# Comparison of the Antigingivitis and Antiplaque Effectiveness of Essential Oil Mouthwash and Chlorhexidine-containing Mouthwash: A Clinical Study

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## Abstract

Gingivitis is an inflammation of gingival tissue which is very common among adulthood and can be found worldwide. Gingivitis can be prevented by good mechanical cleaning procedures. Nowadays, the use of mouthwash adjuncts to brushing and flossing are increasingly popular. Based on several clinical trials, chlorhexidine-containing and essential oil mouthwashes have been accepted by ADA to be used as additional routine to mechanically control plaque. The aims for this study were to compare the effectiveness of essential oil (Listerine®) and chlorhexidine-containing mouthwash and to assess the side effects of the mouthwashes if there was any. Forty-five qualified subjects were selected among patients from Faculty of Dental Medicine, Rangsit University, equally and randomly assigned to one of the three mouthwash groups; Essential oil (Listerine®), 0.12% Chlorhexidine(C -20) and 0.9% normal saline solution (control). A dental prophylaxis program was previously provided. Four indices were measured prior to assigned mouthwashes and after the mouthwash program, as our methodology including; staining index (SI), gingival index (GI), gingival bleeding index (GBI), and plaque index (PI). All informations were statistically analized by using paired-t test, post hoc analysis, and analysis of variance (ANOVA). According to the paired t-test result, there was a statistically significant decrease of GBI when use normal saline solution, a highly statistically significant decrease of GI and PI when use essential oil mouthwash, and also a highly statistically significant decrease of GBI, GI, and PI when use chlorhexidine mouthwash. However, according to (ANOVA) and post hoc analysis results, there were no statistically significant decreases in any of indices from the use of any mouthwashes. In conclusion, mouthwash with either chlorhexidine or essential oil in the composition has the same effect on antiplaque and antigingivitis properties ($p$-value > 0.05).

***Keywords:*** *Antigingivitis, Antiplaque, Chlorhexidine, Essential oil, Mouthwash*

**บทคัดย่อ**

โรคเหงือกอักเสบคือภาวะที่มีการอักเสบของเหงือก สามารถพบได้ทั่วโลกและพบได้บ่อยในกลุ่มประชากรที่เป็นผู้ใหญ่ โรคเหงือกอักเสบสามารถป้องกันได้โดยการแปรงฟันอย่างถูกวิธีร่วมกับการทำความสะอาดบริเวณซอกฟัน ปัจจุบัน การใช้น้ำยาบ้วนปากเสริมจากการแปรงฟันและใช้ไหมขัดฟันได้รับความนิยมเพิ่มมากขึ้นเรื่อยๆ จากการศึกษามากมาย ทำให้น้ำยาบ้วนปากที่มีส่วนผสมของ คลอร์เฮกซิดีน และน้ำยาบ้วนปากเอสเซ็นเชียล ออยล์ ได้รับการยอมรับจากสมาคมทันตแพทย์อเมริกัน หรือ American Dental Association (ADA) โครงการวิจัยนี้จึงจัดตั้งขึ้นเพื่อเปรียบเทียบประสิทธิผลระหว่าง น้ำยาบ้วนปาก เอสเซ็นเชียล ออยล์ (ลิสเตอรีน®) กับน้ำยาบ้วนปากที่มีส่วนผสมของ คลอร์เฮกซิดีนต่อการต้านทานโรคเหงือกอักเสบและคราบจุลินทรีย์บนผิวฟัน รวมไปถึงสังเกตผลข้างเคียงที่อาจเกิดขึ้น ดำเนินการวิจัยโดยการเก็บข้อมูลจากกลุ่มตัวอย่างผู้เข้าร่วมโครงการวิจัย ซึ่งเป็นผู้ป่วยในคณะทันตแพทยศาสตร์ มหาวิทยาลัยรังสิต รวมทั้งสิ้น 45 คน ทั้งนี้ กลุ่มตัวอย่างถูกจำแนกออกเป็น 3 กลุ่ม กลุ่มละ 15 คนด้วยการสุ่ม แต่ละกลุ่มจะได้รับการรักษาทางปริทันต์ก่อนใช้น้ำยาบ้วนปากที่ต่างชนิดกันได้แก่ 0.12% คลอร์เฮกซิดีน (C -20), เอสเซ็นเชียล ออยล์ (ลิสเตอรีน®) และ 0.9% น้ำเกลือ การเก็บข้อมูลในโครงการวิจัยนี้จะใช้ดัชนีทั้งสิ้น 4 ค่า ได้แก่ staining index (SI), gingival index (GI), gingival bleeding index (GBI) และ plaque index (PI) จากนั้นจะนำเอาข้อมูลมาวิเคราะห์ทางสถิติด้วย paired-t test, analysis of variance (ANOVA) และ post hoc analysis ผลการวิจัยพบมีการลดลงอย่างมีนัยสำคัญทางสถิติของ GBI ในกลุ่มน้ำเกลือ 0.9% พบการลดลงอย่างมีนัยสำคัญทางสถิติของ GI and PI ในกลุ่ม เอสเซ็นเชียล ออยล์ และพบการลดลงอย่างมีนัยสำคัญทางสถิติของ GBI, GI และ PI ในกุ่ม คลอร์เฮกซิดีน เมื่อวิเคราะห์ข้อมูลโดย paired-t test อย่างไรก็ตาม เมื่อวิเคราะห์ข้อมูลด้วย ANOVA และ post hoc analysis พบว่าน้ำยาบ้วนปากทั้ง 3 ชนิด ไม่ได้ให้ความแตกต่างอย่างมีนัยสำคัญทางสถิติในค่าดัชนีทุกค่าที่ลดลง สรุปว่าน้ำยาบ้วนปากที่มีส่วนผสมของ คลอร์เฮกซิดีน และ เอสเซ็นเชียล ออยล์ (ลิสเตอรีน®) มีประสิทธิผล ต่อการต้านทานโรคเหงือกอักเสบและคราบจุลินทรีย์บนผิวฟันไม่แตกต่างกัน ($p$-value > 0.05)

***คำสำคัญ:*** *การต้านทานโรคเหงือกอักเสบ การต้านทานคราบจุลินทรีย์บนผิวฟัน คลอร์เฮกซิดีน เอสเซ็นเชียล ออยล์ น้ำยาบ้วนปาก*

## 1. Introduction

Gingivitis is characterized by inflammation and bleeding of the gingiva. The main cause of gingivitis is dental plaque that forms on the tooth surface. Studies in the recent years clearly demonstrated a temporal relationship between the accumulation of plaque and the development of gingivitis, thereby emphasizing the importance of plaque control in a preventive regimen for periodontal diseases as well as for dental caries. It is considered important to motivate patients to correct oral hygiene measures and compensate the hard-to-reach areas as well as inadequate skill, poor motivation and lack of compliance. While it is theoretically possible to maintain a sufficient level of oral hygiene to control gingivitis by mechanical method alone, the majority of people fail to maintain an adequate level of plaque control. Therefore the use of an antimicrobial mouthwash would be meaningful and cost-effective method in addition to mechanical oral hygiene methods (Barnett, 2008). Chemical agents in a mouthwash should be effective at modifying the microbiota by selectively eliminating pathogens without negatively impacting the normal flora that may result in an overgrowth of pathogenic organisms (Osso and Kanani, 2013). A previous study showed that mouthwashes could be recommended for the patients with gingivitis as an adjunct to usual home care routine (Mythri et al., 2011). Studies have demonstrated that these mouthwashes were equally effective in reducing gingival index score and the number of bleeding sites (Marchetti et al., 2011). The safety, effectiveness in delivery of antimicrobials and the antiplaque properties of different mouthwashes have been the subjects of many previous studies (Osso and Kanani, 2013).

Mouthwash is an antiseptic and antiplaque product to be used to enhance oral hygiene. Active ingredients in mouthwash sold commercially include thymol, eucalyptol, hexitidine, methyl salicylate, menthol, chlorhexidinegluconate, benzalkonium chloride, cetylpyridinium, chloride, methylparaben, hydrogen peroxide, and domiphen bromide (Masadeh, 2013).

Among the chemotherapeutic agents used in mouthwash, chlorhexidine is the “gold-standard” due to its proven efficiency (Van Maanen-Schakel et al., 2012). As a result of previous clinical studies, 0.2 % chlorhexidine mouthwash has become the international standard. Chlorhexidine gluconate tightly binds to tooth structure, oral tissues, and dental plaques then release slowly, which resolves in 8 to 12 hours substantively. The mechanism of its action is breaking the bacterial cell membrane, which will result in cell death and will inhibit plaque colonization and pellicle formation. Though effective, chlorhexidine has certain side effects such as brown discoloration of teeth, oral mucosal erosion, and bitter taste. The most common side effect is extrinsic tooth staining, brown discoloration of the tongue, on plastic and composite restorations, and on artificial teeth (Goutham et al., 2013).

Listerine®, on the other hand, was introduced as a mouth rinse in the mid 1970s for prevention of plaque and gingivitis. Listerine® mouthrinse is composed of a highly specific mixture of essential oils that include thymol, eucalyptol, menthol, and methyl salicylate (Fine, 2010; Agarwal and Nagesh, 2011). Several clinical studies showed comparable results when chlorhexidine and Listerine® were compared with each other. The mechanism of action for this mouthwash is by breaking the bacterial cell membrane, which results in cell death and preventing bacterial aggregation and recolonization (Joanna Asadoorian, 2006).

Both chlorhexidine and essential oil demonstrated reductions in supragingival plaque and gingivitis (Depaola and Spolarich, 2007).

Also, essential oil containing mouthwashes may be good alternatives to chlorhexidine in situations, where dental professionals feel that its long-term use would result in abuse or various complications (Neely, 2012).

## 2. Objective

The aim for this study was to compare the effectiveness of essential oil (Listerine®) and chlorhexidine-containing mouthwash and to assess the side effects of the mouthwashes if there was any.

## 3. Materials and Methodology

This clinical study was performed at the Faculty of Dental Medicine, Rangsit University with the human ethic number RSEC 14/2557 approved by Ethical Committe of Research Institute of Rangsit University. A total of 45 subjects who had gingivitis, was within the age range of 18 to 70 years old and had a minimum of 20 teeth in their oral cavities were recruited. The participants were the patients who admitted to the Faculty of Dental Medicine, Rangsit University. Subjects were equally and randomly assigned into one of the three groups with different mouthwashes given to each group. Mouthwashes used in our study were essential oil mouthwash (Listerine® cool mint: thymol-0.064%, eucalyptol-0.092%, menthol-0.042%,

methylsalicylate-0.060%), 0.12% chlorhexidine-containing mouthwash (C-20 Blue Sally: chlorhexidinegluconate-0.12%), and 0.9% normal saline solution (as a negative control).

Plaque index, gingival index, staining index, and gingival bleeding index were initially measured to be used as baseline values. These indices were measured on the periodontal rescale, recheck, or recall visits performed with 14 days intervals. Exclusion criteria consisted of patients with systemic diseases, pregnant women, destructive periodontal diseases (bone loss), allergy to any type of the mouthwashes, and those who are currently taking antibiotics or anti-inflammatory drugs. A fully crowned tooth, a tooth with class ll restoration or orthodontically bonded teeth were not included in our data.

3.1 Study population

A total of 45 subjects participated in the study (3 groups, 15 each). Subjects have randomly received one mouthwash, which was either essential oil (Listerine®) mouthwash, chlorhexidine-containing mouthwash, or 0.9% normal saline solution.

3.2 Data collection

Each index was measured by one observer in order to obtain intraexaminer calibration. Standardization of each index was performed by one investigator.

Indices

Four indices used for assessing plaque and gingivitis were:

3.2.1. Plaque Index (Quigley Hein Index-Modified by Turesky et al., 1970; Panagakos et al., 2005)

An index measuring dental plaque that occurs in the areas adjacent to the gingival margin.

3.2.2. Gingival Index (Löe and Silness, 1963; Rebelo and Queiroz, 2011)

An assessment of a gingival condition by measuring qualitative changes of the gingiva.

3.2.3. Bleeding on Probing Index (Ainamo and Bay, 1975; Rebelo and Queiroz, 2011)

To assess gingival inflammation by bleeding from gingival sulcus.

3.2.4. Staining Index (Lobene, 1968; Macpherson et al., 2000)

An evaluation of the extent and intensity of staining of the tooth.

The study was conducted in 2 phases: pre-treatment phase and experimental phase.

Pre-treatment phase: each subject received a complete prophylaxis, including scaling and professional tooth cleaning to minimize the existing gingivitis prior to using the mouthwash. After complete prophylaxis, subjects were called on periodontal rescale, recheck, or recall. At this point, subjects were informed about the study and signed consent forms were obtained, together with a record of staining index, gingival index, gingival bleeding index, and plaque index respectively. Besides, the subjects were given oral hygiene instructions on brushing, flossing, and mouthwash accordingly. Each subject was given a toothbrush and a tube of toothpaste (Colgate®).

Experimental phase: subjects rinsed with their respective mouthwash; 10 ml for 30 seconds each time, twice daily for fourteen days. After this period, each subject was called for the appointment. The measurement of staining index, gingival index, gingival bleeding index, and plaque index were recorded again. All patients were screened at each visit for oral mucositis and other oral complications. A brief history taking was conducted with attention to any symptoms reported by the patient such as sore mouth, dry mouth, strange sensations, or difficulty eating. All extra information received by the subject was additionally documented.

3.3 Data Analysis

The Kolmogonov-Smirnov test was used to distinguish the normal distribution of the data. Since our data showed a normal distribution, the one-way analysis of variance (ANOVA) was used to analyze the differences in plaque index, gingival index, bleeding on probing index, and staining index among the groups. post hoc analysis was used to analyze the relation between each mouthwash within a similar index. *p*-value of 0.05 or less would be considered as a statistically significant and *p*-value of 0.01 or less was considered as highly statistically significant. *p*-value > 0.05 indicated statistically non-significant difference.

**4. Results**

The means of pre-test and post-test results of participants who were given normal saline solutions did not change significantly, except the gingival bleeding index. Also, the mean values of staining index both pre-test and post-test did not change in any of the 3 groups. The patients in the essential oil group had significant changes in plaque index and gingival index scores after a period of mouthwash use. The participants, who received chlorhexidine mouthwash, had significant changes in the means values of pre-test and post-test of the plaque index, gingival index, and gingival bleeding index scores. None of the subjects had any allergic reaction, discomfort, or signs and symptoms of abnormalities, with an exception of a complaint of unpleasant taste among participants in the chlorhexidine group and salty taste among those who rinsed with normal saline solution.

The paired t-test results are also shown in Table 1. The only significant improvement in the normal saline solution was in the measurements of gingival bleeding index. The essential oil group showed highly statistically significant changes in both plaque index and gingival index scores. The chlorhexidine group showed highly statistically significant changes in plaque index, gingival index, and gingival bleeding index scores. There was no significant change in the staining index scores in any of the groups.

The ANOVA (Table 2) revealed a non-significant variation among normal saline solution, essential oil, and chlorhexidine interventions with *p*-values > 0.05. The post hoc test (Table 3) was intended to analyze the relation between each mouthwash to a similar index. Therefore, *p*-value ≤ 0.05 would consider as statistical significant, however, it has shown that none of these pairing was statistically significant different since the *p*-values were all > 0.05 in our study.

**Table 1** Comparison of the means and standard deviations (S.D.) between pre-test and post-test procedures and paired t-test results in three intervention groups: * Statistical significant at *p*-value of 0.05 ** Highly statistical significant at *p*-value of 0.01 "a" repeating decimal

| Groups | Pre-test | | Post-test | | Index reduction | Paired t-test |
|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | *P*-value |
| Normal Saline Solution | | | | | | |
| Plaque index | 1.158 | 0.429 | 1.163 | 0.373 | -0.005 | 0.904 |
| Gingival index | 1.002 | 0.289 | 0.962 | 0.251 | 0.040 | 0.589 |
| Gingival bleeding index | 0.275 | 0.290 | 0.188 | 0.326 | 0.087* | 0.021 |
| Staining index | .0667[a] | 0.161 | .0667[a] | 0.161 | - | - |
| Essential Oil | | | | | | |
| Plaque index | 1.4785 | 0.250 | 1.224 | 0.192 | 0.254** | 0.000 |
| Gingival index | 1.0142 | 0.324 | 0.797 | 0.314 | 0.217** | 0.000 |
| Gingival bleeding index | 0.1758 | 0.108 | 0.1699 | 0.292 | 0.059 | 0.912 |
| Staining index | .0333[a] | 0.292 | .0333[a] | 0.292 | - | - |
| Chlorhexidine | | | | | | |
| Plaque index | 1.353 | 0.459 | 0.869 | 0.386 | 0.484** | 0.000 |
| Gingival index | 1.109 | 0.345 | 0.806 | 0.247 | 0.303** | 0.003 |
| Gingival bleeding index | 0.264 | 0.257 | 0.197 | 0.250 | 0.067** | 0.003 |
| Staining index | .0278[a] | 0.075 | .0278[a] | 0.075 | - | - |

**Table 2** ANOVA test; Comparison of the means of difference in plaque index, gingival index, and gingival bleeding index among all mouthwashes

| ANOVA | Sum of Squares | df | Mean Square | Sig. |
|---|---|---|---|---|
| Plaque Index | 0.040 | 2 | 0.020 | 0.578 |
| Gingival Index | 0.012 | 2 | 0.006 | 0.697 |
| Gingival Bleeding Index | 0.000 | 2 | 0.000 | 0.961 |

**Table 3** Post Hoc Analysis; Comparison of the means of difference in plaque index, gingival index, and gingival bleeding index between each pairs of the listed three mouthwashes.

Multiple Comparisons

LSD

| Dependent Variable | (I) TREATMENT | (J) TREATMENT | Mean Difference (I-J) | Sig. |
|---|---|---|---|---|
| Plaque Index | Normal saline solution | Essential oil | -.086333 | .583 |
| | Essential oil | Chlorhexidine | -.076667 | .625 |
| | Chlorhexidine | Normal saline solution | .163000 | .315 |
| Gingival Index | Normal saline solution | Essential oil | -.059000 | .584 |
| | Essential oil | Chlorhexidine | -.028667 | .788 |
| | Chlorhexidine | Normal saline solution | .087667 | .423 |
| Gingival Bleeding Index | Normal saline solution | Essential oil | .009333 | .792 |
| | Essential oil | Chlorhexidine | -.002667 | .940 |
| | Chlorhexidine | Normal saline solution | -.006667 | .851 |

**5. Discussion**

The results of our study showed that there was a statistical significant decrease in gingival bleeding index in normal saline solution group. It means that there was a reduction in bleeding on probing quantitatively among those given normal saline solution. There was a highly significant decrease in both plaque index and gingival index in the Listerine® group. This could refer to a certain reduction in both amount of plaque deposition and a severity of gingival inflammation presenting in participants of essential oil group. There was a highly statistically significant decrease in plaque index, gingival index, and gingival bleeding index scores in the chlorhexidine group. Therefore, it may suggest that there was an assured reduction in the plaque deposition amount, severity of gingival inflammation, and the number of sites of bleeding on probing in patients who used chlorhexidine as a mouthwash.These results indicate that the chlorhexidine mouthwash has the highest potential in reducing plaque index, gingival index, and gingival bleeding index compared to essential oil mouthwash and normal saline solution.

An interesting finding of our study was that there was significant decrease in the gingival bleeding index in normal saline solution group, which in fact is a control or a placebo group. The main possible causes this result can be uncontrollable factors or the limitations of our study, which included individual mechanical cleansing capability, oral hygiene, attitudes, and compliances. Even though normal saline solution has an antiseptic property, a total reduction of bleeding on probing sites is not anticipated. Other than the possible causes mentioned previously, it could be also due to better oral hygiene skills among the participants receiving normal saline solutions. Although we applied simple randomization technique to allocate the participants into particular groups, there was an uneven age and sex distribution among the groups. This might have caused the imbalances in oral hygiene measures among the groups.

The only parameter that had no significant correlations with any of the given mouthwashes was staining index. The major reason of using staining index as one of the parameters of our study was to evaluate the possible side effects of the mouthwashes as well. Specifically, chlorhexidine is anticipated to cause an alteration in color of a tooth, which is tooth discoloration. As it has been reported that chlorhexidine causes tooth discoloration, a carefully short-term use of low concentrated chlorhexidine mouthwash with avoidance of any potentially staining food is recommended to minimize the staining risk. In the present study a low concentration chlorhexidine mouthwash, for only short-term and with fully awareness of any possible side effects was applied. As a result, none of the participants in any of the groups was found to have a tooth discoloration.

Limitation

As previously discussed, an important limitation of our study was the small sample size. Since the study protocol required that the subjects must be selected from the patients admitted to the Faculty of Dental Medicine, Rangsit University, the number of qualified subjects was limited. A larger sample size with more diversity would have increased the reliability of the study.

Another limitation of this study was the reliance on the patient's compliance. According to our study design, the participants applied the products at home by themselves. In this case the compliance of each participant was very important. The oral hygiene skills of each subject was generally different, which was likely to cause an imprecision result. Moreover a data collection date of a post-mouthwash-used could not be done as planned.

One other limitation was the randomization technique used in our study. It was a simple randomization technique, which maintained a complete randomness of assigning any subject to a particular group or in other words, the selection was purely made by chance. This randomization approach was simple, easy, and can be reliable when a large sample size was obtained. However when a smaller sample size used, a simple randomization technique could be problematic. These limitations can be considered as uncontrollable factors, which may affect the results of the study.

## 6. Conclusion

There was no statistically significant difference in antiplaque and antigingivitis effects between the use of chlorhexidine-containing and essential oil mouthwash. Both mouthwashes can be regarded as having acceptable antiplaque and antigingivitis properties when used as adjuncts to routine mechanical plaque control procedures.

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