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Efficacy of Essential Oil Strips Containing Thymol, Eucalyptol, Menthol, Methyl Salicylate, and Peppermint Against Dental Caries

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Abstract

Background: Strips containing essential oils are commonly used as an alternative treatment for dental caries prevention. However, their efficacy is unknown. This study aimed to test the efficacy of oral strips containing essential oils on caries prevention. **Methods**: This was a randomized, double-blind, placebo-controlled crossover study with 15 subjects divided into two groups: A and B. In the first week, group A (N = 8) was given strips containing thymol, eucalyptol, menthol, methyl salicylate, and peppermint (TEMMP) three times in 24 hours while group B (N = 7) was given placebo strips. In the second week, after a one-week washout period, each group was given the other product. Pre- and post-treatment evaluation was performed by measuring plaque acidity using a pH plaque indicator and determining the number of (*Streptococcus mutans)* colonies using a hand counter. **Results**: There was no significant difference between plaque pH and *S.mutans* count in subjects given TEMMP strips compared to placebo. **Conclusions**: The use of TEMMP essential oil strips does not significantly inhibit the growth of *S.mutans* or the production of plaque.

Keywords: dental caries, efficacy, plaque, Streptococcus mutans

Introduction

Dental caries is one of the most common oral health problems in Indonesia. According to Indonesian Basic Health Research (Riskesdas) 2013, the prevalence of dental caries in Indonesia is 72.3%, which is quite high.[1] One factor involved in dental caries is plaque accumulation, which is caused by bacteria like (*S. mutans)*.[2,3] These bacteria accumulate into oral biofilms and have the ability to metabolize food residues containing sugar into acid.[4] An acidic environment causes tooth demineralization and, if it is not addressed, it can develop into tooth decay.

Efforts to solve oral health problems include brushing teeth, using dental floss, and cleaning the tongue. Antibacterial mouthwash can also be used. However, mouthwash may have side effects like tooth staining.[5] Nowadays, essential oil strips can be easily found and are believed to kill bacteria. Active ingredients in essential oil strips believed to have antiseptic effects include thymol, eucalyptol, menthol, methyl salicylate, and peppermint (TEMMP).[6,7] However, the efficacy of essential oil strips remains unknown. Research on the efficacy of essential oil strips against tooth caries by measuring plaque pH and counting *S. mutans* colonies is thus needed. This study aimed to address this research gap.

Methods

Research was performed in November 2012 at the Oral Biology Laboratory, Universitas Indonesia. This was a randomized, double-blind, placebo-controlled crossover study of 15 (8 males and 7 female) participants. Participants were Faculty of Dentistry students who met the inclusion criteria and provided informed consent. Participants were asked not to eat for one hour before participation. Approval for the study was obtained from the Dental Research Ethics Committee, Faculty of Dentistry Universitas Indonesia.

Materials used in this research included labels, tissue, petri dish, alcohol lamp, lighter, autoclave, incubator, mask, gloves, plastics, microscope, refrigerator, Eppendorf tubes and rack, pipette, pipette tips, a hand counter, marker, trypticase yeast with sucrose, bacitracin agar media, gas pack, alcohol, 0.9% NaCl, and a plaque pH indicator.

Plaque pH was measured twice per participant, namely before intervention and 24 hours after intervention. The interproximal sides of teeth 35 and 36 and the buccal side of tooth 37 were swabbed using sterilized cotton buds. The swabs from teeth 35 and 36 swabs were immersed in sucrose solution and the color change was assessed after five minutes. Color changes were compared

using the GC plaque pH indicator (GC, Tokyo, Japan) to determine plaque pH. To quantify *S. mutans* colonies, the swab from tooth 37 was first inserted into a sterilized Eppendorf tube containing 0.9% NaCL and homogenized. The specimen was then plated on trypticase soy with sucrose and bacitracin media, placed inside an anaerobic jar along with anaerobic agents, and incubated for 2×24 hours. After 2×24 hours, *S. mutans* colonies were counted using colony counter.

The normality of the dependent variable data, namely *S. mutans* colonies and plaque pH, was assessed by the Shapiro–Wilk test because there were less than 50 samples. The data before intervention had a non-normal distribution, while the data after intervention showed a normal distribution. Thus, the Wilcoxon test was used to evaluate the difference between the paired measures. Statistical significance was set at $p < 0.05$.

Results

As shown in Table 1, the *S. mutans* colonies count in the test group was 6265 CFU/ml before and after intervention it was 7952 CFU/ml, which represents a 26.92% increase. The *S. mutans* colonies count in the control group was 7356 CFU/ml before and 13990 CFU/ml after, which represents an increase of 83.23%.

Before treatment, the test and control groups did not differ significantly in their *S. mutans* colonies count (Mann Whitney test, $p = 0.206$). This is important, as it can be used as a base for the test of the effect of treatment. In the test group, there was not a significant change in *S. mutans* colonies count before and after treatment. However, in the control group, *S.mutans* colonies count increased significantly after placebo ($p < 0.05$). Table 2 shows that plaque pH before treatment in the test group was 6.08 and after treatment it was 5.87, which is a 0.03 decrease. In the control group, the pH was 6.58 before treatment and 6 after treatment 0.09 decrease.

Although there was no significant difference in plaque pH before treatment between the test and control groups (Mann Whitney test, $p = 0.097$), this can be used as a difference in plaque pH before treatment between the test and control groups. There was no significant change in plaque pH before and after intervention in the control or treatment groups ($p > 0.05$).

Table 1. *Streptococcus mutans* Colonies Count in the Test and Control Groups

Group	Mean (SD) Before treatment	Mean (SD) After Treatment
Test	6265 (7050.10)	7952 (6487.38)
Control	7356 (6064.73)	13990 (8944.30)

Table 2. Mean Plaque pH in the Test and Control Groups

Group	Mean (SD) Before treatment	Mean (SD) After Treatment
Test	6.08 (0.86)	5.87 (0.58)
Control	6.59 (0.77)	6 (0.70)

Discussion

To the best of our knowledge, this is the first study on the effects of essential oil strips on tooth caries risk assessed by plaque pH and *S. mutans* count. Previous study showed that use of an essential oil mouthwash reduced plaque acidogeniticy after a sucrose challenge.[8] More mature plaques is expected to have a more acidic (i.e., lower) pH because there are *S. mutans* bacteria inside acidogenic plaque. Acidogenic bacteria ferment glucan and cause pH to decrease, becoming more acid.[9] By contrast, our results showed a non-significant decrease in plaque pH with the use of essential oil strips. However, these results may not be comparable because different methods were used.

Similarly, we found no significant change in *S. mutans* colonies count after using the essential oil strips. This may be due to different amounts of essential oils in strips and mouthwash. However, the non-significant increase showed that essential oil strips have a tendency to inhibit *S. mutans* growth. The increase in *S. mutans* colonies count may have been caused by the lack of mechanical effort to clean plaque (i.e., participants were instructed not to brush their teeth), hence *S. mutans* colonies keep increasing even though the essential oil strips had an antimicrobial effect.

During sleep, saliva production decrease and creates a favorable environment for bacteria multiplication (especially *S. mutans* which has hydrophilic characteristic). Saliva plays a great role in managing the intraoral balance. Saliva has protection effects (salivary clearance of on-adherent bacteria, debris, therefore so acid plaque microorganism growth can be inhibited) and the ability to inhibit demineralization caused by acid produced by bacteria metabolism.[10]

One limitation of this study is that we did not control for food intake, particularly carbohydrates, consumed by subjects. In addition, 24 hours may not be enough time to record changes in plaque pH.

Conclusions

Essential oils strip containing TEMMP do not significantly inhibit *S. mutans* colony growth or plaque formation.

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