Original Article

Antimicrobial Efficacy of Blended Essential Oil and Chlorhexidine against Periodontal Pathogen (*P.gingivalis*)–An *In Vitro* Study

G Kumar, MP Rajula, KS Rao, PL Ravishankar, DH Albar[1], MA Bahammam[2,3], A Alamoudi[4], KJ Alzahrani[5], KF Alsharif[5], IF Halawani[5], FM Alzahrani[5], MM Alnfiai[6], HA Baeshen[7], S Patil[8]

Department of Periodontology, SRM Kattankulathur Dental College and Hospital, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur 603203, Kancheepuram, Tamil Nadu, India, [1]Department of Preventive Dentistry, College of Dentistry, Jazan University, Saudi Arabia, [2]Department of Periodontology, Faculty of Dentistry, King Abdulaziz University. Jeddah, Saudi Arabia, [3]Executive Presidency of Academic Affairs, Saudi Commission for Health Specialties, Riyadh, Saudi Arabia, [4]Department of Oral Biology, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia, [5]Department of Clinical Laboratories Sciences, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia, [6]Department of Information Technology, College of Computers and Information Technology, Taif University, Taif, Saudi Arabia, [7]Department of Orthodontics, College of Dentistry, King Abdulziz University, Jeddah, Saudi Arabia, [8]College of Dental Medicine, Roseman University of Health Sciences, South Jordan, UTAH, USA

Abstract

Background: Essential oils (EOs) have a considerable amount of therapeutic and preventive effect in treating dental diseases due to their wider potential as antibacterial and anti-inflammatory agents. EOs like virgin coconut oil, eucalyptus oil, peppermint oil thyme oil, and clove oil, when used in combination, may further have enhanced antimicrobial effects. However, limited information exists on the synergistic effect of these oils when used in combination, especially on the primary periodontal pathogen *Porphyromonas gingivalis*. **Aim:** The current study aims to compare the antimicrobial efficacy of commercially available EO on the periodontal pathogen, *P. gingivalis*, in comparison to chlorhexidine (CHX). **Materials and Methods:** Antimicrobial efficacy of EO and CHX was assessed at various concentrations against the periodontal pathogen *P. gingivalis*, by evaluating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). **Results:** *P. gingivalis* was seen to be sensitive at a MIC of 100 µg/ml and 50 µg/ml concentration of the EO, which is regarded as the MIC of EO against *P. gingivalis* and CHX effectively inhibited microbial growth at 0.4 µg/ml. **Conclusion:** A combination of EOs possesses a potent antibacterial activity against *P. gingivalis*, and the antibacterial efficacy increases with increasing concentration of EOs.

Keywords: *Clove oil, essential oil, eugenol oil, MIC, P. gingivalis, virgin coconut oil*

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Address for correspondence: Dr. S Patil, College of Dental Medicine, Roseman University of Health Sciences, South Jordan, UTAH-84095, USA.
E-mail: dr.ravipatil@gmail.com

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Introduction

Periodontitis is an inflammatory condition caused due to microbial origin in the oral cavity. A variety of microorganisms were identified as disease-causing microbes and they are referred to as "periopathogens."[1] *Porphyromonas gingivalis* is one of the periopathogen that has piqued the interest of researchers. It is strongly linked with periodontal disease progression since it was isolated from active periodontal lesions.[2] It possesses a plethora of virulence factors and is found in high numbers in periodontitis lesions, making it a dangerous "pathobiont."[3] Conventional periodontal treatments like scaling and root planning were aimed at the reduction of microbial load. However, tissue invasive pathogens like *P. gingivalis* may cause persistent disease progression despite mechanical therapy.[4,5] Hence, it may be necessary to employ adjunctive therapy along with conventional treatment for the control of the disease. Antimicrobial agents against periopathogens have been employed as systemic or local adjuncts to mechanical therapy to enhance clinical outcomes.[4,6] Chlorhexidine (CHX), tetracycline, and metronidazole are of few of the antimicrobial drugs that have been employed successfully for this purpose.[7] These commercial antimicrobials have been used indiscriminately in previous decades, resulting in the emergence of multidrug-resistant microorganisms.[8,9]

Because of these drawbacks, naturally derived antimicrobial compounds, especially herbal preparations, have drawn the attention of researchers for a variety of reasons.[10] Of these natural preparations, essential oils (EOs) have been recently focused more on their antibacterial properties without major hazardous effects.[11] EOs possess anti-microbial, anti-inflammatory, and anti-oxidant action effects owing to the presence of molecules, namely, terpenoids and phenol compounds. EOs are plant-derived volatile secondary metabolites with a characteristic fragrance, flavor, or both.[12] Compounds in the EO are produced in the cytoplasm and plasmids of plant cells through the pathways of mevalonic and malonic acids. Monoterpenes, diterpenes, sesquiterpenes, ketones, esters, alcohols, phenols, and terpenes are a well-known category of EO components.[13] The oxygenated form of terpenoids is the primary component of EO, which is responsible for its antimicrobial property. According to research, terpenoids are lipophilic and diffuse permanently into the cell membrane inducing bacterial cell death.[14] Virgin coconut oil possesses antibacterial, antiviral, and antifungal properties due to the presence of lauric acid. The monoglycerides and monolaurin can penetrate the cell membrane, thus causing physical disruption of the gram-positive and gram-negative bacterial membrane leading to microbial cell death.[15,16]

Along with the analgesic effect, eucalyptus leaves (*Eucalyptus globules* L.) contain phloroglucinol derivatives called macrocarpals that prevented the activity of proteinases specific to Arginine and Lysine in *P. gingivalis*. This led to a reduction in the binding to saliva-coated hydroxyapatite beads, which suggests that macrocarpals lessen *P. gingivalis* adhesion[17] Clove oil (*Syzygium aromaticum* L.) and its primary component, eugenol, is against *Fusobacterium nucleatum* and *Prevotella intermedia*, although another active component, caryophyllene, had noticeably lesser activity.[18] Thyme oil (*Thymus vulgaris* L) inhibits the growth of oral microbes; specifically, the in-vitro study models involving clinical isolates of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* were sensitive to thyme oil, thus establishing its antimicrobial role.[18]

Thus, plant extracts and EOs have sparked interest as specific antimicrobial agents and paved the way for evolving research to establish EO as a potential alternative to pharmaceutical agents. Though individual EOs have been evaluated for their antimicrobial efficacy against *P. gingivalis*, there are very few literature evidences on the combined effect of these EOs on the same. Hence, the current study was undertaken to estimate the antimicrobial effect of EOs when combined, against *P. gingivalis*.

Methodology

This current in-vitro study was conducted to evaluate the antimicrobial efficacy of commercially available blended EO (Cureveda Sparkle Oil Pulling, Climic Health Pvt. Ltd, Mumbai, India) [Figure 1] against cornerstone periodontal pathogen *P. gingivalis* in comparison to CHX (Hexidine®-ICPA Health Products Ltd, Mumbai, India). Clinical isolate of *P. gingivalis* was used as the test organism. The blended EO consisted of a combination of 8.45 g virgin coconut oil, 62.6 mg eucalyptus oil (*E. globules*), 193.7 mg peppermint oil (*Mentha piperita*), 37.4 mg thyme oil (*T. vulgaris*), 171 mg clove oil (*S. aromaticum*), and 46 g of charcoal. The microbiological investigation was conducted at the Department of Periodontology, SRM Kattankulathur Dental College, Potheri, Tamilnadu, India (8529/IEC/2022) in collaboration with the Department of Microbiology, at Maratha Mandal Dental College, Belgaum.

Procedure for minimum inhibitory concentration (MIC) by serial dilution method and minimum bactericidal concentration (MBC): To 380 µl of thioglycollate broth in the first tube, 20 µl of the EO was added.

The following nine tubes each received dilutions by adding 200 µl of thioglycollate broth. Two hundred microliters of the thioglycollate broth were transferred from the initial tube, which was regarded as a dilution of 10–1. This dilution process was repeated until 10–9 dilution was reached. Five microliters was collected from the preserved stock cultures of the *P. gingivalis* and added to the tube containing 2 ml of thioglycollate broth. Each serially diluted tube received 200 µl of the aforementioned culture solution [Figure 2]. The tubes were placed in an anaerobic jar at 37°C for 48–72 h for the appearance of turbidity. Each tube's turbidity was contrasted with that of positive control, which comprised only pure bacterial culture. The MIC was determined to be the lowest concentration of the EO in the tube that did not exhibit any turbidity. From the tubes diluted for MIC, the initial five tubes (tubes that showed sensitivity for MIC) were distributed over the blood agar plate and permitted a drying time of 20 min [Figure 3a]. After drying, a sterile rod was used for dispensing the inoculum completely over the surface of the plate and incubated at 37°C for 24 h, and colony count was taken on the next day [Figure 3b]. The bactericidal effect of EO against *P. gingivalis* was evaluated and this is stated as the MBC of the EO. The lowest concentration of EO that can suppress bacterial growth and reduce the viability of the initial bacterial inoculums is called the MBC.

Results

In the current investigation, combined EO mouth rinse was investigated for antibacterial efficacy on gram-negative, darkly pigmented *P. gingivalis* isolates at varying doses. Bacterial inhibition displayed by EO (at different concentrations) against *P. gingivalis* is depicted in Table 1. *P. gingivalis* had the highest susceptibility to the tested EO at 50 and 100 µg/ml, and then as the concentration of EO was lowered to concentrations of 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 0.8 µg/ml, 0.4 µg/ml, and 0.2 µg/ml, the organism showed resistance. Thus, MIC as the compound gets diluted, the antimicrobial activity decreases. On the other hand, CHX (control agent) was sensitive to *P. gingivalis* from 0.4 µg/ml concentration to 100 µg/ml. Among the dilution tubes tested for MIC, five tubes, which were sensitive to MIC, were plated and subjected for a 48-h anaerobic incubation at 37°C. By using a spot test, the viability of *P. gingivalis* from each well was assessed. On anaerobic basal agar plates supplemented with 5% blood, hemin 5 mg/l, and menadione 1 mg/l, a 10-l solution from each well was spotted in triplicate. The experiments were conducted three times. The maximum EO dilution measured as the MBC value in mg/ml, in the concentration which failed to produce any discernible growth on agar plates. In the current study, there was no growth of

Figure 1: Essential oil

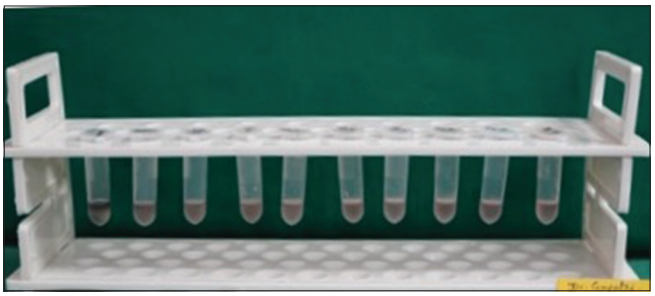

Figure 2: Serial dilutions of the antibacterial agent with bacterial culture concentrations from 0.2 mg/ml to 100 mg/ml are cultured with bacterial isolate and observed for turbidity

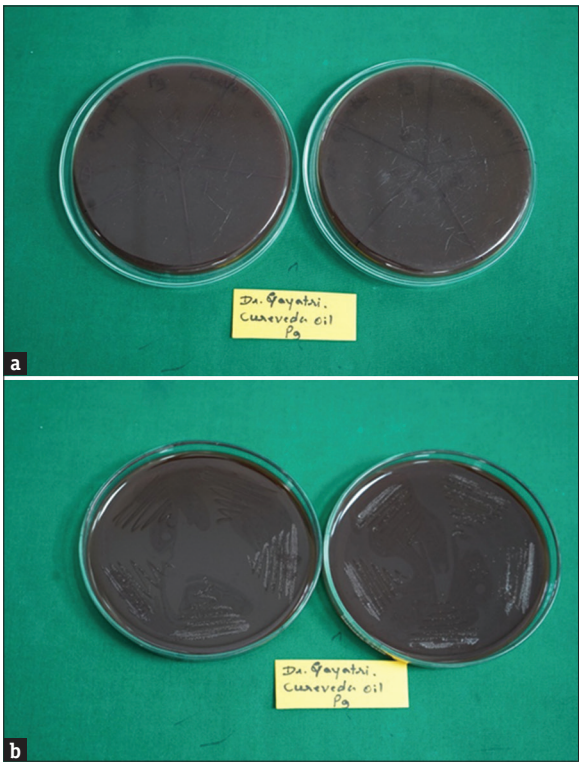

Figure 3: (a) Selected tubes which were sensitive to MIC were plated, (b) Plates were subjected for a 48-h anaerobic incubation at 37°C

Table 1: Minimum inhibitory concentration values of essential oil extract against *Porphyromonas gingivalis*

Sl. no.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
01	Cureveda oil *P. gingivalis*	S	S	R	R	R	R	R	R	R	R
02	Chlorhexidine *P. gingivalis*	S	S	S	S	S	S	S	S	S	R

Table 2: Minimum bactericidal concentration values of essential oil against *Porphyromonas gingivalis*

Sl. no.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
01	Cureveda oil	NG	NG	68	72	94	102	112	168	200	286

bacteria at 50 and 100 µg/ml concentrations of EO as depicted in Table 2, thus reinforcing the effective MBC of the tested EO capable of inhibiting *P. gingivalis* to be 50 and 100 µg/ml. According to the results, with increasing concentration of the EO, its antimicrobial activity against the test pathogen increases.

Discussion

One of the main etiologic factors for periodontal disease is the accumulation of mature adherent biofilm in the oral cavity, which activates host systems and causes an inflammatory response. An effective approach to reducing the microbial load is by employing cosmeceuticals to reduce the formation of dental biofilm, bacterial growth, and subsequent adhesion to the tooth surface.[19] CHX is the major antibiofilm agent because of its well-known antibacterial and antiplaque characteristics. long-term use, however, has been associated with, alterations in taste, tongue flora, and tooth discoloration.[20] Plant-based drugs have a wide variety of potential therapeutic benefits as antibiofilm and antibacterial properties. Studies have reported an effective reduction in gingival inflammation followed by the use of herbal-based preparations in the form of mouthwashes, toothpaste, and gels.[10]

Various studies have tested naturally derived EOs against periodontal pathogens, and the individual antimicrobial efficacy of these EO has been studied.[11] In a systematic review by dos Santos Cardoso *et al*.,[21] individual EOs and alkaloids are the main bioactive substances for the plant species that have been described, and the majority of them have been shown to have antibiofilm actions. However, there are no studies on the synergistic effect of the EOs against *P. gingivalis*, when combined. Hence, the present study was focused on the evaluation of the antimicrobial efficacy of commercially available blended EO at various concentrations against the periodontal pathogen in comparison with CHX. The MIC values and lowest concentration of EO that can potentially suppress the growth of bacteria and reduce the viability of the bacterial inoculums were evaluated. *P. gingivalis* had the highest susceptibility to the tested EO at 50 µg/ml and 100 µg/ml in comparison to CHX which was sensitive at 0.4 µg/ml. This was in accordance with the results obtained from a study by Hans *et al*.[16] as they tested four EOs in varying concentrations individually against *P. gingivalis*. Similarly, Ayob *et al*.[15] in their study observed that the antibacterial activity of fermented VCO was effective against *A. actinomycetemcomitans* and *P. gingivalis* at 50 µg/ml. Similarly, Takarada *et al*.[22] in their study found that periodontopathic bacteria were inhibited completely by tea tree oil and eucalyptus oil at 100 µg/ml and 50 µg/ml. These findings revealed that the individual EOs have antimicrobial effects at higher concentrations than the action of CHX against *P. gingivalis*, thus emphasizing the individual antimicrobial efficacy of these oils against periodontal pathogens. However, the combined effect of these EOs against periopathogens has not been studied. Hence, the current study is the first of its kind to evaluate the combined effect of EOs against *P. gingivalis*. Though the results show the antimicrobial efficacy of the tested oil at higher concentrations only, the synergistic effect of these oils may have potential benefits as an antimicrobial agent against *P. gingivalis*. Therefore, research in the future should be aimed to explore the concentration and exposure time of EO components, to establish strong antimicrobial potential in the oral environment. To conclude, the combination of EOs possesses significant antibacterial activity against *P. gingivalis* and the antibacterial efficacy increases with increasing concentration of EOs.

Ethical approval

The study was approved by the Institutional Review Board of SRM Kattankulathur Dental College & Hospital (8529/IEC/2022) and Maratha Mandal Dental College, Belgaum.

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Conflicts of interest

There are no conflicts of interest.

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