

The Effectiveness of Star Anise (*Illicium Verum*) Extract Nanoemulsion on Fibroblast Density and Collagen in the Skin of Male Wistar Rats (*Rattus Norvegicus*) Exposed to Ultraviolet Light

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ABSTRACT

UV radiation can also cause sun damage to the skin through DNA damage, inflammation, oxidative stress, which is a condition where the production of Reactive oxygen species (ROS) exceeds the body's antioxidant defenses, which causes cell damage.. This research aims to Testing and analyzing the effectiveness of nano emulsion of star anise (*Illicium verum*) extract on the number of fibroblast and collagen density in the skin of male white rats (*Rattus norvegicus*) Wistar strain after exposure to UVB rays. The sample of mice used was 25 in 5 groups (positive and negative controls, treatments 1, 2, and 3) where the extract preparation in the treatment was a nanoemulsion of star anise extract (*Illicium verum*) with a concentration of 10% and 20%. The results were Based on histological assessment, treatment group 2 also showed high collagen density with a score of 3, similar to the normal group and the positive control (vitamin C). In contrast, the negative control group experienced severe collagen damage with a score of 0. This indicates that a concentration of 20% is able to repair collagen tissue damaged by UVB exposure.

Keywords: *Star anise, Nanoemulsion, Skin, UVB, Collagen*

INTRODUCTION

Deeper penetration into the dermis of the skin, is considered to be the dominant factor causing the development of skin aging. (Lan, 2019) Skin aging is characterized by wrinkles, sagging, roughness, and dryness, which occur naturally with age but are also triggered by external factors, with ultraviolet radiation being the most important. A certain amount of UV exposure is essential for human skin vitamin D synthesis. However, excessive exposure to UVB (ultraviolet B radiation), which has various biological effects at wavelengths corresponding to ultraviolet light 280–320 nm, damages DNA, directly and through the formation of free radicals in skin fibroblasts. This disrupts the antioxidant defense system, causes oxidative stress, and increases the activity of matrix metalloproteinases (MMPs), thus leading to photoaging. (Kim, 2024).

The skin is the first barrier to the external environment. Therefore, any damage must be repaired promptly to ensure the restoration of adequate structural resilience. Skin damage repair is a complex, yet well-orchestrated, process in which fibroblasts play a central role, not only providing intercellular signal exchange but also secreting new ECM. Fibroblasts contribute to the secretion and assembly of matrix components necessary for cell migration, provide signals for re-epithelialization, produce bioactive mediators that promote cell differentiation, and regulate the immune response. (Boraldi, 2024).

UV radiation can also cause sun damage to the skin through DNA damage, inflammation, and oxidative stress, a condition where the production of reactive oxygen species (ROS)

exceeds the body's antioxidant defenses, leading to cell damage. is an oxidizing compound derived from oxygen and is highly reactive, consisting of free radicals and non-radicals. In addition to oxygen derivatives, free radicals also come from nitrogen derivatives. Free radicals are atoms or molecules with unpaired electrons, which are highly unstable and highly reactive.

ROS are unavoidable, harmful byproducts of metabolic processes, and ROS accumulation is a common plant response to various environmental stresses. When plants are exposed to unfavorable conditions, ROS production is exacerbated, activating pathways associated with free radical scavenging (Mishra & Prasad, 2021). One such antioxidant-rich plant is star anise.

Star anise is a popular culinary spice in China and Vietnam. Star anise has also been widely used in traditional Chinese medicine for a long time. Phytochemical analysis of star anise reveals high concentrations of essential oils, phenols, flavonoids, and other bioactive compounds that contribute to its diverse pharmacological properties. These properties include antibacterial, anti-inflammatory and analgesic, antioxidant, antiviral, anticancer, antidiabetic, antidiarrheal, and hair growth promotion. (Zhu, 2024)

Star anise (*Illicium verum*), once known primarily for its culinary uses, has emerged as a potent botanical ingredient in the skincare industry, thanks to its rich chemical composition, including polyphenols, flavonoids, and essential oils. These components give star anise remarkable antioxidant properties, offering numerous benefits for the skin. Research conducted by (Krusha Patel, Ms. Urvisha Panchal, 2024) found that star anise extract can fight free radicals, reduce oxidative stress, address problems such as hyperpigmentation and inflammation, and help even out skin tone.

LITERATURE REVIEW

Fibroblasts are typical mesenchymal cells widely distributed throughout the human body, which play a role in synthesizing and maintaining the extracellular matrix, ensuring the structural role of soft connective tissue, secreting cytokines and growth factors, communicating with each other and with other cell types, acting as a signal source for the stem cell niche, and are involved in tissue turnover, wound healing, fibrosis, and cancer (Boraldi et al., 2024).

Collagen is the main structural protein of various connective tissues, constituting 80% of the dry weight of human skin. Collagen is characterized by a triple helix structure formed by repeating glycine every third residue, and particularly by proline and hydroxyproline at the other residues. Collagen, the most common component of the extracellular matrix, provides mechanical support and directs tissue development (Pu et al., 2023). UVB causes DNA damage through the interaction of high-energy photons with DNA. While cells can repair this damage after short exposure, prolonged exposure significantly increases the risk of skin cancer (Scobodova et al., 2012).

METHODS

The type of research used in this study is quantitative experimental, employing a true experiment or laboratory experimental design. Experimental research is conducted by controlling all external variables that could influence the experimental activities. This study used a post-test only control group design.

The sample of this study 25 Wistar rats were used in each experimental group. The test animals were randomly divided into 5 groups. The experimental group was divided into positive and negative control groups, treatments 1 and 2. The test animals were acclimatized for 7 days in the laboratory. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. Research procedures include: acclimatization of test animals, preparation of star anise extract soaking star anise powder using 96% ethanol

solvent with a concentration of 2000 ml for 3 days, phytochemical test of star anise extract to see the content of secondary metabolites in the extract, making star anise extract nanoemulsion with 96% ethanol, Preparation of test animals, administration of treatment, treatment groups 2 and 3 were given extract doses with concentrations of 10% and 20%, for positive control nothing was given, and negative control mice were exposed to UVB rays given base oil, treatment group 1 mice were exposed to UVB rays and smeared with vitamin C. All test animals were given treatment for 14 days. Then, collagen and fibroblasts were observed through mouse skin histopathology. The data from the study were tabulated and analyzed using SPSS (Statistical Package for Social Science).

RESULTS

Phytochemical testing was conducted to identify the secondary metabolite compounds present in star anise extract, which are suspected to have potential as therapeutic agents. Based on the results of the phytochemical tests, it was concluded that star anise extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

Fibroblast evaluation was conducted through histopathological examination of skin tissue under a microscope to identify changes in the number of fibroblasts in the treated burn tissue. The treatment included the application of a base and star anise at concentrations of 4%, 6%, and 8%. After observing the number, histopathological images were also obtained to determine the level of collagen density in the skin of male white rats (*Rattus norvegicus*) of the Wistar strain exposed to UVB light. The data were then analyzed.

Reporting Research Results

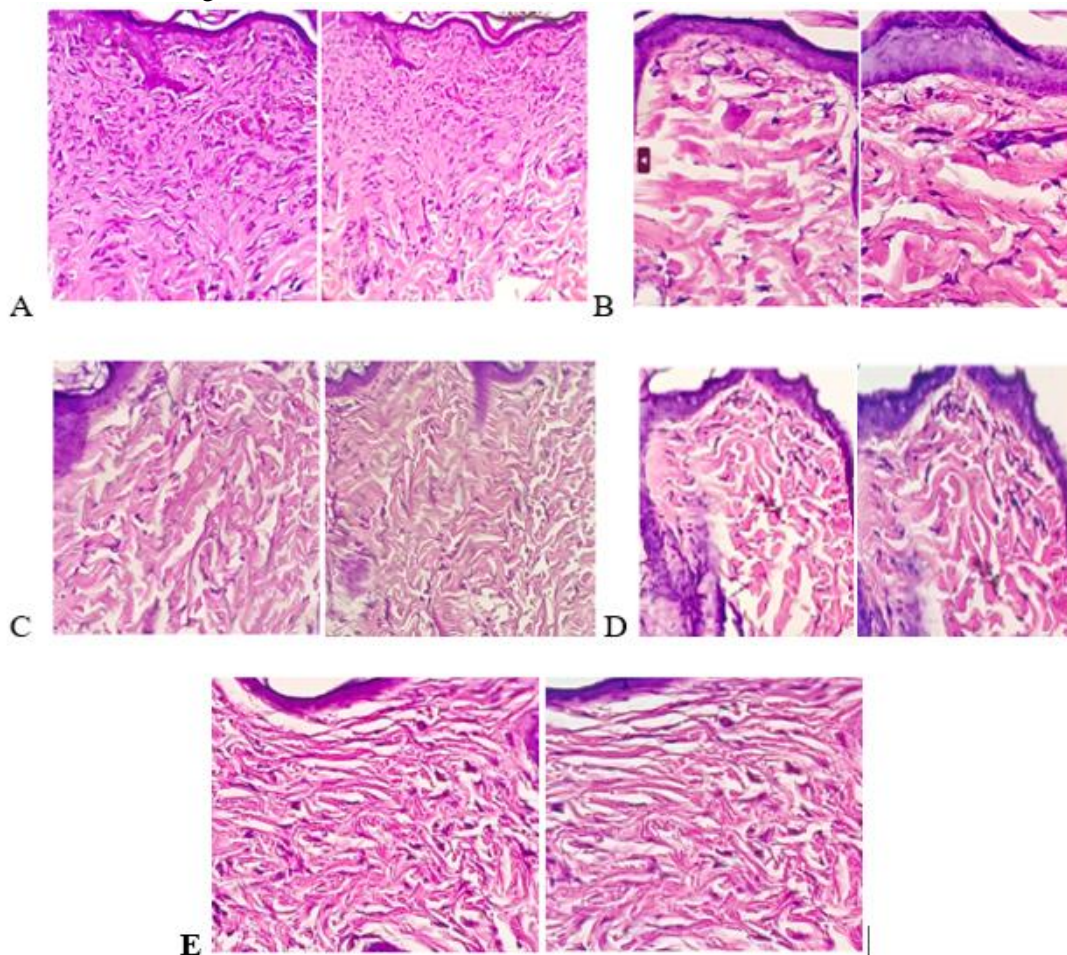
The fibroblast count score refers to the scoring system developed by Karimi et al., (2013), namely: score 0 for tissue without the presence of fibroblasts, score 1 for tissue with 5–10 fibroblast cells, score 2 for tissue with 10–50 cells, and score 3 for tissue with more than 50 fibroblast cells.

Table 1 Fibroblast Observation

Repetition	Group				
	K-1	K-2	K-3	K-4	K-5
1	70	17	49	50	72
2	73	4	45	55	67
3	79	12	41	38	63
4	61	10	55	49	59
5	68	8	51	44	54
6	65	5	58	34	71
Mean	70.2	9.6	48.2	47.2	63
Elementary School	6.61	3.84	5.40	6.45	6.96
Score	3	1	2	2	3

Based on the results of calculating the number of fibroblasts in skin tissue, it is known that the normal group has the highest number of fibroblasts with an average of 70.2 ± 6.61 and is included in the score category 3. The negative control group which was only given distilled water showed the lowest number of fibroblasts, with an average of 9.6 ± 3.84 so it is categorized as score 1. The positive control group which was given vitamin C had an average of 48.2 ± 5.40 and is included in the score 2. Treatment group 1 which received nanoemulsion of star anise extract with a concentration of 10% showed a number of

fibroblasts of 47.2 ± 6.45 . Meanwhile, treatment group 2 which received a concentration of 20% had an average number of fibroblasts of 63 ± 6.96 and is included in the score 3. In histopathological observations of the skin, this effect was evident in the differences in collagen density between the normal, control, and treated groups. Collagen density assessment was performed by observing a single field of view using a microscope at 200x magnification. The results of the collagen density analysis from each group of mice are shown in the following section.



Description: A= Normal Group, B= Negative Control,
C= Positive Control, D= Treatment 1, E= Treatment 2

Figure 1 Skin Histopathology

The normal group, which was not exposed to UVB rays and was not given any treatment, showed an intact and dense collagen structure with an average fiber thickness that exceeded the distance between fibers, so it was categorized into a score of 3. This reflects the optimal integrity of the dermis structure.

The negative control group, which was only given distilled water after UVB exposure, showed a drastic decrease in collagen density, with the fiber structure appearing fragmented and reduced in volume, thus obtaining a score of 1, indicating damage to the collagen structure due to oxidative stress from UVB radiation.

The positive control group, which was given vitamin C as a standard antioxidant, showed significant improvement in collagen morphology, with fiber thickness resembling that of the normal group, and was therefore classified as score 3.

Treatment group 1, which was given a nanoemulsion of star anise extract at a concentration of 10%, showed a moderate level of collagen regeneration, with fiber thickness equivalent to

the distance between fibers, thus obtaining a score of 2. This indicates a partial protective effect against collagen damage.

Treatment group 2, which received the star anise extract nanoemulsion at a higher concentration of 20%, showed a substantial increase in collagen fiber thickness resembling that of the normal group, thus also obtaining a score of 3, indicating the effectiveness of the high dose in maintaining the integrity of the dermal matrix.

Collagen density was then scored. Collagen density was given a score of 0 if collagen growth was very low (<25%); score 1 if collagen growth is low (25%); score 2 if collagen growth is moderate (50%); and score 3 if collagen growth is dense (75-100%). Following are the results of observations of collagen density.

Table 2 Collagen Density Scoring

Repetition	Group				
	K1	K2	K3	K4	K5
1	82	19	70	29	78
2	87	28	65	34	75
3	78	24	61	38	82
4	74	13	59	21	85
5	91	19	74	45	68
Mean	82.4	20.6	65.8	33.4	77.6
Elementary School Score	6.80	5.68	6.22	9.07	6.58
	3	0	2	1	3

Based on the research results, it can be concluded that administration of star anise extract nanoemulsion has an effect on increasing collagen density in the skin of male white rats (*Rattus norvegicus*) of the Wistar strain exposed to UVB light. Histological evaluation showed significant differences in the structure and thickness of collagen fibers between groups, which were analyzed using a collagen scoring system. The normal group received an average score of 82.4 ± 6.80 . Based on this average value, the normal group falls into score 3. For data analysis, The results of the data normality test in this study can be seen in the following table:

Table 3 Results of Fibroblast Normality Test

Group	df	Sig
Normal	5	.987
Negative Control	5	.928
Positive Control	5	.980
Treatment 1	5	.901
Treatment 2	5	.994

Based on the results of the normality test that has been carried out using the Shapiro Wilk test, a significance result of 0.987 was obtained in the normal group. The negative control group had a significance value of 0.928. The positive control group had a significance value of 0.980. Treatment group 1 had a significance value of 0.901. Treatment group 2 had a significance value of 0.994. Data is said to be normally distributed if the $p \text{ value} > 0.05$. Therefore, it can be concluded that the data of the normal group, negative control, positive control, treatment 1, and treatment 2 were normally distributed. After the data was known to be normally distributed, a homogeneity test was continued using the Levene test to

determine whether each variant of this research group came from the same or homogeneous population. Here are the results:

Table 4 Results of Fibroblast Homogeneity Test

<i>Levene static</i>	df1	df2	Sig
.387	4	20	.815

The results of the homogeneity test using the Levene test can be seen in the table above. The significance value is 0.815. The significance value obtained is greater than 0.05, so it can be concluded that the normal group, negative control, positive control, treatment 1, and treatment 2 come from populations that have the same variance, or are homogeneous. Thus, the data from all groups in this study meet the assumption of homogeneity of variance and further analysis can be continued. namely the One-way ANOVA test to test the significant effectiveness between the trial groups, the following results:

Table 5 Results of One Way Anova Fibroblast Test

	Amount	df	Mean square	F	Sig
Intergroup	10962.160	4	2740.540	77,025	.000
In Group	711,600	20	35,580		
Total	11673.760	24			

The results of the One-Way ANOVA test in the table above show that the significance value produced is 0.000 or <0.05. Based on these data, it can be concluded that there is a significant difference between the control group and the treatment group. And the results of the Post Hoc LSD test show that there is no significant difference between treatment group 2 and the normal group ($p = 0.071$), indicating the effectiveness of the treatment in returning the number of fibroblasts to physiological conditions.

DISCUSSION

This research aims to tested and analyzed the effectiveness of star anise (*Illicium verum*) extract nanoemulsion on the number of fibroblasts and collagen density in the skin of male white rats (*Rattus norvegicus*) of the Wistar strain after exposure to UVB rays. Prolonged UVB exposure can cause sunburn, which has a significant impact on all layers of the skin, especially the epidermis and dermis. The dermis shows vascular changes, including enlargement of endothelial cells and edema caused by mast cell degranulation (Guerra & Crane, 2018).

Based on the results of phytochemical tests, it can be concluded that star anise extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids. Star anise's chemical composition is rich in bioactive compounds with antioxidant properties. Star anise also contains vitamins A and C, as well as other nutrients that help fight free radicals. The antioxidants in star anise help reduce dark spots and blemishes while maintaining skin firmness and elasticity by increasing collagen. Vitamin C in star anise has been shown to inhibit melanin synthesis. This can help fade dark spots and even out skin tone (Patel et al., 2024).

Based on the results of calculating the number of fibroblasts in skin tissue, it is known that the normal group has the highest number of fibroblasts with an average of 70.2 ± 6.61 and is included in the score category 3. The negative control group which was only given distilled water showed the lowest number of fibroblasts, with an average of 9.6 ± 3.84 so it is categorized as score 1. The positive control group which was given vitamin C had an average of 48.2 ± 5.40 and is included in the score 2. Treatment group 1 which received nanoemulsion of star anise extract with a concentration of 10% showed a number of

fibroblasts of 47.2 ± 6.45 . Meanwhile, treatment group 2 which received a concentration of 20% had an average number of fibroblasts of 63 ± 6.96 and is included in the score 3.

Further observations were made on collagen density. The normal group and the positive control group (vitamin C) showed the highest scores (3), reflecting a thick and well-organized collagen structure. The negative control group, which was only given distilled water, obtained the lowest score (0), indicating collagen damage due to UVB exposure. Meanwhile, the treatment group given star anise extract nanoemulsion showed an increase in collagen density. Treatment with a 10% concentration showed low results (1), while a 20% concentration was able to restore the collagen structure to near normal conditions (3). Thus, it can be concluded that the administration of star anise extract nanoemulsion, especially at a 20% concentration, has the potential to be an effective topical agent in maintaining and repairing skin collagen structure damaged by UVB exposure.

The fibroblast repair process and increased collagen density are inextricably linked to the bioactive compounds contained in star anise extract. These components, such as flavonoids, polyphenols, and essential oils, play a crucial role in supporting tissue regeneration and collagen synthesis through their antioxidant and anti-inflammatory activities.

CONCLUSION

1. Star anise extract contains various secondary metabolites, including flavonoids, saponins, tannins, alkaloids, and triterpenoids. These active compounds are strongly suspected of increasing antioxidant activity, accelerating fibroblast regeneration, and supporting collagen synthesis.
2. The calculation results showed that treatment group 2 (20% concentration) had a high number of fibroblasts and was close to the normal group. The results of the LSD Post Hoc test showed that there was no significant difference between treatment group 2 and the normal group ($p = 0.071$), indicating the effectiveness of the treatment in returning the number of fibroblasts to physiological conditions.
3. Based on histological assessment, treatment group 2 also showed high collagen density with a score of 3, similar to the normal group and the positive control (vitamin C). In contrast, the negative control group experienced severe collagen damage with a score of 0. This indicates that the 20% concentration is able to repair collagen tissue damaged by UVB exposure.

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