteria that are known to be etiological factors in the development of oral and dental diseases. The phenolic major compounds of essential oils have been suggested to have a potential antifungal activity. There is ample of evidence that plant extracts and essential oils have the potential to be developed into agents that can be used as preventative or treatment therapies of oral diseases.

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Introduction

Periodontal disease and Dental caries are the most important global oral health problems (1). Diseases associated with dental plaque are probably the most common bacterial diseases occurring in man. Dental caries means the destruction of hard dental tissue and, if left untreated, may progress and cause death of vital pulp tissue, with possible spread of infection to the periapical region. The disease process is related to the acidogenic bacterial plaque (*Streptococcus mutans, Streptococcus sobrinus, Lactobacillus spp.*). Periodontal disease is initiated by microorganisms of dental plaque: Aggregatibacter actinomycetemcomitans, Porhyromonas gingivalis, Prevotella intermedia. In the oral cavity, bacteria are mainly accumulated on tooth surfaces above and below the edge of the gingiva, where they form a biofilm composed of bacterial microcolonies, extracellular layers, fluid channels, and communication systems. Adherent, gelatinous biofilm on the teeth, dental restorations, prostheses and implants can contain more than 700 different bacterial species. Dental biofilm can be prevented by regular daily tooth brushing and by effective chemotherapeutic agents (2-5). Also, significant oral health problem is *Candidiasis* caused by *Candida* species. *Candida* species is commensal yeasts in healthy people and can cause systemic infection under immune compromised conditions (6). *Candida albicans,* a fungus normally found in the human body and lives in balance with other microorganisms, is an opportunistic pathogen that can, under certain conditions proliferate and cause local and systemic infections mostly in immune compromised patients and those exposed to long-term therapy with antibiotics to eliminate bacteria, which also eliminates "friendly" *Lactobacillus spp.* that normalize the level of *Candida*. A similar situation occurs during pregnancy, due to hormonal imbalance and improper nutrition. Smokers, diabetics, or patients with partial and total prosthesis often suffer from candidiasis (7,8).

The global need for prevention and alternative forms of treatment and products for oral diseases that are safe, efficient and economical comes from the rise in disease incidence, increased resistance by pathogenic bacteria to currently used chemotherapeutics and antibiotics, opportunistic infections in immune compromised individuals and financial reasons in developing countries. Despite the few agents that are commercially available, these chemicals can alter oral microbiota and have unwanted side-effects such as vomiting, diarrhea and tooth staining. For example, the resistance observed in most (if not all) antibiotics that are commonly used in the treatment of oral infections (penicillins and cephalosporins, erythromycin, tetracycline and derivatives, metronidazole). Other antibacterial agents used in the prevention and treatment of oral diseases, including cetylpyridinium chloride, amine fluorides, chlorhexidine and other products containing such agents may exhibit toxicity, causing staining of the teeth or in the case of ethanol (commonly found in mouthwashes) are associated with increased risk of cancer of oral localization. Chlorhexidine is one of the most widely used biocides in antiseptic products. It can cause brown stains on the teeth, tongue and on restorations, burning sensation at the tip of the tongue and tastes change. In dental practice, the most commonly used antifungals are nystatin and fluconazole. It is believed that the presence of *C. albicans* in subgingival sites is in the form of biofilms, which could explain the resistance to antifungal therapy. Thus, the search for alternative products continues and natural phytochemicals isolated from plants are considered good alternatives to synthetic products (1, 3, 5, 6, 9).

Herbal medications have been used for centuries in the prophylaxis and treatment of many diseases. They have been used for thousands of years and there is numerous archaeological evidence that people in the prehistoric era used medicinal plants so that their application can be considered universal for all time. Some of the earliest known records dealt with the subject of healing with medicinal herbs. In rural areas of developing countries it is still the primary source of medicine care, and to 80% of people use traditional medicines for their health care. The natural products derived from medicinal plants have proven to be an abundant source of biologically active substances, many of which have been the basis for the development of new chemicals for the pharmaceutical industry. Thanks to their active principles, as well as the beneficial effect and efficiency, products based on medicinal herbs are more prevalent in modern phytotherapy (1, 2, 8, 10). With the development of experimental pharmacognosy, these products get scientific proof of its effectiveness, and, in today's world of medicine and dentistry, there is a growing interest in their application due to their therapeutic effect based on completely natural basis.

Effects of essential oil research

Essential oils are complex natural mixtures of volatile secondary metabolites - aliphatic and aromatic, terpinen and phenyl-propane compounds isolated from plants by hydro - or steam distillation and by expression (citrus peel oils). The main constituents of essential oils are terpenes and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones, which are responsible for the fragrant and biological properties of aromatic and medicinal plants. For centuries essential oils have been isolated from different parts of plants and also are used in various applications. Different oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticancerogenic properties. Others are biocides against a broad range of microorganisms such as bacteria, fungi, viruses, protozoa. In dentistry, medical plants with essential oils are particularly used as flavour and odour corrigents in substances for oral hygiene such as toothpastes and mouthrinses. The mechanism of action of terpenes is not fully understood, but it is assumed that the degradation of lipophilic components of cell membranes involved in the antibacterial activity. For the antimicrobial assessment of essential oils, conventional methods of testing antibiotic abilities are usually applied. There are two basic techniques used for the assessment of both antibacterial and antifungal properties of essential oils: the agar diffusion method and the dilution method (2, 8, 10, 11).

Antimicrobial effects of essential oils in vitro

There are numerous *in vitro* studies that dealt with the research activities of natural herbal substances against oral bacteria. These studies have focused on bacteria that are known to be etiological factors in the development of oral and dental diseases. Earlier studies have clearly shown that many substances have the potential to be used in the dental industry, based on their effects on cariogenic bacteria and those that cause periodontal disease. Thus, for example, the researchers examined the effects of Tea tree essential oil in dentistry. During the 1930-ties, clinical efficacy of Tea tree oil was confirmed in different medical fields (8, 12-14). Tea tree essential oil is a highly lipophilic substance. Its mechanism is explained with lipophilic terpene (terpinen-4-ol), which penetrates microorganism cell membranes and acts against its structural permeability. In this way, Tea tree oil can affect the metabolism of some microorganisms with bactericidal or fungicidal effect. Even though the composition of Tea tree oil that provides optimal antimicrobial activity is still uncovered, there is strong evidence that terpinen-4-ol is the most important component (8, 15, 16). Hammer and colleagues presented a study that aimed to test the activity of Tea tree essential oils (*Melaleuca alternifolia*) against 161 isolates of oral bacteria from 15 genera. Minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) ranged from 0.003% to 2% (v/v). MIC values for *Actinomyces spp*, *Lactobacillus spp*, *Streptococcus mitis* and *sanguis* were 1% (v/v) and 0.1% (v/v) for *Prevotella spp*. Isolates of *Porphyromonas*, *Prevotella* and *Veillonella* had the lowest MICs and MBCs, and the isolates of *Streptococcus*, *Fusobacterium* and *Lactobacillus* had the highest. Time kill assays with *Streptococcus mutans* and *Lactobacillus rhamnosus* showed that treatment with more than 0.5% Tea tree caused decreases in viability of more than 3 colonies formed after only 30s, and viable organisms were not detected after 5 min. These studies indicate that a large number of oral bacteria are sensitive to Tea tree oil, suggesting that it can be used for oral hygiene (17).

In addition to Tea tree essential oil study that has been published, numerous other in vitro studies demonstrate the antimicrobial activity of essential oils of various medical herbs. Cha and colleagues have presented a study that aimed to investigate the composition and antibacterial activity of essential oils obtained from Cryptomeria japonica on oral bacteria. The chemical structure of the essential oil was analyzed by gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS). Sixty-eight compounds accounting for 95.82% of the oil were identified. The main components were α-pinene (6.07%), sabinene (8.86%), terpinen-4-ol (9.77%), α-terpineol (6.13%), elemol (11.17%) and 10(15)-cadinen-4-ol (7.16%). The essential oil and some of its major components were tested for antimicrobial activity against 15 different species of oral bacteria. The essential oil of *C. japonica* exhibited considerable inhibitory effects against all bacteria tested (MICs, 0.025 – 0.05 mg/ml; MBCs, 0.025 – 0.1 mg/ml), while its major components showed various degrees of growth inhibition. The study used the following oral bacterial cultures: *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus ratti*, *Streptococcus criceti*, *Streptococcus anginosus*, *Streptococcus gordonii*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromonas gingivalis*. The reference strains used in this study were: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. Brain-heart infusion broth supplemented with 1% yeast extract was used for all bacterial strains except for P. intermedia and P. gingivalis. For them, brain-heart infusion broth containing hemin and menadione was used. The MICs were determined for the essential oil and some of its major components by the broth dilution method, and were carried out in triplicate. The antibacterial activities were assessed after incubation at 37°C for 18 h (facultative anaerobic bacteria), for 24h (microaerophilic bacteria), and for 1–2 days (obligate anaerobic bacteria) under anaerobic conditions. MICs were determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of MICs plates, the MBCs were determined on the basis of the minimum concentration of the essential oil that kills 99.9% of the test bacteria. Ampicillin and gentamicin were used as standard antibiotics in order to compare the sensitivity of the test bacteria to essential oils and some of its major components (18). Takarada and colleagues have published a study which investigated the antibacterial effects of essential oils on the following oral bacteria: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Streptococcus mutans* and *Streptococcus sobrinus*. Cariogenic and periodontopathic bacteria are present in dental plaque as biofilms. They tested manuka oil, tea tree oil, eucalyptus oil, lavandula oil and rosmarinus oil and determined the minimum inhibitory and minimum bactericidal concentration. The essential oils inhibited the growth of the bacteria tested, of which the most effective was manuka oil. MBC values showed that lavandula oil acts bacteriostatically, and the remaining oils, bactericidally. Periodontopathic bacterial strains were completely killed when treated with 0.2% manuka oil, tea tree oil or eucalyptus oil for 30s. Tea tree oil and manuka oil showed significant adhesion-inhibiting activity against *P. gingivalis*, and all the essential oil tested inhibited the adhesion of S. mutans. This study demonstrated that, among the essential oils, manuka oil and tea tree oil had strong antibacterial effects on cariogenic and periodontopathic bacteria. For safety reasons, the authors also investigated the effects of essential oils on cultured human umbilical vein endothelial cells and found that, at a concentration of 0.2% they had little effect on cells (19). Gursoy et al. have published a study whose aim was to investigate the bacterial growth inhibiting and anti-biofilm effects of *Satureja hortensis L.* (summer savory), *Salvia fruticosa M.* (sage), *Lavandula stoechas L.* (lavender), *Myrtus communis L.*, and *Juniperus communis L.* (juniper) essential oils. Chemical compositions of the essential oils were analyzed by gas chromatography–mass spectrometry, minimum inhibitor concentrations with the agar dilution method, and anti-biofilm effects by the "microplate biofilm" assay. They also tested the toxicity of each essential oil on cultured keratinocytes. The bacterial strains used in this study were: *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Porphyromonas gingivalis*; *Parvimonas micra* (formerly *Peptostreptococcus micros*); *Tannerella forsythia* (formerly *Bacteroides forsythus*); *Fusobacterium nucleatum*; *Prevotella intermedia*; *Prevotella nigrescens*. The strains were revived from frozen (-70°C) stocks and subcultured for purity. Bacteria were grown on Brucella blood agar enriched with hemin, and incubated at 37°C in an anaerobic chamber for 5 days. Of the 5 essential oils, *S. hortensis L.* essential oil had the strongest growth inhibition effect. Subinhibitory dose of *S. hortensis L.* essential oil had anti-biofilm effects only against *Prevotella nigrescens*. Essential oils did not affect the viability of keratinocytes at the concentrations of 1–5 µl/ml, but at the concentration of 5 µl/ml epithelial cells detached from the culture well bottom. The results from this study suggest that *S. hortensis L.* essential oil inhibits the growth of periodontal bacteria in the concentration that is safe on keratinocytes, but, in the subinhibitory concentration its anti-biofilm effect is limited (5). In "*in vitro*" studies are also used commercially available solutions based on essential oils such as Listerine TM (Essential Oils Rinse), which are often compared with other available anti-plaque mouth rinses. Pan and colleagues have performed a study that aimed to compare the antimicrobial activity of commercially available antiseptic mouth rinses against saliva-derived plaque biofilms in static and flow-through biofilm systems in vitro. The test treatments were the following commercially available mouth rinses: an essential oil (0.064% thymol, 0.092% eucalyptol, 0.060% methyl salicylate and 0.042% menthol) rinse (EO, Cool Mint, Listerine, Johnson and Johnson); a 0.12% chlorhexidine rinse (CHX, Peridex, 3M Pharmaceuticals), seven 0.05% cetyl pyridinium chloride rinses (CPC1, PLAX global mouth rinses, Colgate Palmolive); a 0.05% cetyl pyridinium chloride/0.05% chlorhexidine rinse (CPC/CHX, Perio-Aid, Dentaid); an amine fluoride/stannous fluoride rinse (AFSF, Meridol, GABA); a 0.07% cetyl pyridinium chloride rinse (CPC2, Crest Pro Health, Clean Mint, Proctor & Gamble); Sterile water or phosphate buffered saline (PBS) and 70% ethanol (Etoh) served as the negative control and positive controls respectively. So, nine mouth rinses were tested in a recirculating flow-through biofilm model (RFTB) with viability assessment by ATP bioluminescence. Five mouth rinses were evaluated in a batch chamber slide biofilm (BCSB) model, using live/dead staining and confocal laser scanning microscopy. In the RFTB model, essential oil and chlorhexidine – containing rinses showed equivalent antimicrobial activity and were more efficient than a range of cetyl pyridinium chloride (CPC1) formulations. In the BCSB model, twice daily mouth rinse exposure demonstrated that the EO rinse was significantly more effective than rinses containing amine and stannous fluorides (AFSF), a combination of CPC/CHX, and another CPC formulation (CPC2). Essential oil showed biofilm kill ability comparable to the CHX rinse (20). Filoche et al. have published a study that aimed to compare antimicrobial effects of essential oils alone and in combination with chlorhexidine digluconate against planktonic and biofilm cultures of *S. mutans* and *Lactobacillus plantarum*. The essential oils included cinnamon, tea-tree (*Melaleuca alternifola*), manuka (*Leptospermum scoparium*), *Leptospermum morrisonii*, *arnica*, *eucalyptus*, *grapefruit*, the essential oil mouth rinse *Cool Mint Listerine* and two of its components, menthol and thymol. Cinnamon had the highest antimicrobial potential (1.25 – 2.5 mg/ml). *Manuka*, *L. morrisonii*, tea-tree oils, and thymol also showed antimicrobial potency but to a lesser extent. The effect of combination of the essential oil–chlorhexidine was greater against biofilm cultures of both *S. mutans* and *L. plantarum* than against planktonic cultures. The amount of chlorhexidine required to achieve a similar growth inhibition against the biofilm cultures was reduced 4–10-fold in combination with cinnamon, manuka, L. morrisonii, thymol, and Listerin (21).

The antimicrobial activities of the essential oil individual components also were published. Carvacrol is one of the most common essential oils components which exhibit antibacterial activity, and is the major component of oregano and thyme (10). Botelho et al. presented a study whose main objective was to examine the composition and antimicrobial activity of *L. sidoides* essential oil. *Verbenaceae*, popularly known as "*Alecrimpimenta*" is a typical shrub commonly found in the Northeast of Brazil. The leaves of *L. sidoides* are extensively used in popular medicine for the treatment of skin wounds and cuts. In this study, the essential oil was obtained by hydro-distillation and analyzed by GC-MS. Twelve compounds were characterized, having thymol and carvacrol as major constituents. The antimicrobial effects of the essential oil and the main components was tested against cariogenic bacterial strains of the genus *Streptococcus* as well as *Candida albicans* using the broth dilution and disk diffusion assays. The essential oil and its major components (thymol, carvacrol) demonstrated potent antimicrobial activity against the microorganisms tested with minimum inhibitory concentrations ranging from 0.625 to 10.0 mg/ml. The most sensitive microorganisms were *C. albicans* and *Streptococcus mutans*. The essential oil of *L. sidoides* and its major components exert promising antimicrobial effects against oral pathogens (22). Didry and colleagues have investigated the antimicrobial activity of thymol, carvacrol, eugenol and cinnamaldehyde alone or combined on eight oral bacteria. The strains were obtained from culture collection or isolated from human clinical samples. Strains used were *Streptococcus mutans*, *S. sanguis*, *S. mitis* and *S. milleri* for aerobes, *Peptostreptococcus anaerobius*, *Prevotella buccae*, *P. oris* i *P. intermedia* for anaerobes. After determination of minimum inhibitory concentration of components, investigators studied the effect of their combinations. The components showed an inhibitory effect on seven bacteria and a synergistic effect was observed with some combinations. The four compounds can be used alone or combined, as eugenol and thymol, eugenol and carvacrol, thymol and carvacrol, during the treatment of oral diseases (23).

Antimicrobial effects of oral care products containing essential oils

Oral hygiene is the key to oral health. Mechanical methods for oral hygiene, brushing and flossing are defined by clinicians as the gold standard methods of plaque control. However, despite this emphasis on mechanical methods of plaque control, gingival inflammation prevalence is still high. Therefore, other agents for the maintenance of oral hygiene such as mouth rinses with anti-plaque and anti-gingivitis properties that can add to the effects of mechanical plaque control may have clinical relevance (24). It is well established that antibacterial mouth rinses are effective in reducing plaque on tooth surfaces. Mouth rinses may contain fluorides, alcohols, detergents and other antimicrobial substances. Such synthetic antimicrobial agents include povidone iodine products, chlorhexidine and cetylpyridinium chloride. Toothpastes also contain fluoride and other substances including triclosan and zinc citrate. Natural antimicrobial substances are now attracting attention as useful antimicrobial agents to be added into mouth rinses and toothpastes. For example, extracts of tea tree oil, peppermint, green tea and manuka honey have recently been added into such products in order to improve their antimicrobial properties. Mouth rinses containing essential oils are generally recognized as safe and effective products and recommend for oral hygiene. Numerous studies have been conducted which included a commercially registered mouth rinses containing essential oils. Listerine TM (Essential Oils Rinse) contains the active ingredients thymol, eucalyptol, methyl salicylate and menthol and has been in widespread use for many years. Thymol and eucalyptol are antimicrobial, while methyl salicylate and menthol act as a cleaning agent and anesthetic respectively. *In vitro* and *in vivo* studies have demonstrated the potential of essential oil mouth rinse use in the control of plaque-related diseases (1, 2, 25).

The clinical efficacy of EO and chlorhexidine rinses in the reduction of plaque and gingivitis has been extensively assessed. Since there is the fact that chlorhexidine eventually leads to the appearance of side effects that have already been mentioned, mouth rinses based on essential oils tend to be increasingly used. A number of recent studies add to the evidence that essential oils may be suitable additives in products used for the maintenance of oral hygiene or prevention of dental disease. Fine DH and colleagues have conducted two studies to determine the antimicrobial effect of rinsing with an essential oil-containing mouth rinse 12 hours after a single rinse and 12 hours after 2 weeks of twice daily rinsing, during the daytime and overnight. It was a randomized, double-blind controlled study with "crossover" design. Following baseline sampling of bacteria from supragingival plaque and the dorsum of the tongue, subjects began twice-daily rinsing with either an essential oil mouth rinse containing 0.09% zinc chloride (Tartar Control Listerines Antiseptic) or a negative control rinse. Bacterial sampling was repeated 12 h after the first rinse and again 12 h after the final rinse 14 days later. Samples were plated on Schaedlers medium (total anaerobes), Schaedlers Nalidixic/Vancomycin medium (Gram-negative anaerobes), and OOPS medium (volatile sulphur compound (VSC)-producing organisms). This study demonstrated that rinsing with the essential oil mouth rinse can have long-lasting effects in reducing anaerobic bacteria overall as well as Gram-negative anaerobes and VSC producing bacteria (26). Fine et al. have published a study that aimed to investigate the effect of rinsing with an essential oil-containing mouth rinse on levels of specific supra and subgingival bacteria in subjects with gingivitis. Fifteen subjects meeting entry criteria completed this randomized, controlled, double-blind, crossover study. Subjects were required to have ≥ 1000 target microorganisms per ml in all samples taken from two subgingival sites. Following sampling of supra and subgingival plaque, subjects began twice-daily rinsing for 14 days with either an essential oil-containing mouth rinse (Cool Mint Listerines Antiseptic) or a negative control. Then, plaque was again sampled on the fifteen day, and the procedure repeated after a week washout period with subjects using the alternate rinse. The study showed that compared to the negative control, the essential oil mouth rinse produced significant reductions in supragingival plaque levels of *Veillonella sp*, *Capnocytophaga sp*, *Fusobacterium nucleatum*, and total anaerobes and respective reductions in subgingival plaque (27). Charles and colleagues have presented a study that aimed to compare the anti-plaque and anti-gingivitis effectiveness and the side-effect profiles of an essential oil-containing mouth rinse and a chlorhexidine-containing mouth rinse. One hundred and eight eligible patients from 20 to 57 years were randomly assigned into 3 groups: essential oil mouth rinse (Listerines Antiseptic); 0.12% chlorhexidine mouth rinse (Peridex); or 5% hydroalcohol negative control. At baseline, subjects received a complete oral soft tissue examination and scoring of the Loe–Silness gingival index, Quigley–Hein plaque index Volpe-Manhold calculus index and Lobene extrinsic tooth stain index. After a complete dental treatment patients began to take twice daily their respective mouth rinse as an adjunct to their usual mechanical oral hygiene procedures. Subjects were reexamined at 3 and 6 months. This six-month controlled clinical study demonstrated that the essential oil mouth rinse and the chlorhexidine mouth rinse had comparable anti-plaque and anti-gingivitis activity. The chlorhexidine mouth rinse group had significantly more calculus and extrinsic tooth stain than either the essential oil mouth rinse group or the control group (28).

The effects of essential oils on Candida species

Candidiasis is a term for a fungal infection caused by Candida species. The spectrum of diseases caused by Candida includes infections of skin, mucous membranes and internal organs. Although over hundred species of Candida are known, not all are pathogenic to humans. Significant pathogens are *Candida albicans*, *Candida tropicalis*, *Candida parapsillosis*, *Candida guilliermondi*, *Candida krusei*, and *Candida stellatoidea*. *C. albicans* is the most common type of Candida species. Candida species are harmless commensals, and are a part of the normal flora of the pharynx, intestine, vagina, perianal skin folds, and mouth. The dorsum of the tongue is the primary oral reservoir for these yeasts, but they also can be on mucosal surfaces and in dental plaque. It was suggested that when *C. albicans* accessed the periodontal tissues, they may be damaged by the production of metabolites by these yeasts (6, 11, 29). The efficacy of essential oils of various medical herbs against Candida species has been extensively assessed (7, 12, 30-32).

Oropharyngeal candidiasis is the most common opportunistic infection that occurs in the oral cavity. The expression of *Candida albicans* virulence in the oral cavity is strongly associated with the decline of the immune system, especially in patients with AIDS. Certain conditions, such as hyposalivation, diabetes mellitus and prolonged antibiotic and corticoid therapy can predispose to oral candidiasis. Specific features of these fungi that contribute to the development of oral candidiasis include its ability to colonize the oral mucosa and form germ tubes. It is accessible a large number of antifungal agents for the treatment of infection caused by Candida. The main agents belong or to the polyenes, such as amphotericin B and nystatin, or to the azoles, such as itraconazole and fluconazole. Studies have shown the resistance of *Candida albicans* to azole, and also on the nephrotoxicity and hepatotoxicity associated with the polyenes, especially with the use of amphotericin B. To avoid all these drawbacks, it is necessary to search constantly for new and effective medicines to treat this fungal infection (29, 33).

The antifungal effect of essential oils of many aromatic plants has been described in various studies. Anticandidal activity is also well described. The phenolic major compounds of essential oils have been suggested to have a potential antifungal activity. Chami and colleagues have published a study that aimed to evaluate the therapeutic efficacy of carvacrol and eugenol, the main components (phenols) of essential oils of some aromatic plants, in the treatment of experimental oral candidiasis caused by *Candida albicans* in immunosuppressed rats. Anticandidal activity was analyzed by microbiological and histopathological techniques, and it was compared with nystatin, which was used as positive control. Microbiologically, carvacrol and eugenol reduced the number of colony forming units sampled from the oral cavity of rats treated for eight consecutive days, compared to the untreated control group of rats. Treatment with nystatin gave similar results. Histologically, the untreated group of animals showed numerous hyphae on the epithelium of the dorsal surface of the tongue. In the group of animals treated with carvacrol hyphae were not seen on the epithelium, whereas in rats treated with eugenol has seen only a few focal areas on the dorsal surface of the tongue. In the group treated with nystatin hyphae were found in the folds of the tongue mucosa. Therefore, the histological data were confirmed by the microbiological tests for carvacrol and eugenol, but not for the group treated with nystatin. Carvacrol and eugenol could be considered as strong antimycotic agents and might be proposed as therapeutic products for oral candidiasis (33). Jandourek et al. have published a study with the objective to evaluate the efficacy of melaleuca oral solution in AIDS patients with fluconazole-resistant oropharyngeal candida infections. The study comprised thirteen patients with AIDS and oral candidiasis documented to be clinically refractory to fluconazole, as defined by failure to respond to a minimum of 14 days of ≥ 400 mg fluconazole per day. In addition, patients had in vitro resistance, which is defined by minimal inhibitory concentrations of ≥ 20 μg/ml. Patients were given 15 ml melaleuca oral solution four times daily to swish and spit for 2-4 weeks. Evaluations were performed weekly for 4 weeks and at the end of therapy for clinical signs of oral candidiasis. A total of 13 patients who participated in the study, 12 were evaluable. After 2 weeks, seven out of 12 patients had improved, none were cured, and six were unchanged. After 4 weeks, eight out of 12 patients showed a response (two cured, six improved), four were non -responders, and one had deteriorated. A mycological response was seen in seven out of 12 patients. A follow up evaluation 2-4 weeks after therapy was revealed that there were no clinical relapses in the two patients who were cured. Therefore, it is estimated that melaleuca oral solution may be an effective alternative in AIDS patients with oropharyngeal candidiasis refractory to fluconazole (34). Shrestha et al. have published *in vitro* study in which they assessed the antifungal effect of mouth rinses containing chlorhexidine and thymol. The fungistatic activities of chlorhexidine - and thymol-containing mouth rinses were assessed by means of the minimum inhibitory concentration and the fungicidal activity was determined by a time-kill assay. It was found that chlorhexidine-containing mouthwash was able to kill all species of *Candida albicans* and *Candida tropicalis* in shorter period compared to the thymol-containing mouthwash. Hexidine showed an MIC of 1:32 for both Candida species, whereas Listerine respectively showed MICs of 1:8 and 1:16 for *C. albicans* and *C. tropicalis*. Antimicrobial agents used in the study had good *in vitro* activity against the two Candida species, but better antifungal and fungicidal activity showed mouth rinses containing chlorhexidine. However, both antimicrobial agents can be proposed for use as topical antimycotic agents (29).

Conclusion

A large number of essential oils have positive characteristics, such as antioxidant, anti-inflammatory and antimicrobial properties. The main components of essential oils, mono - and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones – are responsible for the fragrant and biological properties of aromatic and medicinal plants. Despite the development of antibiotics, bacterial and fungal infections are still a major health problem, and the presence of species that are resistant to multiple products poses great challenge. Recently, there has been a growing interest in natural products due to their accessibility and better biodegradebility. There is ample of evidence that plant extracts, essential oils and purified phytochemicals have the potential to be developed into agents that can be used as preventative or treatment therapies for oral diseases. While it is encouraging to see a number of clinical trials of such products, further studies of the safety and efficacy of these agents will be important to establish whether they offer therapeutic benefits, either alone or in combination with conventional therapies, that can help to reduce oral diseases.

References

1. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid-Based Compl Alt 2009; 2011: 680354. [CrossRef]
2. Allakera RP, Ian Douglas CW. Novel anti-microbial therapies for dental plaque related diseases. Int J Antimicr Ag 2009; 33(1):8-13. [CrossRef][PubMed]
3. More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol 2008; 119(3): 473-7. [CrossRef] [PubMed]
4. Marsh PD. Controlling the oral biofilm with antimicrobials. J dent 2010; 38(1):s11-s15. [CrossRef] [PubMed]
5. Gursoy UK, Gursoy M, Gursoy OV, Cakmakci L, Kononen E, Uitto VJ. Anti-biofilm properties of *Satureja hortensis* L. essential oil against periodontal pathogens. Anaerobe 2009; 15(4):164-7. [CrossRef] [PubMed]
6. Sardi JC, Almeida AM, Mendes Giannini MJ. New antimicrobial therapies used against fungi present in subgingival sites-A brief review. Arch Oral Biol 2011; 56(10):951-9. [CrossRef][PubMed]
7. Duarte MC, Figueria GM, Sartoratto A, Rehder VL, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol 2005; 97(2):305-11. [CrossRef] [PubMed]
8. Marković D, Mirković B, Jovanović T, Knežević A, Nastovski T. The application of Tea tree essential oil in dentistry. Stom Glas S 2007; 54:106-14.
9. Rasooli I, Shayegh S, Taghizadeh M, Astaneh SD. Phytotherapeutic prevention of dental biofilm formation. Phytother Res 2008; 22(9):1162-7. [CrossRef] [PubMed]
10. Schelz Z, Hohmann J, Molnar J. Recent advances in research of antimicrobial effects of essential oils and plant derived compounds on bacteria. In: Chattopadhyay D, editor. Ethnomedicine: A Source of Complementary Medicine. Trivandrum, India: Research Signpost; 2010. p.179-201.
11. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003;10 (10):813-29. [CrossRef] [PubMed]
12. Banes-Marshall L, Cawley P, Phillips CA. In vitro activity of Melaleuca alternifolia (tea tree) oil against bacterial and Candida spp. isolates from clinical specimens. Brit J Biomed Sci 2001; 58(3):139-45. [PubMed]
13. Gustafson JE, Liew YC, Chew S, Markham J, Bell HC, Wyllie SG, et al. Effects of tea tree oil on *Escherichia coli.* Lett Appl Microbiol 1998; 26(3):194-8. [CrossRef] [PubMed]
14. Carson CF, Riley TV. Antimicrobial activity of the essential oil of Melaleuca alternifolia. Lett Appl Microbiol 1993; 16(2):49-55. [CrossRef]
15. 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 alternifolia* (tea tree oil). J Appl Microbiol 2000; 88(1):170-5. [CrossRef] [PubMed]
16. Mann CM, Cox SD, Markham JL. The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). Lett Appl Microbiol 2000;30(4):294-7. [CrossRef] [PubMed]
17. Hammer KA, Dry L, Johson M, Michalak EM, Carson CF, Riley TV. Susceptibility of oral bacteria to Melaleuca Alternifolia (tea tree) oil in vitro. Oral Microbiol Immun 2003; 18(6):389-92. [CrossRef] [PubMed]
18. Cha JD, Jeong MR, Jeong SI, Moon SE, Kil BS, Yun SI, et al. Chemical composition and antimicrobial activity of the essential oil of *Cryptomeria japonica.* Phytother Res 2007; 21(3):295-9. [CrossRef] [PubMed]
19. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immun 2004; 19(1):61-4. [CrossRef] [PubMed]
20. Pan PC, Harper S, Ricci-Nittel D, Lux R, Shi W. In-vitro evidence for efficacy of antimicrobial mouthrinses. J dent 2010; 38(Suppl 1):S16-S20. [CrossRef] [PubMed]
21. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiol Immun 2005; 20(4):221–5. [CrossRef] [PubMed]
22. Botelho MA, Nogueira NA, Bastos GM, Fonseca SG, Lemos TL, Matos FJ, et al. Antimicrobial activity of the essential oil from *Lippia sidoides,* carvacrol and thymol against oral pathogens. Braz J Med Biol Res 2007; 40(3):349-56. [CrossRef] [PubMed]
23. Didry N, Dubreuil L, Pinkas M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharmaceutica Acta Helvetiae 1994; 69(1):25-8. [CrossRef] [PubMed]
24. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J dent 2010; 38(Suppl 1):S6-S10. [CrossRef] [PubMed]

25. Fine DH. Listerine: past, present and future – A test of thyme. J dent 2010; 38(Suppl 1):S2-S5. [CrossRef] [PubMed]
26. Fine DH, Furgang D, Sinatra K, Charles C, McGuire A, Kumar LD. In vivo antimicrobial effectiveness of an essential oil-containing mouth rinse 12 h after a single use and 14 days' use. J Clin Periodontol 2005; 32(4): 335–40. [CrossRef] [PubMed]
27. Fine DH, Markowitz K, Furgang D, Goldsmith D, Charles CH, Lisante TA, et al. Effect of an essential oil-containing antimicrobial mouthrinse on specific plaque bacteria in vivo. J Clin Periodontol 2007; 34(8):652–7. [CrossRef] [PubMed]
28. Charles CH, Mostler KM, Bartels LL, Mankodi SM. Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. J Clin Periodontol 2004; 31(10):878–84. [CrossRef] [PubMed]
29. Shrestha A, Rimal J, Rao A, Sequeira PS, Doshi D, Bhat GK. In vitro antifungal effect of mouth rinses containing chlorhexidine and thymol. J Dent Sci 2011; 6(1):1-5. [CrossRef]
30. Höfling JF, Anibal PC, Obando-Pereda GA, Peixot IA, Furletti VF, Foglio MA, et al. Antimicrobial potential of some plant extracts against Candida species. Braz J Biol 2010; 70(4):1065-8. [CrossRef] [PubMed]
31. Khan A, Ahmad A, Akhtar F, Yousuf S, Xess I, Khan LA, et al. Ocimum sanctum essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. Res Microbiol 2010; 161(10):816-23. [CrossRef] [PubMed]
32. Zambonelli A, Zechini D'aulerio A, Bianchi A, Albasini A. Effects of essential oils on phytopathogenic fungi *in vitro.* J Phytopathol 1996; 144(9-10):491-4. [CrossRef]
33. Chami N, Chami F, Bennis S, Trouillas J, Remmal A. Antifungal treatment with carvacrol and eugenol of oral candidiasis in immunosuppressed rats. Braz J Infect Dis 2004; 8(3):217-26. [CrossRef] [PubMed]
34. Jandourek A, Vaishampayan JK, Vazquez JA. Efficacy of melaleuca oral solution for the treatment of fluconazole refractory oral candidiasis in AIDS patients. AIDS 1998; 12(9):1033–7. [CrossRef] [PubMed]

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ANTIMIKROBNO DEJSTVO ETERIČNIH ULJA NA ORALNE PATOGENE

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Parodontopatija i dentalni karijes, čiji je nastanak povezan sa dentalnim plakom, predstavljaju najčešća bakterijska oboljenja. Takođe, značajni oralni zdravstveni problem predstavlja i kandidijaza. *Candida albicans* je oportunistički patogen koji pod pojedinim okolnostima može da proliferiše i uzrokuje infekciju. Potreba za prevencijom i alternativnim oblicima lečenja i produktima za oralna oboljenja proizilazi zbog povećanja učestalosti oboljenja i povećane rezistencije patogenih bakterija na primenu hemoterapeutika koji se trenutno upotrebljavaju. Dokazano je da su produkti dobijeni od lekovitog bilja izvor biološko aktivnih supstanci, a zahvaljujući svojim aktivnim principima, produkti na bazi lekovitog bilja zastupljeniji su u modernoj fitoterapiji. Eterična ulja su kompleksne prirodne mešavine isparljivih sekundarnih metabolita – alifatičnih i aromatičnih, terpenskih i fenil-propanskih jedinjenja izolovanih iz biljaka. Glavni sastojci etarskih ulja su terpeni i seskviterpeni, uključujući i ugljene hidrate, alkohol, etar, aldehide i ketone, koji su odgovorni za mirisna i biološka svojstva biljaka. Različita ulja imaju brojne farmakološke efekte, kao što su antiinflamatorno, antioksidativno i antikancerogeno dejstvo, ali su takođe i biocidi. Postoje brojne *in vitro* studije koje su se bavile istraživanjem aktivnosti prirodnih biljnih supstanci usmerenih na oralne bakterije za koje je poznato da su etiološki faktori u nastanku oralnih i dentalnih oboljenja. Navedeno je da glavne fenolne komponente eteričnih ulja imaju potencijalnu antigljivičnu aktivnost. Postoje brojni dokazi da biljni ekstrakti i eterična ulja imaju potencijal da se razviju u agense koji se mogu koristiti u preventivi ili u lečenju oralnih oboljenja.

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Ključne reči: *parodontalna bolest, eterična ulja, zubni karijes, kandidijaza, fitoterapija*