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Whitening and Hydrating Property of Cucumber Fruit Extract Cucumis Sativus as A Lightening Serum

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Abstract
Recent skincare trends focused on advanced technologies and innovative treatments. These included dermal fillers, new developments in botulinum toxin, anti-aging cosmeceuticals, and the use of AI for personalized skincare treatments. This shift towards advanced and efficient skincare solutions highlighted the growing demand for innovative approaches to skin health and beauty. The study investigated the whitening and hydrating properties of cucumber (Cucumis sativus) fruit extract as a natural skin-lightening serum. With increasing consumer preference for plant-based skincare alternatives, this research study aimed to determine the effectiveness of cucumber extract in improving skin tone and moisture retention. The study employed High-Performance Liquid Chromatography (HPLC) to analyze the phytochemical composition of the extract, focusing on secondary metabolites such as vitamin C, quercetin as flavonoids, and gallic acid as phenolic acid. Additionally, a bioassay using zebrafish embryos (Danio rerio) was conducted to assess the extract's toxicity, melanin reduction effects, and impact on trans-epidermal water loss (TEWL). Results indicated that cucumber extract significantly reduces melanin production over a 72-hour period while maintaining a non-toxic profile. Furthermore, the formulated serum demonstrates stability over three weeks, supporting its feasibility for cosmetic applications. These findings highlight cucumber extract's potential as a safe, effective, and natural alternative to synthetic skin-lightening products, promoting its use in dermatological formulations.

Keywords: Cucumber Extract, Skin-Lightening Serum, Phytochemical Analysis

1. INTRODUCTION

Skincare has evolved significantly with advancements in technology, including the use of dermal fillers, botulinum toxin, AI-based personalized treatments, and nano emulsions that enhance the delivery of bioactive compounds (Gal et al., 2024). These developments reflect a growing demand for efficient and innovative skincare solutions. Alongside these advancements, there has also been a notable shift towards natural ingredients in cosmetics, as consumers seek safer, plant-based alternatives to synthetic products (Javid et al., 2024).

Cucumber (Cucumis sativus), long appreciated for its high-water content and antioxidant properties, has gained attention for its skin-lightening and hydrating effects (Chowdhury et al., 2021; Amakye et al., 2021). It contains compounds like vitamin C, caffeic acid, and silica, which are known to reduce melanin production, improve skin elasticity, and retain moisture (Siddique et al., 2022). While cucumber is

commonly used in general skincare, its specific potential as a skin-lightening serum has not been extensively explored, creating a research gap in the field of natural cosmeceuticals (Ibrahimi, 2024).
This study investigates the effectiveness of cucumber extract as a natural alternative to synthetic skin-lightening products. By evaluating its whitening and moisturizing properties through phytochemical analysis and biological assays, the research seeks to scientifically validate cucumber's use in skincare. The findings aim to support the development of safe, plant- based cosmetic solutions that align with current consumer preferences and contribute to sustainable dermatological practices.

2. Methodology

2.1 Research Design

This study employed a true experimental research design to evaluate the whitening and hydrating properties of cucumber (Cucumis sativus) fruit extract. By systematically manipulating the independent variable cucumber extract and observing its effects on the dependent variables skin lightening and hydration the research aimed to establish a clear cause-and-effect relationship. Participants were randomly assigned to either an experimental or control group to minimize bias and control for external factors. The inclusion of pre-tests and post-tests further strengthened the validity of the findings by measuring changes in skin condition before and after serum application, thereby supporting the reliability and scientific rigor of the study.

2.2 Research Locale and Sampling Technique

The experimental procedures for this study were primarily conducted at SkinPro Laboratories in San Pedro, Laguna, and in coordination with Mots Animal House Laboratory Research in Sta. Rosa, Laguna. These facilities were chosen due to their capacity to provide controlled laboratory environments, necessary equipment, and ethical research standards for zebrafish embryo experimentation. The sampling technique employed was random sampling within the experimental framework, where zebrafish embryos were randomly assigned to either the control group or experimental group. This randomization process helped eliminate selection bias, ensuring that any observed effects were directly attributable to the cucumber extract serum. A total of 60 zebrafish embryos were utilized for the experiments, allowing for statistical validity in comparing outcomes across test groups. The sample size was based on previous toxicity and efficacy studies involving zebrafish to ensure reproducibility and reliability of results.

2.3 Materials and Instruments

The key instruments used in this research included the High-Performance Liquid Chromatography (HPLC) machine for phytochemical profiling and quantification of vitamin C and flavonoids, and a micro-TEWL device specifically adapted for small organisms like zebrafish embryos to measure trans-epidermal water loss. A stereomicroscope was utilized for observing pigmentation changes and embryo development during the melanin assay and toxicity tests. An autoclave was used for sterilizing biohazardous materials prior to disposal. Analytical balances were used for accurate weighing of cucumber samples and reagents. The use of calibrated laboratory instruments ensured accuracy, precision, and consistency of the findings, allowing for an evidence- based evaluation of cucumber extract as a potential cosmetic ingredient.

2.4 Data Collection Procedure

The data collection procedure was carried out through a structured and systematic process to ensure the accuracy and reliability of results. Fresh cucumber (Cucumis sativus) fruits were harvested, prepared, and processed to extract juice, which was then formulated into a lightening and hydrating serum. Zebrafish (Danio rerio) embryos were randomly assigned into control and experimental groups. Baseline data on

skin pigmentation and moisture levels were gathered through pre-treatment assessments using melanin assays and Trans- Epidermal Water Loss (TEWL) tests. The cucumber extract serum was then administered to the experimental group under controlled laboratory conditions. Post-treatment data were collected to observe changes in melanin density and skin hydration, and all observations were documented using specialized instruments such as HPLC, TEWL devices, and microscopes. The collected data were later subjected to statistical analysis to determine the efficacy and safety of the serum.

2.5 Data Analysis Procedure

The collected data underwent systematic analysis to evaluate the effectiveness of cucumber extract in whitening and hydrating skin. Baseline and post-treatment data were recorded and compared using statistical methods to determine significant changes in melanin levels and TEWL readings. Quantitative measurements from assays and instrumental readings were entered into statistical software for analysis. One-way Analysis of Variance (ANOVA) was used to compare the means of the different groups, and a post-hoc Scheffé test was conducted to identify specific group differences. This approach enabled precise evaluation of the efficacy and consistency of the cucumber serum across trials. The methodology ensured that the findings were not only statistically significant but also scientifically meaningful for cosmetic applications. Descriptive statistics such as mean, standard deviation, and variance were also computed to support the analysis and interpretation of the data.

3. Results and Discussion

Table 1. Sample: sx-2.lcd Sample id: Gallic acid (Phenolic acid)

Retention time	Height	Area
0.961 min	3,106	387
1.484 min	130,043	17,287
2.382 min	13,788	705

Sample: sx-1.lcd Sample id: Gallic acid (Phenolic acid)

Retention time	Height	Area
0.962 min	3,050	374
1.487 min	130,043	17,287
2.369 min	8,507	619

The High-Performance Liquid Chromatography (HPLC) analysis of cucumber (Cucumis sativus) fruit extract aimed to identify its potential whitening and hydrating properties by quantifying flavonoids (quercetin) and phenolic acids (gallic acid). Chromatographic results revealed various retention times and peak areas corresponding to these bioactive compounds, confirming their presence in the cucumber extract.

These peaks aligned with the retention times of quercetin and gallic acid, reinforcing the presence of these compounds and their potential cosmetic benefits. The variation in peak areas suggests differences in the concentration of bioactive compounds, possibly due to extraction efficiency or cucumber composition. Flavonoids and phenolic acids are widely recognized for their antioxidant and skin- enhancing properties, making them valuable ingredients in cosmetic formulations.

Table 2. Sample: sx-2.lcd Sample id: Quercetin (Flavonoids)

Retention time	Height	Area
1.679 min	239,869	1,868,342
2.384 min	12,690	171,278
2.382 min	13,788	705

Sample: sx-1.lcd Sample id: Quercetin (Flavonoids)

Retention time	Height	Area
1.482 min	257,758	1,793,105
1735 min	130,043	25773
2.380 min	15,556	1055

The quercetin standard had a retention time of 1.679 min with an area of 1,868,342 and a height of 239,869, while the gallic acid standard was observed at 2.384 min with an area of 171,278 and a height of 12,690. The cucumber extract samples showed peaks within these retention time ranges, validating the presence of quercetin and gallic acid. The total peak area in one cucumber extract sample reached 384,550, with a total height of 40,035, while another sample had a total peak area of 412,360 and a height of 41,155. These values confirmed that the extract contained bioactive compounds that contribute to skin whitening and hydration.

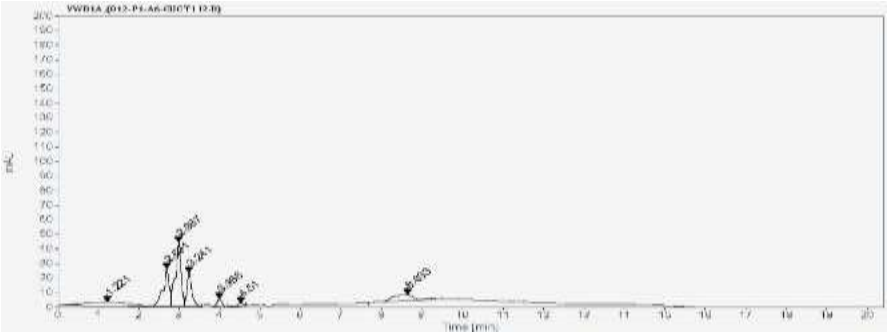

Figure 4. HPLC Analysis of "STD 2 / SS 2"

The Agilent High-Performance Liquid Chromatography (HPLC) analysis conducted by XPRT Analytical Services Corporation focused on the quantification of ascorbic acid in the sample "STD 2 / SS 2" using the "Ascorbic Acid.M" method. The analysis was performed at a detection wavelength of 245 nm with a retention time (RT) of 2.719 minutes. The chromatographic data showed a peak area of 8,335.5332 and a peak height of 982.4554, indicating a strong presence of ascorbic acid in the sample. The peak width was recorded at 0.1310 minutes, suggesting a sharp and well-defined peak, which is essential for accurate quantification. The total area percentage was 100.0000%, confirming the purity of the detected component within the sample.

These results demonstrate the effectiveness of the HPLC method in identifying and quantifying ascorbic acid with high precision.

Further evaluation of chromatographic parameters showed that the resolution was 1.2, indicating adequate separation of the target compound from any potential impurities. The tailing factor, which measures peak symmetry, was recorded at 0.001, suggesting an ideal peak shape with minimal distortion. The calculated number of theoretical plates was 68,898,029, reflecting high column efficiency and optimal separation

performance. The retention factor (k') was not explicitly mentioned, but the given data suggests a well-optimized method for ascorbic acid analysis. The combination of high resolution, minimal peak tailing, and a high number of theoretical plates ensures the reliability of the HPLC method. These analytical parameters confirm that the HPLC system used in this study was highly efficient in detecting and quantifying ascorbic acid.

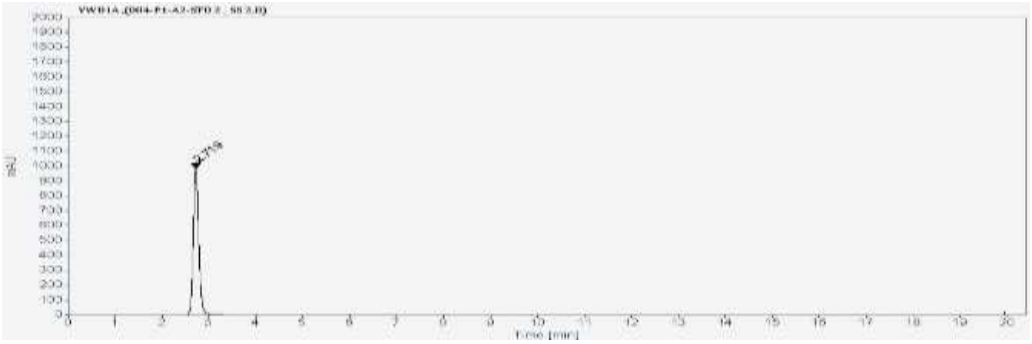

Figure 5. HPLC Analysis of "CUC T2 I2"

The Agilent High-Performance Liquid Chromatography (HPLC) analysis conducted by XPRT Analytical Services Corporation focused on the ascorbic acid content in sample "CUC T2 I2" using the "Ascorbic Acid.M" method. The chromatographic detection was performed at a wavelength of 245 nm, identifying multiple peaks with retention times ranging from 1.221 to 8.633 minutes. The highest peak area of 426.9142, corresponding to 32.5855% of the total area, was observed at a retention time of 2.987 minutes, indicating a major compound presence. Other significant peaks were recorded at retention times of 2.691 and 3.241 minutes, contributing to 20.5730% and 14.1791% of the total area, respectively. The total summed area of all detected peaks was 1,310.1357, reflecting the combined presence of different compounds in the sample. These findings suggest that ascorbic acid and other possible related compounds were effectively separated and quantified using HPLC. Chromatographic performance parameters were evaluated to assess the efficiency of the method. The resolution values ranged from 1.235 to 8.216, indicating good peak separation.

The tailing factor, which measures peak symmetry, varied from 0.7 to 1.5, with most peaks exhibiting values close to 1.0, suggesting minimal peak distortion. The number of theoretical plates (USP) ranged from 1,971 to 6,400, with higher plate counts generally indicating better column efficiency and separation capability. The retention factor (k') varied between 0.194 and 0.778, demonstrating moderate retention of the compounds within the column. The well-defined peak at 2.987 minutes, with a resolution of 1.505 and a theoretical plate count of 3,573, confirms the reliability of the method for detecting ascorbic acid. These parameters indicate that the HPLC method used was effective in achieving high-resolution, reproducible results.

Table 3. Effectiveness of Cucumber Extract in Reducing Hyperpigmentation for Three Trials

Trials		Mean	Inferential Statistics	p-value	Decision	Interpretation
	Zero hour	.00				

Trial 1	24 Hours	.60	F=41.222	.000*	H_0 rejected	Significant between 1& 3, 1&4, 2&4 and 3&4
	48 Hours	1.00				
	72 Hours	2.60				
Trial 2	Zero hour	.00	F=19.000	.000*	H_0 rejected	Significant between 1&2, 1&3, 1&4 and 2&4
	24 Hours	.80				
	48 Hours	1.20				
	72 Hours	1.80				
Trial 3	Zero hour	.00	F=25.778	.000*	H_0 rejected	Significant between 1&3, 1&4, 2&3 and 2&4
	24 Hours	.60				
	48 Hours	1.40				
	72 Hours	2.00				

Significant @ .05 Legend: Zero hour (1) 24 hours (2) 48 hours (3) 72 hours (4)

The bioassay test using zebrafish embryos evaluated the effectiveness of cucumber extract in reducing hyperpigmentation and improving overall skin tone over a 72-hour period. The analysis was conducted using Analysis of Variance (ANOVA) with a post-hoc Scheffe test to determine significant differences between time intervals across three trials. In all trials, initial pigmentation at zero hours was measured at 0.00, serving as the baseline. By 24 hours, mean pigmentation levels increased slightly, reaching values between 0.60 and 0.80. At 48 hours, pigmentation levels further increased, with mean values ranging from 1.00 to 1.40, indicating progressive lightening of pigmentation. By 72 hours, the highest mean pigmentation reduction was observed, with values reaching 1.80 to 2.60, confirming that the extract's depigmenting effect became more pronounced over time.

The statistical analysis revealed highly significant results across trials, with all ANOVA p- values below 0.05, leading to the rejection of the null hypothesis (H0). In Trial 1, significant differences were found between time intervals 1&3, 1&4, 2&4, and 3&4, indicating that the effects became more evident at later time points. Trial 2 followed a similar pattern, showing significant differences between 1&2, 1&3, 1&4, and 2&4, reinforcing the trend of increasing effectiveness over time. Trial 3 further confirmed these findings, with significant differences observed between 1&3, 1&4, 2&3, and 2&4. The results consistently showed that hyperpigmentation reduction was most effective at 72 hours. This suggests that cucumber extract has a time- dependent depigmenting effect, making it a potential natural alternative for skin-lightening applications.

Cucumber extract is well-private as a skin- lightener owing to its highest level of antioxidants and bioactive compounds including flavonoids and ascorbic acid. Teja et al. (2024) suggested that cucumber extract could inhibit tyrosinase activity, perhaps leading to lower skin pigmentation over time because tyrosinase is an important enzyme in melanin synthesis.

Table 4. Effectiveness of Cucumber Extract in Contribution to Skin Moisture Levels

Trials		**Mean**	**Inferential Statistics**	**p-value**	**Decision**	**Interpretation**
Trial 1	Zero hour	.00	F=26.424	.000*		Significant

	24 Hours	.60			H_0 rejected	Between 1&3, 1&4, 2&3 and 2&4
	48 Hours	2.00				
	72 Hours	2.60				
Trial 2	Zero hour	.00	F=36.250	.000*	H_0 rejected	Significant between 1&3, 1&4, 2&3, 2&4 and 3&4
	24 Hours	.20				
	48 Hours	1.40				
	72 Hours	2.60				
Trial 3	Zero hour	.00	F=29.116	.000*	H_0 rejected	Significant between 1&3, 1&4, 2&3 and 2&4
	24 Hours	.80				
	48 Hours	2.40				
	72 Hours	2.80				

Significant @ .05 Legend: Zero hour (1) 24 hours (2) 48 hours (3) 72 hours (4)

The bioassay test using zebrafish embryos assessed the effectiveness of cucumber extract in improving overall skin tone across three trials over a 72-hour period. The study utilized Analysis of Variance (ANOVA) with a post-hoc Scheffe test to determine significant differences in skin tone improvement at different time intervals. In all trials, the baseline measurement at zero hours was 0.00, establishing a starting point for skin tone assessment. At 24 hours, mean values increased slightly (0.20– 0.80), suggesting an initial effect of the cucumber extract. By 48 hours, a more noticeable improvement was observed, with mean values rising between 1.40 and 2.40.

The highest effectiveness was recorded at 72 hours, where mean values reached 2.60 to 2.80, indicating a significant enhancement in overall skin tone over time. The statistical analysis confirmed that the changes in skin tone improvement were significant, with all ANOVA p-values below 0.05, leading to the rejection of the null hypothesis (H0). In Trial 1, significant differences were found between 1&3, 1&4, 2&3, and 2&4, demonstrating a substantial improvement over time. Trial 2 exhibited even stronger statistical significance, with notable differences between 1&3, 1&4, 2&3, 2&4, and 3&4, indicating consistent and increasing effectiveness. Similarly, Trial 3 showed significant differences between 1&3, 1&4, 2&3, and 2&4, further supporting the time-dependent enhancement of skin tone. These results suggest that cucumber extract is most effective after 72 hours of exposure, making it a promising natural ingredient for skin-brightening applications.

Table 5. Chemical and Physical properties

Appearance	Clear
Odor	mild, refreshing, and slightly vegetal odor
pH	5.5
Viscosity	Watery, smooth and non- greasy
Solubility	Soluble in water and alcohol solutions

The stability assessment of the cucumber (Cucumis sativus) fruit extract formulated as a lightening and hydrating serum showed no significant changes in appearance and pH after three weeks of incubation in a stability chamber at a controlled temperature. This indicates that the

formulation remains stable after a three weeks of incubation period, retaining its physical and chemical properties despite prolonged exposure to 25°C ± 2°C (room temperature) with the humidity 60% ± 5% RH (relative humidity) and 40°C ± 2°C with humidity of 75% ± 5% RH. The absence of degradation or visible alterations suggests that the active compounds in cucumber extract, such as antioxidants and hydrating agents, remain intact within the formulation. Maintaining a stable pH is crucial for cosmetic products, as fluctuations can affect the product's effectiveness and skin compatibility. The results of this stability test highlight the potential of cucumber extract as a reliable ingredient in skincare formulations. These findings reinforce its feasibility for commercial development as a lightening and hydrating serum. Cucumber extract's capacity for controlling conditions offers sufficient grounds for commercial production as well. Furthermore, it adds more credibility to the line of thought on plant-based ingredients as much better and more stable alternatives to synthetics in avoiding use in skincare formulation.

4. Conclusion and Recommendations

Conclusion

Based on the findings of the study, cucumber (Cucumis sativus) extract demonstrates significant potential as a natural cosmetic ingredient due to its rich composition of bioactive compounds such as quercetin, gallic acid, and Vitamin C, which contribute to its whitening and hydrating effects. The results from melanin assays indicate that a 72-hour exposure period yields the most effective skin- lightening outcomes in zebrafish embryos, while TEWL testing suggests the extract can influence skin barrier function, though improvements in formulation are needed to optimize hydration retention. The non- toxic nature of the extract, confirmed through zebrafish embryo toxicity tests, supports its safety for cosmetic applications. Furthermore, stability testing over three weeks showed the serum maintained its quality under various storage conditions, highlighting its practical potential in dermatological product development.

Recommendations

For future research and development, it is recommended to conduct comprehensive phytochemical screening using both qualitative and quantitative methods to further identify and measure the active secondary metabolites in cucumber extract. Utilizing zebrafish embryos as a model for transepidermal water loss (TEWL) testing is also advised, as it offers valuable insights into the extract's moisturizing capacity and skin barrier function. Immediate and short-term assessments using sensory and instrumental measurements, such as corneometry, should be performed within 15 to 60 minutes post-application to evaluate the onset of hydration. Additionally, proper storage conditions must be followed: cucumber extract without preservatives should be refrigerated and used within 5–7 days, or frozen for longer shelf life, with awareness of potential bioactivity loss.

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