Comparative Analysis of Antibacterial Effects of Homemade and Commercial Mouthwashes on Bacteria Cultured from a Dirty Kitchen Sponge

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Abstract

This study evaluates the antibacterial effectiveness of five mouthwash types: two commercial formulations (alcoholic and non-alcoholic) and three homemade preparations (sodium chloride, turmeric, and cinnamon). The objective was to determine their relative efficacy in reducing bacterial growth and to assess the potential of homemade alternatives as cost-effective substitutes. A used kitchen sponge was prepared under controlled conditions to promote bacterial growth, swabbed onto a divided tray, and then treated with the different mouthwashes. Bacterial samples were collected pre- and post-treatment, cultured on agar plates, and analyzed quantitatively using colony-forming unit (CFU) counts. A one-way ANOVA revealed significant differences in CFU reduction across treatments ($F(5,12) = 16.02$, $p < 0.001$). Turmeric mouthwash exhibited the greatest reduction, decreasing CFUs by 704, followed by cinnamon (540.44) and alcoholic commercial mouthwash (295.33). Saltwater showed the least effectiveness, with a reduction of 150.56 CFUs. Qualitative observations supported these findings, highlighting differences in bacterial growth density and morphology. The results suggest that while commercial mouthwashes provide consistent antibacterial effects, certain homemade alternatives, particularly turmeric and cinnamon, demonstrate significant potential. These findings underscore the value of natural formulations and contribute to understanding alternative antibacterial treatments.

Introduction

Mouthwash plays a critical role in oral hygiene by targeting bacteria responsible for dental issues such as cavities and gum disease (Radzki et al., 2022). While commercial mouthwashes, both alcoholic and non-alcoholic, are widely used due to their potent antibacterial agents, homemade alternatives have gained popularity for their accessibility and perceived natural benefits (Duane et al., 2023). However, the comparative effectiveness of these options on bacterial growth remains underexplored.

This study investigates the antibacterial effects of five types of mouthwashes, including two commercial formulations and three homemade preparations (sodium chloride, turmeric, and cinnamon). Commercial mouthwashes are widely recognized for their effectiveness, as they contain active ingredients such as chlorhexidine, chlorine dioxide, cetylpyridinium chloride, and essential oils, all of which have demonstrated strong antibacterial properties (Radzki et al., 2022). The primary distinction between the two commercial mouthwashes selected for this study is the presence or absence of alcohol.

Background research informed the choice of the three homemade mouthwashes. Sodium chloride (NaCl), commonly known as table salt, has been recognized for its antimicrobial properties, particularly in food preservation. Its effectiveness stems from its ability to create a hypertonic environment, leading to osmotic stress that inhibits bacterial growth (Li et al., 2021). Turmeric mouthwash was chosen because Alonso-Español et al. (2023) demonstrated that an active ingredient in turmeric, curcumin, showed antimicrobial properties. Similarly, cinnamon, particularly its active component cinnamaldehyde, has demonstrated significant antimicrobial properties. Research indicates that cinnamaldehyde exhibits bactericidal activity against pathogens such as *Listeria monocytogenes*, disrupting bacterial cell membranes and inhibiting essential cellular functions (Gill & Holley, 2004). The findings of these various studies support the inclusion of homemade formulations in this study to evaluate their effectiveness against bacteria from non-oral environments.

Kitchen sponges provide an ideal source of bacteria due to their ability to harbour diverse bacterial populations, simulating a challenging environment for antibacterial agents (Wu et al., 2023). The objectives of this research are to determine the relative effectiveness of these

mouthwash types in reducing bacterial growth and to evaluate the viability of homemade alternatives as cost-effective substitutes for commercial products.

The hypothesis posits that commercial mouthwashes will exhibit superior antibacterial efficacy compared to homemade alternatives due to their targeted formulations. Conversely, the null hypothesis suggests no significant difference among the mouthwash types in reducing bacterial growth. A model of bacterial reduction across different treatment groups is proposed, with bacterial growth quantified through colony-forming unit counts or surface area coverage on agar plates. This study's findings have implications for understanding the effectiveness of alternative antibacterial treatments and may inform consumer choices, particularly in low-resource settings. By comparing commercial and homemade mouthwashes, this investigation bridges a gap in the literature and provides insights into practical applications of accessible antibacterial agents.

Methods

To assess the effectiveness of various mouthwash treatments on bacterial growth, a used kitchen sponge was prepared under controlled conditions to promote bacterial growth. The sponge was regularly provided with nutrients from food residues in the kitchen sink and was kept in a plastic container at approximately 25°C without sanitization for two weeks before the experiment.

We prepared five different types of mouthwashes. For the saltwater treatment, we dissolved 5g of salt in 240 mL of boiling water and stirred until fully dissolved. For the turmeric treatment, we added 5g of turmeric powder to 240 mL of boiling water and stirred thoroughly. Similarly, for the cinnamon treatment, we added 5g of cinnamon powder to 240 mL of boiling

water and stirred until the powder was stirred thoroughly. For both turmeric and cinnamon, their particles were inside the solutions and did not dissolve completely. We then transferred 50 mL of each homemade mouthwash into separate clean containers. Similarly, we transferred 50 mL of the alcohol-based commercial mouthwash into one clean container and 50 mL of the alcohol-free commercial mouthwash into another clean container.

We divided a tray into six equal sections and assigned each section to a specific treatment. Following that, we used the sponge to scrub the surface of the tray for one minute. Using 10 mL Plastic Dropper Pipettes, we treated one section with distilled water and the remaining sections with one of the five mouthwashes. We almost applied a fixed volume of each mouthwash to its designated section and distributed it evenly across the surface (Figure 1).

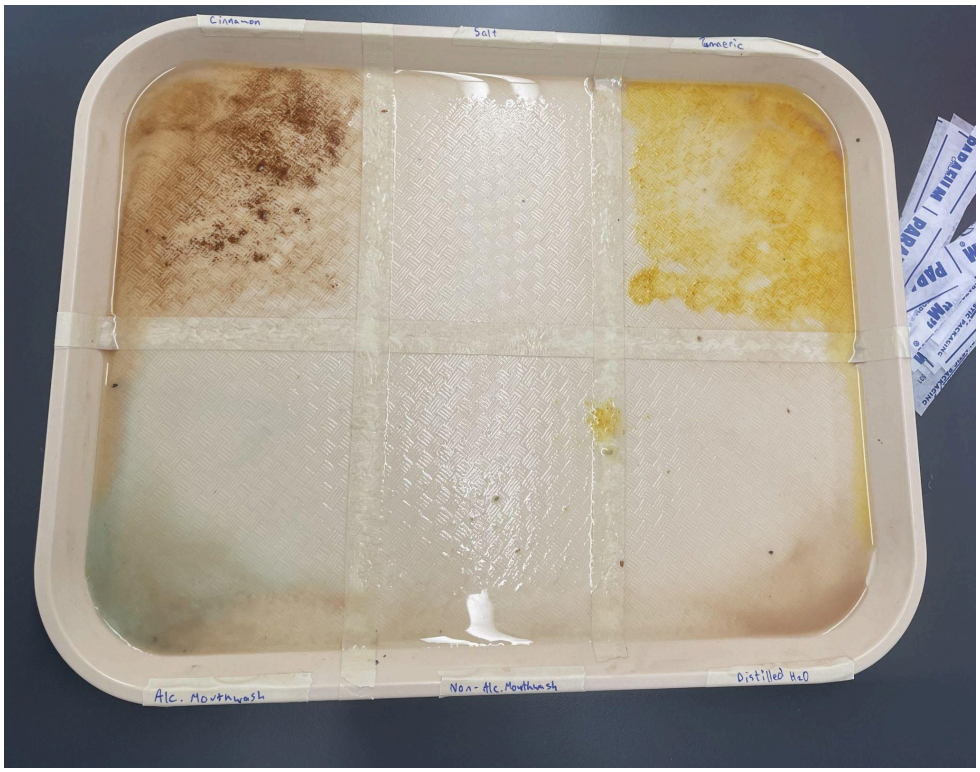

Figure 1. Tray setup displaying six sections, each assigned to a specific treatment: saltwater, turmeric, cinnamon, alcohol-based commercial mouthwash, alcohol-free commercial mouthwash, and distilled water. Visible colour differences are due to the mouthwash treatments applied. This image was captured during the experimental setup phase.

We collected bacterial samples before and after treatment using sterile cotton swabs. After collection, we transferred the samples to labelled agar plates, spread them evenly, and incubated the plates at 37°C. Four plates were prepared per section: one for pre-treatment and three for post-treatment.

After 10 days of incubation, we observed bacterial colonies under a light microscope at 90x magnification. We then counted the number of colonies using OpenCFU and ImageJ software, organized the collected raw data into treatment categories with three replicates per treatment and one control (pre-treatment) group. Subsequently, we calculated the average number of colonies across replicates for each treatment. Following this, we performed a one-way ANOVA test to compare the reduction in colony count across treatments relative to the initial bacterial count on the tray surfaces before the application of the mouthwashes. Since the one-way ANOVA test yielded a p-value of < 0.001, we proceeded with Tukey's HSD post-hoc analysis to identify significant pairwise differences between treatments. Finally, to complete our data collection, we recorded qualitative data such as size, shape, texture, colour, and density.

Results

After completing the one-way ANOVA, the six groups—Saltwater, Turmeric, Cinnamon, Alcoholic Commercial, Non-alcoholic Commercial, and Distilled Water (control)—demonstrated significant differences in average bacterial growth, measured as colony counts ($F(5,12) = 16.02$, $p = 0.00006$).

Figure 2 demonstrates the effectiveness of the five types of mouthwash and distilled water in comparison to their control groups. The treatment groups consistently show lower bacterial colony counts compared to their respective control groups. Moreover, the plot

illustrates varying degrees of reduction among the mouthwash treatments, with turmeric and cinnamon showing the most significant reductions, while saltwater shows the least reduction compared to their respective control group.

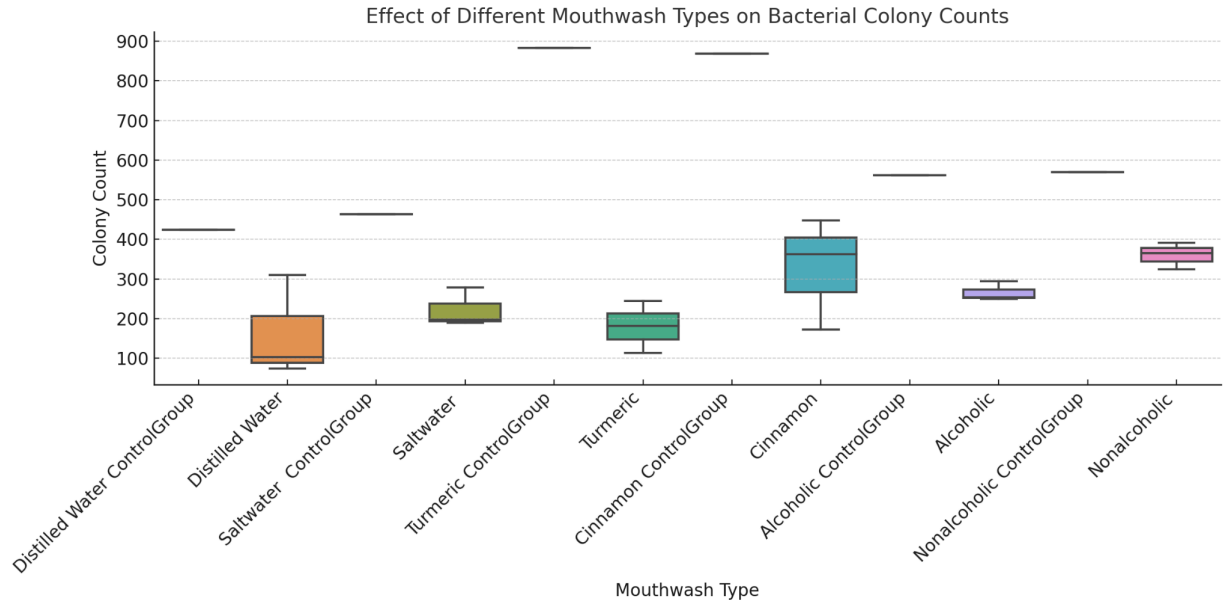

Figure 2. Boxplot shows the effects of different mouthwash treatments on bacterial colony counts. Each box represents the distribution of bacterial growth for treatment and control groups of each mouthwash type. The horizontal line within each box indicates the median colony count, and the interquartile range (IQR) reflects the spread of the data. The whiskers show the full range of colony counts, excluding outliers. Treatment groups exhibit lower bacterial colony counts compared to their respective control groups. Among the treatments, Turmeric Mouthwash demonstrates the greatest reduction in bacterial colonies, while Saltwater Mouthwash showes the least reduction.

To further analyze the data, a Tukey's HSD test was performed. The test suggests significant differences in bacterial growth reduction for several treatments:

- Turmeric Mouthwash showed significant differences in effectiveness compared to Non-Alcoholic Mouthwash ($p = 0.0002$), Saltwater Mouthwash ($p = 0.0003$), Distilled Water ($p = 0.0005$), and Alcoholic Mouthwash ($p = 0.0009$).

• Cinnamon Mouthwash showed significant differences in effectiveness compared to Alcoholic Mouthwash ($p = 0.0415$), Distilled Water ($p = 0.0181$), Non-alcoholic Mouthwash ($p = 0.0055$), and Saltwater Mouthwash ($p = 0.0116$).

No significant differences were observed for comparisons such as Alcoholic Mouthwash vs. Distilled Water ($p = 0.9956$) and Cinnamon Mouthwash vs. Turmeric Mouthwash ($p = 0.2599$).

Figure 4a shows the general colony distribution before treatment, where bacterial colonies are generally small with slight variations in size, circular in shape, and have a smooth texture. The colonies exhibit a translucent appearance and are densely distributed across the agar surface. Figure 4b shows the bacterial colonies after treatment with alcoholic mouthwash. The colonies appear larger, with more variation in size, and they retain a circular shape across the agar surface. The colonies have a smooth texture and a slightly off-white colour. Treatment with the alcohol-free mouthwash showed similar results. Figure 4c was taken after treatment with turmeric mouthwash, which illustrates small to medium-sized colonies, round with smooth edges, and small variations in size. The colour of the colonies is off-white. Treatment with the other homemade mouthwashes produced almost identical results. Figure 4d shows the colonies after treatment with distilled water. The small to medium in size colonies are distributed across the surface. The colony shapes are mostly circular, and the texture is smooth. The colour of the colonies appears off-white to cream.

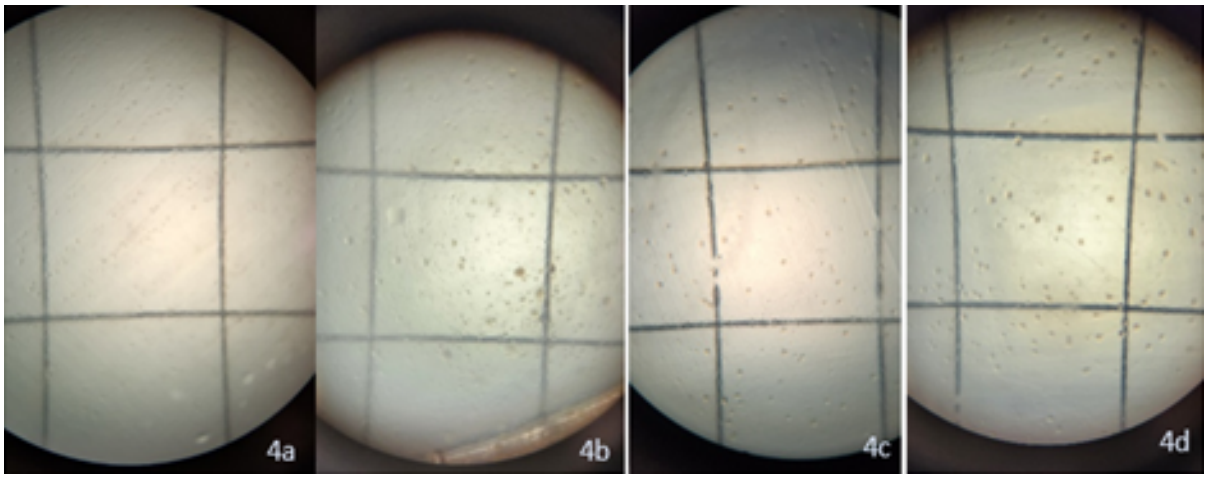

Figure 3. Microscopic observations of bacterial colonies under light microscopy at 90x magnification. Panel 4a shows the bacterial colonies before any treatment, with dense and evenly distributed colonies. Panel 4b displays the colonies after treatment with alcoholic mouthwash, showing an increase in some colony sizes and a change in colony colour. Panel 4c shows the effect of turmeric mouthwash, where most colonies increased in size. Panel 4d depicts the colonies after treatment with distilled water, with colonies appearing larger and more densely packed compared to the treated groups.

Discussion

The null hypothesis that there are no significant differences among the mouthwash types in reducing bacterial growth was rejected. The statistical test, one-way ANOVA, presented a p-value below 0.05 indicating a statistical significance. The observed differences partially refuted the hypothesis that commercial mouthwashes would exhibit superior antibacterial efficacy compared to homemade alternatives. Turmeric and cinnamon mouthwashes demonstrated the greatest ability to reduce the bacterial colony forming units (CFU), while saltwater was the least effective. So the turmeric and cinnamon mouthwashes were more effective in reducing bacterial growth than the commercial mouthwashes.

These results could be due to the antibacterial properties that cinnamon and turmeric contain due to the active components, curcumin and cinnamaldehyde, which align with previous research (Gill & Holley, 2004). Another possible explanation is that commercial mouthwashes

are designed to target oral bacteria, as mentioned in previous studies (Mensitieri et al., 2023). A third possible explanation could be that the sponge contained multiple different bacterial populations. The bacteria found in the oral environment could not have been present on the sponge which would explain the higher bacterial growth on the plates with commercial mouthwash. Contrastingly, turmeric and cinnamon's active compounds target a broader range of bacteria, which could be the reason it was better than the commercial forms (Hussain et al., 2022).

Saltwater mouthwash being the least effective could be due to the 2% concentration because a higher concentration could demonstrate more antibacterial properties on the bacteria present (Fabrizio et al., 2024). Additionally, the bacteria present on the tray may have been more adaptable and resilient than those found in the mouth, previous literature mentions saltwater's effectiveness on oral bacteria (Ballini et al., 2020).

Several sources of variation could have influenced the results. There were most likely a lot of different types of bacterial populations on the kitchen sponge, and scrubbing the tray may have distributed different types of bacteria unevenly across the sections. Variation could have also arisen from the different techniques in mouthwash application and swabbing, due to multiple experimenters, which could have also affected the results.

The study also faced limitations such as the inability to utilize human oral samples to mimic the bacteria in an oral setting. If the study were to be replicated again, it would be beneficial to utilize bacteria found in the mouth to improve the accuracy. Another limitation was the CFU counting method, which may have underestimated the bacterial count due to the difficulty in seeing the colonies in the microscope images. Human error when taking pictures or

utilizing the software may have also contributed to the inaccuracies. Future experiments should find a more precise way to take pictures to minimize counting errors.

Conclusion

In conclusion, cinnamon and turmeric mouthwashes demonstrated the least bacterial growth, as opposed to commercial mouthwashes and saltwater. These results challenge the hypothesis that commercial mouthwashes would be more effective than traditional forms. This study suggests that homemade traditional mouthwashes, particularly turmeric and cinnamon, may be a more cost-effective and accessible alternative to commercial mouthwashes.

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Appendix

Table 1: Raw Data for Bacterial Colony Count Across Treatments

Treatment Group	Colony Count 1	Colony Count 2	Colony Count 3	Control Group Average Colony Count
Saltwater	278.67	197.67	190.00	464.00
Turmeric	113.00	181.33	244.67	883.67
Cinnamon	448.00	362.33	172.33	868.00
Alcoholic	254.00	294.00	250.00	561.33
Nonalcoholic	325.00	364.67	391.33	570.33
Distilled Water	74.67	310.67	103.67	423.67

***Table 1*:** Table presents the raw bacterial colony counts for six different treatments: Saltwater, Turmeric, Cinnamon, Alcoholic Commercial, Nonalcoholic Commercial, and Distilled Water, along with their respective control groups. The colony counts represent three replicates for each treatment group. The "Control Group Average Colony Count" column shows the average colony count for the corresponding control group.

Table 2: ANOVA Results for Bacterial Growth Comparison Among Mouthwash Treatments

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	5	599923.2401	119984.648	16.0246	0.00005998
Error (within groups)	12	89850.5925	7487.5494		
Total	17	689773.8327	40574.9313		

***Table 2*:** Table presents the results of a one-way Analysis of Variance (ANOVA) test conducted to compare bacterial growth (measured by colony counts) across six different mouthwash treatments: Saltwater, Turmeric, Cinnamon, Alcoholic Commercial, Nonalcoholic Commercial, and Distilled Water (control). The table includes the degrees of freedom (DF), sum of squares (SS), mean square (MS), F statistic, and p-value for the between-group (treatment) variance, within-group (error) variance, and total variance. A p-value of 0.00005998 indicates statistically significant differences in bacterial growth among the mouthwash treatments.

Table 3: Tukey's HSD Pairwise Comparisons

Group1	Group2	Mean Diff	p-value	Lower CI	Upper CI	Reject
Alcoholic	Cinnamon	245.1111	0.0415	7.7968	482.4255	TRUE
Alcoholic	Distilled Water	-34.6667	0.9956	-271.981	202.6477	FALSE
Alcoholic	Nonalcoholic	-85.3333	0.8252	-322.648	151.981	FALSE
Alcoholic	Saltwater	-53.4444	0.97	-290.759	183.8699	FALSE
Alcoholic	Turmeric	408.6667	0.0009	171.3523	645.981	TRUE
Cinnamon	Distilled Water	-279.778	0.0181	-517.092	-42.4634	TRUE
Cinnamon	Nonalcoholic	-330.444	0.0055	-567.759	-93.1301	TRUE
Cinnamon	Saltwater	-298.556	0.0116	-535.87	-61.2412	TRUE
Cinnamon	Turmeric	163.5556	0.2599	-73.7588	400.8699	FALSE
Distilled Water	Nonalcoholic	-50.6667	0.976	-287.981	186.6477	FALSE
Distilled Water	Saltwater	-18.7778	0.9998	-256.092	218.5366	FALSE
Distilled Water	Turmeric	443.3333	0.0005	206.019	680.6477	TRUE
Nonalcoholic	Saltwater	31.8889	0.997	-205.426	269.2032	FALSE
Nonalcoholic	Turmeric	494	0.0002	256.6857	731.3143	TRUE
Saltwater	Turmeric	462.1111	0.0003	224.7968	699.4255	TRUE

Table 3: Table shows the results of Tukey's Honest Significant Difference (HSD) test for pairwise comparisons between six treatment groups: Alcoholic, Cinnamon, Distilled Water, Nonalcoholic, Saltwater, and Turmeric. For each pairwise comparison, the mean difference in values, p-value, 95% confidence intervals (CI), and statistical significance (Reject or Fail to Reject null hypothesis) are provided. Statistical significance is indicated by a p-value of less than 0.05 (denoted as "TRUE" in the "Reject" column). Significant pairwise differences are observed between the following treatment pairs: Alcoholic vs. Cinnamon, Alcoholic vs. Turmeric, Cinnamon vs. Distilled Water, Cinnamon vs. Nonalcoholic, Cinnamon vs. Saltwater, Distilled Water vs. Turmeric, Nonalcoholic vs. Turmeric, and Saltwater vs. Turmeric. No significant differences were found in the other comparisons.

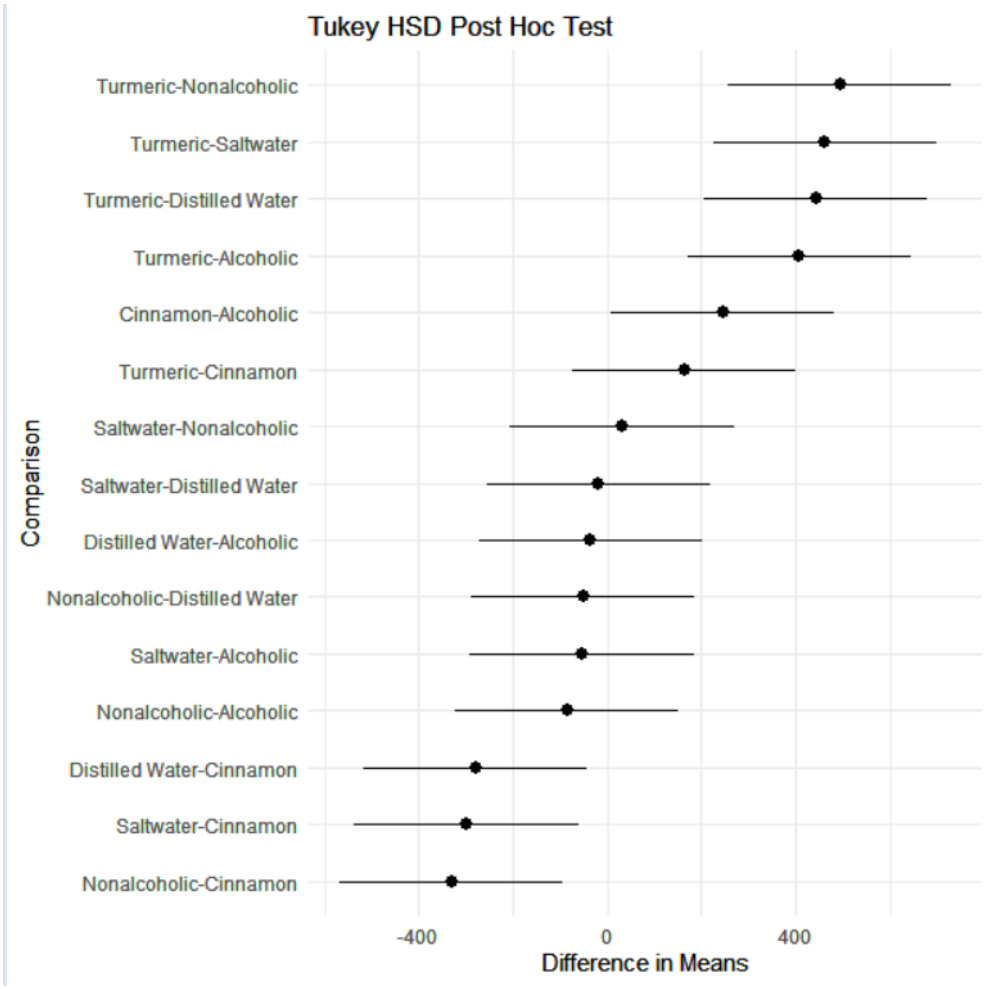

Figure 4. Tukey's HSD Post-Hoc Test Results showing pairwise comparisons of mouthwash treatments. The plot displays the difference in means for each comparison, with confidence intervals. Significant differences are indicated where the confidence intervals do not cross zero. The treatments compared include Turmeric, Cinnamon, Alcoholic, Nonalcoholic, Saltwater, and Distilled Water.