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An Investigation on The Impact of Orally Administered Celery and Orange Juices on The Production of Collagen in Rats Exposed to Ultraviolet-B Light

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ABSTRACT

At the correct dosage, ultraviolet (UV) radiation has many positive health effects and is essential for the body. The flip side is that exposure to excessive amounts of ultraviolet light (UV) can be harmful. Repetitive exposure to UVB light causes reactive oxygen species (ROS) to be produced, reducing the production of antioxidant enzymes, increasing oxidative protein modification, and causing glycation and lipid peroxidation products to accumulate. Because of this, it's essential to take precautions to guard the skin from free radical damage and slow the aging process by employing antioxidants in various forms. In this study, rats were exposed to UVB light and then given either celery juice (Apium graveolens) or orange juice (Citrus) orally to see if it would increase the quantity of collagen in the rats. True experimentation, or study in a controlled laboratory setting, is the method of choice. Each of the three groups consisted of eight white rats, for a total of twenty-four rats. Due to their high concentrations of antioxidant components, the phytochemicals found in celery and orange juices were determined to have therapeutic potential in the study's conclusions. With a collagen density value of 3+ and a density of collagen fibers in the skin ranging from 50 to 90% per field of view, the group given orange juice had a more substantial impact on the collagen growth of rat skin tissue after exposure to UVB light compared to the group given celery juice.

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1. INTRODUCTION

The skin is one of numerous tissues and organs that make up an aging body; it is the organ closest to the body's surface. An organ that covers every part of the body, the skin also acts as a barrier to keep harmful substances out. Skin is one of the body's most vulnerable organs to the detrimental impacts of pollution and UV radiation (Rosaini et al., 2019). Protecting and maintaining skin health is important because free radicals, such as UV radiation, can harm the skin and impact its appearance and health.

Indonesia is a tropical nation that enjoys sunshine throughout the year. About eighty percent of skin aging is caused by ultraviolet radiation. Pigments and spots like elastin and Antigo can be caused by long-term exposure to ultraviolet radiation. There are three distinct bands of ultraviolet light: ultraviolet A (320–400 nm), ultraviolet B (290–320 mm), and ultraviolet C (270–290 nm). According to Siahaan et al., UVB rays have a more substantial impact on increasing skin pigmentation (Siahaan et al., 2017). Excellent UVC protection isn't enough to prevent skin damage. UVB rays are a thousand times more damaging to skin than UVA rays since they can reach the skin's outermost layer. Skin cancers, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), can develop as a result of free radical generation, early aging, and exposure to ultraviolet B (UVB) radiation (Veronica et al., 2021).

Long-wavelength ultraviolet A (UVA) radiation can reach the lip's outer layer and even the dermis, the skin's deepest layer. According to Azzahra et al., the uppermost layer of skin absorbs most

ultraviolet B (UVB) radiation (Azzahra et al., 2014). Compared to UVA rays, UVB rays are more likely to produce skin burns, which might affect fertility. According to Rosita (2017), skin can be damaged by UVA radiation, which can penetrate deeper layers of the skin and even alter its DNA, leading to aging (Rosita et al., 2010).

Aging is an inevitable process that all living things on Earth must undergo. The slow loss of a tissue's capacity to self-repair and preserve its form and function causes aging (Madonna et al., 2016). Skin collagen is reduced due to radical oxygen species (ROS) released during skin aging brought on by UV radiation exposure. UV exposure can cause reactive oxygen species (ROS) to develop, which can cause genes and proteins that cause skin damage and skin cancer to express (Ekawati & Wulandari, 2021). Repeated exposure to UVB light causes ROS to grow, which in turn causes an increase in oxidative protein modification, a decrease in antioxidant enzyme production, and an accumulation of glycation products and lipid peroxidation. The collagen formation is impeded by ROS generated during UV exposure, as they block the transforming growth factor (TGF)-β. Additionally, they promote the production of matrix metalloproteinase (MMP)-1, an enzyme that breaks down collagen (Chou et al., 2016).

Since collagen is a constituent of the skin's structure, its breakdown leads to wrinkles and aging. Collagen, the human body's primary extracellular protein (polypeptide), is present in nearly every organ. Currently, 21 different forms and quantities of collagen have been identified in various human organs. Over 71% of skin comprises the significant molecular protein collagen. Coagents contribute to skin elasticity, firmness, and regeneration of skin cells. A lack of collagen will result in early aging symptoms such as wrinkles, fine lines on the skin, and decreased skin suppleness (Reilly & Lozano, 2021). The collagen breakdown in photoaged skin is caused by MMP-1, the type most affected by solar UV light induction. Melanoma, basal cell carcinoma, mite-like sunspots, solar keratosis, automatic fibrosis, coronary optic cheilitis, and squamous cell carcinoma are among the dermatological conditions that can arise from uncontrolled skin photoaging (Subchan et al., 2022).

Collagen gives the skin its firm appearance by supporting it. Skin loses its suppleness and begins to droop and wrinkle as a result. Antioxidants boost collagen creation, which means they can prevent this. An additional effective method of avoiding skin aging is the usage of antioxidants. According to She et al., antioxidants are commonly utilized to lessen the effects of UV radiation and shield the skin from its potential hazards (She et al., 2019). Fewer adverse effects are associated with naturally occurring antioxidants in fruits and bodies than synthetic ones (Ribeiro et al., 2018).

Celery, scientifically known as Apium graveolens, is one plant with antioxidant characteristics. One of the most common uses for the herb Apium graveolens L. is in cooking and as a nutritional supplement. Analgesia, dry eyes, high blood pressure, coughs, reduced cholesterol, and hair fertilization are just some of the many ailments that celery leaves have traditionally been used to treat (Jubaidah et al., 2018). Among celery's medicinal uses are antispasmodic, antirheumatic, sedative, antihypertensive, antimicrobial, and uric acid-lowering agents (Rahayu et al., 2022).

The flavonoids found in celery have a variety of medicinal uses, including reducing inflammation, fighting viruses, warding off cancer, and possibly even preventing free radical generation (Djajanti & Asfi, 2018). The plant known as celery has a variety of nutrients, including essential oils, tannins, aspirin, apigenin, choline, asparagine, bitter compounds, vitamins A, B, and C, iron, calcium, sulfur, and phosphor, as well as other components (Hutauruk et al., 2020; Mentari et al., 2020; Rahayu et al., 2022). You may use any section of the celery for any purpose because it has all of these elements.

In addition to celery, oranges are another fruit that contains antioxidants. Protecting skin from free radical damage, which can lead to aging, is one of the many functions of orange antioxidants. Eat an orange daily to keep your skin looking fresh even after hitting 50. Oranges are rich in vitamin C and contain a lot of water in their flesh. Malina and Mujahid say orange juice is rich in essential minerals like potassium, vitamin C, and folate. Collagen production is one of vitamin C's basic functions. The synthesis of hydroxyproline, a crucial component in collagen creation, requires vitamin C for the hydroxylation of proline and lysine (Marlina & Mujahid, 2020).

The information demonstrates that antioxidants found in celery and orange leaves help prevent skin damage from free radicals, which can accelerate aging. This research aims to determine and test the effect of oral administration of celery juice (*Apium graveolens*) and orange juice (*Citrus*) on increasing the amount of collagen in rats (*Rattus Norvegicus*) Wistar strain that were given Ultra Violet-B light since previous studies on the topic have not yet revealed this to be the case.

2. METHOD

Laboratory experimental research, also known as an actual experiment, is what this study is describing (Notoatmodjo, 2022). The researchers utilized a post-test-only group design to examine how giving Wistar mice Ultra Violet-B light and then administering celery juice (*Apium graveolens*) and orange juice (Citrus) affected the mice's collagen amount.

The rats used in this study were adult male Wistar rats (*Rattus Norvegicus*), which means they were between 160 and 200 grams, two to three months old, and deemed healthy because they exhibited standard movement patterns and had no visible abnormalities. This study utilized twenty-four white rats, with eight allocated to each of the three groups. Researchers conducting in vivo studies must adhere to the "3R Principle" (Replacement, Reduction, and Refinement), which dictates that the number of animals employed in the study must be minimized without compromising the validity of the data. This decision was made after considering the "Reduction" point (Kendall et al., 2018). Two types of variables are used in this study: independent and dependent (Suwarno & Nugroho, 2023). In this study, orange juice and celery juice are independent variables. This study's dependent variable was the amount of collagen increased in male Wistar rats (*Rattus Norvegicus*) exposed to UV-B radiation.

The tools used in this research include rat cages, digital scales, juicers or blenders, filters, packaging bottles, masks, gloves, mini surgical tools (stainless still tray, scalpel, blade, scissors, and tweezers), syringes, blunt-tipped sonde, UVB and UV-meter lamps, preparation tools, LC Evolution camera and Olympus Bx51 microscope, laptop, and Adobe Photoshop CS2 device. Meanwhile, the ingredients used include celery leaves, sweet oranges, distilled water, anesthetic (ketamine), xylazine, male white rats, rat food, and drink.

Then, the acclimatization procedure for the test animals was carried out for one week at the Animal House, Faculty of Mathematics and Natural Sciences, Medan State University. Then make Celery Juice and Orange Juice. Then, the treatment procedure was carried out in the control group (P-0), given exposure to UV-B light and distilled water—treatment group 1 (P-1): 4 ml of celery juice orally and exposure to UV-B light. Treatment group 2 (P-2): 4 ml of orange juice orally using a gastric probe and exposure to UV-B light. SPSS was used to do the statistical analysis of the research data. The Kolmogorov-Smirnov test was used to examine the normality of the data ($p > 0.05$) (Ghozali, 2018). The t-test, also known as the independent sample T-test, was used to determine whether or not there was a statistically significant difference in the levels of efficacy between the trial groups (p 0.05) (Sugiyono, 2018).

3. RESULTS AND DISCUSSION

Research Result

Celery and orange content and phytochemical tests were studied at the University of North Sumatra Faculty of Mathematics and Natural Sciences. Celery and oranges from Berastagi, Karo Regency, and North Sumatra were used. Phytochemical experiments on celery and orange extracts in 90% ethanol solvent can reveal secondary metabolite chemicals. Because it matches the compound's polarity, 90% ethanol solvent is used.

Table 1 shows that the compounds or chemical substances contained in celery extract are flavonoids, alkaloids, and tannins. These results are from research by Gustiana (2022), who also found the content of flavonoids, steroids, terpenoids, alkaloids, and tannins in celery extract (*Apium graveolens*) (Gustiana et al., 2022). From Table 1, it can also be observed that the compounds or chemical substances contained in orange fruit extracts are flavonoids and phenolics. These results are based on research by Fahrurroji (2020), who also found the content of flavonoid and phenolic compounds in orange fruit water extract (Fahrurroji & Riza, 2020).

Table 1. Results of Phytochemical Tests

Compound	Celery Extract		Orange Extract	
	Result	Note	Result	Note
Flavonoids	Orange solution	+	Yellow-orange solution	+
Saponin	No foam is formed.	-	No foam is formed.	-
Alkaloids	A brown precipitate is formed.	+	No brown or yellow precipitate is formed.	-
Tanin	Green solution	+		
Phenolic			Red color solution	+

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

Table 2 shows that between day 1 and day 14, the average weight of the control group of mice (P0) exposed to UVB light and provided only distilled water grew by 43.00 grams. A 29.13-gram gain in average body weight was seen in P1 mice after they were exposed to UVB light and given 4 milliliters of celery juice. In addition, after being exposed to UVB light and fed 4 ml of orange juice, the average body weight of P2 mice increased by 22.50 grams. The average overall body weight of the mice was 31.54 grams, or from 215.67 ± 16.39 grams to 247.21 ± 17.71 grams, according to the evidence in Table 2.

Table 2. Average Rat Body Weight (grams)

Groups	Observations of Rats Day to		
	1	8	15
Control (P0)	211.63	233.63	254.63
Celery Juice (P1)	208.50	233.38	237.63
Orange Juice (P2)	226.88	245.13	249.38
Mean	**215.67**	**237.38**	**247.21**
SD	9.83	6.71	8.70

The results of the collagen density test using Image J software for each group of experimental mice are presented in Table 3.

Table 3. Collagen Density Test Results in Rat Skin Tissue

Mice	Collagen Density (%)		
	P0	P1	P2
1	38.56	47.92	51.03
2	39.67	46.32	54.97
3	37.85	45.18	53.18
4	38.26	44.87	50.28
5	39.28	44.28	50.33
6	37.88	48.15	52.34
7	38.30	45.80	52.30
8	39.41	46.350	55.15
Mean	**38.65**	**46.11**	**52.45**
SD	0.71	1.38	1.91
Skor	+2	+2	+3

Note : P0 = control, only exposed to UVB light, P1 = giving 4ml celery juice and exposure to UVB light, P2 = giving 4ml orange juice and exposure to UVB light

Table 3 shows that the average percentage of collagen density in the control group of mice (P0), which were only exposed to UVB light, was 38.65 ± 0.71. The average percentage of collagen density in treatment group I (P1), namely those exposed to UVB light and given 4ml celery juice, was 46.11 ± 1.38. The average percentage of collagen density in treatment group II (P2) mice, namely those exposed to UVB light and given 4ml orange juice, was 52.45 ± 1.91.

Table 4. Normality Test (Shapiro-Wilk Technique)

Group Extract Dose	Statistical	Significance
Control (P0)	0,896	0,265
Celery Juice (P1)	0,936	0,576
Orange Juice (P2)	0,903	0,310

Data normalcy is crucial because data that follows a normal distribution is considered representative of the population as a whole. The data is said to be normally distributed if the p-value is more significant than 0.05 and not normally distributed if the p-value is less than 0.05 (Ghozali, 2018).

Table 4 shows the results of an SPSS normality test, which demonstrated that from day 1 through day 14, both the control and treatment groups had statistically significant values for the collagen density % variable. The control group (P0) had a significance value (p) of 0.265 in the Shapiro-Wilk Test, the group given celery or celery juice (P1) had a p-value of 0.576, and the group given orange juice (P2) had a p-value of 0.310. Therefore, the data on collagen density percentage follows a normal distribution, according to the Shapiro-Wilk normality test**.**

Table 5. Test Of Homogeneity of Variances

Results Category	Levene Statistical	df1	df2	Sig.
Based on Mean	2.666	2	21	0.093
Based on Median	2.385	2	21	0.117
Based on Median and with adjusted df	2.385	2	14.869	0.126
Based on trimmed Mean	2.596	2	21	0.098

The percentage of collagen density, which reflects the increase in collagen in the skin in each group P0, P1, and P2 after 14 days of therapy, was assessed for homogeneity using the One Way ANOVA Test. Table 5 shows that the research data variance of the P0, P1, and P2 groups is 0.093 (p>0.05).

Table 6. ANOVA Test

Comparison of wound healing percentages	Number of Comparisons	df	Significance Value
Between Groups	763.015	2	0.000
In Group	42.345	21	
Total	805.360	23	0.000

On top of that, we used Table 6 to compare the three groups that were either observed or studied for differences in average percentage of collagen density. The results are derived from the "Sig" column of the above table. A p-value of 0.000 was achieved. Therefore, we can conclude that there is a significant difference in the average percentage of collagen density between the three groups since Ho is rejected at the actual level = 0.05.

Additional tests utilize the Bonferroni Post Hoc Test, with results shown in Table 7. White rats (*Rattus Norvegicus*) of the Wistar strain show differences in the average percentage of collagen density across all groups, as demonstrated by comparing groups I and J. These differences are indicated by an asterisk ("*"). SPSS for Windows was used for the Bonferroni Post Hoc Test on groups (Campbell & Stanley, 2015). On the fifteenth day, after the mice had been sacrificed by inhalation with chloroform, the skin was obtained by cutting the skin at a length of 2.5 cm, a thickness of ± 3 mm up to the sub-cut, and removing any hair that was beginning to grow back from the back area (NRC, 2011).

Table 7. Post Hoc Bonferroni Test Results

Test	Groups (I)	Groups (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Control (P0)	Celery Juice (P1)	-7.45750*	.71000	.000	-9.3045	-5.6105
		Orange Juice (P2)	-13.79625*	.71000	.000	-15.6432	-11.9493
	Celery Juice (P1)	Control (P0)	7.45750*	.71000	.000	5.6105	9.3045
		Orange Juice (P2)	-6.33875*	.71000	.000	-8.1857	-4.4918
	Orange Juice (P2)	Control (P0)	13.79625*	.71000	.000	11.9493	15.6432
		Celery Juice (P1)	6.33875*	.71000	.000	4.4918	8.1857

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

Hematoxylin-stained tissue was washed for 5 minutes and 10 minutes with running water. After staining with eosin for 2 minutes, the sample was placed in a graded alcohol solution, cleared with sill, and covered with an adhesive-coated cover glass. A light microscope coupled to a digital camera and OptiLab Viewer 2.2 software was used to observe collagen density using red hematoxylin-Eosin staining. 400-times magnification was used to view the preparations in 5 fields. Press the camera icon on the taskbar of OptiLab Viewer 2.2 to save images (Chen et al., 2017).

What follows is a picture of the results of the histological preparations used to observe the collagen density in the skin tissue of the mice in each group. We could watch the extensive reading procedure for collagen density using the Image J software application.

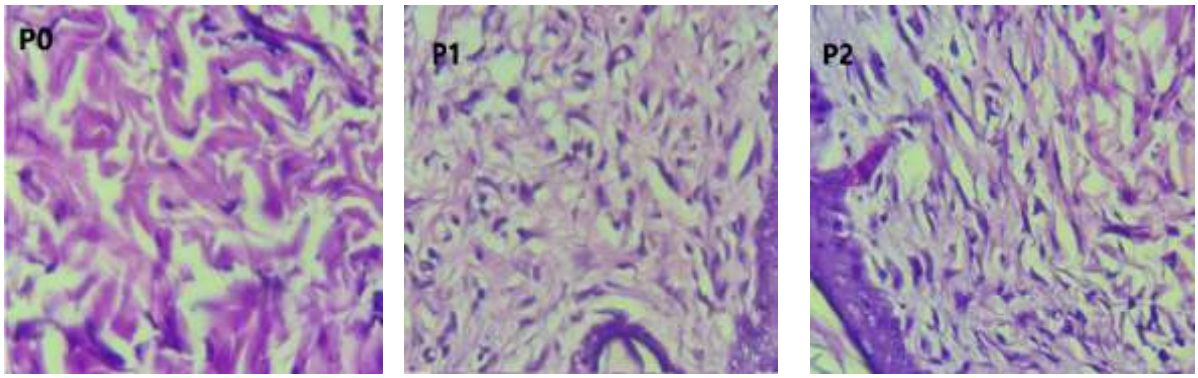

Figure 1: Control–P0 Collagen density is moderate and unusual (scoring +2). P1 (Celery Juice) Collagen density is medium and becoming dense (score +2). Orange Juice (P2) High collagen density (score +3)

As shown in Figure 1, the collagen density preparations were seen using Image J software with the area fraction method. Five fields of view were used, and the magnification was set at 400x. Compared to the other groups, the standard control group (P0), consisting of individuals exposed solely to UVB light, exhibited the lowest percentage of collagen density. The snapshot shows that the P0 group (blue) has lower collagen density than the other groups.

Those in the P2 treatment group, who received 4 milliliters of orange juice, had higher collagen density than those in the P1 group, which was exposed to UVB light and given celery juice. The reason behind this is that compared to celery juice or celery, orange juice has a higher chemical level.

Research Discussion

Extrinsic skin aging, known as photoaging, encompasses avoidable alterations in the structure and function of the skin, primarily resulting from unprotected exposure to ultraviolet light (Gromkowska-Kępka et al., 2021; Guan et al., 2021; She et al., 2019). UVB radiation is the primary cause of direct DNA damage and inflammation in photoaging. UVB radiation can penetrate both the outermost layer of the skin (epidermis) and the layer beneath it (upper dermis) (Fernandez et al., 2014; Juzeniene & Moan, 2012; Matsumura & Ananthaswamy, 2004). As a result, in addition to triggering typical aging processes, UVB radiation also leads to the breakdown of collagen and the creation of elastotic substances in the skin.

The percentage area of collagen density, as measured using Image J software, was used to determine the amount of collagen that grew in this investigation. In this work, we aimed to determine if using celery and orange juice orally or gastrically in experimental mice will improve collagen density following UVB light exposure. We hypothesized that these natural ingredients would have this effect.

Celery juice and celery that were positive in the phytochemical test contain chemicals such as tannins, alkaloids, and flavonoids. The orange color reaction demonstrated the flavonoid concentration in celery extract in the test. Once a brown precipitate was observed in the solution reaction, it was determined that the celery extract contained alkaloids. The celery tannin test was likewise deemed positive after the chemical reaction, which produced a greenish-brown solution.

Consistent with earlier work by Gustiana, the phytochemical test results show that celery extract (Apium graveolens) contains flavonoids, steroids, terpenoids, alkaloids, and tannins (Gustiana et al., 2022).

According to the phytochemical test results, this study found that orange juice contains active or beneficial components, such as flavonoids and phenolics. The orange-yellow color response in the test indicates that orange juice contains flavonoids. The chemical reaction yielded a red solution, meaning the orange juice had passed the phenolic test. The findings of the phytochemical tests were consistent with those of earlier studies by Fahrurroji; those researchers had previously discovered the presence of flavonoid and phenolic components in orange juice extract (Fahrurroji & Riza, 2020).

Because they are familiar, manageable, and physiologically and anatomically comparable to humans, male white rats served as the experimental animals for this study. Each group consisted of 8 mice, for a total of 24 mice. For the mice to be ready for treatment, they were given a week to acclimate. Separated and labeled were the following groups of rats: P0, which served as a control; P1, which was subjected to UVB light in addition to 4 milliliters of celery juice taken orally; and P2, which was subjected to UVB light in addition to 4 milliliters of orange juice taken orally. The mice had an average weight of 215.67 ± 16.39 grams on the first day and 247.21 ± 17.71 grams on the fifteenth day.

Afterward, samples of skin and other tissues were collected on the fifteenth day before the mice's inhalation with chloroform for sacrifice. To prepare the skin for collection, the area to be sampled was cleansed of hair that was beginning to regrow, and the skin was then cut with a thickness of ± 3 mm up to the subcut and a length of 2.5 cm. Samples of tissue were immersed in hematoxylin for five minutes before being washed under running water for ten. After two minutes of eosin staining, the sample was engaged in a graded alcohol solution and rinsed with silol. Finally, the tissue slide was covered with an adhesive-coated cover glass.

It can be seen from the results of calculating the percentage of collagen density that the control group of mice (P0), who were exposed to only UVB light, had an average percentage of 38.65% ± 0.71, which is a score of +2, or medium density of collagen fibers in the skin. Collagen fiber density in the skin was medium, with an average percentage of 46.11% ± 1.38 in treatment group I (P1), which included individuals exposed to UVB light and given 4ml of celery juice. The mice in treatment group II (P2), exposed to UVB light and fed 4 ml of orange juice, had an average collagen density percentage of 52.45% ± 1.91, indicating dense collagen fibers in the skin or a score of +3.

From day 1 through day 14, the collagen density % variable exhibited significant values for the control and treatment groups, according to the normality test results using Shapiro-Wilk. The control group (P0) had a significance value (p) of 0.265 in the Shapiro-Wilk Test, the group given celery or celery juice (P1) had a p-value of 0.576, and the group given orange juice (P2) had a p-value of 0.310. Therefore, the data on collagen density percentage follows a normal distribution, according to the Shapiro-Wilk normality test.

Research data homogeneity testing revealed that all three groups' variables (P0, P1, and P2) had the same variance (0.093, $p>0.05$), suggesting that the data was either generated from the same population or was otherwise homogeneous. In addition, additional tests utilizing the Bonferroni Post Hoc Test led to the conclusion that the Wistar strain of white rats (*Rattus Norvegicus*) had a different average percentage of collagen density compared to all other groups.

When looking at collagen density preparations, the group that was only exposed to UVB light (P0), the usual control group, had the lowest percentage of collagen density compared to the other groups. The P0 group (blue) appears to have lower collagen density in the photo than the other groups. Those in the P2 treatment group, who received 4 milliliters of orange juice, had higher collagen density than those in the P1 group, which was exposed to UVB light and given celery juice. This is because orange juice's chemical composition is more influential than celery or celery juice. This provides support for the theory that drinking Citrus juices, such as orange and celery, can increase collagen production in the skin of mice exposed to ultraviolet B light.

The fact that this study only utilized 32 white mice, or eight mice per group, as a sample size is lower than in other research is another potential confounding issue. Since the likelihood of

generalization errors decreases as the number of samples used increases, research that uses a large sample size will inevitably have an impact (Weichbrod et al., 2018).

4. CONCLUSION

The research concludes that the phytochemical tests revealed the presence of flavonoids, alkaloids, and tannins in celery juice. Additionally, it was discovered that orange juice contains flavonoids and phenolics. Therefore, it can be inferred that celery juice and orange juice possess phytochemicals that can serve as therapeutic components due to their abundance of antioxidant substances. The study concluded that there was a significant disparity in the average percentage of collagen growth between the control group (P0) and the P1 and P2 treatment groups after exposure to UVB light. The absence of applications or treatments containing active compounds in the control group (P0) hindered the acceleration of collagen development in skin tissue. The study demonstrated that the administration of orange juice had a more pronounced impact on collagen development in rat skin tissue following exposure to UVB radiation, compared to the group administered celery juice and the group just exposed to UVB light. Nevertheless, the mean collagen density percentage was similar between the group that issued orange juice and the group that administered celery juice. Research utilizing and comparing various concentration levels or doses of celery and orange juice should be conducted in the future. To determine the optimal dosage for stimulating collagen formation in rat skin. We need to do additional studies on safer and more effective ways to prepare celery and oranges in the future. Researchers interested in studying the effects of drinking celery juice or a combination of celery and orange juice, mainly to speed up collagen formation following UVB radiation exposure, should compare the results of this study to those of other studies.

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