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COMPARISON OF THE EFFECTIVENESS OF RED AND YELLOW WATERMELON EXTRACTS AS NATURAL TOOTH BLEACHING AGENTS ON DISCOLORED TEETH

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ABSTRACT

Background: Red and yellow watermelon (*Citrullus lanatus*) cultivars may differ in their metabolite composition. Such differences could be relevant to the biological properties of their extracts, although the contribution of individual compounds to tooth-colour change has not been established.**Objective:** To compare tooth-colour changes following treatment with red watermelon extract, yellow watermelon extract, and 15% carbamide peroxide, and to report the malic-acid concentrations stated in the source manuscript.

Methods: Thirty extracted permanent premolars were stained in black tea for seven days and assigned to three groups (n=10). The buccal surfaces were treated for four hours daily over 14 days. Tooth colour was measured before and after treatment using a VITA Easyshade spectrophotometer. Color difference (ΔE) was compared using the Kruskal–Wallis test and pairwise Mann–Whitney U tests with Holm adjustment. The source manuscript reports HPLC-derived malic-acid concentrations of 12,893.39 mg/kg in red watermelon extract and 12,007.76 mg/kg in yellow watermelon extract; the underlying chromatograms and validation records were not supplied for independent verification.

Results: Mean ΔE was 14.04 (SD 3.02) for red watermelon extract, 17.44 (SD 4.00) for carbamide peroxide, and 7.95 (SD 4.42) for yellow watermelon extract. The overall group difference was significant (H=14.56, df=2, p=0.0007; epsilon-squared=0.465). Red and yellow extracts differed (Holm-adjusted p=0.0146), as did carbamide peroxide and yellow extract (p=0.0030); the red-extract and carbamide-peroxide comparison was not significant (p=0.0640).

Conclusion: The extracts were associated with different tooth-colour changes in this *in vitro* model. The reported malic-acid values suggest a modest difference between the extracts but do not demonstrate that malic acid caused the observed effects. Further validated phytochemical profiling and direct testing of enamel safety are required.

KEYWORDS: *Citrullus lanatus*; Red watermelon; Yellow watermelon; Malic acid; Phytochemical composition; Tooth-colour change; *In vitro* study

INTRODUCTION

Watermelon (*Citrullus lanatus*) is cultivated in varieties that differ in flesh colour and biochemical composition. Differences among cultivars may be reflected in their metabolite profiles, and comparative metabolomic studies have reported distinct profiles in red- and yellow-fleshed watermelon. However, cultivar-level metabolite differences should not be assumed to explain a biological effect unless the relevant compounds are measured and their contributions are experimentally evaluated [1].

Plant-derived extracts contain mixtures of organic acids and other metabolites. Their composition can vary with cultivar, growing conditions, maturity, processing, extraction solvent, and storage. Consequently, a description such as "100% extract" is not a reproducible chemical specification [2-3]. Comparative studies should report extraction yield, solvent removal, final vehicle, analytical standards, chromatographic conditions, and the concentration of each identified compound. Where High-Performance Liquid Chromatography (HPLC) is used, the analytical method should include appropriate calibration, specificity, precision, recovery, limits of detection and quantification, and representative chromatograms [4-6].

Tooth discoloration may be extrinsic or intrinsic, and bleaching commonly involves hydrogen peroxide or carbamide peroxide. Natural preparations have also been investigated for tooth-colour modification, but their effects and safety depend on the specific preparation and exposure conditions. In particular, acidic formulations may affect enamel surface properties; therefore, colour change alone cannot establish safety or suitability for clinical use [7].

Red watermelon extract has previously been investigated in relation to tooth whitening, while metabolomic research has compared red and yellow watermelon cultivars. Nevertheless, the available literature does not establish that a particular watermelon metabolite, including malic acid, is responsible for any observed tooth-colour change [8]. A comparison of extract composition alongside a controlled *in vitro* bleaching experiment may provide preliminary information, but a causal relationship requires additional analytical and mechanistic evidence [9].

The study does not include genetic analysis or gene-level investigation. The present study compared changes in the colour of tea-stained extracted premolars treated with red watermelon extract, yellow watermelon extract, or 15% carbamide peroxide [10]. The manuscript also reports malic-acid concentrations attributed to HPLC analysis. Because the HPLC source report was not available with the supplied dataset, those concentrations are presented as values reported by the authors

MATERIALS AND METHODS

Study design and specimens

This laboratory experimental study used a pretest–posttest design with a positive-control group. Thirty extracted permanent premolars were randomly allocated into three treatment groups (n=10 per group): 100% red watermelon extract, 15% carbamide peroxide, and 100% yellow watermelon extract [11]. Inclusion criteria were permanent premolars with intact crowns. Exclusion criteria were teeth with calculus, caries, restorations, or fractures. Ethical approval was obtained from the Research Ethics Committee of Universitas Prof. Dr. Moestopo (Beragama) (approval No. 98/KEPK/FKGUPDMB/VI/2026. All tooth samples were immersed in 20-ml containers filled with black tea solution for 7 days. The solution was replaced every 24 hours, and the samples were rinsed with distilled water between solution changes. After 7 days, the teeth samples were removed, cleaned, and dried. [12-15].

Preparation of watermelon extracts

Red and yellow watermelon fruits were washed, cut with the rinds, and dried in an oven at 60°C. The dried material was ground to powder. Each powder was macerated in 96% ethanol at a ratio of 100 g powder to 900 mL ethanol for 3 × 24 hours [16]. The mixture was filtered, the residue was remacerated, and the liquid extracts were combined and concentrated using a rotary evaporator[17].

HPLC analysis of malic acid

The source manuscript reports malic-acid concentrations of 12,893.39 mg/kg in red watermelon extract and 12,007.76 mg/kg in yellow watermelon extract [18-20]. The HPLC report was not included in the supplied raw-data workbook.[21].

Tooth staining and treatment

The specimens were immersed in black tea solution for seven days. The solution was replaced every 24 hours, and the teeth were rinsed with distilled water at each change [22]. After staining, the teeth were cleaned, dried, and mounted upright in gypsum with the buccal surface facing forward. Initial colour was measured using a calibrated VITA Easyshade spectrophotometer. The buccal surfaces were treated with the assigned material for four hours daily over 14 days and rinsed with distilled water after each application [23-24].

Colour measurement and statistical analysis

Lightness (L), chroma (C), and hue (H) were recorded. For each specimen, ΔE was calculated from the recorded differences as $\sqrt{[(L_2-L_1)^2 + (C_2-C_1)^2 + (H_2-H_1)^2]}$, as stated in the supplied workbook. Descriptive statistics were calculated for each group [25,26]. Pre–post comparisons were evaluated using paired t-tests. The group comparison of ΔE used the Kruskal–Wallis test, followed by pairwise Mann–Whitney U tests with Holm adjustment [27]. Two-sided $p<0.05$ was considered statistically significant.[28-30].

RESULTS

Reported malic-acid concentrations

The original manuscript reports malic-acid concentrations of 12,893.39 mg/kg in red watermelon extract and 12,007.76 mg/kg in yellow watermelon extract, a difference of 885.63 mg/kg. These figures are reproduced as author-reported results; the underlying HPLC report was not available in the supplied workbook and therefore could not be independently checked [31].

Changes in tooth-colour parameters

The supplied workbook contained 10 specimens per group. Lightness and chroma increased in all three groups. Hue increased numerically in all groups, but the paired comparison was statistically significant only in the red watermelon extract group [32]. The group summaries are presented in Tables 1 and 2.

Table 1: Pre- and post-treatment colour parameters. Values are mean ± standard deviation. P-values are paired t-test results recalculated from the supplied specimen-level data.

Group	Parameter	Before, mean ± SD	After, mean ± SD	Paired t-test p
Red watermelon extract	Lightness (L)	7.78 ± 3.29	16.88 ± 2.12	<0.001
Red watermelon extract	Chroma (C)	6.88 ± 2.00	17.07 ± 4.11	<0.001
Red watermelon extract	Hue (H)	-1.80 ± 0.91	-0.78 ± 1.09	0.0455
15% carbamide peroxide	Lightness (L)	5.63 ± 2.96	20.86 ± 2.84	<0.001
15% carbamide peroxide	Chroma (C)	5.88 ± 1.70	13.09 ± 1.14	<0.001
15% carbamide peroxide	Hue (H)	-2.92 ± 2.66	-1.49 ± 1.91	0.2209
Yellow watermelon extract	Lightness (L)	11.36 ± 5.75	16.70 ± 2.25	0.004

Yellow watermelon extract	Chroma (C)	11.04 ± 4.87	15.83 ± 2.89	0.0016
Yellow watermelon extract	Hue (H)	-1.83 ± 1.00	-1.41 ± 1.09	0.2877

Table 2: Colour difference (ΔE) by treatment group, recalculated from the supplied workbook

Group	n	ΔE mean ± SD	ΔE median	Range	Shapiro–Wilk p
Red watermelon extract	10	14.04 ± 3.02	13.64	8.85–20.73	0.242
15% carbamide peroxide	10	17.44 ± 4.00	18.35	9.76–22.43	0.371
Yellow watermelon extract	10	7.95 ± 4.42	7.65	2.13–15.61	0.772

The Kruskal–Wallis test showed an overall difference in ΔE among the three groups (H=14.56, df=2, p=0.0007; epsilon-squared=0.465). Pairwise Mann–Whitney U tests with Holm adjustment are shown in Table 3.

Table 3: Pairwise comparisons of ΔE. Holm-adjusted p-values control the family-wise error rate across the three comparisons

Comparison	Mann–Whitney U	Unadjusted p	Holm-adjusted p	Interpretation
Red watermelon extract *vs.* 15% carbamide peroxide	25	0.064	0.064	Not significant
Red watermelon extract *vs.* Yellow watermelon extract	86	0.0073	0.0146	Significant
15% carbamide peroxide *vs.* Yellow watermelon extract	94	0.001	0.003	Significant

DISCUSSION

In this *in vitro* experiment, all three treatment groups showed increases in mean lightness and chroma after treatment. Mean ΔE was largest in the 15% carbamide-peroxide group, followed by red watermelon extract and yellow watermelon extract. The overall difference among groups was statistically significant. Post-hoc testing showed that the yellow extract group differed from both the red extract and carbamide-peroxide groups, whereas the red-extract and carbamide-peroxide comparison was not statistically significant after adjustment.

The reported malic-acid concentration was higher in red than in yellow watermelon extract. This observation may be relevant to future investigations of cultivar-specific extract composition, but it does not establish that malic acid caused the larger colour change observed with red extract. The study did not isolate malic acid, manipulate its concentration, quantify the full metabolite profile, or test a mechanistic pathway. However, the reported malic acid concentrations should be interpreted cautiously, as the underlying HPLC data were not available for independent verification.

Published metabolomic work has reported differences between red and yellow watermelon cultivars. Such research provides a rationale for comprehensive chemical profiling, but it cannot substitute for profiling the exact fruit batches and extracts used in this experiment. Future studies should include validated chromatographic or mass-spectrometric analyses of the study extracts to identify and compare their phytochemical constituents. Any proposed relationship between a compound and bleaching activity should be tested using appropriate controls, dose-response experiments, and, where feasible, compound-specific or mechanistic experiments.

The absence of a statistically significant difference between red watermelon extract and carbamide peroxide does not demonstrate equivalence or non-inferiority. Such conclusions require a pre-specified margin and an appropriate study design. The sample size of 10 teeth per group also limits precision. Further, colour measurements do not establish enamel safety. The pH of each preparation and changes in enamel microhardness, surface roughness,morphology, or mineral content were not measured.

Other limitations include the *in vitro* design, possible variation among extracted teeth, incomplete reporting of randomization and specimen storage, and the lack of a reproducible definition of the extract concentration. The ΔE calculation should be checked against the instrument's colour-space output and accepted colour-difference convention. These limitations should be considered when interpreting the findings of this study. Therefore, the results should be interpreted cautiously, and further studies with improved methodological standardization and comprehensive extract characterization are warranted.

CONCLUSION

Within the limitations of this *in vitro* study, red and yellow watermelon extracts were associated with changes in the measured colour of tea-stained premolars. Red watermelon extract produced a larger mean ΔE than yellow watermelon extract, while the difference between red watermelon extract and 15% carbamide peroxide was not statistically significant in the adjusted pairwise comparison. The reported malic-acid values suggest a difference in extract composition, but they do not establish a causal mechanism. Additional validated phytochemical profiling, reproducible extract characterization, and enamel-safety testing are required. The present dataset does not support genetic or molecular conclusions.

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