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Cosyn J, Princen K, Miremadi R, Decat E, Vaneechoutte M, De Bruyn H. A double-blind randomized placebo-controlled study on the clinical and microbial effects of an essential oil mouth rinse used by patients in supportive periodontal care.

A double-blind randomized placebo-controlled study on the clinical and microbial effects of an essential oil mouth rinse used by patients in supportive periodontal care

Abstract: *Aim:* This 3-month double-blind randomized placebo-controlled study evaluated the clinical and microbial effects of an essential oil mouth rinse used as an adjunct to mechanical plaque control by patients in supportive periodontal care. *Material and methods:* Fifty patients were randomly allocated to an essential oil group (Listerine® Coolmint; Johnson & Johnson, New Brunswick, NJ, USA) or placebo group to rinse twice per day as an adjunct to mechanical plaque control. At baseline and after 3 months, plaque index (PI), gingivitis index (GI), probing pocket depth, bleeding on probing (BoP) and clinical attachment level were registered. Subgingival plaque samples were collected for the detection and quantification of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Micromonas micros, Prevotella intermedia, Fusobacterium* genus and *Streptococcus mutans* by means of real-time PCR (qPCR). Patient's compliance, satisfaction and side effects were registered. *Results:* Twenty-three patients in the essential oil group (mean age: 57) and 21 in the placebo group (mean age: 55) with acceptable oral hygiene at intake (mean PI <1.5 on a scale of 5) adhered to the study protocol. Gingivitis index, PI and BoP significantly reduced over time ($P \leq 0.029$); however, between group analyses revealed no significant differences. There was no significant change over time neither in detection frequency nor load for any of the microbiota. Daily rinsing with an essential oil rinse was found safe and perceived beneficial by the patients. *Conclusion:* Patients in supportive periodontal care who are fairly compliant with oral hygiene may not benefit from additional mouth rinsing using an essential oil solution.

Key words: clinical; essential oil; microbiology; oral hygiene; periodontitis; randomized controlled study; supportive care

Introduction

Chronic periodontitis is a common infectious disease characterized by progressive attachment loss and alveolar bone resorption, which may lead to tooth loss. The ultimate goal of periodontal therapy is to prevent this

endpoint. When a strict supportive care programme is implemented following active therapy, subsequent tooth loss is limited to a mean of about 0.1 per patient per year (1, 2). In contrast, three to six times as many teeth may be lost if the disease is left untreated (3, 4).

The objective of supportive care is to prevent disease recurrence, which is accomplished by strict home care and professional plaque control at regular intervals depending on the patient's needs. Evidently, not all patients are optimally compliant and motivated. In fact, the majority of adults may not follow an adequate home care routine, which includes the use of interdental devices such as dental floss, brushes or toothpicks (5). This may be explained by the fact that interdental cleaning is technically demanding and time-consuming. Therefore, chemical aids could be considered to supplement mechanical plaque removal (6). Antimicrobial mouth rinses have also been recommended when mechanical oral hygiene is difficult or even impossible (7–9).

Chlorhexidine, which is a cationic bis-guanide with a broad antimicrobial spectrum, attacks the bacterial cell membrane causing leakage or precipitation of the cellular contents (10). It is by many still considered the gold standard anti-plaque agent. However, because of a number of side effects, chlorhexidine may not be indicated as an adjunct to daily mechanical plaque control. These side effects essentially include tooth staining (11), discoloration of teeth and mucosae (12), taste alterations (13) and less commonly, ulcerations (14).

In 1987, an essential oil mouth rinse (Listerine® Coolmint; Johnson & Johnson, New Brunswick, NJ, USA) was approved by the American Dental Association. The brand currently comes with six different flavours and contains menthol, thymol, methylsalicylate and eucalyptol as active agents. Essential oil exerts a lethal effect on microbiota by disrupting the cell wall and inhibiting enzymatic activity (10, 15, 16). They prevent commensal bacteria from aggregating with bacterial and fungal pathogens (17). Essential oil inhibits bacterial multiplication and extracts endotoxins from Gram-negative species (18). As a result, bacterial load is reduced and plaque maturation is slowed down, hereby decreasing the plaque mass (10). Clinical studies have shown an additional anti-plaque and anti-gingivitis effect to mechanical plaque control without relevant side effects (19–22). However, clinical and microbial data relating to the use of essential oil solutions in periodontitis patients are scarce. Clearly, these subjects could benefit from optimal plaque control to limit disease progression.

The objective of this 3-month double-blind randomized placebo-controlled study was to evaluate the clinical and microbial effects of an essential oil mouth rinse used as an adjunct to mechanical plaque control by patients in supportive periodontal care. It was hypothesized that patients rinsing with the essential oil solution would have superior clinical and microbial outcomes when compared with a placebo solution.

Material and methods

Study design

This 3-month double-blind randomized placebo-controlled study included chronic periodontitis patients attending a supportive care programme (two to four times per year) at the Department of Periodontology and Oral Implantology of the University Hospital in Ghent. All were recruited from March until May 2010, based on specific selection criteria.

Inclusion criteria were as follows:

1 At least 30 years old.
2 Good general health.
3 Maintenance care for at least 1 year.
4 Presence of at least one 4–6-mm pocket per quadrant.

Exclusion criteria were as follows:

1 Use of antibiotics within 3 months prior to or during the study.
2 Patients undergoing orthodontic therapy.
3 Patients wearing removable prostheses.
4 Presence of 7-mm pockets or deeper.

All subjects signed an informed consent form after they had received detailed information on the objective and study-related procedures. Random allocation to the essential oil rinse ($n = 25$) and placebo rinse group ($n = 25$) was performed by means of a computer-generated randomization scheme. The study protocol was approved by the ethical committee of the University Hospital in Ghent.

Essential oil rinse group and placebo rinse group

Patients in the essential oil rinse group were given a commercially available mouth rinse (Listerine® Coolmint; Johnson & Johnson) to use twice a day (2 × 20 ml) following daily mechanical plaque removal. Patients in the placebo rinse group were given a negative control solution, also to use twice per day (2 × 20 ml) following daily home care. The placebo rinse solution, made at the Pharmacy Department of the University Hospital in Ghent, consisted of sorbitol 15%, ethanol USP 21.6%, sodium saccharin 0.05%, benzoic acid 0.1%, mint flavouring QS, sodium benzoate, dye green QS and water QSF 11 as previously described by Cortelli *et al.* (23). Both solutions had indistinguishable taste, smell and colour and were delivered in neutral and identical vials, labelled as A and B. The label corresponding to the essential oil rinse, respectively, placebo rinse, was revealed by a member of the Pharmacy department following all statistical analyses. Patients were motivated to comply with the study protocol and received one phone call after 2 weeks of rinsing.

Clinical examination

A complete periodontal examination was carried out at baseline and after 3 months by one and the same blinded, calibrated clinician (PK). Calibration was performed prior to

the start of the study and on the basis of duplicate clinical recordings in three patients.

Gingivitis index (GI) (24) and plaque index (PI) (25) were considered primary outcome variables. Gingivitis index was measured at six sites (mesial, central, distal; buccally and orally) per tooth, using a manual probe (PCPUNC 15; Hu-Friedy®, Leimen, Germany). The scores ranged from 0 to 5. Plaque index was measured at the same sites following plaque disclosure (Rondell Red; Svenska Dental Instrument AB, Upplands Vasby, Sweden). The scores ranged from 0 to 5.

Probing pocket depth (PPD), bleeding on probing (BoP) and clinical attachment level (CAL) were considered secondary outcome variables. Probing pocket depth was measured to the nearest mm at six sites (mesial, central, distal; buccally as well as orally) per tooth using a manual probe (PCPUNC 15; Hu-Friedy®). Bleeding on probing was evaluated 15 s following pocket probing at the same sites. A dichotomous score was given. Location of the gingival margin in relation to the cemento-enamel junction was measured to the nearest mm at the same sites using the same manual probe. Recession was given a positive value, whereas pseudo-pockets a negative value. Clinical attachment level was calculated for each site as the sum of PPD and gingival recession or overgrowth.

Microbial examination

Detection frequencies (patients positive or negative for a given species) and bacterial load were analysed for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Micromonas micros*, *Prevotella intermedia*, *Fusobacterium* genus and *Streptococcus mutans* by means of real-time PCR (qPCR). Subgingival plaque samples were collected from the deepest pocket per quadrant at baseline. The same sites were sampled again after 3 months. A sterile paper point was inserted following supragingival plaque removal and left *in situ* for about 20 s. The paper points were collected in 200 µl of a 20 mM TRIS–HCl, pH 8 solution (Merck, Darmstadt, Germany) and stored at −20°C until DNA extraction.

Samples were pretreated with 200 µl of a proteinase K buffer (9.5 ml 20 mM TRIS–HCl, pH 8 + 0.5 ml 10% SDS) and 2 µl of 25 U mutanolysin μl^{-1} (Sigma-Aldrich, Bornem, Belgium), followed by 15-min incubation at 37°C. Thereafter, 10 µl of a 25 mg ml^{-1} proteinase K solution (Merck) was added, the mixture was vortexed and incubated for 15 min at 55°C, with vortexing every 5 min. Finally, 1600 µl Nuclisens Easymag Lysis buffer (BioMérieux, Brussels, Belgium) was added, and the mixture was incubated for 10 min at room temperature. Pretreated samples were extracted by the Easymag system (BioMérieux) according to the manufacturer's prescriptions. The elution volume containing the purified DNA was 110 µl. The four DNA extracts obtained per patient were pooled per time point (baseline and 3 months) to have the overall microbial status on a patient level.

Primer sequences and concentrations are listed in Table 1. The qPCR assays were all run with the same thermocycling programme: initial denaturation (+ activation of hot start enzyme) for 10 min at 95°C, 40 cycles of 15 s at 95°C and 1 min at 60°C on an ABI 7300 real-time PCR system (Applied Biosystems, Halle, Belgium) using the SybrGreen Core kit (Eurogentec, Seraing, Belgium). All reactions were performed according to the manufacturers' guidelines. Primer concentrations were the same for all assays, *that is,* 0.3 µM. Detection limits of each assay were evaluated using a 10-fold dilution series of the type strain of each of the tested species. Dependent on the assay, detection limits varied between 2 and 37 chromosomes per reaction, except for the *T. denticola* assay, which had a detection limit of 150 chromosomes/reaction. Specificity of all primer pairs was tested on a panel of strains of 35 oral bacterial species, belonging to the genera *Actinomyces*, *Aggregatibacter*, *Bacteroides*, *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella* and *Streptococcus*. Using

Table 1. **Primer sequences and concentrations**

Species	Primers	Target gene	Concentration (µM)	References
Aggregatibacter actinomycetemcomitans	F: CTTACCTACTCTTGACATCCGAA RV: ATGCAGCACCTGTCTCAAAGC	16S rRNA	0.3	Maeda *et al.* (46)
Porphyromonas gingivalis	F: TGGTTTCATGCAGCTTCTTT R: TCGGCACCTTCGTAATTCTT	*waaA*	0.3	Hyvarinen *et al.* (47)
Tannerella. Forsythia	F: GGGTGAGTAACGCGTATGTAACCT R: ACCCATCCGCAACCAATAAA	16S rRNA	0.1	Shelburne *et al.* (48)
Treponema denticola	F: CCTTGAACAAAAACCGGAAA R: GGGAAAAGCAGGAAGCATAA	*waaG*	0.3	Hyvarinen *et al.* (47)
Micromonas micros	F: AAACGACGATTAATACCACATGAGAC R: ACTGCTGCCTCCCGTAGGA	16S rRNA	0.3	Bartz *et al.* (49)
Prevotella. Intermedia	F: TCCACCGATGAATCTTTGGTC R: ATCCAACCTTCCCTCCACTC	16S rRNA	0.3	Maeda *et al.* (46)
Fusobacterium genus	F: AAGCGCGTCTAGGTGGTTATGT R: TGTAGTTCCGCTTACCTCTCCAG	16S rRNA	0.3	Martin *et al.* (50)
Streptococcus mutans	F: AGCCATGCGCAATCAACAGGTT R: CGCAACGCGAACATCTTGATCAG	*gftB*	0.3	Yano *et al.* (51)

standard curves, obtained by performing qPCR on a 10-fold dilution series of the type strain of each of the eight bacterial species, the loads of each species present in the samples could be calculated. In the qPCR assay, a negative control (DNA-/RNA-free HPLC water) was included and the 10-fold dilution series of the type strains were considered as positive controls.

Supportive periodontal treatment

Following clinical and microbial examination and irrespective of the group, patients received supportive care by one and the same clinician (PK). Scaling was performed using ultrasonic devices and hand curettes. Subgingival debridement was performed for remaining (4–6 mm) bleeding pockets. All teeth were polished, and oral hygiene instructions were given using mechanical aids (tooth brush and interdental aids).

Questionnaire

Patient's compliance, satisfaction and side effects were registered by means of a questionnaire after the 3-month rinsing period. All patients completed the questionnaire without the clinician being present to avoid bias.

Statistical analysis

Data analysis was performed with the patient as the experimental unit. Intra-examiner repeatability for PI, PPD and CAL was evaluated using Spearman correlation coefficient and Wilcoxon signed ranks tests. Mean values and standard deviations were calculated for all clinical parameters and bacterial load per subject and per time point (baseline and 3 months). To evaluate the time effect on clinical parameters and bacterial load, the Wilcoxon signed ranks test was adopted (within group comparison). The treatment effect was evaluated using the Mann–Whitney test (between group comparison). Within group comparison of microbial detection frequency was performed using the McNemar test. The Fisher's exact test was adopted to compare groups in terms of this parameter. Primary outcome variables (GI and PI) were also analysed as frequency distributions. Data were re-categorized for this purpose (clinically healthy: GI score 0–1/mild gingivitis: score GI 2–3/heavy gingivitis: GI score 4–5; low plaque: PI score 0–1/moderate plaque: PI score 2–3/heavy plaque: PI score 4–5), and analyses were performed using the Fisher's exact test. The level of significance was set at 0.05.

Results

Fifty eligible Caucasian patients were recruited for the study of which six terminated their participation during the first week. Two were given the essential oil rinse, and 4 were given the placebo rinse. Reported reasons for these early drop-outs included 'strong flavour' (3), 'a burning feeling during and/or after rinsing' (1) and 'intolerance to the product' (2). Thus, full data on 23 patients in the essential oil rinse group (eight men; 15 women; mean age, 57 [30; 77]; five smokers) and 21 in the placebo rinse group (nine men; 12 women; mean age, 55 [25; 87]; five smokers) were available at the end of the study period. A patient was considered a smoker if he/she smoked at least 10 cigarettes a day (26).

Duplicate recordings on PI, PPD and CAL were registered prior to the start of the study in three patients with a total of 378 sites. Intra-examiner repeatability was favourable for PI (Spearman correlation coefficient, 0.882; $P < 0.001$ – Wilcoxon signed ranks test, $P = 0.483$), PPD (Spearman correlation coefficient, 0.875; $P < 0.001$ – Wilcoxon signed ranks test, $P = 0.248$) and CAL (Spearman correlation coefficient, 0.851; $P < 0.001$ – Wilcoxon signed ranks test, $P = 0.695$).

As shown in Table 2, the degree of periodontal destruction at baseline was comparable between the groups. There was no significant difference in terms of GI, PI, PPD, BoP or CAL.

Clinical results

Table 3 presents the time effect for both groups. Gingivitis index, PI and BoP showed a significant drop over time ($P \leq 0.029$). For the essential oil rinse group, these reductions were 0.3, 0.7 and 18%, respectively. In the placebo rinse group, GI, PI and BoP dropped by 0.3, 0.3 and 12%, respectively. The aforementioned changes did not differ significantly between the groups ($P \geq 0.121$). Probing pocket depth and CAL did not change over time.

The time effect with respect to GI is further illustrated in Fig. 1. On average, 89% of the sites in the essential oil rinse group showed a clinically healthy gingiva (score 0–1) at baseline. After a 3-month rinsing period, 96% of the sites were clinically healthy ($P < 0.001$). Corresponding data for the control group

Table 2. **Baseline characteristics sorted per group**

Group	Gingivitis index	Plaque index	Probing pocket depth (mm)	Bleeding on probing (%)	Clinical attachment level (mm)
Essential oil rinse	0.6 (0.3)	1.3 (0.8)	2.7 (0.3)	39 (16)	3.4 (0.7)
Range	[0.1; 1.2]	[0; 2.5]	[2.1; 3.6]	[8; 71]	[2.1; 4.8]
Placebo rinse	0.5 (0.3)	1.0 (0.6)	2.8 (0.3)	38 (17)	3.6 (1.0)
Range	[0.1; 1.3]	[0.1; 1.9]	[2.3; 3.6]	[8; 81]	[2.3; 5.3]
P-value	0.786	0.176	0.611	0.605	0.621

Mean (standard deviation).

Table 3. **Time effects on clinical parameters sorted per group**

	Gingivitis index		Plaque index		Probing pocket depth (mm)		Bleeding on probing (%)	
Group	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Essential oil rinse	0.6 (0.3)	0.3 (0.2)	1.3 (0.8)	0.6 (0.5)	2.7 (0.3)	2.8 (0.4)	39 (16)	21 (13)
Pre-post	0.3 (0.3)		0.7 (0.6)		−0.1 (0.2)		18 (11)	
P-value	<0.001		<0.001		0.737		<0.001	
Placebo rinse	0.5 (0.3)	0.3 (0.2)	1.0 (0.6)	0.7 (0.5)	2.8 (0.3)	2.9 (0.4)	38 (17)	25 (12)
Pre-post	0.3 (0.3)		0.3 (0.6)		−0.1 (0.2)		12 (17)	
P-value	0.002		0.029		0.263		0.002	

Mean (standard deviation).

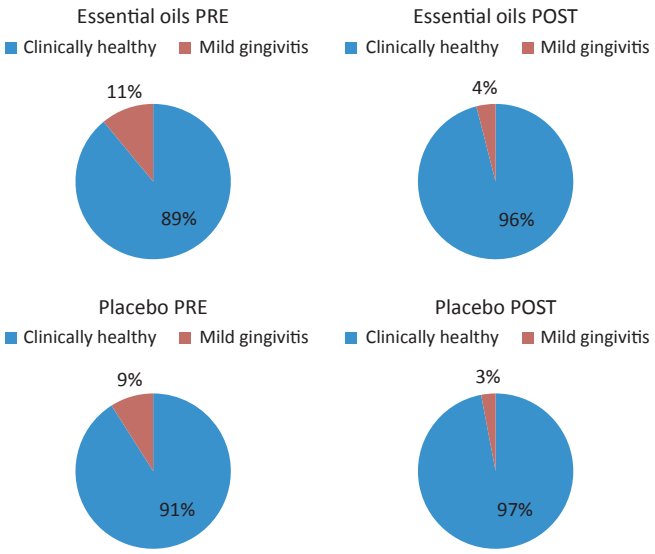

Fig. 1. Time effects: gingivitis index.

were 91% (baseline) and 97% (3 months) ($P = 0.001$). Sites with heavy inflammation (score 4–5) were never recorded.

The time effect concerning PI is shown in Fig. 2. At baseline, on average 61% of the sites in the essential oil rinse group showed low plaque levels (score 0–1). After a 3-month rinsing period, 85% of the sites had low plaque levels ($P < 0.001$). Corresponding data for the control group were 73% (baseline) and 84% (3 months) ($P < 0.001$).

Although GI, PI and BoP improved over time, between group analyses revealed no significant differences (GI: $P = 0.972$ – PI: $P = 0.663$ – PPD: $P = 0.265$ – BoP: $P = 0.121$).

Microbial results

There was no significant difference between the groups neither in terms of detection frequency ($P \geq 0.196$), nor in terms of bacterial load at baseline ($P \geq 0.058$). There was no significant change over time in detection frequency for any of the species ($P = 1.000$). Similarly, bacterial load did not alter significantly over the 3-month rinsing period, as shown in Table 4 ($P \geq 0.074$). As a result, detection frequencies

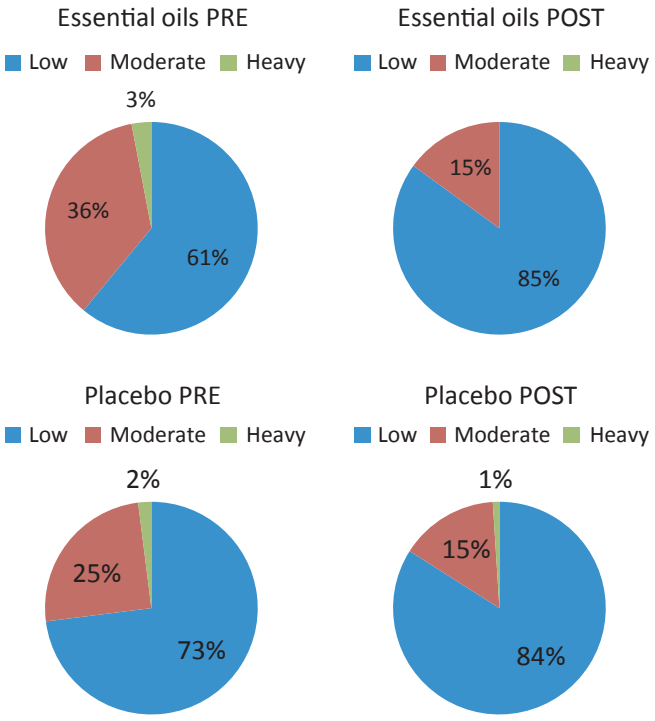

Fig. 2. Time effects: plaque index.

($P \geq 0.197$) and bacterial load ($P \geq 0.111$) were similar between the groups at study termination.

Questionnaire

None of the patients reported any side effect after a 3-month rinsing period supporting the safety of the essential oil mouth rinse. Table 5 presents the outcome of the questionnaire. Given the similar taste and colour of the essential oil rinse and placebo rinse, we made no distinction between the groups. The vast majority of the patients expressed a refreshing feeling after rinsing and felt that rinsing had a positive effect on oral hygiene. Fifty-seven per cent of the patients would like to continue rinsing and would recommend it to family and friends.

Table 4. Time effects on bacterial load sorted per group

	*Aa**		*Pg**		*Tf**		*Td**		*Mm**		*Pi**		*Fn**		*Sm**	
Group	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)	Pre (SD)	Post (SD)
Essential oil rinse	1 (3)	5 (19)	1271 (2259)	724 (1517)	2690 (2823)	2368 (3198)	21 (44)	7 (13)	647 (995)	391 (635)	594 (902)	454 (1451)	2117 (2271)	2001 (2300)	292 (656)	191 (454)
Range	[0; 10]	[0; 80]	[0; 8000]	[0; 5000]	[30; 9000]	[1; 10000]	[0; 162]	[0; 50]	[10; 4000]	[7; 2000]	[0; 3000]	[0; 6000]	[90; 8000]	[50; 8000]	[0; 2130]	[0; 1520]
P-value	0.655		0.074		0.422		0.204		0.133		0.814		0.477		0.104	
Placebo rinse	0 (0)	2 (5)	4808 (13041)	2688 (5363)	3405 (6918)	3599 (6846)	44 (92)	35 (64)	2235 (5130)	1409 (2850)	488 (1200)	156 (340)	1913 (2178)	1420 (1318)	1508 (6025)	589 (2278)
Range	[0; 1]	[0; 20]	[0; 50000]	[0; 20000]	[0; 20000]	[0; 20000]	[0; 358]	[0; 208]	[0; 20000]	[0; 10000]	[0; 4000]	[0; 1000]	[20; 7000]	[3; 5000]	[0; 24100]	[0; 9130]
P-value	0.180		0.678		0.470		0.597		0.255		0.400		0.220		0.342	

*10^4 cfu ml^{-1}: *Aa, Aggregatibacter actinomycetemcomitans*; *Pg, Porphyromonas gingivalis*; *Tf, Tannerella forsythia*; *Td, Treponema denticola*; *Mm, Micromonas micros*; *Pi, Prevotella intermedia*; *Fn, Fusobacterium* genus; *Sm, Streptococcus mutans*.

Discussion

Accurate plaque control and patient adherence to prescribed treatments are fundamental for long-term clinical success (27). Clinical practice may show that proper home care is sometimes difficult to achieve, which calls for additional oral hygiene measures. Given a low risk for side effects, essential oil could be considered for this indication.

Essential oil has a well-documented anti-plaque and anti-gingivitis effect in individuals without periodontal disease (21, 28–33). In these 6-month studies, rinsing with an essential oil solution reduced supragingival plaque by 14–57% compared with a control solution. Fine *et al.* (34) found significant reductions of supra- and subgingival bacterial levels in gingivitis patients as a result of 14 days of twice-daily rinsing with an essential oil solution.

Essential oil mouth rinse also showed beneficial effects in the treatment of chronic periodontitis. One study showed significant reductions in periodontopathogens in the subgingival biofilm following subgingival irrigation (35). In another study of the same group, subgingival levels of *P. gingivalis*, *F. nucleatum*, *Veillonella species* and total anaerobes were significantly lower after 14 days of twice-daily rinsing with an essential oil solution by patients with mild to moderate periodontitis (36). Cortelli *et al.* (23, 37) investigated in a 6-month randomized controlled study the clinical and microbial effects of an essential oil solution used as chemotherapeutic agent in the so-called one-stage full-mouth disinfection protocol, originally introduced by Quirynen *et al.* (38). Fifty patients received either full-mouth disinfection using an essential oil solution or placebo solution (37). Albeit the rinsing period was only 2 weeks, the essential oil rinse group showed superior clinical improvement in terms of plaque and gingivitis scores after 6 months of observation. In their preliminary study on 20 patients, PPD was even additionally reduced by essential oil (23). In terms of microbial alterations, however, no consistent results could be found. Three other studies have compared the efficacy of subgingival irrigation with an essential oil solution to that of a control solution in periodontal pockets. Feng *et al.* (39) performed a 24-week study in which subjects received non-surgical periodontal therapy. After approximately 1 month, residual pockets ($\geq$5 mm) received ultrasonic instrumentation irrigated with essential oil or ultrasonic instrumentation irrigated with a placebo. The latter was repeated twice afterwards with a time interval of 1 week. The results did not demonstrate any significant differences between the test and control group in terms of PI, BoP, full-mouth PPD and full-mouth CAL. However, when considering only deep pockets ($\geq$7 mm), clinical results favoured essential oil irrigation. The second study is a 3-month trial by Yilmaz *et al.* (40) comparing the possible adjunctive effects of irrigation with essential oil in scaling and root planing to chlorhexidine and distilled water. Patients specifically with class II furcation involvement were recruited to the study and underwent a session of full-mouth ultrasonic debridement using either chlorhexidine, essential oil, or water as irrigant. All treatments tended to be equally

Table 5. **Questionnaire**

Questions	Not at all *n* (%)	Rather not *n* (%)	Neutral *n* (%)	Rather yes *n* (%)	Definetely *n* (%)
Did you experience a refreshing feeling after rinsing?	2 (5)	3 (6)	6 (14)	17 (39)	16 (36)
Did you experience an unpleasant irritant or burning sensation during or after rinsing?	4 (9)	5 (11)	7 (16)	18 (41)	10 (23)
Did rinsing have a positive effect on mouth odor?	2 (5)	0 (0)	23 (52)	12 (27)	7 (16)
Did you experience an altered taste after rinsing?	12 (27)	4 (9)	17 (39)	9 (20)	2 (5)
Did you experience discoloration of tongue or teeth?	12 (27)	8 (18)	21 (48)	1 (2)	2 (5)
Did rinsing have a positive effect on your oral hygiene?	1 (2)	0 (0)	12 (27)	18 (41)	13 (30)
Would you like to continue rinsing with this mouth rinse?	5 (11)	4 (9)	10 (23)	11 (25)	14 (32)
Would you recommend this mouth rinse to family or friends?	3 (7)	4 (9)	11 (25)	15 (34)	11 (25)

effective in terms of PPD and CAL reduction at both 1- and 3-month evaluations. Howbeit, the essential oil group, showed significant reduction in BOP scores compared with the other groups at 1 month. In a similar study by Cosyn *et al.* (41), 35 chronic periodontitis patients underwent ultrasonic root debridement with either water or essential oil as coolant. After a 3-month period, despite the significant clinical improvements in both groups, no statistically significant inter-group difference was observed.

Albeit rinsing may have an impact on supragingival plaque development, the microbiota were studied in the subgingival area in this study. This relates to the fact that our sample included periodontitis patients. In fact, only one study has been published on the clinical and microbial effects of an essential oil rinse when used by chronic periodontitis patients in supportive care whereby the same approach was followed (42). As in the present study, patients rinsed twice a day during a 3-month period in this randomized controlled trial. It was concluded that all mouth rinses (two herbals, essential oil rinse and chlorhexidine rinse) produced shifts in the composition of subgingival microbiota, although the results differed among the groups. Plaque levels were mainly reduced by chlorhexidine rinsing and to a lower degree by essential oil. The present study failed to show a relevant impact of essential oil on clinical or microbial parameters, and therefore, the research hypothesis could not be confirmed. However, the significant time effects we found suggest that rinsing may help to motivate patients in terms of daily home care. This is substantiated by the results of the questionnaire indicating that most patients felt a positive effect of rinsing on oral hygiene. On the other hand, time effects could also reflect a novelty and/or Hawthorne effect. Especially in short-term studies, the impact of these should not be underestimated.

Chlorhexidine may still be considered the gold standard in chemical plaque control. In three randomized controlled studies, the impact of low-concentration chlorhexidine (0.05%) rinsing was investigated in patients attending a supportive periodontal care programme (43–45). Santos *et al.* (43) demonstrated additional reductions in plaque accumulation and subgingival bacterial counts following 2 weeks of chlorhexidine rinsing. In the study by Quirynen *et al.* (44), patients were allocated to one of 2 chlorhexidine rinse groups (0.05% chlorhexidine + 0.05% cetyl pyridinium chloride versus 0.2% chlorhexidine) or a placebo rinse group following initial periodontal therapy. After a 6-month rinsing period, chlorhexidine reduced plaque levels, gingivitis levels and subgingival bacterial counts beyond these observed by the placebo, yet with high degree of staining irrespective of the chlorhexidine concentration. More recently, Escribano *et al.* (45) demonstrated similar findings on plaque reduction by low-concentration chlorhexidine rinsing in non-compliant patients with high plaque levels (mean 3 on a scale of 5) attending a supportive care programme. *F. nucleatum* and *P. intermedia* showed significantly lower counts in subgingival plaque samples following chlorhexidine rinsing when compared with placebo rinsing. In contrast to the study by Escribano *et al.* (45), patients in our study were more compliant showing acceptable plaque levels at intake (mean PI < 1.5 on a scale of 5). Because patients with high plaque levels may show (a higher) margin for improvement and therefore differences to be detected, it would be valuable to do the present study again in patients lacking oral hygiene. After all, these subjects would benefit more from chemical plaque reduction than the ones under investigation. Given the high degree of staining as shown by Quirynen *et al.* (44), chlorhexidine may not be the ideal chemotherapeutic agent for this indication, even at low concentration.

At the time, the present investigation was set up, there were no studies available on an essential oil rinse used by patients in supportive periodontal care. Hence, a proper sample size calculation could not be performed. However, *post-factum* calculations demonstrated that the present study was clearly not underpowered. We found statistical power of 87% based on an arbitrarily chosen clinically relevant mean difference of 0.5 in plaque levels between the groups, standard deviation of 0.65 for the essential oil rinse group (sample size: 23), standard deviation of 0.55 for the placebo rinse group (sample size: 21) and alpha error of 0.05.

In conclusion, this 3-month double-blind randomized placebo-controlled study showed that daily rinsing with an essential oil solution was safe and perceived beneficial by the patients. Plaque and gingivitis levels improved over time irrespective of the group, implying no additional benefit of essential oil. Future research should focus on less-compliant patients with high plaque levels.

Conflict of interest and funding

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