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REVIEW ARTICLE

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The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: *a systematic review and meta-analysis*

Abstract: *Objective:* The purpose of this review was to systematically evaluate the effects of an alcohol vehicle solution (V-Sol) compared with an essential-oils mouthwash (EOMW) and if available with a water-based control (WC) on plaque, gingival inflammation parameters and extrinsic tooth staining. *Materials and Methods:* The PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE databases were searched. Where appropriate, a meta-analysis was performed, and difference of means (DIFFM) as calculated. *Results:* In total, 971 unique papers were found of which five met the eligibility criteria. The DIFFM of the meta-analysis of four 6-month studies showed that the EOMW provided significantly better plaque control (DIFFM = 0.39, $P < 0.00001$) and gingival inflammation reduction as measured by the Löe and Silness Index (DIFFM = 0.36, $P = 0.00001$) as compared to the V-Sol. Regarding extrinsic tooth staining, a small but significant difference (DIFFM = −0.08, $P = 0.03$) was observed. *Conclusion:* Limited data, but with a low risk of bias, were available to assess the potential benefit of the alcohol-containing V-Sol. 'High'- and 'moderate'-quality data were available for the analysis of plaque and gingivitis, respectively. Within these limitations, EOMW appears to provide a significant oral health benefit during the 6 months of use. The data retrieved for this review suggest that the essential oils produce an effect on plaque and gingivitis that extends beyond the V-Sol. Furthermore, the V-Sol proved to be no different from a WC.

Key words: alcohol; bleeding; essential oils; extrinsic tooth stain; gingivitis; listerine; mouthrinse; plaque; systematic review

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Introduction

Dental plaque is a key factor in the aetiology of gingival inflammation. Gingivitis represents a risk factor for periodontal attachment loss and tooth loss (1). Therefore, it is important to encourage patients to perform accurate oral hygiene procedures aimed at removing dental plaque and preventing gingival inflammation. Antiseptic chemical agent use may supplement oral hygiene programmes and compensate for hard-to-reach areas as well as inadequate skill, poor motivation and lack of compliance (2). The antiplaque potential of multiple antimicrobial agents has been assessed. These agents include stannous fluoride (3), essential oils (4),

cetylpyridinium chloride (CPC) (5), hexetidine (6), hydrogen peroxide (H_2O_2) (7), triclosan (2) and chlorhexidine (CHX) (8). Among these agents, chlorhexidine is considered to be the gold standard (8, 9).

A previous systematic review Stoeken *et al.* (4) established that a standardized formulation of an essential-oils mouthwash (EOMW) was significantly more effective with regard to plaque and gingivitis reduction than a control mouthwash. In a recent systematic review of Van Leeuwen *et al.* (10), the effects of CHX and the EOMW were compared on plaque and gingival inflammation parameters. CHX mouthwash provided significantly better plaque control than the EOMW, but no significant difference in the reduction of gingival inflammation was observed.

Essential-oils mouthwash was initially marked and commonly known as Listerine®, with a fixed formula containing the essential oils thymol (0.06%), eucalyptol (0.09%), menthol (0.04%) and methyl salicylate (0.05%) with either a 21.6 or 26.9% hydro-alcohol as a vehicle solution (11). Alcohol is in general used to both dissolve and stabilize certain active ingredients and to improve the product's shelf life (12). Alcohol also adds to the flavour and provides a 'strong taste perception' to the mouthwash. It has been suggested that not only the essential oils but also the alcohol vehicle solution contributes to the antibacterial effect (2).

Previous systematic reviews regarding the evaluation of essential oils in a fixed formula compared this to a placebo, a (5%) hydro-alcohol control (4) or as a positive control CHX mouthwash (4, 10). To our knowledge, no systematic review available has evaluated the effect of the alcohol-containing vehicle solution of 'over-the-counter' available EOMW on plaque and gingivitis. Therefore, the aim of this review was to evaluate to what extent an alcohol-containing (21.6–26.9%) vehicle solution as compared to an standardized essential-oils mouthwash affected plaque, gingival inflammation parameters and extrinsic tooth staining in patients with gingivitis. The hypothesis is that there is no significant difference between a fixed essential-oils mouthwash and its vehicle solution nor between the vehicle solution and a water-based control.

Materials and methods

This systematic review was conducted in accordance with the guidelines for the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA-statement) (13).

Search strategy

Three electronic Internet databases were used to search for appropriate papers that satisfied the study purpose: the National Library of Medicine's (Washington, D.C.) PubMed-MEDLINE, the Cochrane Central Register of Controlled Trials, and the Excerpta Medica Database (EMBASE). The databases were searched for studies conducted during the period up to and including September 2013. This comprehensive search was designed to include any published paper that evaluated the effects of an essential-oils[1] mouthwash compared with its alcohol vehicle solution. For the detailed search strategies, see Fig. 1. In addition, the manufacturer[2] was contacted for unpublished data.

The eligibility criteria for suitable articles were as follows:

- Randomized controlled clinical trials (RCTs) or controlled clinical trials (CCTs)
- Conducted in humans
  - ≥18 years of age
  - Good general health (no systemic disorders);
- Mouthrinses were used as an adjunct to self-performed daily mechanical oral hygiene
- Treatment
  - Standardized essential-oils mouthwash formulation (EOMW)
- Comparison
  - Vehicle solution from a fixed formula of essential oils containing between 21.6 and 26.9% hydro-alcohol (V-Sol)
  - When available a water-based control (WC)
- The parameters of interest were retrieved from the following study types:
- Short-term studies (duration ≤4 weeks) (7)
  - Plaque scores;
- Intermediate length studies (>4 weeks to <6 months) (7)
  - Primary outcome, plaque and gingivitis scores
  - Secondary outcome, extrinsic tooth staining.
- Long-term studies (duration ≥6 months) (7)
  - Primary outcome, plaque and gingivitis scores
  - Secondary outcome, extrinsic tooth staining.

Screening and selection

Only papers with the English and Dutch language were accepted. Case reports, letters and narrative or historical reviews were not included in the selection. Two reviewers (GAW and MPC) independently screened the papers by title and abstract to select studies that potentially met the inclusion criteria. If the search keywords were present in the title, the paper was selected. If none of the keywords were mentioned in the title, the abstract was read in detail to search for keywords. After selection, full-text papers were read in detail by two reviewers (DES and MPC). Papers that fulfilled all of the selection criteria were processed for data extraction. Disagreements concerning eligibility were resolved by consensus, or if a disagreement persisted, by arbitration through a third reviewer (GAW). All reference lists of the selected studies were hand-searched by two reviewers (DES and MPC) for additional published work that could possibly meet the eligibility criteria of this study. In addition, the manufacturer of Listerine® was contacted for unpublished data.

[1]A standardized essential-oils mouthwash formulation – Listerine® Antiseptic.
[2]Johnson & Johnson, Skillman, NJ, USA.

{<ingredient> AND <vehicle>}

{<*ingredient:* phenol OR phenols OR oils, volatile [Mesh] OR tartar control Listerine [Substance Name] OR LISTERINE OR essential oils OR essential oil OR phenol OR phenols [text words]>

AND

<*vehicle:* mouthwashes [Mesh] OR mouthwashes OR mouthwash OR mouthwash* OR mouthrinses OR mouthrinse [text words]>}

Fig. 1. The search strategy was customized according to the search requirements of the individual databases. The following terms were used in the PubMed-MEDLINE, Cochrane Central Register of Controlled Trials and EMBASE search strategy.

Assessment of heterogeneity

The following factors were used to evaluate the heterogeneity of the outcomes of the different studies: the study design, evaluation periods, the subject characteristics, comparison and regimen, and industry funding.

Quality assessment

The methodological quality of the included studies was independently scored by two reviewers (DES and MPC). For the criteria listed, see online Appendix S2. In short, a study was considered to have a 'low risk' of bias when 'random allocation, defined inclusion/exclusion, blinding to patient and examiner, balanced experimental groups, report of follow-up criteria and an identical treatment between groups except for intervention' were present. Studies that met five of these six criteria were considered to have a potential 'moderate risk' of bias, and the absence of two or more of these six criteria was considered to represent a potential 'high risk' of bias, as proposed by Van der Weijden (14).

Data extraction

From the collection of papers that met the inclusion criteria, data were extracted with regard to the EOMW compared with the V-Sol as an adjunct to self-performed oral hygiene. When feasible, the mean values and standard deviations (SD) were extracted by two reviewers (DES and MPC) concerning data from baseline, end-trail and specific increments with respect to the parameters of interest. When present in the selected papers, the authors of this review specifically used the data concerning the results of an EOMW and its V-Sol as well as a WC. When intermediate assessments regarding the use of EOMW and the V-Sol were presented, the baseline and final evaluations were used in this review. If applicable, any other data were neglected. Some of the studies provided standard errors (SE) of the mean. When possible, the authors calculated the standard deviation based on the sample size ($SE = SD/\sqrt{N}$).

Data analysis

The Cochrane Collaboration's statistical guidelines were followed to determine the choice of summary statistics and estimates of the overall effect (15). Regarding plaque and gingivitis scores, studies II (16), IV (17) and V (18) provided baseline data and end-trial assessments, while study I (19) and III (20) only presented end data.

Considering the above, a meta-analysis was performed using only the available data from the end-of-trial assessments. Differences of means (DIFFM) were calculated with Review Manager[3] using a random-effect model or a fixed-effect model in case there are <4 studies included (8). Not all studies could be included in the meta-analysis (i.e. cases of non-comparable indices, instances of inappropriate data presentation or unknown SDs were excluded). Heterogeneity was tested by chi-squared test and the I^2 statistic. A chi-squared test resulting in a $P < 0.1$ was considered an indication of significant statistical heterogeneity. As a rough guide for assessing the possible magnitude of inconsistency across studies, I^2 statistic of 0–40% was interpreted as not be important, and above 40% moderate to considerable heterogeneity may be present.

Grading the 'body of evidence'

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system, as proposed by the GRADE working group (21, 22), was used to grade the evidence that emerged from this review. Two reviewers (DES and MPC) rated the quality of the evidence and strength of recommendations. Any disagreement between the two reviewers was resolved after additional discussion, and if a disagreement persisted, the judgement of a third reviewer (GAW) was decisive.

Results

Search and selection

The PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE searches identified 940, 159 and 224 papers, respectively (Fig. 2). In total, 971 unique papers were found. The screening of titles and abstracts initially identified 25 full-text articles. Fifteen papers were excluded because the alcohol-containing control rinse had a low hydro-alcohol concentration of 0.02% (23, 24) or 5% (25–37), and in one study, the concentration of the hydro-alcohol was not provided (38). In addition, in one study, the control rinse was either a chlorhexidine or sanguinarine rinse (39), and in two another, a water-based control was used (40, 41). Furthermore, in two studies, the participants were periodontitis patients (42, 43). By hand-searching the reference lists of the selected studies, one additional paper was identified (20) (study III), which was found in Gordon *et al.* (16) (study II). Additionally, the manufacturer[4] provided

[3]Review Manager, version 4.2 for Windows, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark.

[4]Johnson & Johnson, Skillman, NJ, USA.

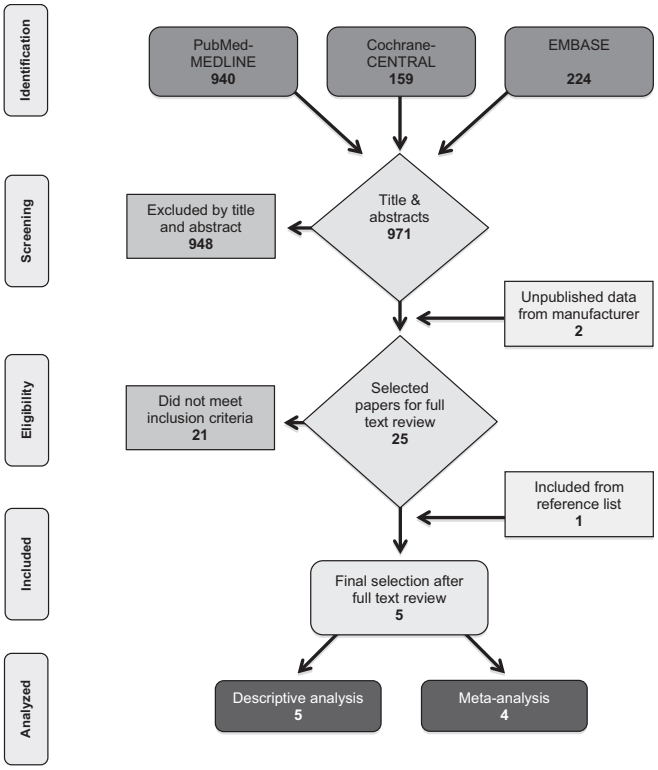

Fig. 2. Flowchart of the search and selection process, including the results.

two reports with unpublished data, studies IV and V. Eventually, five studies (16–20) were processed for data extraction, which displayed variations in the study design, participant age range, evaluation period, oral prophylaxis, treatment, industry funding involvement, comparisons and regimens. Furthermore, the gender distribution of the participants varied between the studies or was unknown. Detailed information regarding the study characteristics is provided in Appendix S1.

Study design and subject characteristics

The study populations of all selected studies were participants with gingivitis but without periodontitis. All of the studies were conducted as randomized controlled clinical trials and were double-blind. Participants in studies I, II, IV and V underwent oral prophylaxis before the experiment, while no oral prophylaxis was provided in study III. The evaluation periods varied between 3 weeks and 6 months among the selected brushing studies. The participants in the short-term brushing study (I) were scored in the upper jaw where one quadrant (#1) was not brushed, while the other (#2) was brushed. As only the upper left quadrant was brushed in addition to the rinsing, data obtained from this quadrant were used. In study II (originally a 9-month study), most of the participants did not participate in the last assessment. Therefore, end scores at 6 months were used.

Comparison and regimen

The V-Sol with a hydro-alcohol concentration of 26.9% was used in five studies, while in one study (I), a 22% concentration was used. In two studies (II and III), additional groups rinsed with WC. The rinsing regime, performed twice daily for all five studies, included 30-second rinses with 20 ml of the EOMW, V-Sol and WC. In all studies, participants received a toothbrush and dentifrice and in addition to rinsing continued their usual oral hygiene. Participants in study I also received dental floss, while in study III, it was explicitly mentioned that flossing was allowed.

Quality assessment

Quality assessment parameters, including external, internal and statistical validity, are presented in Appendix S2. Based on a summary of these criteria, the estimated risk of bias was low. However, four of the five studies were either funded by the manufacturer or involved contributing authors who were employed by the same manufacturer.

Study outcomes

Differences between the baseline and end scores for parameters of interest within groups are shown in Appendix S3 (A–C). Outcomes are presented for brushing studies. Table 1 summarizes the differences between the V-Sol, EOMW and WC in a descriptive manner.

Meta-analysis

Meta-analyses were performed to compare the effect of the V-Sol and essential oils as adjunct to self-performed daily oral hygiene. A summary is shown in Table 2A,B. The 6-month brushing studies that evaluated the plaque scores at the end of the trial [Quigley & Hein (44) modified by Turesky (45)] showed a significant effect in favour of the EOMW

Table 1. **A descriptive analysis of the comparison of an essential-oils mouthwash, alcohol-containing vehicle solution or water control as an adjunct to daily brushing**

Treatment	Study #	Plaque scores	Gingival index	Staining scores	Comparison
EOMW	I (19)	○	■	■	V-Sol
	II (16)¶	+	○	?	V-Sol
	III (20)	+	+	○	V-Sol
	IV (17)¶‡	+	○	○	V-Sol
	V (18)¶‡	+	+	–	V-Sol
V-Sol	II (16)¶	○	○	?	WC
	III (20)	○	○	○	WC

+: in favour of treatment, –: Treatment significantly less effective, ○: no difference, ■: no data available, ?: not reported/unclear, EOMW: Listerine® mouthwash, V-Sol: alcohol-containing vehicle solution (21.6%–26.9%), WC: water control and ¶: professional prophylaxis at baseline, rendering the panellist with zero visible plaque.

when compared to the V-Sol, with a difference in means of 0.39 [95% CI = (0.30; 0.47), $P < 0.00001$]. There was also a significant effect in favour of the EOMW for gingivitis reduction according to the Löe and Silness Index (46), with a DIFFM of 0.36 [95% CI = (0.26; 0.62), $P = 0.0001$], and the Modified Gingival Index (47), with a DIFFM of 0.17 [95% CI = (0.08; 0.25), $P < 0.001$]. Regarding extrinsic tooth staining Lobene extrinsic tooth stain index (48), a small but significant difference, with a DIFFM of −0.08 [95% CI = (−0.16; −0.01), $P = 0.03$], was shown between the rinses.

When the V-Sol was compared to WC, no significant difference was found for either the plaque scores or the gingivitis reduction, DIFFM = 0.04 [95% CI = (−0.09; 0.18), $P = 0.51$] and DIFFM = 0.03 [95% CI = (−0.06; 0.13), $P = 0.51$], respectively.

The majority of meta-analysis showed non-important heterogeneity (I^2 value: 0–9%).

Grading the body of evidence

Table 3 shows a summary of the various aspects that were used to rate the quality of the evidence and the strength of the recommendations according to GRADE (22).

The study results were generalizable. The strength of the recommendation appeared to be dependent on the consistency of the outcome parameter. Consequently, the plaque score data were 'strong', the evidence for gingivitis was 'moderate', while the evidence of the side effect of tooth staining was 'weak'.

Table 3. **GRADE evidence profile assessing the strength of the recommendation that an alcohol vehicle control is different from an essential-oils mouthwash**

GRADE	Plaque score	Gingivitis index	Stain index
Risk of bias	Low	Low	Low
Consistency	Consistent	Moderately consistent	Inconsistent
Directness	Generalizable	Generalizable	Generalizable
Precision	Precise	Precise	Precise
Publication bias	Possible	Possible	Possible
Strength recommendation	**Strong**	**Moderate**	**Weak**

Discussion

The available evidence emerging from this review shows that the reduction in plaque and gingivitis between the V-Sol and WC was not significantly different, whereas the vehicle solution was significantly less effective when compared to the

Table 2. **Meta-analysis: A comparison of essential-oils mouthwash, alcohol-containing vehicle solution or water control as an adjunct to daily brushing. (A) An alcohol-containing vehicle solution compared with an essential-oils mouthwash as adjunct in brushing studies (random or fixed effect where appropriate) and (B) alcohol-containing vehicle solution compared with a water control**

					Test for overall effect		Test for heterogeneity	
Model	Index	Study #		DIFFM	95% CI	*P*-value	I^2-value	*P*-value
(A)				(random effect)				
6-month brushing	Quigley and Hein (44) modified by Turesky (45)	II (16) III (20) IV (17)‡ V (18)‡	End	0.39	(0.30; 0.47)	<0.00001	0%	0.41
				(fixed effect)				
	Gingival index Löe and Silness (46)	II (16) III (20)	End	0.36	(0.26; 0.62)	0.00001	92%	0.0004
	Lobene modified gingival index (47)	IV (17)‡ V (18)‡	End	0.17	(0.08; 0.25)	<0.0001	0%	0.92
	Lobene extrinsic tooth stain index (48)	III (20) IV (17)‡	Base	0.02	(−0.06; 0.10)	0.65	0%	0.7
		V (18)‡	End	−0.08	(−0.16; −0.01)	0.03	9%	0.33

					Test for overall effect		Test for heterogeneity	
Model	Index	Study #		DIFFM	95% CI	*P*-value	I^2-value	*P*-value
(B)				(fixed effect)				
6-month brushing	Quigley and Hein (44) modified by Turesky (45)	II (16) III (20)	End	0.04	(−0.09; 0.18)	0.51	0%	0.94
	Gingival index Löe and Silness (46)	II (16) III (20)	End	0.03	(−0.06; 0.13)	0.51	0%	0.46

DIFFM, difference of means; CI, Confidence Interval.

EOMW. These findings suggest that active agents in the EOMW formulation effectively contribute to plaque reduction and gingival inflammation. Subsequently, the antiseptic effect of the hydro-alcohol solution seems negligible. Therefore, the essential oils should be considered as the active ingredient (49, 50). The apparent ineffectiveness of the hydro-alcohol solution as an antiplaque agent and inhibitor of dental plaque bacteria conflicts with the well-established high sensitivity of bacteria to hydro-alcohol and its use as an effective preservative at 10–12% (50). One possible explanation for this disparity is that bacterial biofilms have greater resistance than dispersed bacteria (49–52).

The clinical evaluation of the mouthrinses included one 3-week brushing study and four studies with a duration of 6 months. The brushing model was only used to evaluate the plaque scores of these products. The limitation the 3-week brushing study (53) is that the long-term efficacy of the product, which would more accurately reflect the patient's actual use of the mouthrinse, cannot be evaluated. Subsequently, 6-month brushing studies have been used to evaluate the efficacy of mouthrinses (9, 54), as required according to the guidelines of the American Dental Association (ADA) (55).

This systematic review included only papers that provided data concerning the mouthwash when used as an adjunct to self-performed oral hygiene. Given that mouthrinses can be used and prescribed for short duration, data about their efficacy over shorter periods are of interest (58). Consequently, studies with an evaluation period of <4 weeks were included for the evaluation of plaque scores in this review. However, in concordance with ADA requirements, it was not intended to extract gingival inflammation data from short-term studies (<4 weeks). Concerning adjunctive devices for controlling plaque and gingivitis, the ADA requires an evaluation period of ≥4 weeks (59). Therefore, selected studies with the duration of 4 weeks or more were considered for extraction of both plaque and gingivitis data. All but one included study had the duration of 6 months, while Preus *et al.* (19) evaluated the product over 3 weeks.

Regarding heterogeneity, the composition of the used controls needs to be considered. Testing the efficacy of the original essential products should be carried out against its alcohol vehicle as the negative control. The alcohol content of the VC solution in four studies (II, III, IV and V) was identical to the commercial product with a hydro-alcohol concentration of 26.9%. The exception was Preus *et al.* (19) in which the hydro-alcohol solution was made from 96% ethanol diluted with water to a final concentration of 22%. Table 1 shows that in all but the study by Preus *et al.* (19), the essential-oil product was significantly more effective regarding plaque scores than the vehicle control. An explanation for this disparate finding could be due to the model, the limited evaluation time of 3 weeks and/or the composition of the hydro-alcohol control solution.

As incremental data were sparsely presented and in some instances lacking (SDs), the best way to perform a meta-analysis was to use baseline and end-trail data separately. Where appropriate and feasible, separate meta-analyses were performed.

Limitations

- A rigorous search was conducted across various electronic databases. Titles and abstracts were screened by two reviewers in an effort to locate all relevant papers. Despite these efforts, some papers may have been missed. The search eventually yielded five studies with a high quality of evidence.
- The formal testing for publication bias proposed by Egger *et al.* (56) could not be used because fewer than 10 studies were included in the meta-analysis (15).
- Furthermore, a sensitivity analysis, intended to examine the effects of random sequence generation, allocation concealment and blind outcome assessment on the overall estimates of the effects, could not be conducted because of the limited number of included studies.
- The studies were considered as being double blind. However, some of the participants may have unravelled their group assignment by recognizing the EOMW taste as opposed to those, who rinsed with the V-Sol or WC (57).
- The number of studies that was included in this systematic review was limited because only those with a true control V-Sol that contained a hydro-alcohol concentration between 21.6 and 26.9% were selected.
- As suggested by the Cochrane handbook, unpublished data were searched. The manufacturer provided two unpublished papers. Including data of unpublished studies can in itself introduce bias. For instance, they may be of lower methodological quality than published studies or may be an unrepresentative sample of all unpublished studies (15). The methodological quality of the unpublished studies was assessed (Appendix S2), and those studies were considered to have a low estimated risk of bias. Moreover, this systematic review concerned one specific product of one manufacturer. Therefore, underrepresentation is no item of concern.

Conclusion

Limited data, but with a low risk of bias, were available to address the potential benefit of the alcohol-containing V-Sol on plaque and gingivitis scores. 'High'- and 'moderate'-quality data separately were available for the analysis. Within these limitations, EOMW appears to provide a significant oral health benefit during the 6 months of use. The data retrieved for this review suggest that the essential oils have an effect on plaque and gingivitis parameters that extends beyond the V-Sol. Furthermore, the V-Sol proved to be no different from WC.

Practical implications

- This systematic review is applicable for patients with gingivitis
- The alcohol containing vehicle solution of the essential oil mouthwash alone does not contribute to the efficacy in reducing plaque scores and gingivitis when compared to a water control.
- The essential oils themselves effectively contribute to the reduction of plaque and gingival inflammation.

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Conflict of interest and source of funding statement

The authors have declared no conflict of interests. This study was self-funded by the authors and their institutions. D.E. Slot and G.A. Van der Weijden have received lecture fees from Johnson & Johnson to present their comprehensive work on chemical plaque inhibitors.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Overview of the studies processed for data extraction.

Appendix S2. Methodological quality scores of the included studies.

Appendix S3. (a) Extracted data of the selected studies by plaque scores. (b) Extracted data of the selected studies by gingivitis scores. (c) Extracted data of the selected studies by extrinsic tooth staining.