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Original Article

Efficacy of antioxidant mouthwash in the reduction of halitosis: A randomized, double blind, controlled crossover clinical trial

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Abstract *Background/purpose:* Halitosis is the unpleasant and offensive odour in exhaled air, which is linked to the presence of volatile sulphur compounds (VSC). Different mouthwashes have been used to treat halitosis. The objective of this study was to test the effect of an antioxidant (AO) mouthwash, and mouthwash containing [0.05% chlorhexidine, 0.05% cetylpyridinium chloride, and 0.14% zinc lactate (CHX-CPC-Zn)] on VSC.
Material and methods: Thirty-five subjects with halitosis participated in this clinical trial. At the baseline visit, a breath sample was taken and analyzed for the level of hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide (CH_3SCH_3) using portable gas chromatography (OralChroma™). Two mouthwashes were randomly provided to each subject in addition to saline solution (NaCl 0.9%) as control. Subjects were instructed to rinse with 20 ml of the mouthwash for 1 min twice daily for 2 weeks. At second visit, post-treatment breath sample was taken. Afterward, the patient was asked to refrain from using mouthwash for a washout period of 1 week. A similar procedure was repeated for each mouthwash interval.
Results: No significant differences in VSC level between all three groups were detected at baseline. A significant reduction in VSC level was obtained after using CHX-CPC-Zn mouthwash. On other hand, both AO mouthwash and saline had no significant impact on the level of VSC.
Conclusion: CHX-CPC-Zn mouthwash has a significant effect on VSC level reduction in subjects with confirmed halitosis. Besides, using AO mouthwash regularly for 2 weeks did not have any impact on improving the level of halitosis.

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Introduction

Halitosis is the unpleasant and offensive odour in exhaled air; that is linked with the existence of volatile sulphur compounds (VSC). Halitosis can have an important effect on normal social interactions.[1] The principal cause of halitosis originates intraorally (90%), while only 10% of its cause is considered to be extraoral.[2,3] The main cause of halitosis is the bacterial formation of the odorous volatile sulphur compounds in the oral cavity. There are three major volatile sulphur components which lead to oral malodour, they are: hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide (CH_3SCH_3). VSCs form as a result of bacterial putrefaction of amino acids that contain sulphur molecules such as cysteine and methionine, which are usually found in exfoliated epithelial cells, and white blood cell debris.[4]

Different methods can be used to diagnose halitosis, such as organoleptic method, sulphide monitoring, and gas chromatography. Management of halitosis focuses upon to the elimination of the detected causal factors. In the majority of the cases, halitosis is treated by reducing the buildup of bacterial biofilm and food debris. This can be achieved by improving the oral hygiene status through mechanical or chemical methods, or a combination of both.

Several mouthwashes have been used to treat halitosis. Mouthwashes containing chlorhexidine, cetylpyridinium chloride, triclosan, or essential oil have been reported to treat oral malodour by decreasing the quantity of VSC-producing microorganisms in oral cavity.[5–7] Moreover, metal ions such as zinc chloride, iminium chloride were added in an attempt to neutralize VSCs. Zinc ion can reduce the expression of the VSCs by binding to sulphur radicals, which will convert, the volatile H_2S and CH_3SH into non-volatile Zn-sulphides.[7] The effectiveness of CHX-CPC-Zn in reducing VSC levels could be explained by the combined antibacterial and VSC neutralizing actions.[8] Neutralizing VSC with zinc ion is more effective for immediate action (masking effect), which is not stable and will be deteriorated with time, While reduction of the microorganisms using the antibacterial properties of the mouthwash is more effective for long-term action (therapeutic effect).[9] Recently, dental manufacturers have included antioxidants into mouth rinses. However, there are no studies done to find the effect of AO mouthwashes on halitosis; therefore, this study aimed to test the effect of an antioxidant (AO) mouthwash, and mouthwash containing (CHX-CPC-Zn) on halitosis.

Materials and methods

The present study is a single-center, randomized, crossover, double-blind clinical trial conducted at the College of Dentistry at King Saud University from February to May 2016. The present clinical trial was reported following Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Ethical consideration

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved ethically by the College of Dentistry Research Center (CDRC), King Saud University (PR 0039); the study was also approved by the Saudi FDA and registered at The Saudi Clinical Trials Registry (SCTR) under number 15101202. An informed written consent containing details of the nature of the study was given to all participants.

Study population

A total of 53 subjects who complained of oral halitosis were initially screened, which resulted in the inclusion of 37 participants (16 females, 21 males) who met the inclusion criteria.

Inclusion criteria

Volunteers aged 18—60 years old who were diagnosed with halitosis were recruited to participate into the study. Halitosis was defined as the VSC level recorded in parts per billion (ppb) of the breath sample being equal to or greater than the following threshold: hydrogen sulphide (H_2S) $\geq$ 112 ppb, or methyl mercaptan (CH_3SH) $\geq$ 26 ppb.[10]

Exclusion criteria

Subjects were excluded from this study if they have any of the following conditions: Presence of respiratory tract diseases, tonsillitis, stomach disorders, antibiotic use in the previous 3 months, pregnancy, or presence of less than 20 natural teeth.

Study protocol

Participants who enrolled in the study and met the inclusion criteria received a detailed questionnaire about their medical conditions. At the initial visit, a pre-treatment breath sample was collected from each subject and then an oral hygiene kit containing a dentifrice and soft toothbrush was provided to each subject to be used throughout the study. Instruction on the use of mouthwashes and oral hygiene were provided, and participants were asked to follow their normal diet and daily oral hygiene activity. In addition, plaque index (PI),[11] and gingival index (GI)[12] were recorded at baseline visit. Furthermore, probing depth (PD) was recorded for the Ramfjord teeth[13] at baseline visit in order to reflect the periodontal condition of the included participants. The same qualified examiner performed all measurements. The three mouthwashes used in this clinical trial are listed in (Table 1). The following mouthwashes were used in this clinical trial:

1. Antioxidant mouthwash (AO ProRinse®) containing ferulic acid, tetrahydrocurcuminoids, and epigallocatechin gallate (PerioSciences, Dallas, TX, USA)
2. Halita® mouthwash containing 0.05% chlorhexidine, 0.05% cetylpyridinium chloride, and 0.14% zinc lactate (Dentaid, Barcelona, Spain) considered positive control
3. NaCl 0.9% as a negative control

Table 1 Active ingredients of the different mouthwashes.

Code	Mouthwash	Manufacturer	Active ingredients
A	AO ProRinse®	PerioSciences, Dallas, TX, USA	Ferulic acid, tetrahydrocurcuminoids, epigallocatechin gallate
B	Halita®	Dentaid, Barcelona, Spain	Chlorhexidine 0.05%, cetylpyridinium chloride 0.05% zinc lactate 0.14%
C	Saline	PSI, Riyadh, Saudi Arabia	(NaCl 0.9%)

AO = Antioxidant.

All mouthwashes were provided to participants in identical bottles that were coded as A, B and C by another investigator (HA) to ensure the blindness of the study, neither participants nor examiner were aware of the codification. Along with the mouth rinse, an individual plastic measuring device (20 ml) was provided to each participant. Coded bottles were containing mouthwashes given to the participants following a computer-generated randomization schedule, which was performed by an independent investigator. Subjects were directed to rinse with 20 ml of the assigned mouthwash for 1 min two times a day for 2 weeks. At the second visit, a post-treatment breath sample and PI were taken. Afterward, the patients were asked to refrain from using mouthwash for a washout period of 1 week to avoid any carryover effects. After a week of washout period, clinical measurements were repeated and compared to the previous baseline clinical values. If the measurements were similar, the subject was provided with the second MW. The same procedure was applied for the third mouth rinse (Fig. 1).

VSC assessment

Portable gas chromatography, (OralChroma CHM-1, ABIMEDICAL Corporation, Kawasaki City, Japan), was utilized to assess H_2S, CH_3SH, and CH_3SCH_3 in breath samples from study subjects. Subjects were asked to refrain from eating, brushing, mouth rinsing, and smoking for 3 h before their breath sample assessment. Before obtaining the breath sample, the participants were instructed to keep their mouth closed for 60 s. Then, 1 ml of breath sample was obtained using a specific syringe. Afterward, the collected oral breath sample was injected immediately into the OralChroma device. Concentration of VSC was recorded in ppb.

Statistical tests

Power of the study

The sample size was determined using Cohen (1988) procedure, by assuming an effect size (f) of 0.80 and with power of 90% ($1-\beta = 0.90$) and at $\alpha = 0.05$; the minimal number of subjects was calculated as 10 to establish a statistical significant difference between the 3 study groups (saline and 2 mouthwash products). As the study design is a crossover randomized, double-blind study, the minimal subjects were increased due to an anticipated loss to follow up.

Statistical analysis

Statistical calculation was performed using SPSS Statistics version 21 (Chicago, IL, USA). Descriptive statistics (frequency and proportions) were used to describe the categorical study variables. Taking into consideration the small sample size and skewness of the data, Non-parametric statistical test was performed. Wilcoxon sign rank test was used to compare the mean ranks of outcome variables PI, H_2S, CH_3SH, and CH_3SCH_3 between pre and post-intervention stages. Also, the Kruskal–Wallis test was used to compare the mean ranks of skewed values of outcome variables across the three types of interventions (AO, Halita, and Saline). A p-value of <0.05 was used to report the statistical significance of results.

Results

Two patients were excluded from the study one due to antibiotics use and the other due to personal circumstances. Therefore, the data of 35 study subjects, which included 20 men and 15 women, were analyzed. The mean age of all subjects was 20.94 (±3.3) years (ranged 19–32 years). Results of the completed questionnaire showed that 40% of the participants visited a dentist every 6 months, while 48.6% of subjects used a medium type brush, and 65.7% brushed twice a day. Mouthwash frequency was reported as "never" in 48.6% and "rarely" in 34.3%. Most of the study subjects, 81.5%, used waxed dental floss as a dental aid to clean between teeth. Tongue-scraping frequency was reported as "never" in 77.1% of subjects (Table 2). At baseline visit, the mean PI was 36.1, GI was 0.6, and PD was 1.98 mm.

The comparison of H_2S, CH_3SH and CH_3SCH_3 values between pre and post intervention of antioxidant mouthwash showed no significant changes in the values of all VSC (Table 3). After CHX-CPC-Zn treatment, the level of H_2S concentration decreased from 234 ppb to 32 ppb, the CH_3SH concentration decreased from 41 ppb to 7 ppb and the CH_3SCH_3 concentration decreased from 16 ppb to 5 ppb. Using CHX-CPC-Zn for 2 weeks has shown a significant decrease in values of H_2S, CH_3SH and CH_3SCH_3 in the breath samples compared to the pre-treatment levels ($p < 0.001$, $p < 0.001$, and $p = 0.004$) (Table 4). Saline showed no significant impact on the level of H_2S, CH_3SH and CH_3SCH_3 (Table 5). When comparing H_2S, CH_3SH, and CH_3SCH_3 across the three interventions, AO and CHX-CPC-Zn showed statistically significant differences in the reduction of H_2S and CH_3SH ($p < 0.001$, $p = 0.009$). Whereas, there was no statistically significant difference found between all 3 interventions in the reduction of CH_3SCH_3 ($p = 0.105$) (Table 6).

Discussion

Recently, numerous mouthwashes have been introduced to the market, claiming effectiveness in reducing halitosis.

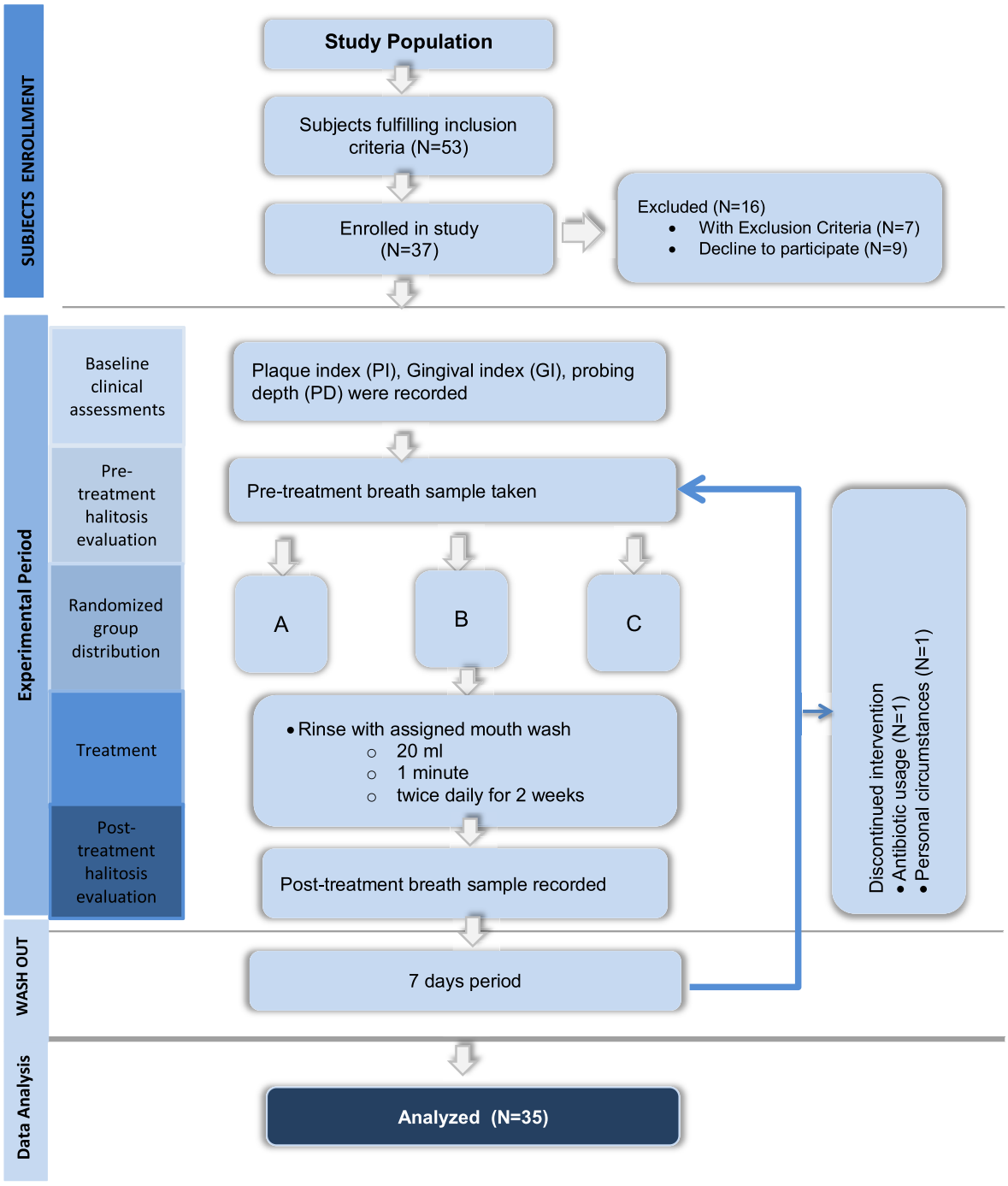

Figure 1 (CONSORT) Flow chart of the study design.

The present study aimed to test and compare the effect of a new mouthwash AO on the reduction of halitosis. The design of this study is a crossover trial, where each individual acts as his or her own control. It was used to decrease the chance of interindividual variations influencing the study's outcome such as: presence of dental plaque biofilm, or the severity of gingival inflammation. Unlike the parallel design, the intersubject variation is much greater than the intrasubject variation. Furthermore, a crossover study design is inherently more powerful than a parallel design for the same number of subjects. To avoid any carryover effects of treatment intervention, a washout period was considered in this crossover clinical trial designed study. The appropriate washout period for products would depend on their efficacy and/or their mode of action. For regular oral care products, one-week washout periods are considered to be an appropriate timescale to ensure no carryover effects.[14,15]

Gas chromatography was used in this study to assess H_2S, CH_3SH, and CH_3SCH_3 in breath samples from study subjects. Portable gas chromatography has major advantages, which have very low detection limit, it can easily detect and

Table 2 Socio-demographic characteristics and the responses towards oral hygiene practices of study subjects (n-35).

Socio-demographic characteristics	No (%)
Age (Mean, SD)	20.94 (3.3)
Gender	
Male	20 (57.1)
Female	15 (42.9)
Oral hygiene practices	**No (%)**
Frequency of dental visits	
Rarely	13 (37.1)
Every 6 months	14 (40.0)
Every 12 months	8 (22.9)
Brushing type	
Soft	16 (45.7)
Medium	17 (48.6)
Hard	2 (5.7)
Brushing frequency	
1/day	12 (34.3)
2/day	23 (65.7)
Flossing type ($n = 27$)	
Waxed	22 (81.5)
Un waxed	4 (14.8)
Supra floss	1 (3.7)
Flossing frequency	
Never	8 (22.9)
Rarely	8 (22.9)
1/day	9 (25.7)
2/day	2 (5.7)
2–3 times/week	8 (22.9)
Mouthwash frequency	
Never	17 (48.6)
Rarely	12 (34.3)
1/day	1 (2.9)
2/day	4 (11.4)
2–3 times/week	1 (2.9)
Tongue scraping frequency	
Never	27 (77.1)
Rarely	6 (17.1)
2/day	1 (2.9)
2–3 times/week	1 (2.9)

Table 3 The effect of antioxidant mouthwash on the levels of H_2S, CH_3SH, and CH_3SCH_3 in breath samples.

	Antioxidant		*P*
	Pre	Post	
H_2S	165 (429)	192 (321)	0.752
CH_3SH	68 (101)	24 (62)	0.059
CH_3SCH_3	20 (109)	13 (42)	0.171

Values represent median (interquartile range) in parts per billion.
P values for the statistical comparison using Wilcoxon sign rank test.

Table 4 The effect of CHX-CPC-Zn mouthwash on the levels of H_2S, CH_3SH, and CH_3SCH_3 in breath samples.

	CHX-CPC-Zn		*P*
	Pre	Post	
H_2S	234 (229)	32 (59)	<0.001*
CH_3SH	41 (86)	7 (21)	<0.001*
CH_3SCH_3	16 (36)	5 (16)	0.004*

Values represent median (interquartile range) in parts per billion.
P values for the statistical comparison using Wilcoxon sign rank test. *statistically significant.

Table 5 The effect of saline mouthwash on the levels of H_2S, CH_3SH, and CH_3SCH_3 in breath samples.

	Saline		*P*
	Pre	Post	
H_2S	194 (362)	136 (217)	0.174
CH_3SH	42 (79)	26 (63)	0.768
CH_3SCH_3	19 (97)	10 (23)	0.174

Values represent median (interquartile range) in parts per billion.
P values for the statistical comparison using Wilcoxon sign rank test.

distinguish between oral and extra-oral halitosis. It is also extremely easy to use. Unlike other halitosis, measurement devices e.g., Halimeter, which measure the total sulphur content of the patient's breath, but it is not suitable for detecting extra-oral halitosis because it cannot differentiate between the three VSCs. Moreover, its sensitivity to H_2S is more than CH_3SH, and it is almost insensitive to CH_3SCH_3.[16]

To our knowledge, this was the first study done to test the effect of an antioxidant mouthwash on halitosis. The results the current study have shown that there is no significant reduction in all three VSCs with an AO mouthwash. The concept of this mouthwash is the use of specific antioxidants in the proper combination, which neutralize damaging free radicals that produce disease states. One of the antioxidants mouthwash components is epigallocatechin gallate (EGCG), which can be found in green tea. The effect of green tea extract mouthwash on VSC was studied by Farina et al., they observed that green tea had an immediate inhibitory effects on the production of VSC with no residual inhibitory effects at 90 and 180 min[17] different studies concluded that green tea is effective in reducing halitosis temporary immediately after administration and up to 30 min with no reduction in halitosis at 1, 2 and 3 h after use.[18,19]

In this study, the data showed the beneficial impact of CHX-CPC-Zn mouthwash on reducing the VSC comparing to AO mouthwash and saline, which have no significant impact on VSC. The effectiveness of CHX-CPC-Zn in reducing halitosis is comparable with previous studies.[8,9,14,20,21]

Table 6 Comparison of values of H_2S, CH_3SH and CH_3SCH_3 among the three types of interventions.

Type of intervention	H_2S		CH_3SH		CH_3SCH_3	
	Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*
AO	192 (321)[2]	<0.0001*	24 (62)[2]	0.009*	13 (42)	0.105
CHX-CPC-Zn	32 (59)[1]		7 (21)[1]		5 (16)	
Saline	136 (217)[2]		26 (63)[2]		10 (23)	

AO = antioxidant; IQR = interquartile range.
Values represent Median (IQR) in parts per billion.
P value for the statistical comparison using Kruskal–Wallis test; *statistically significant.
[1]Significantly lower than AO and saline ($p < 0.001$); [2]No significant difference between saline and AO ($p > 0.05$).

Dadamio 2013 et al., compared the masking effect of CHX-CPC-Zn on halitosis using Halimeter and organoleptic method in different time periods, and they found that CHX-CPC-Zn were effective in both short and extended period of time. CHX mouthwash known to have antibacterial activity,[22] the electrostatic attraction between cationic CHX and the anionic bacterial surfaces cause membrane disruption and increased permeability and death of the cell. As a result, it may lead to a reduction in bacterial load and malodour.[23] Chlorhexidine mouth rinses are available in the form of 0.2% and 0.12%.[24] Chlorhexidine appears to be quite useful in managing oral malodour. However, many studies suggested that using chlorhexidine for long term may lead to brownish discolouration of the teeth and tongue, taste alterations, increased desquamation of oral mucosa, and calculus formation.[23,25,26] therefore, low doses of CHX (0.05%) have been used to decrease these sides effects.[27,28] To reduce these side effects, a reduction in the CHX concentration[29] and combination with other active agents [e.g. cetylpyridinium chloride (CPC)][30] has been recommended. In fact, the use of mouth rinses containing low-concentration CHX (0.05%) combined with 0.05% CPC has shown efficacy in the management of gingivitis.[31,32]

Moreover, zinc ions have shown the ability to reduce VSC in the oral cavity.[33,34] The mechanism of action of zinc is that zinc ions which has two positive charges (Zn++) binds to the twice-negatively charged sulphur radicals, which then convert, the volatile H_2S and CH_3SH into non-volatile Zn-sulphides which lead to a reduction in the expression of the VSCs in the breath.[5] The combination of CHX-CPC-Zn was found to be effective against halitosis with almost no noticeable side effects.[27,34,35] In this study, there was a statically significant difference in reducing the level of VSCs except in dimethyl sulphide (CH_3SCH_3). This could be explained by the fact that dimethyl sulphide was reported as the most common VSC associated with extra-oral halitosis.[36]

The current study focused on the effect of the tested mouthwashes on halitosis for a follow up period of 2 weeks. Future studies should be conducted for longer follow up period and to assess the effect of AO on periodontal condition and related pathogens.

In conclusion, this study has shown that using antioxidant mouthwash regularly for 2 weeks had no impact upon improving halitosis. In contrast, CHX-CPC-Zn mouthwash did exhibit a significant effect upon the reduction of VSC levels in subjects with confirmed halitosis.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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