Effectiveness Of Applying Anti-Aging Cream With Lemon Peel Extract (Citrus Limon) In Inhibiting The Skin Aging Process Of Wistar Rats (Rattus Norvegicus) Exposed To Ultra Violet-B Rays

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Abstract.

Aging is a gradual physiological change in an organism that results in senescence or a reduction in biological function and the ability of the organism to respond to metabolic stress. Aging occurs in cells, organs, or the entire organism over time; the study of the aging process seeks to understand and regulate all elements that contribute to an individual's life constraints. The predominant acute consequence of UV radiation on normal human skin is sunburn-induced inflammation (erythema). The overall goal of this study is to examine and test the efficacy of applying an anti-aging cream containing lemon peel extract (Citrus limon) in preventing the aging process of mouse skin. Wistar strain (Rattus norvegicus) exposed to ultraviolet-B light. The specific goal of this study is to investigate the efficacy of applying lemon peel extract (Citrus limon) cream at 5%, 10%, and 15% concentrations in preventing the aging process of rat skin exposed to UV-B radiation.

Keywords: *UV Radiation, Citrus Limon Extract and Anti-Aging.*

I. INTRODUCTION

Everyone wants healthy, young skin, especially ladies. Environmental factors like sun exposure can accelerate aging. Wrinkles and fine lines signify premature aging. There are also dark spots and facial elasticity issues [1]. Premature aging causes skin to dry and darken, and acne scars take longer to erase. Anti-aging skincare is becoming popular, especially among women, to treat these skin issues [2]. Bleach is also used to brighten and cure dry skin.Senescence, or a decline in biological function and metabolic stress adaptation, results from aging [3]. Aging occurs in cells, organs, or the whole body. Every living thing goes through this as an adult. Gerontology studies the aging process and aims to understand and regulate all the elements restricting a person's life [4]. It addresses a far broader issue than weakness, which is prevalent in human experience. In each species, the individual's life span is related to reproductive life span, reproductive systems, and development. How these interactions formed is relevant to gerontology and evolutionary biology. It's also vital to distinguish between purely physicochemical aging and unintended organismic sickness or injury that kills [5].Everyone ages differently, but skin aging commonly causes fine lines and wrinkles, loss of elasticity, rough texture, and discoloration. As we age, collagen and elastin break down, making skin looser and wrinkled. UV rays and lifestyle choices like smoking and drinking can accelerate skin aging [6]–[8]. Certain skin care products with active components can slow aging. Anti-aging moisturizers and serums cannot stop aging.

Skin treatment using retinol and hyaluronic acid can slow aging and improve skin look [9]–[11].Sunlight emits UV radiation that causes cancer. UV from sunshine and therapy lights are dangerous to human health. Sunburn, tanning, and immunosuppression are the main acute effects of UV radiation on normal human skin. Nucleotide excision repair (NER) repairs DNA damage from UV radiation, such as cyclobutane pyrimidine dimers and photoproducts [12]. UV exposure over time causes photoaging, immunosuppression, and photocarcinogenesis. Photocarcinogenesis causes skin cancer through genetic alterations and immune system regulation. In clinics, UVB (280–320 nm) and UVA (320–400 nm) lights and

chemical medications treat numerous skin illnesses, including psoriasis and vitiligo. This therapy is helpful but has adverse effects. UV radiation has both a negative and a positive impact [12], [13].The skin alterations caused by persistent UVA and UVB exposure are called photoaging or dermatoheliosis: aging causes biological function and metabolic stress management to decline. Complex and progressive aging causes skin functional and cosmetic changes.

Extrinsic (environmental) and intrinsic (genetic) variables can cause this process. Long-term, continuous UV exposure of 300-400 nm, natural or manufactured, causes photoaging in naturally aging skin [14], [15].Anshori reported that oral lemon peel extract reduced MMP-1 expression and increased collagen in male white Wistar rats (Rattus norvegicus) exposed to UVB light [16]. Instead, this study made cream from lemon peel extract. Many people use anti-aging and whitening creams to defend against UV rays. Anti-aging and whitening lotions are often contested, even though they prevent UV-induced skin aging. Even though the two cream products are on the market, their promotional ads often have similar benefits. Not to mention that a higher concentration of lemon peel extract may better protect skin from UVB rays to prevent aging.Thus, more research is needed to determine the efficacy of anti-aging creams, particularly those containing lemon peel extract, in delaying skin aging. Based on this background, this researcher aims to examine the efficiency of an anti-aging cream with lemon peel extract (Citrus limon) in suppressing skin aging in Wistar rats (Rattus norvegicus) exposed to ultraviolet-B light. To test the antioxidants of lemon peel extract cream (Citrus limon) at 5%, 10%, and 15% doses in slowing rat skin aging after UVB exposure.

II. METHODS

This study is a pure laboratory experimental study with a post-test with a control group design [17] to examine the effectiveness of administering an anti-aging cream containing lemon peel extract (Citrus limon) in inhibiting the aging process of the skin of Wistar rats (Rattus norvegicus) exposed to light. UVB (ultraviolet). This study's variables include both independent and dependent factors [18]. Giving ultra violet-B light and giving lemon peel extract cream are the independent variables. The dependent variable is the rate of delay in mouse skin aging.The Animal House, Faculty of Mathematics and Natural Sciences, University of North Sumatra, will conduct this research. From January to February 2023, this investigation was conducted. This study used healthy adult Wistar rats (Rattus norvegicus) weighing 150-300 grams and aged 2-3 months to ensure objective research results. Wistar rats were chosen because they are one of the most commonly used experimental animals in biomedical research.

They have approximately the same traits and physiology as humans, are more significant than mice, and can adapt to a lab setting.The number of rat samples utilized in this investigation was 20, divided into four (four) groups of five rats each. Determining the number of animals follows the "reduction" point, which is to minimize the number of animals used in research without compromising the validity of the results, by the "3R Principle" (Replacement, Reduction, and Refinement), which researchers must follow in vivo research [19].The information gleaned from each observation parameter (variable) was recorded and organized in a table. The gathered quantitative data (independent variables) were examined for significance on the effect of the treatment group (dependent variable) using a computer statistical tool, mainly the statistical product and service solution (SPSS) program. If the normality and homogeneity tests show that they are not statistically different, then the Anova test is performed; if the Anova test reveals that they are significantly different ($p > 0.05$), then a Post Hoct Bonferroni analysis test at the 5% level is performed. If the data is not normally distributed or homogeneous ($p < 0.05$), non-parametric tests will be used to analyze it [20].

III. RESULTS AND DISCUSSION

Result

Phytochemical experiments on lemon peel extract in 96% ethanol solvent can reveal secondary metabolite chemicals. Because it matches the compound's polarity, 96% ethanol solvent is used. Table 1 shows that lemon peel extract contains flavonoids, saponins, phenolics, and tannins from the phytochemical test.

Table 1. Results of Phytochemical Tests for Lemon Peel Extract

Compound	Result	Note
Flavonoids	Orange and yellow solution	+
Saponin	The solution forms foam.	+
Phenolic	Blackish green solution	+
Tanin	Green solution	+

Note: (+) = positive or contains secondary metabolites, (-) = negative or does not contain secondary metabolites

Table 2 compares lemon peel extract cream antioxidant activity before and after cycling. According to the antioxidant activity test, the IC_{50} value of lemon peel extract cream before cycling for F1 was 141,353 ppm, F2 was 139,188, and F3 was 141,896. This displays lemon peel cream's antioxidant properties. Lemon peel extract IC_{50} values have dropped when manufactured into creams. Before being converted into cream, lemon peel extract has an IC_{50} of 103.572 ppm and a 35.956% decrease

Table 2. Lemon Peel Extract Cream Antioxidant Activity Test Results

Sample	Antioxidant Activity				IC_{50} Change (%)
	Before Cycling test		After Cycling test		
	IC_{50} (ppm)	AAI	IC_{50} (ppm)	AAI	
F1	141,353	1,132	172,886	0,925	22,308%
F2	139,188	1,150	169,023	0,947	21,435%
F3	141,896	1,128	175,930	0,909	23,985%

The cycling test raised the lemon peel extract cream's IC_{50}. Preparations with higher IC_{50} values have lower antioxidant action. The IC_{50} value of lemon peel extract cream for F1 was 172,886 ppm (a 22.308% drop), F2 was 169,023 (a 21.435% decrease), and F3 was 175,930 (a 23.985% decrease). After finding the IC_{50}, compute the AAI. AAI classifies the extract's antioxidant capabilities. According to antioxidant activity testing, lemon peel extract cream has an AAI of 1.132 for F1, 1.150 for F2, and 1.128 for F3. This indicates that lemon peel extract cream (Citrus limon L.) has considerable antioxidant activity (AAI = 1-2). After the cycling test, lemon peel extract cream AAI dropped.A lower preparation AAI suggests lower antioxidant activity. F1, F2, and F3 lemon peel extract cream AAI values are 0.925, 0.947, and 0.909. This reveals that the lemon peel extract cream's antioxidant activity reduced following the cycling test and became moderate (AAI = 0.5-1). Table 3 shows that the P3 group, which received 15% lemon peel extract cream, had the highest collagen density. The normal control group (K) or no treatment had the worst average collagen density %.

Table 3. Rat Skin Tissue Collagen Density Test Results

Collagen Density (%)				
Group	**K**	**P1**	**P2**	**P3**
1	42.707	46.428	50.254	56.012
2	39.968	40.416	53.320	55.328
3	42.198	43.152	48.688	52.268
4	43.414	42.736	47.325	50.825
5	41.560	43.268	48.842	51.842
Mean	41.969	43.200	49.686	53.255
SD	1.171	1.918	2.040	2.038
Score	+2	+2	+2	+3

Note: K = Normal control (no treatment), P1: 5% lemon peel cream, P2: 10% lemon peel cream, P3: 15% lemon peel cream.

According to Table 4, a normality test using SPSS showed that the control group was normal, and the treatment group had significant collagen density values from day 1 to day 14. The Shapiro-Wilk Test significant value (p) exceeds the conventional margin of $p>0.05$, 0.816 for Group K, 0.561 for Group P1, 0.495 for Group P2, and 0.335 for Group P3. Shapiro-Wilk normality tests show the data is normally distributed.

Table 4. Normality Test Results

Group Extract Dose	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Normal Control (K)	.177	5	.200*	.961	5	.816
Treatment P1	.287	5	.200*	.925	5	.561
Treatment P2	.244	5	.200*	.914	5	.495
Treatment P3	.268	5	.200*	.885	5	.335

*. This is a lower bound of the true significance

a. Lilliefors Significance Correction

Table 5 shows collagen growth or density increase in groups K, P1, P2, and P3 after 14 days of therapy, assessed for homogeneity using the One Way ANOVA Test. The data variance from the research outcomes of the normal control group (K), groups P1, P2, and P3 is 0.571 (p>0.05).Table 6 was used to test whether the five research or observation groups had different collagen density percentages, according to the "Sig" column. The p-value is 0.000. Thus, Ho is rejected at the fundamental level = 0.05, indicating that the five groups have a significant variation in collagen density.

Table 5. Test of Homogeneity of Variances

	Levene Statistical	df1	df2	Sig.
Base on Mean	.691	3	16	.571
Base on Median	.265	3	16	.850
Based on the Median & the adjusted df	.265	3	13.673	.849
Based on trimmed mean	.674	3	16	.580

Table 6. Results of the ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	430.412	3	143.471	34.347	.000
Within Groups	66.835	16	4.177		
Total	497.247	19			

Table 7. Post Hoc Bonferroni Test Results

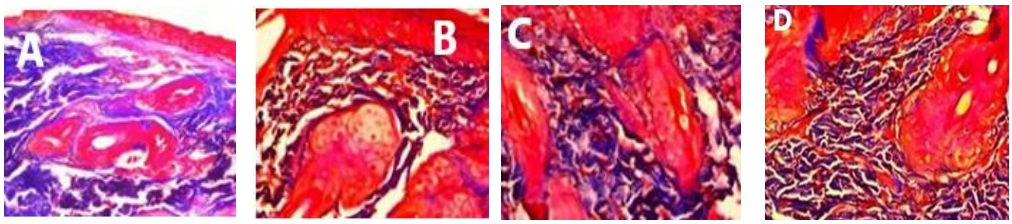
Experimental Group (I)	Experimental Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Normal Control (K)	Treatment P1	-1.230600	1.292619	1.000	-5.11923	2.65803
	Treatment P2	**-7.716400***	1.292619	.000	-11.60503	-3.82777
	Treatment P3	**-11.285600***	1.292619	.000	-15.17423	-7.39697
Extract Dosage 5% (P1)	Normal Control (K)	1.230600	1.292619	1.000	-2.65803	5.11923
	Treatment P2	**-6.485800***	1.292619	.001	-10.37443	-2.59717
	Treatment P3	**-10.055000***	1.292619	.000	-13.94363	-6.16637
Extract Dosage 10% (P2)	Normal Control (K)	**7.716400***	1.292619	.000	3.82777	11.60503
	Treatment P1	**6.485800***	1.292619	.001	2.59717	10.37443
	Treatment P3	-3.569200	1.292619	.083	-7.45783	.31943
Extract Dosage 15% (P3)	Normal Control (K)	**11.285600***	1.292619	.000	7.39697	15.17423
	Treatment P1	**10.055000***	1.292619	.000	6.16637	13.94363
	Treatment P2	3.569200	1.292619	.083	-.31943	7.45783

*. The mean difference is significant at the 0.05 level.

From the Bonferroni Post Hoc Test (Table 7), a comparison of group I and group J shows that the average percentage of collagen density in mouse skin tissue differs between all groups, marked with an asterisk "*": the normal control group (K) and the P2 and P3 groups. P1 has a different average than P2 and P3. P2 has an average other than K and P1. The standards of groups P3, K, and P1 differ. In mouse skin tissue, groups K and P1, P2 and P3, and P3 and P2 had similar collagen density percentages.

The normal control group (K) had the lowest collagen density when seen using Image J software with the area fraction approach in 5 fields of vision at 400x magnification. The photo shows that group K (blue) has less collagen density than the others.

Fig 1. Histopathological Collagen Density Results at 400x Magnification.



Note: A: Blue represents normal group collagen density (K), B: 5% dosage treatment group collagen density (P1), C: 10% dosage treatment group collagen density (P1), D: 15% dosage treatment group collagen density (P1).

After UVB light exposure, group P3, given 15% lemon peel extract lotion, had higher collagen density. Lemon peel extract cream has more compounds due to its higher dose.

Discussion

Photoaging—extrinsic skin aging—is induced by unprotected UV light and involves preventable structural and functional alterations. UVB causes most DNA damage and inflammation during photoaging. UVB rays can penetrate the epidermis and higher dermis, causing aging collagen degradation and elastotic skin formation [21], [22].This study measured collagen growth using Image J's collagen growth area percentage. The lemon plant extract was employed in this investigation. Making lemon peel extract requires maceration. By blending 1000 grams of dried lemon peel, placing it in a vessel, adding 7500 ml of 70% ethanol, and stirring for five days, maceration is completed. Scrub/stain, squeeze, and wash the dregs with 2500 ml 70% ethanol. Next, concentrate at 500C with a rotary evaporator until the water stops dripping. The liquid extract was evaporated in a 500C water bath. We are manually controlling the temperature with a thermometer. Ethanol meets extracting requirements. Ethanol dissolves most polar and non-polar compounds. Lemon peel extract is then made into cream. The more solvents utilized, the higher the yield.Lemon peel extract that passed the phytochemical test contains flavonoids, saponins, phenolics, and tannins. Adding 1 mL of thick lemon peel extract to a test tube, 2 mg Magnesium powder, and three drops of concentrated HCl produces an orange and yellow solution that shows flavonoids. A foam of 3.8 cm was visible after shaking 10 mL of the thick lemon peel extract vertically in a test tube for 10 seconds and leaving it for 10 seconds to determine its saponin component concentration.Tannin and phenolic metabolic compound tests involved pouring 1 mL of thick lemon peel extract into a test tube and adding a few drops of 10% iron (III) chloride solution to produce a dark blue or greenish-black color.

Male white rats were utilized in this study because they are easy to handle and have human-like physiology and anatomy. Twenty mice—five per group—were employed. To adapt, mice were acclimatized for one week before treatment. Each rat was grouped and marked as follows: normal control group (K) without treatment, treatment group 1 (P1), which received 5% topically lemon peel extract cream; treatment group 2 (P2), which received 10% topically, and treatment group 3 (P3), which received 15% topically.Before sacrificing mice with chloroform, skin/tissue samples were taken on the 15th day. The back area was cleaned of hair that was growing back, and the skin was cut with a thickness of ± 3 mm up to the subcutaneous and a length of 2.5 cm—hematoxylin-stained tissue for 5 minutes and washed for 10 minutes with running water. After staining with eosin for 2 minutes, the sample was placed in a graded alcohol solution, cleared with still, and covered with an adhesive-coated cover glass.The results of observing collagen density preparations using Image J software with the area fraction method using five fields of view at 400x and analyzing the results above show that the Shapiro Wilk test significance value for each group is > 0.05, so the test is significant. Further statistical analysis was conducted using the parametric ANOVA test with a p-value < 0.05. Parametric ANOVA yielded 0.000 significance.The significance value is ($p<0.05$), indicating substantial differences among groups.Statistical analysis proceeded with Bonferroni.The normal control group (K) had the lowest collagen density in the Bonferroni post hoc difference test.

Significant differences ($p<0.05$) were observed in the normal control group (K) with groups P1 and P2. The collagen density score in the normal control group (K) was +2 with an average of 41.969% ± 1.171, lower than the P1, P2, and P3 groups.

Since the normal control group was not exposed to UVB rays or administered lemon peel extract lotion, collagen production was more persistent.Groups P1, P2, and P3 treated with lemon peel extract cream had average collagen densities of 43.200%, 49.686%, and 53.225% or higher than the normal control group. The collagen density scores in groups P1 and P2 were +2, indicating that the three formula groups had moderate collagen fiber density (10-50% per field of view). In the P3 group, the collagen density score was +3, indicating dense collagen.The vast difference in results between groups P1, P2, and P3 and the normal control group (K) reveals that extract cream increases collagen in mouse skin after UVB light destroys mouse skin tissue. The rise in lemon peel extract in cream preparations—5%, 10%, and 15% in groups P1–P3—influenced this condition. Thus, the collagen fiber density between P1, P2, and P3 increases with the lemon peel extract dose in the cream. Lemon peel extract cream contains flavonoid and polyphenol compounds, which have antioxidant and anti-inflammatory properties due to flavonoids, polyphenols, carotenoids, vitamins C and E, and extracts. It can prevent or accelerate collagen degradation or production.As an astringent, the cream's tannin and phenolic content reduce mucosal permeability and strengthen mucosal connections, preventing irritants. Tannins indirectly shrink and kill germs by affecting their permeability. These findings suggest that lemon peel's phenolic component prevents free radical-induced cell damage and inflammation.

IV. CONCLUSION

This research shows that lemon peel extract contains flavonoids, saponins, tannins, and phenolics, making it a medicinal ingredient with high antioxidant and anti-inflammatory compounds. This study found that UVB light exposure normalized collagen development in the control group (K) compared to the P1, P2, and P3 treatment groups. This is because the typical control group (K) was not given any cream with active ingredients to promote collagen development in skin tissue.This study found that the normal control group had no collagen growth and was stable. This was because the typical control treatment was not exposed to UVB rays; hence, collagen formation was stable. The 15% lemon peel extract cream group exhibited a more substantial effect on rat skin tissue collagen development following UVB light exposure than the 5% and 10% groups. This is because a higher dose of lemon peel extract includes more chemical components that promote mouse skin collagen formation.urther research could examine higher and better concentrations or doses of lemon peel extract as a treatment preparation and compare them to the positive control group (bioplacenton).

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