Original Article

Effectiveness of Alcohol-free Mouth Rinse Containing Essential Oils and Fluoride as an Oral Hygiene Adjunct among Pregnant Thai Women: A Randomized Clinical Trial

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Aim: This 3-month, double-blind, two-center, parallel, randomized controlled clinical trial compared the improvement of oral hygiene status from alcohol-free essential oils (EO) with 0.05% fluoride mouthwash to the control (0.05% fluoride mouthwash). Materials and Methods: One hundred and fifty-four pregnant women were clinically examined to determine Modified Gingival Index (MGI), Plaque Index (PI), and Winkel Tongue Coating Index (WTCI) at baseline, 2 weeks, and 3 months by calibrated examiners. After supragingival scaling and provision of a tooth brushing method, participants were randomly assigned to daily use of alcohol-free EO or the control rinse for 30 s at bedtime. Repeated measures of analysis of variance (ANOVA) were performed to assess the effectiveness of alcohol-free EO with 0.05% fluoride mouthwash on MGI, PI, and WTCI scores. Results: One hundred and forty subjects completed the study. The dropout rate of 9.1% (n = 14) was mainly due to loss of follow-up. At baseline, no significant differences were observed between the intervention and the control groups for MGI (1.19±0.57 vs. 1.11±0.48, P = 0.371), PI (1.53±0.56 vs. 1.47±0.48, P = 0.439), and WTCI (0.88±0.48 vs. 0.88±0.50, P = 0.990). There was a statistically significant reduction of MGI, PI, and WTCI scores over time (P < 0.001). However, no significant differences were observed for between-group comparisons for all measured indices at any time point. No adverse effect was reported in either group. Conclusion: At the end of 3-month period, improvement of oral hygiene of pregnancy women in this study was evidence. However, the use of alcohol-free EO mouthwash as supplements to the daily oral hygiene did not provide a significant improvement in terms of plaque, gingival, and tongue coating indices.

Keywords: Gingivitis, mouth rinse, plaque, pregnancy, tongue coating, tooth brushing

INTRODUCTION

Pregnancy is a specific and critical period in a woman’s life due to hormonal, physical, and emotional changes. Maternal oral diseases, especially gingivitis and periodontitis, during pregnancy are important public health problems as they are prevalent and have a positive relationship with adverse pregnancy outcomes. Although plaque biofilm is responsible for the development of periodontal disease, previous studies have shown that estrogen and progesterone

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changes during pregnancy could aggravate the inflammatory response to plaque biofilm, resulting in severe gingival inflammation.\(^4,3\) Furthermore, some pregnant women face difficulty when performing effective oral hygiene care due to pregnancy-related nausea and vomiting symptoms during the first trimester.\(^6\)

Gingival inflammation as measured by bleeding when probed could be reversible at the end of pregnancy or after parturition.\(^6\) The daily removal of supragingival dental plaque and nonsurgical periodontal therapy provided up to 24th week of pregnancy improved quality of life.\(^10\) However, it was unclear whether periodontal treatment may reduce adverse pregnancy outcomes.\(^11\)

It was also reported that greater gingival inflammation during pregnancy was observed among women with low educational attainment, poor awareness of the importance of oral hygiene, and those having barriers to dental care.\(^7,12-15\) The importance of oral health promotion interventions during the prenatal period has been emphasized; however, there are few evidence-based intervention programs addressing oral-related symptoms, hygiene behaviors, and potential oral-systemic health implications.\(^12\)

The prevention and control of periodontal disease, physical disruption, and elimination of supragingival biofilms can be accomplished by mechanical means, for example, tooth brushing, dental flossing, and professional scaling. However, mechanical approaches alone may not be sufficient to prevent periodontal disease in the general population, especially with high-risk individuals, for example, pregnant women. The efficacy of using chemical anti-plaque mouth rinses as an adjunctive measure to reduce plaque biofilm and gingivitis has been documented in clinical studies.\(^16-18\)

Various anti-plaque agents with different formulation such as chlorhexidine, stannous fluoride, essential oils (EO), cetlypyridinium chloride (CPC), and tricosan have been assessed.\(^19-23\) Systematic review and meta-analysis have also indicated that mouth rinses significantly improved gingival inflammation, bleeding on probing, and plaque scores.\(^20,21\) In addition, several studies have shown that EO containing mouth rinse has superior anti-plaque and antigingivitis effects than other mouth rinses and could be an alternative to chlorhexidine rinse as adjuncts to oral hygiene.\(^20,22,24\)

The effectiveness of alcohol-based EO on plaque and gingival inflammation parameters did not differ from water-based EO.\(^17,19,24\)

Anti-plaque mouth rinse is an attractive method to improve maternal oral health as it is affordable and easy to implement by oneself.\(^17,18\) It has also been advised by US oral health care during pregnancy expert workgroup to rinse every night with a fluoridated, alcohol-free mouth rinse for maintaining oral hygiene during pregnancy.\(^25\) However, a few studies have been conducted with pregnant women and have reported promising results such as the indication that antiseptic mouth rinse intervention improves periodontal health as well as pregnancy outcomes.\(^18,26\) The aim of this study was, therefore, to compare the improvement of plaque scores, gingival health, and tongue cleanliness after the usage of an alcohol-free EO with 0.05% fluoride mouth rinse to the placebo control (green alcohol-free 0.05% fluoride mouth rinse) among pregnant women.

**Materials and Methods**

**Study population and study samples**

The study was approved by the ethics committee of the Faculty of Dentistry, Prince of Songkla University (EC 5808-23-L-HR), and registered to Thai Clinical Trials Registry (TCTR 20191223005). This study was a 3-month, double-blind, multi-centered, parallel, randomized controlled clinical trial that compared the improvement of gingival health, plaque control, and tongue cleanliness of a commercial mouth rinse, an alcohol-free EO with 0.05% fluoride mouthwash (LISTERINE Natural Green Tea, Johnson & Johnson Consumer, USA) to the placebo control (green alcohol-free 0.05% sodium fluoride mouthwash). EO mouthwash’s active ingredients contained 0.096% eucalyptol, 0.068% thymol, 0.041% menthol, and 0.070% methyl salicylate. Two districts from Trang Province and one district from Pattani Province in Southern Thailand were chosen. The sample size calculation showed 140 subjects (70 per treatment group) would be expected to complete the study at 3 months after baseline. This number of subjects provides 90% power to detect a difference of 0.21 with respect to a plaque index (PI) and 0.10 with respect to a modified gingival index (MGI), assuming two-sided tests at the 0.05 significance level. As the dropout rate was assumed to be 10%, the study was designed to include 154 participants.

**Inclusion and exclusion criteria**

Each eligible participant was informed about the nature of the study protocol and provided with a written consent form. They were enrolled in the study according to the following inclusion and exclusion criteria: pregnant women aged 15–40 years at 12–18 weeks of gestation who had at least 20 natural teeth. Exclusion criteria were those who had dental caries, exposed pulp, or had been diagnosed with periodontitis, second-degree tooth mobility, or had fixed orthodontic...
apartments. In addition, individuals who currently had a systemic disease (e.g., hypertension, diabetes mellitus, cardiovascular disease, epilepsy, or asthma), received immunosuppressant drugs and antibiotics or had a history of significant adverse effects from oral hygiene products such as toothpastes or mouth rinses were also excluded from the study.

Participants who were using mouth rinses containing chlorhexidine, triclosan, EO, or CPC were instructed not to use these products for 2 weeks before baseline examination and during the study.

Baseline examination and group allocation
Qualified participants were evaluated for baseline examinations after assessment of inclusion/exclusion criteria with binding of the treatment groups for the examiners and the recorders. Baseline examinations involved a self-administered questionnaire and an oral examination, including the assessment of plaque level, gingival status, and tongue cleanliness. Participants then received a complete dental prophylaxis to remove plaque, stains, and supragingival calculus in addition to oral hygiene instruction by trained dental personnel. Participants were instructed to brush their teeth for at least two minutes in their usual manner and brush their tongues twice daily. They were provided a toothbrush (Reach, High Ridge Brands, La Palma, CA, USA) and a 1000 ppm fluoride dentifrice (Systema, Lion Co. Limited, Bangkok, Thailand) to be used during the study period. The allocation sequence was conducted by the site manager of each center. The sequence was concealed until completion of the baseline examination of each participant. The participants were randomly assigned to one of two treatment groups by another staff member in each center following a random list.

Questionnaires
A self-administered questionnaire comprising 29 questions with 9 questions on socio-demographic status (e.g., age, gestational age, religion, and educational attainment), 7 questions pertaining to medical and dental history (e.g., systemic diseases, dry mouth symptom, nausea and vomiting, and dental visit during pregnancy) and 13 questions on oral hygiene practices (tooth brushing, tongue cleaning, flossing, fluoride toothpaste, and mouthwash) was applied for the assessment of the presence of tongue coating. The dorsum of the tongue was divided into six sextants. The coating density of each sextant was evaluated as follows: no coating = 0; light coating = 1; and severe coating = 2. The tongue coating value was calculated by the sum of all six scores, ranging from 0–12.\[29\]

The pregnant women were examined by ten trained and experienced dental hygienists who had worked in the Dental Public Health Unit for at least 2 years. The didactic portion of training session included an instruction of examiner and recorder roles, procedure for obtaining informed consent, questionnaire collection, and examination procedures. Inter-examiner reproducibility was assessed in field setting using kappa statistics which were 0.5–0.8 for MGI; 0.5–0.9 for PI; and 0.5–1.0 for WTCI. Fifteen volunteer subjects were used for training and calibration session. Following the training period, a group discussion was carried out with three dental school faculty members to review and resolve any differences or disagreements. Images with various scores of MGI, PI, and WTCI were also provided to the examiners for review 2 weeks before the beginning of study.

Intervention and control groups
The subjects were provided one bottle containing 450mL of mouth rinse according to their treatment groups (an alcohol-free EO with 0.05% fluoride mouth rinse for the intervention group and a green alcohol-free 0.05% fluoride mouth rinse for the control group). Both mouth rinses were green in color and were put into the same types of bottles. Rinsing instructions were explained and shown for each participant: rinsing properly with 10–20mL of full-strength assigned mouthwash for 30s daily after brushing before bedtime. During the first follow-up at 2 weeks, the same oral examinations were performed. New bottles of the mouth rinse according to each treatment group

Assessments and outcomes
Gingival status and plaque level were examined on six Ramfjord sample teeth using the MGI and PI.\[27,28\] The MGI was assessed as follows: 0 = normal (absence of inflammation; 1 = mild inflammation; 2 = moderate inflammation; and 3 = severe inflammation. The plaque accumulation was coded as follows: 0 = no plaque; 1 = plaque covering less than 1/3rd of the area; 2 = plaque covering more than 1/3rd, but less than 2/3rd of the area; 3 = plaque covering 2/3rd or more of the area. The Winkel tongue coating index (WTCI) was applied for the assessment of the presence of tongue coating. The dorsum of the tongue was divided into six sextants. The coating density of each sextant was evaluated as follows: no coating = 0; light coating = 1; and severe coating = 2. The tongue coating value was calculated by the sum of all six scores, ranging from 0–12.\[29\]
were provided. Finally, 3 months after the baseline period, both self-administered questionnaires and oral examinations were conducted in the same manner as at the baseline. At 2 weeks and 3 months after the baseline, subjects were asked to return their mouth rinse bottles to the study site. Compliance was estimated by measuring the volume of remaining mouth rinse in each bottle. Side effects and safety information in relation to their mouth rinses were also monitored throughout the study. Local adverse events relating to mouthwash such as oral burning, ulcer, whitish slough, taste alteration, and oral paresthesia were recorded by examiners.

**Statistical analysis**

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, USA). Socio-demographic characteristics as well as oral health behaviors of the study participants were compared across groups using a chi-square test or Fisher’s exact test for dichotomous and categorical variables, and an independent t-test for continuous variables. Effectiveness analysis, using per-protocol analysis, was performed using repeated measures of analysis of variance (ANOVA) to compare means in plaque, gingival, and tongue coating scores between the intervention and the control groups at baseline, 2 weeks, and 3 months. Estimated marginal means and 95% confidence intervals (CIs) in plaque, gingival, and tongue coating scores corresponding to each group and examined time point were calculated. Subgroup analysis was performed to investigate if the outcomes was influenced by examiner variability (kappa values ≥ 0.7). For all the tests, two-sided test at the 0.05 level of significance was performed.

**Results**

Total of 140 pregnant women with an average of 26.8 years old completed the study and were included in the analyses. The drop-out rate of 9.1% (n = 14) was mainly due to a lack of follow-up. There were no protocol violations during the study. [Figure 1] There was no statistical difference between the drop-out group and the analytical group in terms of socio-demographic characteristics and oral health behaviors (data not

![Figure 1: Flow chart of study design](image-url)
shown in the tables). At baseline, the intervention and the control groups had comparable average ages, gestational periods, and daily oral health care practices. Most pregnant women in this study reported good daily oral hygiene practices, for example, using fluoride toothpaste (83%) and brushing teeth daily in the morning (86%) and before bedtime (73%). However, only one-fourth (26%) reported tooth brushing after meals and very few (5%) regularly used dental floss. In addition, about 13% of study participants reported an average tooth-brushing time of less than one minute. Approximately half of the participants reported regular tongue cleaning. Lastly, more than two-thirds (77%) of the study participants reported vomiting. This experience was slightly greater for the control group (77%) than the intervention group (69%).

At baseline, there were no significant differences between the intervention and the control groups for MGI (1.19±0.57 vs. 1.11±0.48, P = 0.371), PI (1.53±0.56 vs. 1.47±0.48, P = 0.439), and WTCI (0.88±0.48 vs. 0.88±0.50, P = 0.990). Repeated measures of ANOVA revealed statistically significant reductions of gingival, plaque, and tongue coating scores over a 3-month period (time effect) (P < 0.001). However, there were no statistically significant reductions of these three indices for between-group comparisons at any time point. Estimated marginal means of gingival scores were decreased by 36% from 1.18 (95% CI =1.05–1.31) at baseline to 0.75 (95% CI = 0.65–0.85) at 3 months for the intervention group and by 34% from 1.11 (95% CI = 0.99–1.24) at baseline to 0.73 at 3 months (95% CI =0.63–0.83) for the intervention group.

### Table 1: Sociodemographic characteristics, oral health behaviors, and oral hygiene status at baseline of participants who completed the study (N = 140)

| Characteristics                                      | Total (N = 140) | Intervention (n = 68) | Control (n = 72) | P Value* |
|------------------------------------------------------|-----------------|-----------------------|------------------|----------|
| Age (years), mean ± SD                               | 26.76 ± 5.13    | 26.74 ± 5.21          | 26.78 ± 5.08     | 0.961    |
| Gestational age (weeks), mean ± SD                   | 15.54 ± 3.39    | 15.87 ± 4.01          | 15.23 ± 2.66     | 0.266    |
| Regular tooth brushing in the morning (missing = 2)  |                 |                       |                  |          |
| Sometimes                                            | 20 (14.5)       | 7 (10.6)              | 13 (18.1)        | 0.214    |
| Everyday                                             | 118 (85.5)      | 59 (89.4)             | 59 (81.9)        |          |
| Regular tooth brushing after meals (missing = 1)     |                 |                       |                  |          |
| Sometimes                                            | 103 (74.1)      | 46 (68.7)             | 57 (79.2)        | 0.158    |
| Everyday                                             | 36 (25.9)       | 21 (31.3)             | 15 (20.8)        |          |
| Regular tooth brushing before bed (missing = 3)      |                 |                       |                  |          |
| Sometimes                                            | 37 (27.0)       | 17 (25.8)             | 20 (28.2)        | 0.751    |
| Everyday                                             | 100 (73.0)      | 49 (74.2)             | 51 (71.8)        |          |
| Regular dental flossing (missing = 3)                |                 |                       |                  |          |
| Sometimes                                            | 130 (94.9)      | 61 (93.8)             | 69 (95.8)        | 0.598    |
| Everyday                                             | 7 (5.1)         | 4 (6.2)               | 3 (4.2)          |          |
| Regular tongue cleaning (missing = 2)                |                 |                       |                  |          |
| Sometimes                                            | 64 (46.4)       | 31 (46.3)             | 33 (46.5)        | 0.980    |
| Everyday                                             | 74 (53.6)       | 36 (53.7)             | 38 (53.5)        |          |
| Average tooth brushing time (missing = 1)            |                 |                       |                  |          |
| < 1 min                                               | 18 (12.9)       | 6 (9.0)               | 12 (16.7)        | 0.066    |
| 1–2 min                                               | 83 (59.7)       | 37 (55.2)             | 46 (63.9)        |          |
| >2 min                                                | 38 (27.3)       | 24 (35.8)             | 14 (19.4)        |          |
| Using of fluoride toothpaste                         |                 |                       |                  |          |
| Yes                                                   | 118 (84.3)      | 58 (85.3)             | 60 (83.3)        | 0.750    |
| No                                                    | 22 (15.7)       | 10 (14.7)             | 12 (16.7)        |          |
| Mouth rinse use in the past week                      |                 |                       |                  |          |
| Yes                                                   | 19 (13.6)       | 12 (17.6)             | 7 (9.7)          | 0.171    |
| No                                                    | 121 (86.4)      | 56 (82.4)             | 65 (90.3)        |          |
| Self-reported vomiting (missing = 1)                 |                 |                       |                  |          |
| Yes                                                   | 102 (73.4)      | 47 (69.1)             | 55 (77.5)        | 0.266    |
| No                                                    | 37 (26.6)       | 21 (30.9)             | 16 (22.5)        |          |
| Modified gingival index at baseline, mean ± SD       | 1.15 ± 0.53     | 1.19 ± 0.57           | 1.11 ± 0.48      | 0.371    |
| Plaque index at baseline, mean ± SD                  | 1.50 ± 0.52     | 1.53 ± 0.56           | 1.47 ± 0.48      | 0.439    |
| Winkel tongue coating index at baseline, mean ± SD    | 0.88 ± 0.49     | 0.88 ± 0.48           | 0.88 ± 0.50      | 0.990    |

*The significance of differences between mean values was tested using independent t-test. Chi-square or Fisher’s exact test was used to compare the proportion of pregnant women between the intervention and the control groups.

SD = standard deviation
group. No significant difference was seen in the estimated marginal means and reduction in plaque (44% vs. 40%) and tongue coating (28% vs. 32%) scores, comparing between the intervention and the control groups. [Table 3] Subgroup analysis showed that there was no evidence of heterogeneity for all outcomes with regard examiners’ variability [Supplemental Table 1]. At the end of the study, no adverse event was reported, nor did any subjects withdraw from our study due to an adverse event.

**Discussion**

Findings from the present study highlight important implications regarding pregnant women and effective oral hygiene practices. Our study also showed that daily use before bedtime of alcohol-free EO-0.05% fluoride mouthwash during pregnancy did not significantly improve oral hygiene status compared to 0.05% fluoride mouthwash when used as an adjunct to self-performed oral hygiene.

Most pregnant women in this study reported proper oral hygiene practices at baseline. However, on average, they had visible plaque deposits and mild-to-moderate gingivitis at the initial examination. Overall, study participants in the control and intervention groups showed gradually improving oral hygiene levels, which was consistent with other studies. The reductions of plaque, levels of gingival inflammation, and tongue-coating scores in the present study may be a combined effect of mechanical and chemical plaque controls as well as part of the “Hawthorne effect.” It seems that effective calculus and plaque removal by means of supragingival scaling at the initial visit along with two-minute toothbrushing and tongue brushing played important roles that were observed at 2 weeks. However,

| Table 2: Tests of between-subject and within-subject effects on gingival, plaque, and tongue coating scores comparing between control and intervention groups at baseline, 2 weeks, and 3 months (N = 140) |
|-----------------|--------|--------|-------|
| Source          | df     | Mean square | F     | P Value*   |
| **Modified gingival index** |        |            |       |            |
| Time            | 2      | 5.966      | 69.244 | <0.001     |
| Group           | 1      | 0.045      | 0.274  | 0.601      |
| Time x group    | 2      | 0.027      | 0.318  | 0.728      |
| **Plaque index** |        |            |       |            |
| Time            | 2      | 14.478     | 102.15 | <0.001     |
| Group           | 1      | 0.017      | 0.113  | 0.737      |
| Time x group    | 2      | 0.065      | 0.462  | 0.631      |
| **Tongue coating index** |        |            |       |            |
| Time            | 2      | 2.501      | 25.05  | <0.001     |
| Group           | 1      | 0.035      | 0.241  | 0.624      |
| Time x group    | 2      | 0.040      | 0.404  | 0.668      |

*Repeated measures of analysis of variance (ANOVA) compared means in plaque, gingival, and tongue coating scores between the intervention and the control groups at baseline, 2 weeks, and 3 months

| Table 3: Estimated marginal means, standard errors (SE), and 95% confidence intervals (CIs) of gingival, plaque, and tongue coating scores at baseline, 2 weeks and 3 months for control and intervention groups (N = 140) |
|-----------------|--------|--------|-------|
| Source          | Intervention (n = 68) | Control (n = 72) |
| **Mean ± SE**   | 95% CIs         | Mean ± SE | 95% CIs         |
| **Modified gingival index** |        |            |       |            |
| Baseline        | 1.18 ± 0.064   | 1.05–1.31  | 1.11 ± 0.062  | 0.99–1.24  |
| 2 weeks         | 1.02 ± 0.057   | 0.91–1.14  | 1.01 ± 0.055  | 0.90–1.12  |
| 3 months        | 0.75 ± 0.051   | 0.65–0.85  | 0.73 ± 0.050  | 0.63–0.83  |
| **Plaque index** |        |            |       |            |
| Baseline        | 1.53 ± 0.064   | 1.41–1.66  | 1.47 ± 0.061  | 1.34–1.59  |
| 2 weeks         | 1.12 ± 0.060   | 1.00–1.24  | 1.10 ± 0.058  | 0.99–1.22  |
| 3 months        | 0.85 ± 0.057   | 0.74–0.97  | 0.87 ± 0.055  | 0.76–0.98  |
| **Tongue coating index** |        |            |       |            |
| Baseline        | 0.88 ± 0.060   | 0.76–0.99  | 0.88 ± 0.057  | 0.76–0.99  |
| 2 weeks         | 0.73 ± 0.052   | 0.62–0.83  | 0.66 ± 0.050  | 0.56–0.76  |
| 3 months        | 0.63 ± 0.056   | 0.52–0.74  | 0.60 ± 0.053  | 0.49–0.71  |

SE = standard error; CIs = confidence intervals
it should not be noted that the present study was not set up to examine the effect of brushing.

The adjunctive use of chemical plaque control helps to inhibit the proliferation rate of bacteria in plaque biofilm or prevent bacterial attachment to tooth surfaces, and thus reduces the amount of biofilm. Evidence from the literature shows the short-term and long-term efficacy of using adjunctive anti-plaque chemical agents. A systematic review of clinical studies also confirmed the improvement of gingival health and the safety of alcohol-free, EO mouth rinses in comparison to other mouth rinses. The efficacy of EO mouth rinse with and without alcohol did not show a significant difference concerning the reduction of plaque and gingivitis.

However, in the present study, the additional benefits of using alcohol-free EO containing 0.05% fluoride mouth rinse as an adjunctive measure for plaque control and reducing gingival inflammation were not observed at 3 months when compared to 0.05% fluoride mouthwash. The small effects of alcohol-free EO containing 0.05% fluoride mouth rinse in reduction of plaque and gingivitis scores shown in this study may also be due to the floor effect or shorter follow-up time compared to a recent study by Lynch et al. In that study, participants had whole-mouth baseline values of MGI and PI ≥ 1.95 and were followed for 6 months, while the baseline values of MGI and PI in the present study were 1.15 and 1.50, respectively.

A previous study that compared the effects of sodium fluoride, EO, and chlorhexidine mouth rinses on plaque and gingivitis has shown that all three mouth rinses significantly reduced PI scores in comparison to negative control rinse (sodium chloride). Fluoride and CHX mouth rinse groups showed greater reductions of PI score than EO group. All tested mouth rinses showed equally effective in reducing GI scores. Our study observations are in line with the previous study. Moreover, it appeared that the higher frequency of rinsing showed the greater effect. Twice-daily rinsing of EO mouthwash have shown statistically and clinically additional benefits on plaque deposit and gingival health when used in addition to mechanical oral hygiene care and was a common recommendation to patients. In contrast, rinsing every night with a fluoridated, alcohol-free EO mouth rinse was advised to participants in the present study as this frequency has been proposed for pregnancy women to maintain oral hygiene.

While mechanical plaque removal is mandatory for prevention and control of gingivitis and periodontitis, this method alone may not be sufficient for pregnant women. The straightforward explanation is that pregnancy-related nausea and vomiting symptoms lead to difficulties in performing effective regular oral hygiene care. Compared with pregnant women who did not vomit, women who vomited during pregnancy were more likely to have a higher gingival index score and probing pocket depth. In our study, about 73% of pregnant women reported vomiting during the first trimester. Moreover, hormonal changes during pregnancy could provoke an inflammatory response to plaque biofilm, resulting in severe gingivitis.

Insufficient brushing time and limited use of interdental cleaning also play an important role for gingival health. In this study, only about 5% and 54% reported routine use of dental flossing and tongue cleaning, respectively. In addition, after receiving oral hygiene education at the initial visit, the proportion of pregnant women who reported 1-2-minutes tooth-brushing time increased from 87% at baseline to 94% at 3 months. Although there was no statistical significance between the average tooth-brushing time between the two groups, pregnant women in the intervention group were more likely to report tooth brushing times longer than two minutes (42.2% vs. 30.6%) (data not shown in the tables). Moreover, previous research reported dental service usage during pregnancy ranged from 16 to 83%. Many factors were associated with dental use during pregnancy such as demographic, socioeconomic, psychological, behavioral factors, and perceived need. A study conducted among pregnant women in rural China showed that improved periodontal health, measured by periodontal disease scores, of those who used an alcohol-free antimicrobial mouth rinse containing CPC throughout the whole pregnancy was greater than that of those who brushed teeth only. In this study, the pregnant women were required to rinse their mouths twice a day after regular tooth brushing.

A previous study had shown that EO mouth rinses were more effective in reducing interproximal plaque accumulation compared to dental flossing at 2 weeks. However, there was no difference in the reduction of interproximal gingival inflammation and bleeding. The use of an EO mouth rinse has been suggested as a complement for individuals who are unable to floss effectively. This study may have a positive bias due to the fact that the participants were third-year dental hygiene students. They tended to more effectively use dental floss than the general population.

In Thailand, oral hygiene instruction and supragingival calculus removal during prenatal periods are given to pregnant women as part of the national oral health promotion and prevention program. Fluoride mouth
This study has some limitations and a potential bias, which may reduce the difference between the alcohol-free EO with 0.05% fluoride mouth rinse and the fluoride mouth rinse. First, to assess the primary outcomes (plaque accumulation and gingivitis), we used Ramfjord index teeth, not a whole-mouth evaluation. Furthermore, the assessments were mainly based on visual examinations of the buccal or lingual sides of the index tooth. This may result in underestimation of the findings, especially the gingival health of interproximal areas. In addition, as pregnant women in this study had mild to moderate plaque accumulation and gingivitis at baseline, they may not be representative of the general population. The external validity of this study may be compromised. Finally, this clinical study was implemented as part of the routine work of dental hygienists in providing oral health services for pregnant women. All oral health behavior parameters were collected using a self-administered questionnaire. Therefore, the results may be subject to recall bias and incompleteness of the responses due to a lack of on-going feedback from the examiners.

CONCLUSION
A mouth rinse once a day with alcohol-free EO with 0.05% fluoride does not provide additional benefits to reduce plaque, gingivitis, and tongue coating during pregnancy compared to 0.05% fluoride mouthwash in pregnancy who brush teeth regularly.

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CONFLICT OF INTEREST
There are no conflicts of interest.

AUTHORS CONTRIBUTIONS
Study design, concepts, clinical studies, and data acquisition (JH, ST, SN). Data analysis and manuscript preparation (SN). Literature search, manuscript editing, and manuscript review (JH, ST, SN).

ETHICS POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT
Ethical approval was obtained from the institutional review board at the Faculty of Dentistry, Prince of Songkla University (EC 5808-23-L-HR). Written consent was obtained from all participants in the study.

DECLARATION OF PATIENT CONSENT
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/ her/their clinical information to be reported in the journal. The patient understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author (SN), upon reasonable request.

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**Supplementary Material**

Supplemental Table 1: Tests of between-subject and within-subject effects on gingival, plaque, and tongue coating scores comparing between control (N = 25) and intervention (N = 24) groups at baseline, 2 weeks, and 3 months

| Source            | df | Mean square | F      | P-value* |
|-------------------|----|-------------|--------|----------|
| **Modified gingival index** |    |             |        |          |
| Time              | 2  | 3.162       | 56.202 | <0.001   |
| Group             | 1  | 0.007       | 0.057  | 0.812    |
| Time x Group      | 2  | 0.025       | 0.450  | 0.639    |
| **Plaque index**  |    |             |        |          |
| Time              | 2  | 4.433       | 44.456 | <0.001   |
| Group             | 1  | 0.108       | 0.750  | 0.391    |
| Time x Group      | 2  | 0.161       | 1.615  | 0.204    |
| **Tongue coating index** |    |             |        |          |
| Time              | 2  | 0.220       | 3.746  | 0.027    |
| Group             | 1  | 0.031       | 0.188  | 0.667    |
| Time x Group      | 2  | 0.058       | 0.981  | 0.379    |

*Repeated measures of analysis of variance (ANOVA) compared means in plaque, gingival, and tongue coating scores between the intervention and the control groups at baseline, 2 weeks, and 3 months. Subgroup analysis was carried out to investigate if the outcomes was influenced by the examiner variability (examiners with kappa value ≥ 0.7.).