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Role of Kefir Mouthwash in Halitosis Reduction. A Triple-Blind Randomized Clinical Trial

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Abstract

Background: Bad breath or halitosis is a prevalent issue that adversely effects social life and general well-being. Chlorhexidine is commonly used to manage it, but its prolonged use is associated with undesirable side effects. Natural probiotic alternatives like kefir may help reduce halitosis by restoring oral microbial balance. **Aim**: This research aimed to investigate the efficacy of kefir mouthwash, a probiotic-based natural alternative, compared to chlorhexidine (CHX) in reducing halitosis **Materials and Methods:** A triple-blind, randomized, controlled clinical study involved 60 systemically healthy adults whose ages ranged 18-28 years diagnosed with moderate gingivitis, recruited from dentistry students at Bilad Al-Rafidain University and training dentists from a specialized dental center in Diyala, Iraq. Subjects were randomly assigned to either control group (30 subjects) using 0.12% chlorhexidine mouthwash or study group (30 subjects) using kefir mouthwash twice daily for 28 days. Halitosis was assessed objectively via volatile sulfur compound (VSC) levels, concentration, and odor detection distance using a Halimeter, and subjectively using a Visual Analog Scale (VAS) at baseline, day 14, and day 28. **Results:** Both groups exhibited significant reductions in halitosis parameters over 28 day time ($p < 0.001$). The kefir group showed slightly greater improvements in VSC concentration, odor detection distance, and VAS scores at follow-up points, although these differences compared to the control group were small. **Conclusion:** The findings of this study indicate that both kefir and chlorhexidine mouthwashes are effective options for reducing halitosis over the 28-day period. Although the kefir mouthwash led to slightly better improvements, which may be due to its effects on the oral microbiota, these differences compared to the control group were small. Additionally, kefir's good safety and biocompatibility profile imply that it might be an effective natural substitute for people looking for long-term halitosis treatment without the negative side effects of chlorhexidine. Further research with longer follow-up and larger sample sizes is recommended to confirm its efficacy and mechanisms.

Keywords: Halitosis, probiotics, kefir mouthwash, chlorhexidine, volatile sulfur compounds, randomized controlled trial.

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Introduction

Bad breath, or halitosis, is a prevalent condition characterized by considerable community and psychological consequences. Although it is not typically a life-threatening medical issue, it impacts a significant percentage of the individuals. Notably, the site of origin is the oral cavity for over 90% of cases (Karbalaei et al, 2021; Huang et al., 2022). The issue mainly results from anaerobic oral bacteria that metabolize proteins into volatile sulfur compounds (VSCs), such as methyl mercaptan and hydrogen sulfide which are the key contributors to the distinctive unpleasant odor associated with halitosis (Hampelska et al., 2020). *Porphyromonas gingivalis* and *Fusobacterium nucleatum* are examples of anaerobic bacteria tend to colonize areas that are difficult to access, including the periodontal pockets and the dorsum of the tongue. Conventional treatment strategies typically involve mechanical debridement and the use of chemical agents like chlorhexidine (CHX). Although effective, these interventions are associated with several significant drawbacks such as dental discoloration, taste disturbances, and disruption of the natural oral microbiome (McCoy et al., 2008; James et al., 2017; Tartaglia et al., 2019). Therefore, probiotics (live microorganisms that provide health benefits by influencing microbial populations and boosting immune responses) have gained scientific interest (Williams, 2010). The most frequently utilized bacterial species in probiotic preparations are bacteria that produce lactic acid, notably those species of *Bifidobacterium* and *Lactobacillus* (Santosa et al, 2006).

Kefir is a fermented dairy product containing lactic acid bacteria, yeasts, and acetic acid producing bacteria, has gained recognition as a potential probiotic carrier, exhibiting notable antimicrobial and immunomodulatory properties within the oral environment (Vieira et al., 2021). Specific strains found in kefir, including *Lactobacillus acidophilus*, have the ability to suppress volatile sulfur compound-producing pathogens and enhance the growth of beneficial oral microbiota by producing bacteriocins and through competitive exclusion (Zhao et al., 2019). Enzymatic activity in kefir (such as the bioconversion of lactose into galactooligosaccharides) can enhance flavor and reducing undesirable odors, thereby improving product acceptability without causing the adverse side effects often associated with alcohol-based components (Kaczyński and Cais-Sokolińska, 2018). Although kefir exhibits promising properties, its application as a

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mouthwash in the clinical management of halitosis has been insufficiently investigated. Consequently, this study aims to assess kefir's mouthwash effectiveness as a natural alternative to chlorhexidine (CHX) mouthwash in the management of halitosis, using both objective and subjective measurements. Specifically, volatile sulfur compound (VSC) levels, concentration and distance as an objective indicator using a Halimeter device, and Visual Analogue Scale (VAS) scores to assess patient-perceived oral malodor**,** as a subjective indicator**.**

Materials & Methods

Study design

A triple-blind randomized controlled clinical study was carried out using two parallel groups: Group A the control group received 0.12% chlorhexidine mouthwash (CHX), whereas Group B the study group received kefir mouthwash. Outcomes were assessed following the use of kefir in comparison to chlorhexidine. Randomization and blinding procedures were implemented to ensure methodological rigor, minimize bias, and enhance the reliability of results.

Sample Size Calculation:

To determine the appropriate sample size, a power analysis was conducted using G*Power (version 3.1.9.7) incorporating a large effect size ($\eta p^2 = 0.90$) derived from the comparison between the probiotic and chlorhexidine (CHX) groups as reported by (Kandaswamy et al., 2018). Assuming a two-sided significance level of $\alpha = 0.05$ and a statistical power of 95%, the analysis indicated that a total of 60 participants (30 per group) would be required. This estimation is consistent with the standards proposed by (Cohen, 1988; Faul et al., 2009).

Subjects

The study involved 60 systemically healthy adults diagnosed with moderate gingivitis aged 18–28 years, recruited from dentistry students at Bilad Al-Rafidain University and training dentists from a specialized dental center in Diyala, Iraq. Participants were divided into two equal groups by random assignment (n=30 each group). 0.12% chlorhexidine mouthwash was given to the control group, and the study group utilized kefir mouthwash. Each group comprised 18 males and 12 females. The study spanned 28 days and included data collection at three time points: baseline (Day 0), mid-point (Day 14), and endpoint (Day 28).

Inclusion Criteria:

1. Participants were aged 18–28 years.
2. Systemically healthy.

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3. Diagnosed with moderate gingivitis with a gingival index score (1.1 to 2) were included in the study to ensure uniformity in baseline oral health status.
4. Individuals had not taken antibiotics in the previous three months.

Exclusion Criteria:

1. Individuals with systemic diseases.
2. Pregnancy or lactation.
3. Recent antibiotic use.
4. Mouth breathing.
5. Orthodontic/prosthodontics appliances.
6. Smoking.
7. Non-compliance with the study protocol.

Ethical Approval

Ethical approval was obtained from Mustansiriyah University, College of Dentistry research ethics committee with research ethics reference number (REC165), valid from November 1, 2024, to November 1, 2025. The trial was registered with ClinicalTrials.gov (NCT06900881). Additional permissions for sample collection were granted by Bilad Al-Rafidain University and the Specialized Dental Centre in Diyala, Iraq. All participants provided written informed consent. The study was conducted in accordance with Declaration of Helsinki.

Subject Allocation & Blinding Protocol

An experienced dentist who was not participating in the trial used Research Randomizer (v2.0; https://www.randomizer.org) to randomly allocate 60 individuals into control and study groups (n = 30 each). To reduce potential bias, a triple-blind strategy was used to make sure that group allocations were unknown to participants, the researcher, and data analysts. To maintain blinding, all bottles had the same color and shape and were labeled with code A or B.

Halitosis Measurement Methods

A dual-method approach was used to assess oral malodor (halitosis). Oral malodor was examined subjectively through participant-reported Visual Analog Scale (VAS) scores and objectively through measurements obtained by a Halimeter device (YRY Smart Breath Odor Detector, China)Figure 1.Volatile sulfur component identification helped to assess halitosis levels from mild to severe. Oral malodor objectively was measured using a Halimeter (YRY, China), that has the ability to identify volatile sulfur compounds (VSCs), including hydrogen sulfide and methyl mercaptan. The device quantifies VSC

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concentration was measured in part per million (ppm), which corresponds to odor intensity classified into four levels (slight, moderate, strong, intense) and the approximate social distance was measured in centimeter (cm) at which the odor is perceivable. At least an hour before the assessment, participants abstained from eating, drinking, and practicing good dental hygiene (Seemann et al., 2014). To conduct the test, the Halimeter was activated, and once the device indicated readiness (vibration and flashing blue light), participants were instructed to exhale 1 cm from the sensor for five seconds. The resulting odor level, concentration and distance was observed on the display, and results were saved.

Additionally, participants used a Visual Analog Scale (VAS) to score their perceived oral odor, ranging from 0 (no odor) to 10 (severe odor). Assessments were conducted at (0, 14, and 28 days) to evaluate mouthwash efficacy.

Kefir Mouth Wash Preparation

Kefir mouthwash was prepared by adding 3 g of kefir starter (Yogourmet, France) per 1 liter of boiled and then cooled at (25°C) of 3% fat milk (Pegah, Iran). Figure 1. To avoid any contamination, the kefir mouthwash was prepared in a clean, controlled household setting. The inoculated milk was transferred into a clean airtight container, and the lid was sealed, the container was left to ferment at room temperature for 24 hours, then refrigerated for 8 hours to stop fermentation. It was subsequently transferred into sterile bottles for later use in the study. The kefir starter includes *Lactobacillus acidophilus, L. diacetylactis, L. cremoris, Lactococcus lactis, maltodextrin, Saccharomyces cerevisiae,* and *Kluyveromyces lactis*, and met ISO 9001 and FSSC 22000 standards.

Intervention

Participants used their assigned mouthwash for one minute, twice daily for 28 days, rinsing with 30 mL of kefir mouth wash or 15 mL of 0.12% chlorhexidine mouth wash (Biofresh LIC, Scitra, U.A.E) each time.

The rinse was conducted 30 minutes after brushing, and participants were prohibited from eating or drinking for one hour. The instructions came in sealed packages and were standardized.

Monitoring of compliance

Participants recorded their usage in diaries and returned the empty bottles at follow-up visits. WhatsApp group made it easier to remind participants of the correct time to use mouthwash and to report any side effects.

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Statistical Analysis

For statistical analysis, IBM Corp, Chicago, IL, USA, SPSS version 22.0 was utilized. The clinical and demographic data were generated using descriptive statistics such means, standard deviations, frequencies, and percentages, on days 0, 14, and 28, respectively. The data distribution's normality was evaluated using the Shapiro-Wilk Test. Mixed design two-way ANOVA demonstrate the effect of time on the different groups. After significant ANOVA findings, post hoc comparisons were conducted using Bonferroni**.**

Results

Shapiro-Wilk Test for Normality Assessment

The Shapiro-Wilk test was used to assess the normality of all study variables. As shown in Table 1, all *p*-values across different time points for both the chlorhexidine (CHX) and kefir groups exceeded 0.05. This shows that the assumption of normality was met and the data were distributed properly.

Halitosis evaluation

Both kefir and control groups showed significant decrease in halitosis over the28 day period using the Halimeter device analysis and Visual Analog Scale (VAS); kefir produced slightly better results at day 28. At baseline (day 0), Comparable initial halitosis conditions were confirmed by the lack of significant variations between the kefir and control groups. All parameters decreased significantly at 14 and 28 days in both groups ($P < 0.001$), although the kefir group's improvements in VSC concentration, odor detection distance, and VAS scores were marginally larger at follow-ups. As shown in Table 2, 3. Figure 2-5.

Table 4 shows the progressive changes in Halimeter level scores for the CHX and Kefir groups. Bonferroni pairwise comparisons showed statistically significant within-group decreases at all-time points ($P = 0.000$) which indicates continuous breath odor improvement in both groups. Kefir showed slightly greater reduction by day 14, but both groups had similar improvements from day 14 to 28. By day 28, both treatments had the greatest reductions, with no significant intergroup differences.

Table 5 presents multiple pairwise comparisons of volatile sulfur compound (VSC) concentrations over time within the CHX and Kefir groups using Bonferroni test. At all-time points, both groups showed statistically significant reductions ($P = 0.000$). The Kefir group observed a marginally higher mean decrease from day

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0 to day 14. (0.497 ± 0.066) than the CHX group (0.477 ± 0.066), and between days 14 and 28, Kefir again showed a marginally higher reduction (0.323 ± 0.019 vs. 0.300 ± 0.019). Overall, from day 0 to day 28, total reductions were 0.820 ± 0.065 for Kefir and 0.777 ± 0.065 for CHX.

Table 6 displays multiple pairwise comparisons of odor detection distance over time within the CHX and Kefir groups using Bonferroni test. At all-time points both groups showed statistically significant reductions ($P < 0.001$). The Kefir group observed a slightly higher mean decrease from day 0 to day 14. (32.33 ± 1.87 cm) than the CHX group (25.33 ± 1.87 cm). Overall, from day 0 to day 28, the Kefir group achieved slightly a larger total reduction (71.67 ± 2.39 cm) compared to the CHX group (59.67 ± 2.39 cm), indicating consistently slightly greater improvement with Kefir.

Table 7 presents multiple pairwise comparisons of VAS scores for halitosis over time using Bonferroni test. Both the Kefir and CHX groups showed statistically significant improvements at all intervals ($p = 0.000$). The Kefir group observed a marginally higher mean decrease from day 0 to day 14. (3.77 ± 0.14) compared to the CHX group (3.10 ± 0.14). Overall, from day 0 to Day 28, the Kefir group achieved slightly a greater total reduction (5.47 ± 0.17) than the CHX group (5.03 ± 0.17), indicating slightly more substantial improvement in self-reported halitosis.

Discussion

Halitosis remains a prevalent concern in dental practice, often treated with chlorhexidine, despite its undesirable side effects (Athar et al., 2022). This study evaluates kefir, a natural probiotic alternative with no reported side effects, comparing its efficacy to chlorhexidine in reducing halitosis using both objective and subjective measures. Both kefir and control groups showed significant reductions in halitosis over the 28-day period, as measured by Halimeter device readings and Visual Analog Scale (VAS) scores. However, the kefir group consistently demonstrated slightly better outcomes across all parameters, including perceived odor VAS, VSC concentration, and odor detection distance. By day 28, the kefir group's VAS score decreased to 0.20 compared to 0.67 in the control group, suggesting kefir's slightly superior efficacy in managing halitosis. This may be attributed to the antimicrobial activity of kefir's probiotic strains, such as *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*, against

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pathogens associated with moderate gingivitis. , which generate VSCs responsible for bad breath (Fakruddin et al., 2017; Bueno et al., 2023). Unlike the chemical antiseptic action of chlorhexidine, kefir exerts a biological effect by promoting microbial balance, which may contribute to longer-lasting breath freshness and fewer side effects (Soares et al., 2023).Significant reductions were observed in all halitosis parameters for both groups (p = 0.000), with the kefir group showing slightly greater decreases in VSC concentration (0.103 ± 0.016 vs. 0.187 ± 0.027; P = 0.010), odor detection distance (12.667 ± 2.086 cm vs. 24.333 ± 3.413 cm; P= 0.005), and visual analog scale VAS scores were (0.200 ± 0.074 compared to 0.667 ± 0.111; P = 0.001). Halimeter levels did not differ significantly (P > 0.05). Subjective improvement is particularly important, as patient compliance and satisfaction often rely more on perceived benefits than solely on clinical outcomes (Jabbareh et al., 2025). The current findings align with those of Georgiou et al. (2018), who reported that probiotics are highly effective in reducing volatile sulfur compounds (VSCs) when compared to mechanical and chemical treatments. Moreover, kefir mouthwash demonstrated a slightly more sustained effect on rebalancing malodor-producing oral microbiota than chlorhexidine, reflected by a slightly greater reduction in both VSC concentrations and odor perception distance and as measured by visual analogue scale (VAS) scores. These findings corroborate prior studies reporting probiotic benefits for halitosis. Mayanagi et al. (2009) and Seemann et al. (2014), demonstrated probiotics reduce VSC levels and gram-negative anaerobic bacteria. Sudhakaran et al. (2021), found probiotics improve oral malodor and plaque by fostering a healthier biofilm bacterial contents species, while Huang et al. (2023), confirmed the inhibitory effect of *Bifidobacterium lactis* and *Lactobacillus acidophilus* probiotics on halitosis-related bacteria. These studies support the current results and highlight kefir's probiotic potential in oral care. Anaerobic bacteria metabolize sulfur-containing amino acids from inflamed tissues into VSCs, the primary cause of halitosis (Tonzetich, 1977; Persson et al, 1989). Furthermore, Enioutina et al. (2024), demonstrated that probiotics decrease pro-inflammatory cytokines (IL-1β, IL-8, and TNF-α) and increase anti-inflammatory IL-10 to reduce halitosis and tongue coating.

Soares et al. (2019), founds that a 90-day oral intake of *Lactobacillus* strains, including *L. acidophilus*, significantly

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reduced periodontal issues and halitosis ($P < 0.01$). This supports the role of *L. acidophilus* in controlling bad breath and improving oral health. Our kefir product also contains this bacterium, which may partly explain the positive effects observed in our study.

Conclusion

Both intervention groups demonstrated statistically significant reductions in halitosis parameters over the 28-day period. The kefir group exhibited slightly greater improvements in volatile sulfur compound (VSC) concentration, odor detection distance, and visual analog scale (VAS) scores at follow-up assessments, although these differences compared to the control group were small. The kefir mouthwash may confer a marginally more sustained reduction in halitosis compared to chlorhexidine, potentially due to its capacity to modulate the oral microbiota and promote a more stable and balanced microbial ecosystem. The consistent improvements observed with the kefir intervention indicate its potential as a safe, natural, and effective alternative to chlorhexidine for the management of halitosis. Nevertheless, further studies with longer follow-up and larger sample sizes are necessary to validate these results and clarify the underlying biological mechanisms.

Conflict of interest

The authors reported that they have no conflicts of interest.

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Tables

Table 1 Shapiro-Wilk test results evaluating the normality of every variable investigated.

Variables	Time	CHX		Kefir	
		Statistic	*p*-value	Statistic	*p*-value.
Level	Baseline	0.958	0.331	0.940	0 .150
	14 days	0.980	0.882	0.967	0.450
	28 days	0.951	0.178	0.961	0.330
Concentration	Baseline	0.955	0.181	0.930	0.062
	14 days	0.966	0.550	0.944	0.073
	28 days	0.947	0.096	0.940	0.093
Distance	Baseline	0.933	0.066	0.948	0.077
	14 days	0.965	0.338	0.955	0.222
	28 days	0.980	0.882	0.967	0.450
VAS	Baseline	0.950	0.177	0.961	0.330
	14 days	0.977	0.556	0.954	0.222
	28 days	0.957	0.101	0.944	0.116

Significant: $p < 0.05$; Non Significant: $p > 0.05$.

Table 2: Tests of descriptive statistical analysis of Halimeter measurements among groups and time

Variables.	Time	CHX		Kefir	
		Mean	±SD	Mean	±SD
Level	Baseline	2.367	0.102	2.333	0.100
	14 days	1.500	0.093	1.400	0.091
	28 days	0.833	0.069	0.733	0.082
Concentration	Baseline	0.963	0.080	0.923	0.069
	14 days	0.487	0.015	0.427	0.025
	28 days	0.187	0.027	0.103	0.016
Distance	Baseline	84.000	2.473	84.333	2.656
	14 days	58.667	1.496	52.000	2.510
	28 days	24.333	3.413	12.667	2.086
VAS	Baseline	5.700	0.160	5.667	0.182
	14 days	2.600	0.123	1.900	0.111
	28 days	0.667	0.111	0.200	0.074

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Table 3: Multivariate test effect of time, groups and their interaction on studied variables.

Variables.	Effect		F	P value
Level	Between subjects	Groups	0.528	0.471
	Within subject	Time	311.398	**0.000**
		Time*Groups	0.234	0.792
Concentration	Between subjects	Groups	1.594	0.212
	Within subject	Time	19.426	**0.000**
		Time*Groups	0.159	0.853
Distance	Between subjects	Groups	3.942	0.052
	Within subject	Time	789.359	**0.000**
		Time*Groups	7.424	**0.001**
VAS	Between subjects	Groups	7.355	**0.009**
	Within subject	Time	973.513	**0.000**
		Time*Groups	6.488	**0.003**

Significance is indicated by $p < 0.05$, $p = 0.000$* meaning $p < 0.001$ (highly significant). * Marks significant results. F-value is the ANOVA statistic for changes over time.

Table 4: Multiple pairwise comparison of Level among time and groups using Bonferroni.

Groups	Time periods		Mean Difference (I-J)	Std. Error	*P value'*	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CHX	Baseline	14 days	0.867	0.073	**0.000***	0.686	1.047
		28 days	1.533	0.092	**0.000***	1.307	1.760
	14 days	28 days	0.667	0.088	**0.000***	0.451	0.882
Kefir	Baseline	14 days	0.933	0.073	**0.000***	0.753	1.114
		28 days	1.600	0.092	**0.000***	1.374	1.826
	14 days	28 days	0.667	0.088	**0.000***	0.451	0.882

The difference between two time points is represented by the mean difference (I–J); Standard Error reflects the precision of this difference. A *p*-value below 0.05 indicates statistical significance, with * denoting significant differences. The 95% Confidence Interval provides the range likely containing the true mean difference, defined by the Lower and Upper Bounds. A *p*-value of 0.000* signifies $p < 0.001$, indicating a highly significant difference.

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Table 5: Multiple pairwise comparisons of Concentration among time using Bonferroni.

Groups	Time periods		Mean Difference (I-J)	Std. Error	*P value*	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CHX	Baseline	14 days	0.477	0.066	**0.000***	0.314	0.639
		28 days	0.777	0.065	**0.000***	0.618	0.936
	14 days	28 days	0.300	0.019	**0.000***	0.253	0.347
Kefir	Baseline	14 days	0.497	0.066	**0.000***	0.334	0.659
		28 days	0.820	0.065	**0.000***	0.661	0.979
	14 days	28 days	0.323	0.019	**0.000***	0.276	0.370

Table 6: Multiple pairwise comparison of distance among time using Bonferroni.

Groups	Time Periods		Mean Difference(I-J)	Std. Error	*P value*	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CHX	Baseline	14 days	25.333	1.870	**0.000***	20.724	29.943
		28 days	59.667	2.387	**0.000***	53.782	65.551
	14 days	28 days	34.333	2.506	**0.000***	28.156	40.511
Kefir	Baseline	14 days	32.333	1.870	**0.000***	27.724	36.943
		28 days	71.667	2.387	**0.000***	65.782	77.551
	14 days	28 days	39.333	2.506	**0.000***	33.156	45.511

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Table 7: Multiple pairwise comparison of VAS among time using Bonferroni .

.	Time periods		Mean Difference (I-J)	Std. Error	*P value*	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CHX	Baseline	14 days	3.100	0.140	**0.000***	2.755	3.445
		28 days	5.033	0.167	**0.000***	4.622	5.445
	14 days	28 days	1.933	0.102	**0.000***	1.681	2.185
Kefir	Baseline	14 days	3.767	0.140	**0.000***	3.422	4.112
		28 days	5.467	0.167	**0.000***	5.055	5.878
	14 days	28 days	1.700	0.102	**0.000***	1.448	1.952

Figures

Figure 1: Materials and device used in the study

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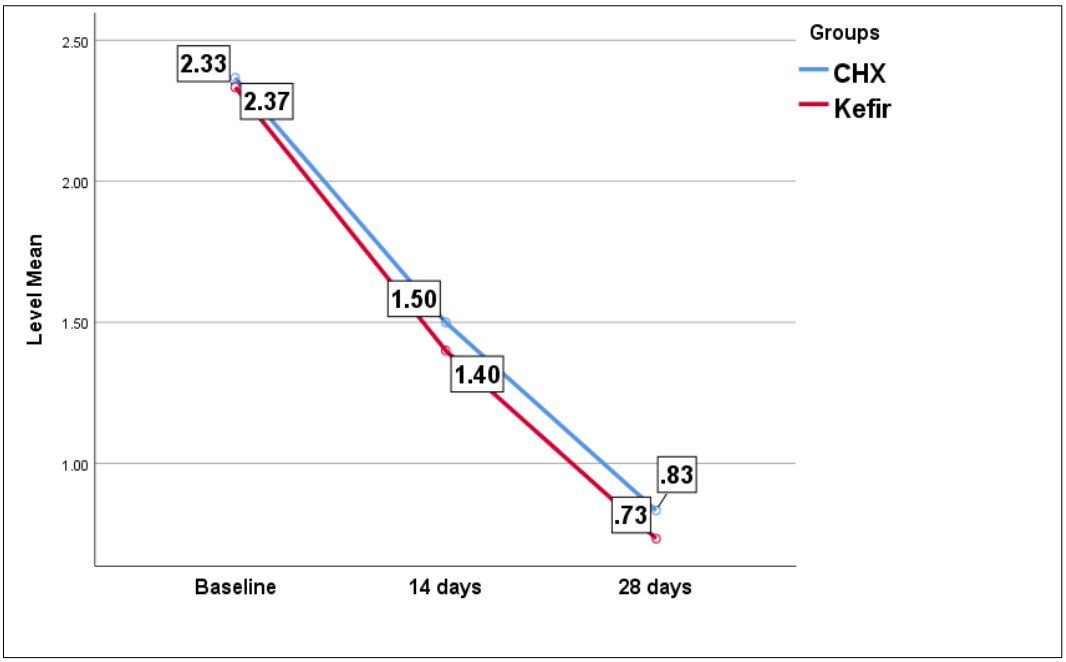

Figure 2: Line chart representation for mean values level of halitosis of both study groups

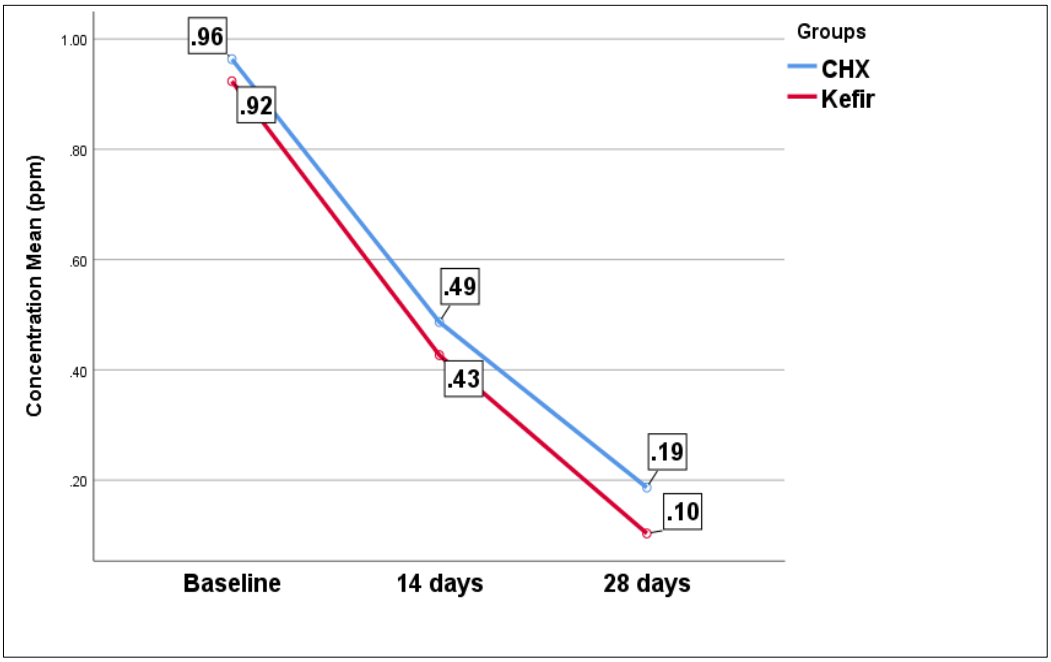

Figure 3: Line chart representation for mean values concentration of halitosis of both study groups

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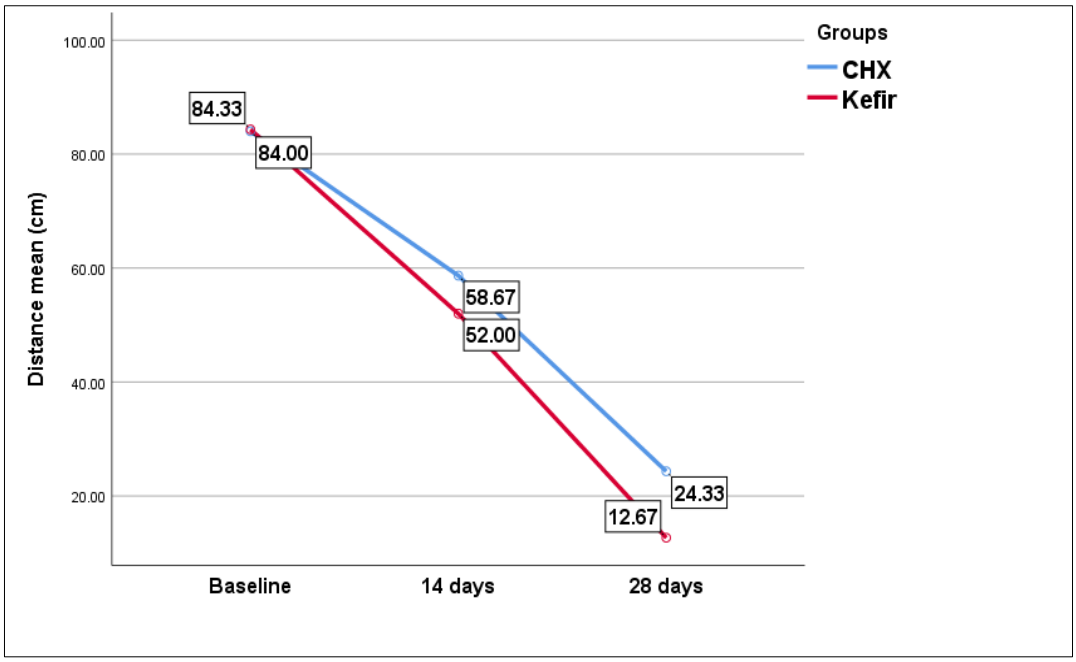

Figure (4): Line chart representation for mean values distance of halitosis of both study groups

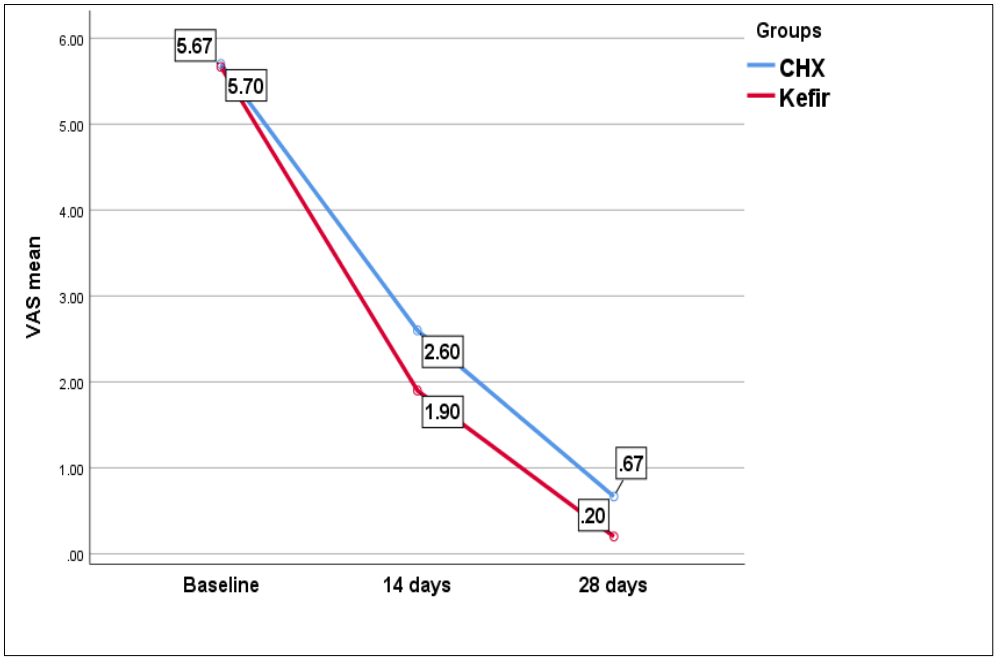

Figure 5: Line chart representation for mean values VAS halitosis of both study groups.