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Clinical Efficacy of a New Mouthwash Containing Essential Oil of Cardamom in Reducing Volatile Sulphur Coumpounds Concentration

Suci Erawati[1], Anton Rahardjo[2], Sondang Pintauli[3]

[1]*Doctoral Student, Graduate School of Faculty of Dentistry, University of Sumatera Utara, Medan,*
[2]*Department of Preventive Dentistry, Faculty of Dentistry, University of Indonesia, Jakarta,*
[3]*Department of Preventive Dentistry, Faculty of Dentistry, University of Sumatera Utara, Medan, Indonesia*

Objective: The aim of this study was to assess the clinical efficacy of new mouthwash based on kapulaga fruit (*Amomum cardamommum L.*) containing 0.5% essential oil and compared to 0.5% Listerine® as a positive control and placebo in reducing volatile sulphur compounds (VSCs) concentration of halitosis subjects.

Methods: A randomized, double blinded, cross-over design was conducted among 20 healthy volunteers in Medan, Indonesia. Subjects were divided into 3 groups (Cardamom, Listerine®, placebo group) and instructed to rinse 10 ml of experiment and control mouthwash twice per day for 5 days. After one week washout periods, each group used the opposite mouthwash. The concentration of VSCs, hydrogen sulfide, methyl mercaptan, and dimethyl sulfide were assessed twice daily for 5 days (morning at 5 a.m. and afternoon at 11 a.m.) by using oral chroma and organoleptic measurement. The three mouthwash were analyzed statistically by repeated measures ANOVA.

Results: Either Cardamom or Listerine® was effective in reducing VSCs for up to 4 to 5 hours after mouth rinsing, while placebo not. An analysis showed significant differences of cardamom essential oil in mouthwash and positive control group ($p<0.05$) from 1st and 5th day while no significant difference in placebo group ($p>0.05$). Results showed that Cardamom more effective than the other two mouthwashes. Unlike other mouthwash in general, the advantage of cardamom essential oil in mouthwash is alcohol-free, that could avoid from oral pain and sensitivity.

Conclusion: It can be concluded that 0.5% cardamom containing essential oil in mouthwash is useful to overcome halitosis.

Keywords: essential oils, volatile oils, halitosis

Corresponding author **Anton Rahardjo**
Department of Preventive Dentistry, Faculty of Dentistry, University of Indonesia, Jalan Salemba Raya No. 4, Jakarta Pusat. 10430, Indonesia. Tel: +62-816774416, Fax: +62-213191412, E-mail: antonrahardjo@gmail.com

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Introduction

Today, the problem of bad breath or halitosis has gained attention among health professionals', especially dental health as well as the common people [1,2]. Bad breath can be experienced by everyone, and can arise without realizing it. If this situation is not treated immediately, it could reduce communication and cause low self-esteem, sense of embarrassment, social inter-

action difficulties as well as loss of self-confidence. Those who are not aware of bad breath would disturb people around them, therefore, it can have an extensive impact on their job and personal life [1-4]. Halitosis is the third cause that makes someone visit the dentist after dental disease and dental aesthetic problems. This suggest that disruption caused by bad breath disturbs personal life very much that should be concerned by dental professional [3,4].

Tonzetich from British Colombia University in Canada was the first person who successfully found that the compound of volatile sulphur compounds (VSC) as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide $(CH_3)_2S$ contributed to the onset of halitosis [1]. The amount of the three VSCs is quite a lot and evaporates very easily that it causes odor [1,5,6].

To date, information about halitosis is not so much because of epidemiological studies is still very limited. In Japan, a study was conducted on 2.672 subjects by measuring the concentration of VSCs. The result of this study showed that 6% to 23% of the subject suffered from halitosis with an average of 75 ppb (parts per billion) VSCs going out of the subject's mouth for one day. Of the surveys measuring the level of VSC in Indonesia, it was found that VSC of the subject for one day reached 105 ppb [7].

Some descriptive epidemiological research on halitosis reported that prevalence of halitosis was found in 31% of 270 American adults. It was also reported that in France, 22% of 4,815 subjects suffered from halitosis. Conducted a descriptive epidemiological study on halitosis in Bern, Switzerland on 419 adults showed the prevalence of halitosis obtained by measuring organoleptic with score of >3 was 11.5% and the prevalence of VSC with the level of >75 ppb was 28% [3,8]. In China, 27% of 2,000 people aged 15 to 64 years suffered from severe halitosis [4].

There are many ways to overcome halitosis, one of the methods is by using mouthwash. Many mouthwashes or breath freshener products are freely sold in the market. Most of these products try to control halitosis by reducing the existing odors. There is mouthwash with delicious and fragrant aroma; however, it only lasts for a short time. After the aroma of breath fresher is gone, the odor becomes increasing [8-10].

Mouthwash containing essential oils, triclosan, cetylpyridinium chloride, chlorhexidine, or chlorine dioxide, have been clearly demonstrated effective in reducing malodour. Shinada et al. [11] demonstrated that chlorine dioxide (ClO_2) mouthwash was effective in reducing morning malodour of 15 healthy male subjects for up to 4 hours after mouth rinsing. However, many mouthwashes also containing alcohol as a preservative and antiseptic agent. From the literature, it was stated that the addition of alcohol is considered makes mouth dryness and sensitivity [12].

Recently, an alternative herbal mouthwash was developed to overcome oral malodor with a little side effect. The mouthwash originated from fruit of kapulaga, which is usually used as cooking spice to scent the food. Kapulaga (Cardamom) also known as *Amomum cardamomum* Solad ex Maton, or *Elletaria cardamom* Maton belongs to Zingiberaceae family, is rich in chemical content including '*terpinol*', '*alfaborneol*', '*betakamper*', protein, sugars, fats, and silicates. Cardamom has a slightly bitter taste and warm; however, the savory aroma of kapulaga has made the Englishmen take it as '*grains of paradise*'. This savory aroma comes from the essential oil content which contains five main substances. namely 'borneol' that smells like camphor, 'alphaterpinil asetat' with the fragrance of orange pettigrain, 'limonere' with the fragrance of tangerines, 'alphaterpinen' with the fragrance of lemon and 'cineol' which gives warmth [13,14].

Kaushik et al. [13] conducted a study to determine whether various crude extracts of cardamom has inhibitory activity on some pathogenic bacteria isolated from different clinical samples. The Kaushik study was depicted that the oil, leaves and fruit extract of *Elettaria cardamomum* Maton can be used as potential source of novel antimicrobial agents used for different types of bacteria in different infections. Medical insurance claims data showed hopeful results as some of the extracts exhibited significant inhibitions of bacteria even at concentrations as low as 512 μg/ml [15].

This study aimed to clinically evaluate the effect of mouthwash of kapulaga fruit (*Amomum Cardamommum L.*) containing essential oil in decreasing the concentration of main malodor-causing substances, H_2S, CH_3S, $(CH_3)_2S$ and VSCs in halitosis subjects using oral chroma and organoleptic measurements compared to Listerine® (PT Johnson & Johnson, Jakarta, Indonesia) and placebo.

Materials and Methods

Cardamom is a type of plants which were collected from one of the garden village in Sumatera Utara, ±2 hours from Medan, Indonesia Taxonomic identification of plants was performed by botanists of Central Botanies in Herbarium Bogoriense. Bogor as *A. cardamommum L.* The standardized cardamom essential oil in mouthwash was produced at the Traditional Medicine Laboratories, Pharmacy Faculty of University of Sumatera Utara in Medan, Indonesia. In pre-clinical trial, 0.25% cardamom essential oil in mouthwash was obtained as minimum inhibitory concentration and 0.5% as minimum bactericidal concentration against *P. gingivalis* ATCC 33277, a bacteria which

is dominant to cause halitosis.

Subjects consisted of 20 volunteers, 10 males and 10 females, aged 18 to 21 years (mean age 19.7±0.91 years), recruited from Raudhatul Hasanah, one of religion-based school in Medan. They had general good health, no pulp inflammation, no tongue coating. not smoking and drinking alcohol and no certain drugs usage in the last 4 months such as antibiotic, anti-parkinson and anti-hypertension. Female subjects in menstrual period as well as subjects that used any type of antibacterial mouth wash were excluded from the study. An informed consent was provided for subjects who participated in study protocol approved by the Ethical Committee of Medical Faculty University of Sumatera Utara.

The present study was a randomized, double-blind cross over design (neither the subjects nor the clinicians were aware of the rinsing solution used) with three days of period wash out. In first day (base line) at 5:00 a.m. in the morning, all subject divided in three parallel groups (experimental, positive control and placebo group) were assessed for the first halitosis measurement using Oral Chroma™ (Abimedical, Abilit Corp., Osaka, Japan). Following breath analysis, all subjects were instructed to rinse for approximately 1 minute with 10 ml of rinsing solution twice daily (once after breakfast and once before sleeping) during a period of 5 days clinical trial and to refrain from eating or drinking for 1 hour afterwards. The experimental group received 0.5% Cardamom mouthwash (alcohol-free) and the positive control group received the same regimen, Listerine® (an alcohol-based antiseptic mouth rinse which also containing essential oils). The second halitosis measurement was taken every noon at 11:00 a.m.

The organoleptic measurement was measured by two trained judges. Subjects were instructed to close their mouth for 1 minute and breath slowly. The measurement was taken from distances of 10 cm from the oral cavity. Five grades or 0 to 5 scale (Rosenberg scale) were assigned as follows: 0: no oral malodor; 1: barely noticeable odor; 2: slight but clearly noticeable odor; 3: moderate oral malodor; 4: strong oral malodor; 5: exteremely strong oral malodor.

The following parameters were assessed: the mean concentration of VSC, H_2S, CH_3SH, and $(CH_3)_2S$ by a blinded and calibrated examiner. Statistical analyses were performed using ANOVA repeated measurement to test for significant differences within the three groups. Differences with p-value of <0.05 were considered significant.

Results

All selected subjects completed the study. Table 1, 2 show the mean concentration and standard deviation of H_2S. CH_3SH. $(CH_3)_2S$, and VSC (μg/10 ml) over the five days clinical trial period which obtained from gas chromatography (GC) measurement. GC is one of the objective methods to be considered by panelist as the method of choice for differentiating and quantifying VSC.

Table 1. Changes in mean concentration of VSC of Cardamom based-essential oil mouthwash, Listerine® and placebo group in the morning time over 5 days clinical trial

	1st day	2nd day	3rd day	4th day	5th day	Statistical analysis	
H_2S							
Cardamom	1.046±0.638	0.980±0.773	0.831±0.662	0.619±0.545	0.499±0.401	p=0.002	F=3.714 p=0.030
Listerine®	1.312±1.212	0.976±0.964	0.801±0.876	0.637±0.690	0.493±0.577	p=0.000	
Placebo	1.057±0.947	1.059±0.927	0.987±0.894	0.992±0.899	0.916±0.649	p=0.842	
CH_3SH							
Cardamom	1.312±1.212	0.976±0.964	0.801±0.876	0.637±0.690	0.493±0.577	p=0.000	F=10.699 p=0.0001
Listerine®	1.430±1.387	1.225±1.197	1.039±1.070	0.795±0.789	0.693±0.797	p=0.018	
Placebo	0.813±0.777	0.957±0.879	0.960±0.818	0.917±0.757	0.745±0.533	p=0.822	
$(CH_3)2S$							
Cardamom	0.755±0.837	0.473±0.492	0.443±0.597	0.301±0.333	0.248±0.278	p=0.023	F=9.849 p=0.0001
Listerine®	0.745±0.772	0.621±0.540	0.539±0.494	0.429±0.457	0.323±0.347	p=0.001	
Placebo	0.413±0.297	0.398±0.295	0.394±0.331	0.358±0.258	0.550±0.683	p=0.919	
VSC							
Cardamom	3.363±1.773	2.691±1.156	2.332±1.128	1.763±0.842	1.380±0.707	p=0.000	F=21.771 p=0.0001
Listerine®	3.488±2.002	2.823±1.610	2.380±1.464	1.859±1.138	1.502±1.079	p=0.000	
Placebo	2.284±1.136	2.468±1.399	2.319±1.235	2.267±1.260	2.213±1.253	p=0.676	

Values are presented as $\bar{x}$ ± standard deviation (10 μg/ml). VSC: volatile sulphur compounds, H_2S: hydrogen sulfide, CH_3SH: methyl mercaptan, $(CH_3)2S$: dimethyl sulfide.

Table 2. Changes in mean concentration of VSC compound after taking of Cardamom based-essential oil mouthwash, Listerine® and placebo group in the noon time over 5 days clinical trial

	1st day	2nd day	3rd day	4th day	5th day	Statistical analysis	
H_2S							
Cardamom	1.027±0.787	0.895±0.670	0.724±0.605	0.535±0.473	0.370±0.296	p=0.003	F=6.268 p=0.003
Listerine®	1.052±0.956	0.885±0.883	0.733±0.796	0.560±0.680	0.391±0.446	p=0.001	
Placebo	1.051±0.906	1.040±0.924	0.966±0.783	0.986±0.773	0.936±0.651	p=0.591	
CH_3SH							
Cardamom	1.052±0.956	0.885±0.883	0.733±0.796	0.560±0.680	0.391±0.446	p=0.000	F=11.515 p=0.000
Listerine®	1.270±1.255	1.043±1.031	0.936±0.980	0.708±0.725	0.496±0.578	p=0.017	
Placebo	0.915±0.863	0.946±0.925	0.951±0.890	0.780±0.693	0.880±0.814	p=0.352	
$(CH_3)_2S$							
Cardamom	0.575±0.624	0.422±0.446	0.340±0.417	0.285±0.305	0.209±0.231	p=0.009	F=6.422 p=0.003
Listerine®	0.699±0.610	0.625±0.547	0.437±0.436	0.418±0.439	0.289±0.327	p=0.001	
Placebo	0.413±0.292	0.369±0.302	0.399±0.309	0.528±0.773	0.448±0.411	p=0.430	
VSC							
Cardamom	2.952±1.270	2.461±1.083	2.005±0.943	1.549±0.761	1.118±0.597	p=0.000	F=38.842 p=0.000
Listerine®	3.022±1.711	2.553±1.511	2.107±1.303	1.687±1.107	1.177±0.766	p=0.000	
Placebo	2.379±1.363	2.401±1.325	2.346±1.393	2.295±1.292	2.267±1.336	p=0.317	

Values are presented as $\bar{x} \pm SD$ (10 μg/ml). VSC: volatile sulphur compounds, H_2S: hydrogen sulfide, CH_3SH: methyl mercaptan, $(CH_3)_2S$: dimethyl sulfide.

1. Oral chroma measurement

1) H_2S

The first measurement in the morning showed the changes of mean concentrations in experiment group was 1.027±0.787 and become 0.370±0.296 in the fifth day. It is also seen in the positive control group (Listerine®), 1.052±0.956 in the first day and 0.391±0.446 in the fifth day. Both groups showed significant differences statistically ($p<0.05$). Rinsing with placebo showed the mean concentration was reduced to 0.936±0.651 in the fifth day, however, there was no statistically significant different in this group ($p>0.05$). Average levels of H_2S in the experiment and both control group (Listerine® and placebo) tended to decrease from the first day until the fifth day (Table 1). The same tendency was noted in the second measurement in the afternoon at 11.00 a.m. (Table 2).

2) CH_3SH

In the morning time of first day, mean concentration of experimental and positive control group were 1.052±0.956 and 1.270±1.255, respectively and reduced to 0.391±±0.446 and 0.496±0.578 in the fifth day (Table 1). The measurement which is made in the noon time showed the same tendency, the concentration was reduced to 0.493±0.577 and 0.693±0.797 in the fifth day (Table 2). Concentrations after five days rinsing were much lower compared to the first day. Statistically significant differences were noted in both groups ($p<0.05$) while there was no statistically significant difference in placebo group ($p>0.05$).

3) $(CH_3)_2S$

The same tendency was noted on the mean concentration of $(CH_3)_2S$ both in the experiment and positive control group in the morning and noon time. The mean concentration of experiment group and positive control group were 0.575±0.624 and 0.699±0.610 in the first day and reduced to 0.209±0.231 and 0.289±0.327 in the fifth day. On the contrary, the mean concentration in placebo group on first measurement (morning time) was 0.413±0.292 and rose to 0.448±0.441 in the fifth day (Table 1) and 0.413±0.297 rose to 0.550±0.683 on second measurement (noon time) (Table 2) . There were statistically significant differences in the experiment and control group ($p<0.05$), while no statistically significant in placebo group ($p<0.05$).

4) VSC

The mean concentration of experiment group was 3.363±1.773 and declined to 1.380±0.707 in the fifth day and in the Listerine® group from 3.488±2.002 reduced to 1.502±1.079, both with statistical differences ($p=0.000$) while in the placebo group 2.284±1.136 to 2.213±1.253 without any statistical difference ($p=0.317$) (Table 1). In the second measurement (noon time), all groups showed reduction of the VSCs concentration (Table 2). Statistically significant differences also noted only in the experiment and control group ($p<0.05$). Figure 1, 2 show the line graph reduction both in the experiment

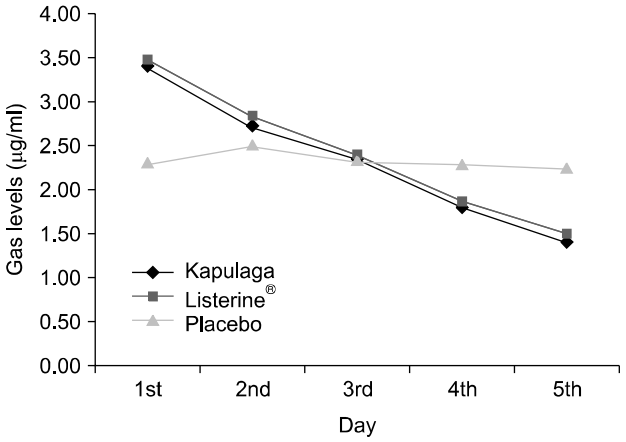

Figure 1. Changes in volatile sulphur compounds concentration after rinsing mouthwash containing 0.5% Cardamom, Listerine® and placebo in the morning time over 5 days clinical trial.

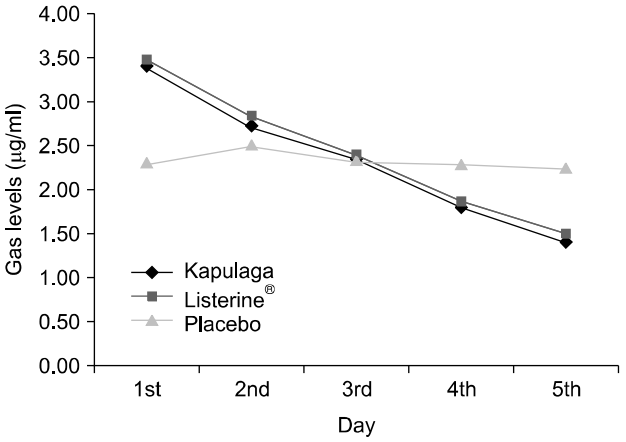

Figure 2. Changes in volatile sulphur compounds concentration after rinsing mouthwash containing 0.5% Cardamom, Listerine® and placebo in the noon time over 5 days clinical trial.

and control group over five days clinical trial, and it was not seen in the placebo group.

2. Organoleptic measurement

Changes in organoleptic measurement were shown in Table 3. In the experiment group was 2.60, control group 2.75 and placebo group 2.05 in the first day. The mean concentration reduced to 0.5, 1, and 1.7 respectively in the fifth day. No differences are noted between experiment and control group ($p>0.5$, adjusted mean 95% confidence interval [CI]) and between experiment and placebo group ($p>0.5$, adjusted mean 95% CI) in the first day. However, there were statistically significant differences between groups in the fifth day ($p<0.05$).

Table 3. Statistical analysis of the three groups based on organoleptic measurement

Group	Morning (1st day)	Afternoon (5th day)
Cardamom	2.60±0.86	0.50±0.42
Listerine®	2.75±0.90	1.00±0.68
Placebo	2.05±0.77	1.70±2.10
Kapulaga vs. Listerine®		
Adjusted mean	p=0.633	p=0.049*
95% confidence interval	−0.77±0.47	−1.00±0.00
Listerine® vs. placebo		
Adjusted mean	p=0.029*	p=0.007*
95% confidence interval	0.08±1.32	−1.20± −0.20
Kapulaga vs. placebo		
Adjusted mean	p=0.083	p=0.0001*
95% confidence interval	−0.07±1.17	−1.70± −0.70

*Significant.

Discussion

Many antibacterial agents such as chlorhexidine, eucalyptol (comprises up to 90% essential oil), and ClO_2 have been tested for its efficacy in the treatment of oral malodor. This study showed a significant effect of a new herbal mouthwash, 0.5% Cardamom in the treatment of halitosis compared to Listerine®, as the positive control and placebo. Natural resources and traditional medicine is national assets that should be developed and optimized. Research in traditional medicine aims to support the development in the field of traditional medicine that are of high quality and safe as well as having real efficacy scientifically tested and utilized extensively by the society and formal health service [16].

In this study, rinsing with a mouthwash containing 0.5% Cardamom and Listerine® showed effective in reducing of halitosis-related outcome variables (H_2S, CH_3SH, $[CH_3]2S$, and VSC) since the mean concentration of all variables dropped slightly day by day over 5 days clinical period. Statistically significant differences noted only in the Cardamom and Listerine® group ($p<0.05$), while it is not seen in placebo. Not only reduction of the compound concentration but also killing the bacteria. According to Anyanwu et al. [12], it has the antibacterial activity due to the presence of eucalyptol and thymol in Listerine®, and Cardamom maybe with the presence of terpinol and cineol. Several research has been carried out and it is proved that essential oil of Cardamom effective in inhibiting bacteria of gram negative (*P. gingivalis*) as the main cause of halitosis. The main element of essential oil is β-terpineol (13.4%), β-pinene (9,4%) and α-pinene (6,9%) [13]. Therefore, it is stated that Cardamom and Listerine® is effective as oral antibacterial agent in reducing halitosis. However, high alcohol concentration in Listerine® is considered to have side effects by many researchers such as re-

duces taste sensation and oral pain [11-13]. On the other hand, Cardamom is alcohol-free, one of the advantages of Cardamom mouthwash. A review of a study carried out in Cuba, Argentina, and Brazil which is published on December 2008 in the *Australian Dental Journal* stated that the use of mouthwashes that do not contain alcohol may be equally effective. It is recommended for the use of alcohol-containing mouthwash only for a particular situation and for a limited and controlled period of time [12].

In 2004 Carvalho et al. (requoted from Malhotra and Yeltihar, 2011 [15]) demonstrated that rinsing with four commercially available mouthwash (0.03% triclosan, 0.12% chlorhexidine gluconate, 0.05% cetylpyridinium chloride and essential oils) could significantly reduce VSC level in the absence of mechanical plaque control. Our results showed that kapulaga (essential oil) and Listerine® mouthwash were significantly more effective in reducing the methyl mercaptan and dimethyl sulfide $(CH_3)_2S$ levels ($p<0.05$), (Table 1, 2) even H_2S not much decline compared to CH_3SH and $(CH_3)_2S$. However, Malhotra and Yeltihar [15] showed that when comparing chlorhexidine with the essential oil mouth rinse, the reduction in VSCs was highly significant in the chlorhexidine group ($p<0.01$). The difference in the methodology applied and essential oil mouthwash in the study might explain the differences in the results.

Organoleptic measurements are often not reproducible as they depend on the subjective judgement of the panelist. In the present study, the measurements were conducted by two trained panelist. The decrease in organoleptic scores was significant between the groups ($p<0.05$) and the reduction was found to be statistically high significant ($p<0.001$). Proper instruction of the participants prior to measurements will minimize errors in the results. To exclude confounding factors such as temperature and humidity, measurements were always taken in the same room under constant conditions. Not only the intensity but also the type of halitosis could changes with the time of day, saliva flow, and oral hygiene. In addition, hormonal fluctuations can play a great role [17]. However, this method should be conducted as a '*gold standard*' for halitosis measurement.

Conclusion

The results showed that 0.5% Cardamom mouthwash containing essential oil could overcome halitosis through reducing the concentrations of H_2S, CH_3SH, $(CH_3)_2S$ and VSC for up to 4 to 5 hours after mouth rinsing. Organoleptic measurement as a gold standard confirm the results. However, future studies are still needed to examine long-term effects of the mouthwash.

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