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Evaluation of Photoprotection and Anti-Aging Properties of Calamansi (Citrus microcarpa) Rind Extract in Sunscreen Formulation

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Abstract

The sun releases ultraviolet (UV) radiation, which, with prolonged exposure, may cause damaging effects on human skin. This led to the invention of Sunscreens used for photo-protection and for anti-aging from skin damage. However, most sunscreens readily available in the market are made from synthetic materials that have an environmental impact. This study investigates the photoprotective and anti-aging properties of calamansi (*Citrus microcarpa*) rind extract for potential sunscreen formulation. The research aims to explore the natural bioactive compounds present in the Calamansi rind that contribute to UV protection and anti-aging benefits, thereby offering a sustainable and eco- friendly alternative to synthetic sunscreens. High-Performance Liquid Chromatography (HPLC) and Ultraviolet-Visible (UV-Vis) Spectroscopy were employed to analyze the bioactive components and assess the extract's UV absorption capabilities. The primary bioactive compounds identified were Vitamin C and the flavonoid Quercetin, both known for their antioxidant and photoprotective properties. The anti-aging potential was evaluated through collagen assay using zebrafish (Danio rerio) as the model organism, while the toxicity of the extract was determined through zebrafish toxicity tests. The results revealed that the calamansi rind extract demonstrated significant photoprotective activity comparable to standard sunscreens in specific UV wavelengths (292, 294, and 296 nm). Furthermore, the collagen assay indicated the extract's effectiveness in promoting collagen production, confirming its anti-aging potential. The toxicity test showed no mortality in zebrafish up to a concentration of 2000 mg/L, indicating the extract's safety for potential cosmetic applications. Additionally, the stability test of the formulated sunscreen showed no significant changes in pH or physical appearance over a three-week period. This study concludes that calamansi rind extract is a promising natural ingredient for developing eco-friendly sunscreen with photoprotective and anti- aging properties. Future research is recommended to further optimize the formulation, extend stability testing, and explore the exact Sun Protection Factor (SPF) of the product.

Keywords: Cosmetics, Sunscreen, Calamansi, HPLC, UV-Vis Spectroscopy, Zebrafish, Collagen Assay, Toxicity Tests, Stability, Formulation

Introduction

The sun emits ultraviolet (UV) radiation, which can cause significant harm to human skin with prolonged exposure. These effects include sunburn, premature aging, and increased risk of skin cancer. To combat these risks, the use of sunscreen has become a common and recommended skincare practice.

However, recent studies have raised environmental concerns about synthetic UV filters used in commercial sunscreens. For instance, research conducted in the Mediterranean Sea reported adverse effects of these chemicals on marine bacteria, prompting the need to explore safer, eco-friendly alternatives.

Natural plant-based substances, particularly those rich in flavonoids and polyphenols, have demonstrated promising UV-filtering and antioxidant properties. Citrus fruits like lemons and oranges have been extensively studied for their photoprotective and anti-aging effects. Notably, Calamansi (*Citrus microcarpa*), a citrus fruit native to Southeast Asia, contains high levels of Vitamin C, flavonoids, and essential oils, which contribute to its antioxidant and UV-protective capabilities. Previous research found that Calamansi extracts, especially those that were sundried, exhibited strong antioxidant activity, suggesting potential use in skincare to prevent sun damage and aging.

Despite this potential, the use of Calamansi rind extract in actual sunscreen formulation remains largely unexplored. Most studies have focused on the fruit's nutritional and culinary applications or have used crude extracts without developing them into finished skincare products. This study seeks to fill that gap by examining the photoprotective and anti- aging potential of Calamansi rind extract when formulated into sunscreen. The research evaluates its effectiveness as a natural alternative to synthetic UV filters through laboratory techniques such as UV-Vis Spectrophotometry and High-Performance Liquid Chromatography (HPLC), along with biological assays on zebrafish to test for collagen production and toxicity.

Methodology

Research Design

This study adopted an experimental research design to explore the photoprotective and anti-aging effects of Calamansi rind extract when formulated into a sunscreen. This design allowed the researcher to control and manipulate variables systematically, ensuring the reliability and replicability of the results. It involved a step-by-step scientific approach that included extraction, formulation, testing, and evaluation of the sample. The experiment enabled comparison with a commercial sunscreen to establish efficacy through UV-Vis Spectrophotometry. The experimental approach also facilitated biological testing using zebrafish for toxicity and collagen production. The findings derived from this design were grounded in observable and measurable outcomes, establishing a cause-and-effect relationship between Calamansi rind extract and its skin- protective potential.

Research Locale and Sampling Technique

The research was conducted in various scientific settings including the UPH–Dr. Jose G. Tamayo Medical University's PCR Laboratory and a certified zebrafish facility. The calamansi samples were sourced from a local farm in Maragondon, Cavite, ensuring natural and fresh plant material for the study. The study employed purposive sampling in selecting the calamansi fruits based on their quality and ripeness, which are critical for ensuring maximum extract yield. For bioassay testing, random sampling was used to assign zebrafish to experimental groups to minimize bias and ensure validity in results. These carefully selected research sites and sampling methods supported the accuracy, safety, and scientific rigor of the entire experimentation process.

Materials and Instruments

Key materials in this study included fresh calamansi fruits, ethanol for extraction, and ingredients for sunscreen formulation such as coconut oil, shea butter, and beeswax. Laboratory instruments used were a UV-Vis Spectrophotometer for measuring UV absorption, and High-Performance Liquid Chromatography (HPLC) for analyzing bioactive compounds such as flavonoids and Vitamin C. A blender, double boiler, and syringe filters were used during the extraction and formulation phases. Zebrafish were kept in controlled aquariums for bioassay and collagen tests. Microscopes and Sirius Red solution were used for histological analysis. These tools and materials ensured precision and scientific reliability in testing the sunscreen's effectiveness and safety.

Data Collection Procedure

Data collection began with the preparation of calamansi rind extract using maceration and ethanol evaporation. The resulting extract was subjected to various analyses including HPLC for compound profiling and UV-Vis Spectroscopy to evaluate UV absorbance. Comparative data were gathered by testing both the formulated calamansi sunscreen and a commercial counterpart under identical conditions. Zebrafish were used in both toxicity and collagen assays, with their responses and tissue changes being carefully documented. Throughout the study, standardized laboratory methods were employed to ensure the accuracy, consistency, and replicability of the data collected. Documentation and storage of results were maintained in lab notebooks and digital files for proper analysis and verification.

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.

Results and Discussion

Table 1. Percentage Yield Determination

Plant Sample	Plant Extract	Percentage Yield
14,000 grams	4,667 grams	33.3%

The results indicated that a total of 4,667 grams of extract was obtained from 14,000 grams of calamansi rind. Using the formula for percentage yield, it was determined that the researcher extracted 33.3% of the total sample. Percentage yield was used to determine how well a reaction performed, detect potential faults in the experiment, and optimize reaction parameters for future experiments. This result suggests that the calamansi rind extract is a favorable alternative active ingredient to be used on pharmacological and cosmeceutical purposes due to the very high yield of 33.3% from the ethanolic extract.

Table 2. High-Performance Liquid Chromatography (HPLC)

Trial	Sample	Retention Time (RT) [minutes]	Peak Area	Concentration [mg/mL]	Result
Trial 1 Injection 1	Vitamin C	2.675	8313.8721	0.104	0.83 mg/mL
	Calamansi rind extract	2.701	1173.2871	0.02	
Trial 1 Injection 2	Vitamin C	2.675	8313.8721	0.104	
	Calamansi rind extract	2.718	1279.8079	0.02	
Trial 2 Injection 1	Vitamin C	2.719	8335.5332	0.104	
	Calamansi rind extract	2.716	1425.1094	0.02	
Trial 2 Injection 2	Vitamin C	2.719	8335.5332	0.104	
	Calamansi rind extract	2.729	1412.6738	0.02	

The results indicated that 0.83 mg/mL of ascorbic acid was present in the calamansi rind extract. Based on this finding, it can be concluded that the calamansi rind exhibited anti-aging activity due to the antioxidant properties of Vitamin C. HPLC is used to identify and quantify active pharmaceutical ingredients (APIs), as well as to assess their purity and stability. The procedure used in this instrumentation is based on the United States Pharmacopeia (USP) standard. Since the extract has obtained a presence of 0.83 mg/mL of Ascorbic Acid, it supports the theory that Calamansi rind is high in antioxidants which leads to the extract's ability to stimulate collagen synthesis and neutralize free radicals. Therefore, this study highlights the extract's use in anti-aging skincare products. The average concentration of flavonoid Quercetin present in the calamansi rind extract was calculated using data from Trials 1 and 2. The values obtained from the peak areas and concentrations of the standard and sample were computed using the formula stated in Chapter 3, Section 2.

Table 3. Flavonoid (Quercetin) Assay using HPLC

Trial	Sample	Retention Time (RT) [minutes]	Peak Area	Concentration [mg/mL]	Result
Trial 1	Quercetin	1.679	1868.342	4	1,208.156 mg/mL
	Calamansi rind extract	1.560	2165.8611	0.003948	
Trial 2	Quercetin	1.678	1763.928	4	
	Calamansi rind extract	1.562	2165.2956	0.003948	

Based on this finding, it can be concluded that the calamansi rind is an effective sunscreen agent due to the photoprotective properties of Quercetin. HPLC was used to identify and quantify active pharmaceutical ingredients (APIs), as well as to assess their purity and stability. The procedure used in this instrumentation is based on the United States

Pharmacopeia (USP) standard. Since the extract has an extraordinarily high content of Quercetin (1,208.156 mg/mL), it has the capacity to absorb UV rays and reduce inflammation which establishes it as a formidable natural sunscreen component and as a natural alternative to synthetic UV filters.

Table 4. UV-Vis Spectroscopy

Table 4.3

Difference in the Photoprotective Activity of the Calamansi Rind Extract and Standard Sunscreen

Wavelength	Types	Mean	Test Statistic	p-value	Decision	Interpretation
290	Calamansi rind strength	3.63	t=-3.198	.054	Failed to reject H_0	Not Significant
	Standard sunscreen	4.13				
292	Calamansi rind strength	3.57	t=-4.525	.034*	H_0 rejected	Significant
	Standard sunscreen	4.00				
294	Calamansi rind strength	3.63	t=-3.881	.039*	H_0 rejected	Significant
	Standard sunscreen	3.95				
296	Calamansi rind strength	3.64	t=-4.525	.016*	H_0 rejected	Significant
	Standard sunscreen	3.97				
298	Calamansi rind strength	3.58	t=-3.881	.145	Failed to reject H_0	Not Significant
	Standard sunscreen	2.76				
300	Calamansi rind strength	3.58	t=-4.137	.176	Failed to reject H_0	Not Significant
	Standard sunscreen	2.78				
302	Calamansi rind strength	3.96	t=2.227	.072	Failed to reject H_0	Not Significant
	Standard sunscreen	2.76				
304	Calamansi rind strength	3.91	t=2.637	.079	Failed to reject H_0	Not Significant
	Standard sunscreen	2.78				
306	Calamansi rind strength	3.68	t=2.658	.111	Failed to reject H_0	Not Significant
	Standard sunscreen	2.74				
308	Calamansi rind strength	3.66	t=2.352	.140	Failed to reject H_0	Not Significant
	Standard sunscreen	2.77				
310	Calamansi rind strength	4.11	t=3.241	.075	Failed to reject H_0	Not Significant
	Standard sunscreen	2.80				
312	Calamansi rind strength	3.56	t=1.284	.323	Failed to reject H_0	Not Significant
	Standard sunscreen	2.91				
314	Calamansi rind strength	3.97	t=2.472	.081	Failed to reject H_0	Not Significant
	Standard sunscreen	2.81				
316	Calamansi rind strength	3.71	t=1.789	.206	Failed to reject H_0	Not Significant
	Standard sunscreen	2.88				
318	Calamansi rind strength	3.72	t=1.435	.275	Failed to reject H_0	Not Significant
	Standard sunscreen	2.96				
320	Calamansi rind strength	3.69	t=1.779	.206	Failed to reject H_0	Not Significant
	Standard sunscreen	2.87				

*Significant at .05

Results of the t-test for independent variables shows that there are significant differences between the calamansi extract and the standard sunscreen in the 292, 294 and 296 wavelengths. This means that standard sunscreen in these wavelengths have better photoprotective activity. For the rest of the wavelengths, no significant differences were found. With this, we can assume that the calamansi rind extract produced a photoprotective activity with the same quality as the standard sunscreen on certain wavelengths (292, 294 and 296 wavelengths).

Table 5. Collagen Assay

NORMAL	PRESENCE OF COLLAGEN	
	YES	NO
Zebrafish 1	/	
Zebrafish 2	/	
Zebrafish 3	/	
Zebrafish 4	/	
Zebrafish 5	/	
Zebrafish 6	/	
Zebrafish 7	/	

AFTER 96 HOURS	PRESENCE OF COLLAGEN	
	YES	NO
Zebrafish 1	/	
Zebrafish 2	/	
Zebrafish 3	/	
Zebrafish 4	/	
Zebrafish 5	/	
Zebrafish 6	/	
Zebrafish 7	/	

Results from the collagen assay of the histopathology of the zebrafish shows that even after 96 hours after exposure, the collagen is still present. The ability of the extract to retain the presence of collagen after 96 hours suggests that it supports skin regeneration and that it can exhibit its anti-aging activity for up to 96 hours.

Table 6. Toxicity Test

DOSAGE	ZEBRA FISH GROUP	0-96 HRS TOXICITY TEST	RESULTS
100 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
250 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
500 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
750 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
1000 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
1500 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC
2000 mg	1 2 3	DONE DONE DONE	NON-TOXIC NON-TOXIC NON-TOXIC

From this data we can confirm the safety of the calamansi rind extract to the zebrafish. It can be seen that even at 96 hours of exposure on concentrations ranging from 100 mg to 2000 mg of the extract, no death has been recorded from the zebrafish used in the assay. Therefore, we can assume that the LD50 is greater than 2000 mg since no toxicity has been observed.

Table 7. Formulation of the sunscreen

Appearance	Description
Color	Beige
pH	Acidic
Odor	Citrus
Formulation	Cream Sunscreen

The tinted sunscreen is beige in color. The pH is Acidic. Upon smelling, the note of citrus odor is evident. The formula is a cream-type sunscreen that is soft and smooth upon application to the skin.

Table 8. Stability Test

Table 4.6.2
Stability Data of Formulated Calamansi Sunscreen

Date	Time	Room Temperature				
		Color	Odor	Appearance	pH	Viscosity
27 Jan 2025	1 week	0	0	0	4.79	n/a
03 Feb 2025	2 weeks	0	0	0	4.85	n/a
10 Feb 2025	3 weeks	0	0	0	4.81	n/a
Date	**Time**	**Over 40°C**				
		Color	**Odor**	**Appearance**	**pH**	**Viscosity**
27 Jan 2025	1 week	0	0	0	4.79	n/a
03 Feb 2025	2 weeks	0	0	0	4.98	n/a
10 Feb 2025	3 weeks	0	0	0	4.92	n/a

SCORE:
0 – no change
1 – slight change
2 – moderate change
3 – extreme change

From this, we can observe that there were no changes on the color, odor and appearance of the tinted sunscreen both at room temperature and at over 40 C. There are minor changes in the pH of the sunscreen but since the pH is less than 7, we can conclude that this is an acidic formulation.

Conclusion

This study concluded that Calamansi rind extract, with an average yield of 33.34% from the total raw material, contains key bioactive compounds such as Vitamin C and Quercetin, which are linked to anti- aging and photoprotective properties. The extract demonstrated collagen-stimulating effects in zebrafish due to its antioxidant activity and showed no toxicity up to a concentration of 2000 mg/L, indicating a favorable safety profile. While the commercial sunscreen outperformed the extract in select UV wavelengths (292, 294, and 296 nm), no significant differences were observed in other wavelengths, suggesting comparable photoprotective potential overall. Additionally, the formulation remained stable in appearance and quality over a three-week observation period, underscoring its viability as a natural sunscreen candidate. These findings support the potential of calamansi rind as a sustainable, natural ingredient in cosmetic sunscreen products with dual photoprotective and anti-aging benefits.

Recommendations

To build on these findings, future research should explore alternative extraction methods and evaluate other parts of the calamansi plant. Advanced screening tools such as LC-MS are recommended to identify additional bioactive compounds, while the Sun Protection Factor (SPF) should be determined to quantify UV-blocking efficacy and ideal reapplication intervals. Researchers are encouraged to investigate the synergistic effects of combining calamansi extract with other natural antioxidants for enhanced benefits. A more thorough toxicity assessment beyond 2000 mg/L and dermatological testing on animal models should be pursued to ensure product safety. Lastly, stability testing should be extended to the industry- standard 40 weeks to validate the product's long-term shelf life and market readiness.

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