Potential of Curcuma Temulawak Extract on Melatonin and Hyaluronidase Levels in Human Skin Fibroblast Cell Line Exposed to UV Light as an Aging Model

Eva Christine Natalia Lumbantobing[1], Linda Chiuman[2], Edy fachrial[3]

[1]*Biomedical Science master's Study Program, Faculty of medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, eva.tobing@rocketmail.com*
[2]*Biomedical Science master's Study Program, Faculty of medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, lindachiuman@unprimdn.ac.id*
[3]*Biomedical Science master's Study Program, Faculty of medicine, Dentistry and Health Sciences, Universitas Prima Indonesia edyfachrial@unprimdn.ac.id*
**Corresponding E-mail: eva.tobing@rocketmail.com*

KEYWORDS

Skin aging, UV light, Melatonin, Hyaluronidase, Javanese ginger

ABSTRACT

Skin aging is characterized by a decrease in the skin's ability to regenerate and maintain its structure, which is influenced by endogenous factors (genetic mutations, hormonal factors) and exogenous factors (UV exposure, pollution). UV radiation accelerates skin aging by generating reactive oxygen species (ROS) that can activate hyaluronidase and induce inflammation, leading to cell apoptosis. Natural antioxidants such as melatonin play an important role in skin protection, but melatonin levels decrease with UV exposure. The temulawak plant contains compounds that can be utilized as antioxidants. This study aims to determine the potential of Curcuma xanthorrhiza (temulawak) natural extract to reduce UV-induced skin aging by increasing melatonin levels and reducing hyaluronidase activity. Extraction was performed using maceration method with 70% ethanol solvent. Aging model cells were prepared from human skin fibroblast cell cultures that were exposed to UV light 300J/cm2 for 75 minutes. The treatment given to the aging model cells, namely temulawak extract in 3 different concentrations 12.50; 6,25; 3.13 µg/mL. The results showed that temulawak extract significantly increased melatonin level in fibroblasts exposed to UV light, with an optimal concentration of 12.25 µg/mL (147.71 pg/mL metionin). In addition, temulawak extract also significantly reduced hyaluronidase levels with the same optimal concentration (95.78 ng/mL). Curcuma longa extract may serve as an effective natural agent for anti-aging skin care products by increasing melatonin levels and reducing hyaluronidase activity, thus potentially improving skin health and reducing the effects of aging caused by UV exposure.

1. Introduction

Skin aging is a decrease in the skin's ability to regenerate and maintain skin structure (Marta et al., 2016). One of the characteristics of aging is skin folds caused by poor hydration, loss of body mass, epidermal junctions, and dermis disintegration. Skin aging triggers changes caused by a combination of endogenous factors (genetic mutations, cellular metabolism, and hormonal changes) and exogenous factors (UV exposure, chemicals, pollution, and toxins) (Chaudhary et al., 2020). Intrinsic aging occurs due to biological factors, while extrinsic aging is influenced by sun exposure, pollution, and lifestyle habits (Zhang & Duan., 2018).

Sun exposure contains high UV intensity that accelerates the skin aging process (de Paula Corrêa et al., 2021). UV radiation is a natural component of sunlight that is invisible to the naked eye. UV is classified into three types based on wavelength: UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm). The Earth's stratospheric ozone layer fully filters out UV-C, but is unable to filter out UV-A and UV-B rays, allowing them to reach the Earth's surface (Panich et al., 2016). The mechanism of premature aging triggered by UV light triggers free radicals and oxidative stress, which then triggers inflammation that can eventually lead to cell death (apoptosis). UV light stimulates various oxidizing

molecules and free radicals, which due to their chemical reactivity can damage lipids and alter molecular structures (Brand et al., 2018).

In skin protection, the body has natural antioxidants that can clear harmful compounds. One type of natural antioxidant in the body on the skin is melatonin (Fischer et al, 2008). When UV light penetrates the skin, melatonin binds to ROS to protect the skin so that its levels decrease (D'Orazio et al, 2013). However, the more frequent exposure to UV light, melatonin levels continue to decrease. Meanwhile, UV light induces an increase in hyaluronidase which plays a role in degrading hyaluronic acid, resulting in dry and wrinkled skin (Jusri et al., 2019; Weber et al, 2019). Hyaluronic acid functions to bind water and keep the body's skin smooth, watery and lubricated (Roy et al., 2013). Nowadays, skin health and beauty is considered an indicator of one's health (Shanbag et al., 2019). Treatments such as *botulinum toxin* or botox are often used for preventive skin care (Salsabila et al., 2024). However because the effects of botox are *short acting*, patient compliance and repeated injections are a problem. Botulinum toxin works locally, where large muscle groups may require multiple injection sites to achieve the desired effect, making the cost very high (Verheyden & Blitzer, 2002).

People's alternative is to use skin care products to prevent aging, hence the demand for "anti-aging" care products continues to increase (Shanbag et al., 2024). However, it is important to consider the ingredients contained in skincare products. Some synthetic chemicals often used in anti-aging products are considered harmful to human health and the environment (Ramalhete et al., 2018). One example is parabens, which are used as cosmetic preservatives. Continuous use of parabens can cause allergic reactions and skin rashes on sensitive skin (Chermahini & Sarmidi, 2011). Prevention of the effects of skin aging due to UV rays can utilize natural ingredients that have potential as anti-aging and antioxidants (Verma et al., 2024) while triggering an increase in melatonin to inhibit aging (Bocheva et al., 2019). Natural products have the advantages of specialized resources and few side effects. Recently, many compounds or extracts from natural products that slow down aging have been reported (Ding et al., 2017).

Indonesia has abundant natural resources, namely medicinal plants, most of which have not been scientifically proven to be useful. One of the potential natural ingredients is temulawak. Temulawak has the Latin name *Curcuma xanthorriza* is a traditional plant that is utilized throughout the local distribution area as an ingredient of jamu (Indonesian herbal medicine and supplements) or for the treatment and control of various diseases and disorders since ancient times (Rahmat et al., 2021). Recorded in 2019, *C. xanthorrhiza* is widely cultivated in Indonesia with a harvest area of approximately 13,042,873 m^2 producing a yield of 29,637,119 kg (BPS statistic Indonesia, 2019). Temulawak has a distinctive compound, xanthorrizol, which distinguishes it from turmeric (Erpina et al., 2017).

Curcumin and terpenoid compounds are the most abundant phytochemicals in temulawak rhizomes (Minarni et al., 2023). Compounds that play an active role in the biological activity of temulawak include: curcuminoids, xanthorrhizol, ar-turmerone, and α-curcumene (Widyastuti et al., 2021). Previous research reported that temulawak has anti-aging (Wargasetia et al., 2023), antioxidant (Laksmitawati et al., 2022), hepato- and neuroprotector (Rahmat & Kang, 2021), and wound healing (Malau et al, 2024) activities. The compound content and biological activity of temulawak have the potential to be used as a therapeutic material for the future, especially as an anti-aging agent.

Increased hyaluronidase enzyme causes skin aging which is the result of excessive UV exposure. Alternative prevention for this needs to be done, one of which is by increasing melatonin production. Metabolite compounds in temulawak have potential as anti-aging agents. This study was conducted to analyze the potential of temulawak extract on hyaluronidase enzyme activity, melatonin levels, human skin fibroblast cell lines exposed to UV as a model of aging.

2. Methodology

The type of research in this study is *experimental design* with *posttest only control group* method. Place General and Cell Culture Laboratory, PT Aretha Medika Utama, Bandung the independent variable used

is various concentrations of temulawak extract as a treatment for human skin fibroblast cell lines exposed to UV light. The study began with the extraction of temulawak rhizomes in a CPOTB standardized factory. The research was continued with fibroblast cell culturing, UV light exposure and treatment with extracts. Determination of the best 3 concentrations for treatment has been tested cytotoxic first by previous studies. Therefore, after 70-80% confluence cells, the research continued directly to UV light exposure. UV light exposure was given at an intensity of 300J/cm^2 for 75 minutes. Cells were then given 3 concentrations of extracts and incubated for 24 hours in an incubator. After treatment, the cells were then centrifuged, and the supernatant was taken for ELISA testing of melatonin and hyaluronidase levels. The research flow can be seen in figure 1. The independent variables used in this study are various concentrations of temulawak extract (ETL) given to UV-induced fibroblast cells.

3. Results and discussion

Research on the administration of temulawak extract to measure melatonin and hyaluronidase levels was tested on aging model cells. Aging model cells are human skin fibroblast cell lines that are exposed to UV light given UV light exposure for 75 minutes. The following are the results of research on the administration of temulawak extract to melatonin and hyaluronidase levels in aging model cells. Melatonin levels were quantified using the ELISA method based on the absorbance value of the enzyme activated by an antibody that binds to melatonin-specific antigens. Statistical analysis was performed on IBM SPSS software (version 20). The data obtained from the ELISA test were tested for normality to determine the distribution of ELISA data. The method used in the normality test was the Shapiro-Wilk method because the samples in each treatment group did not reach 50 samples. The homogeneity test was conducted to determine whether the ELISA result data was homogeneous or not. The results of the normality test (Appendix 3.1) and homogeneity (Appendix 3.2) showed $p>0.05$ in the measurement of melatonin levels of aging model cells given temulawak extract. Differences in the mean values of the various treatments were obtained from one-way ANOVA analysis. The results of ANOVA analysis (Appendix 3.3) showed a value of $p<0.05$, so it was concluded that H_0 was rejected and there was an effect of temulawak extract on melatonin levels in human skin fibroblast cell lines exposed to UV light as a model of aging. Furthermore, to determine which treatment group showed statistically significant differences, the Post Hoc test was conducted with the Tukey HSD method (Appendix 3.4). The results of research on the potential of temulawak extract on melatonin levels in human skin fibroblast cell lines exposed to UV light as a model of aging (Table 4.1) and (Figure 4.1).

Table 4.1 Melatonin levels in aging model cells

Treatment	Melatonin Level (pg/mL)
KN	164,83 ± 12,17^{c}
KP	61,38 ± 8,63^{a}
ETL1	74,96 ± 5,88^{a}
ETL2	118,96 ± 7,61^{b}
ETL3	147.71 ± 22.56 ^{bc}

Note: data are presented as mean ± standard deviation. Different superscripts (a, b, bc, c) indicate significant differences ($p<0.05$) between treatments on melatonin levels in aging model cells. KN: negative control, KP: positive control, ETL1: treatment with 3.13 µg/mL temulawak extract, ETL2: treatment with 6.25 µg/mL temulawak extract, ETL3: treatment with 12.25 µg/mL temulawak extract.

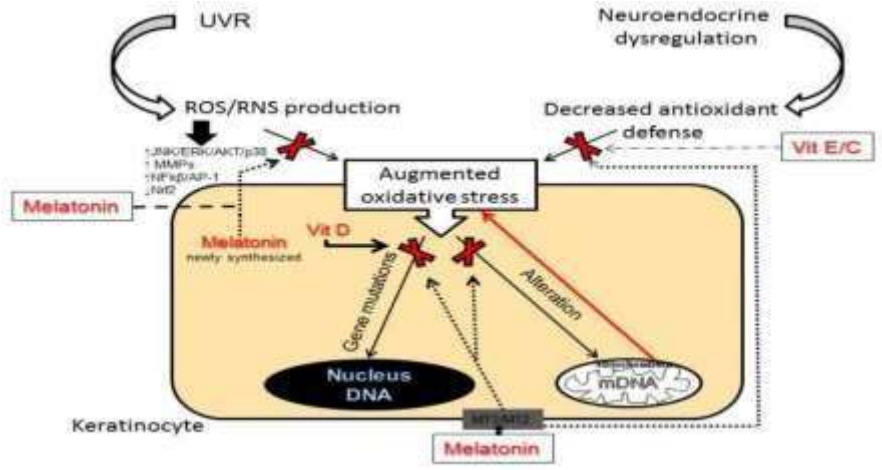

Figure 4.1 Melatonin Levels in Aging Model Cells

Note: data are presented as mean ± standard deviation. Different superscripts (a, b, bc, c) indicate significant differences ($p<0.05$) between treatments on melatonin levels in aging model cells. KN: negative control, KP: positive control, ETL1: treatment with 3.13 µg/mL temulawak extract, ETL2: treatment with 6.25 µg/mL temulawak extract, ETL3: treatment with 12.25 µg/mL temulawak extract.

Based on the data from the measurement of melatonin levels in Table 4.1 and Figure 4.1, it shows that the administration of temulawak extract can increase melatonin levels in aging model cells. The positive control treatment (KP) or fibroblast cells exposed to UV light with an average value of 61.38 pg/mL showed a decrease in melatonin levels which was significantly different from the negative control treatment (KN) or fibroblast cells that were not exposed to UV light with an average value of 164.83 pg/mL. The administration of temulawak extract with a fairly small dose, namely 3.13 µg/mL with an average value of 74.96 pg/mL has not shown a significant increase in melatonin levels compared to the KP treatment. The effect of giving temulawak extract with a concentration of 6.25 µg/mL with an average value of 118.96 pg/mL and a concentration of 12.25 µg/mL with an average value of 147.71 pg/mL showed a significant increase in melatonin levels compared to the KP treatment. The results showed that fibroblast cells given UV exposure can reduce melatonin levels, then the administration of temulawak extract can increase melatonin levels again.

Hyaluronidase levels were measured using the ELISA method based on the absorbance value of the enzyme that has been activated by an antibody that binds to hyaluronidase-specific antigens. Statistical analysis was performed on IBM SPSS software (version 20). Data obtained from the ELISA test were tested for normality using the Shapiro-Wilk method because the number of samples in each treatment group did not reach 50 samples. The normality test was carried out to determine the distribution of normally distributed ELISA data. Homogeneity test is conducted to determine whether the ELISA result data is homogeneous or not. The results of the normality test (Appendix 5.1) and homogeneity (Appendix 5.2) showed $p>0.05$ in the measurement of hyaluronidase levels of aging model cells treated with temulawak extract. Each treatment was subjected to one-way ANOVA analysis to determine the difference in mean values in the treatments tested. The results of ANOVA analysis (Appendix 5.3) showed a value of $p < {}_0.05$, so H_0 is rejected and it can be concluded that there is an effect of temulawak extract on hyaluronidase levels in human skin fibroblast cell lines exposed to UV light as a model of aging. Post Hoc test with Tukey HSD method was then conducted to determine which treatment group showed meaningful or statistically significant differences (Appendix 5.4). The results of the research on the potential of temulawak extract on hyaluronidase levels in human skin fibroblast cell lines exposed to UV light as a model of aging can be seen in Table 4.2 and Figure 4.2.

Table 4.2 Hyaluronidase Levels in Aging Model Cells

Treatment	Hyaluronidase Level (ng/mL)
KN	79,36 ± 1,98[a]
KP	149,52 ± 9,17[d]
ETL1	119,85 ± 3,11[c]
ETL2	107.42 ± 3.71 [bc]
ETL3	95,78 ± 5,05[b]

Note: data are presented as mean ± standard deviation. Different superscripts (a, b, bc, c, d) indicate significant differences ($p<0.05$) between treatments on hyaluronidase levels in aging model cells. KN: negative control, KP: positive control, ETL1: treatment with 3.13 µg/mL temulawak extract, ETL2: treatment with 6.25 µg/mL temulawak extract, ETL3: treatment with 12.25 µg/mL temulawak extract.

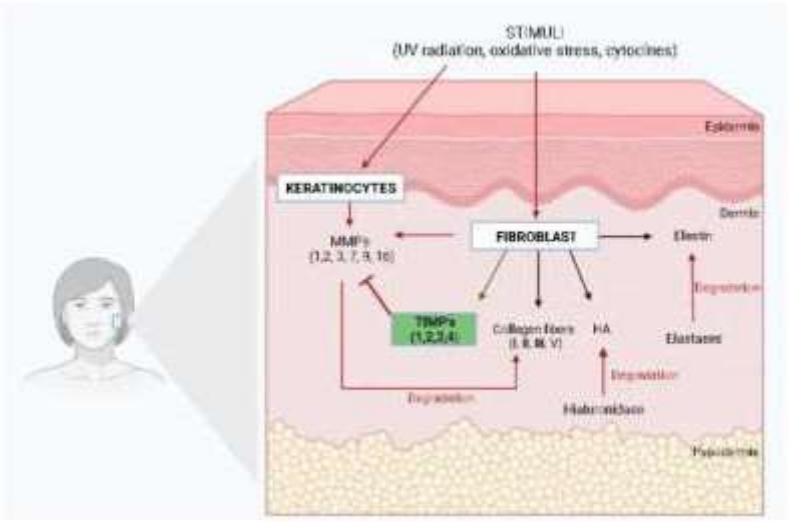

Figure 4.2 Hyaluronidase Levels in Aging Model Cells

Note: data are presented as mean ± standard deviation. Different superscripts (a, b, bc, c, d) indicate significant differences ($p<0.05$) between treatments on hyaluronidase levels in aging model cells. KN: negative control, KP: positive control, ETL1: treatment with 3.13 µg/mL temulawak extract, ETL2: treatment with 6.25 µg/mL temulawak extract, ETL3: treatment with 12.25 µg/mL temulawak extract.

Based on the data from the measurement of hyaluronidase levels in Table 4.2 and Figure 4.2, it shows that the administration of temulawak extract can reduce hyaluronidase levels in aging model cells. The positive control treatment (KP) or fibroblast cells exposed to UV light with an average value of 149.52 ng/mL showed an increase in hyaluronidase levels which was significantly different from the negative control treatment (KN) or fibroblast cells that were not exposed to UV light with an average value of 79.36 ng/mL. Treatment with temulawak extract with a concentration of 3.13 µg/ml with an average value of 119.85 ng/mL, a concentration of 6.25 µg/mL with an average value of 107.42 pg/mL, and a concentration of 12.25 µg/mL with an average value of 95.78 ng/mL showed a significant decrease in hyaluronidase levels compared to the KP treatment. The results showed that fibroblast cells given UV exposure can increase hyaluronidase levels, then the administration of temulawak extract can reduce hyaluronidase levels again.

Discussion

In this study, fibroblast cells were exposed to UV light to create an aging cell model. Fibroblast cells synthesize several components such as hyaluronic acid and are cells commonly found in connective tissue (Yoshimura et al., 2023). Skin organs that are commonly exposed to the sun's UV rays contain fibroblast cells. The occurrence of skin aging can be characterized by a decrease in the number of

fibroblast cells (Yusharyahya, 2021). The use of fibroblast cells as aging model cells has been done in previous studies because they can show decreased proliferation and morphological changes after exposure to oxidative stress (Widowati et al., 2022).

Prolonged exposure to UV radiation can cause various impacts on skin cells. UV absorbed by the skin can cause DNA damage and activate ROS. This can cause genetic mutations and lead to abnormal cell growth that causes skin cancer and premature aging (Fitraneti et al., 2024). UV exposure to fibroblasts and skin cells can activate free radicals and then increase oxidative stress (Ansary et al., 2021). Oxidative stress is a condition of imbalance between antioxidants and pro-oxidants that occurs due to excessive formation of ROS (Mulianto, 2020). Therefore, consumption of food ingredients that are high in antioxidant content can help protect the body from free radicals. Plants are one source of antioxidants with minimal side effects (Gunarti et al., 2022).

Melatonin levels in fibroblast cells exposed to UV light decreased. The decrease in melatonin levels can indicate an increased activity of melatonin in fighting free radicals. Melatonin is hydrophilic and lipophilic, so it can cross cell membranes and protect cells from free radicals (Suyanto & Widianti, 2023). The protective effect provided by melatonin can maintain cell physiology and tissue homeostatis, especially in UV-exposed skin cells (Bocheva et al., 2022). Melatonin indirectly affects the skin through its metabolites, which are hydroxyl derivatives of melatonin produced in response to UV light (Rusanova et al., 2019). Another study showed that melatonin administration to UV-irradiated human fibroblast cells can inhibit oxidative stress-induced skin damage (Yuksel Egrilmez et al., 2022). Therefore, increasing melatonin levels is essential in maintaining skin quality. The use of temulawak extract can help the skin in increasing melatonin levels, thus preventing aging.

Hyaluronidase levels showed an increase in UV-irradiated fibroblast cells. An increase in hyaluronidase levels indicates an increase in free radicals that induce hyaluronidase enzyme activity. Excessive amounts of free radicals induce hyaluronidase enzyme function to excessively degrade hyaluronic acid (Papaemmanouil et al., 2022). One of the key components in maintaining skin quality is hyaluronic acid which plays a role in keeping the skin moist and smooth (Khojah et al., 2024). Efforts to prevent aging due to increased levels of the enzyme hyaluronidase which causes a decrease in hyaluronic acid levels can be done by consuming antioxidants. One source of antioxidants can be obtained from plants (Kusumawulan et al., 2022). Therefore, reducing hyaluronidase enzyme levels is very important in maintaining skin quality. The use of temulawak extract can help the skin in reducing hyaluronidase levels, thus preventing aging.

The use of temulawak extract on fibroblast cells irradiated with UV light showed an increase in melatonin levels and a significant decrease in hyaluronidase levels. This shows that temulawak extract can act as antiaging by reducing the levels of free radicals present in aging model cells. The curcuminoid content in temulawak acts as a strong antioxidant, so it can neutralize free radicals that damage skin cells (Panjaitan et al., 2022). Previous research has begun to develop temulawak-based instant powder drinks (Solikhah et al., 2023). Several other products that have been developed from temulawak, namely sweets, pastries, noodles, dodol, crackers, and jelly candy have begun to be made to increase public interest in consuming temulawak (Khamidah et al., 2017). In addition to being developed into food or beverage products, temulawak has also begun to be used as a basic ingredient for skin beauty products as an anti-aging agent. Previous research has developed a lotion made from temulawak combined with strawberries (Alfian et al., 2023). Temulawak was also developed into a gel mask for the face that serves to prevent aging (Muslikh et al., 2024). The development of products made from temulawak has been widely done, but basic research using aging cell models to prove that temulawak has the potential as anti-aging has not been done. The results of this study are expected to be the basis for using temulawak in products that can prevent the aging process. These results support the results of a previous study which showed that temulawak extract on UV-irradiated fibroblast cells proved efficacious for mediating UVinduced skin aging (Rahmat et al., 2021).

4. Conclusion and future scope

The Temulawak extract can significantly increase melatonin levels in human skin fibroblast *cell lines* exposed to UV light with the best results at a concentration of 12.25 µg/mL (147.71 pg/mL melatonin). Temulawak extract can significantly reduce hyaluronidase levels in *human skin fibroblast cell lines* exposed to UV light with the best results at a concentration of 12.25 µg/mL (95.78 ng/mL hyaluronidase).

Advice

Future research can test other parameters related to skin aging, such as collagenase, elastase, and tyrosinase levels. Other parameters tested include gene expression levels associated with skin aging, such as MMP. Further research is needed at the in vivo level using mice or rats as animal models induced by UV and free radicals to test anti-aging and anti oxidantactivities.

Reference

Alfian, M., Maulana, M. L., & Mustainin, M. (2023). Formulation and Physical Stability of Temulawak (Curcuma xanthorhiza Roxb.) Antiaging Lotion with Natural Colorant from Strawberry Extract (Fragaria vesca L). Jurnal Mandala Pharmacon Indonesia, 9(1), 20-26.

Al-Halaseh, L. K., Al-Jawabri, N. A., Tarawneh, S. K., Al-Qdah, W. K., Abu-Hajleh, M. N., Al-Samydai, A. M., & Ahmed, M. A. (2022). A review of the cosmetic use and potentially therapeutic importance of hyaluronic acid. Journal of Applied Pharmaceutical Science, 12(7), 034-041.

Amin, M. M., Harisudin, M., & Setyowati. (2016). Analisis Marjin Pemasaran temulawan Di Kabupaten Wonogiri. AGRISTA, 4 (3), 550 -559.

Anggowarsito , J. (2014). Aspek Fisiologi Penuaan Kulit . Jurnal Widya Medika Surabaya , 56 - 61.

Ansary, T.M., Hossain, M.R., Kamiya, K., Komine, M., & Ohtsuki, M., 2021. Inflammatory Molecules Associated with Ultraviolet Radiation-Mediated Skin Aging. International Journal of Molecular Sciences 22, 1–14. https://doi.org/10.3390/ijms22083974

Azevedo Martins, T. E., Sales de Oliveira Pinto, C. A., Costa de Oliveira, A., Robles Velasco, M. V., Gorriti Guitiérrez, A. R., Cosquillo Rafael, M. F., & Retuerto-Figueroa, M. G. (2020). Contribution of topical antioxidants to maintain healthy skin—A review. Scientia Pharmaceutica, 88(2), 27.

Bocheva G, Slominski RM, Slominski AT. (2019). Neuroendocrine Aspects of Skin Aging. International Journal of Molecular Sciences.20(11):2798.

Bocheva, G., Slominski, R. M., Janjetovic, Z., Kim, T. K., Böhm, M., Steinbrink, K., ... & Slominski, A. T. (2022). Protective role of melatonin and its metabolites in skin aging. International journal of molecular sciences, 23(3), 1238.

Bonté, F., Girard, D., Archambault, J. C., & Desmoulière, A. (2019). Skin changes during ageing. Biochemistry and cell biology of ageing: Part II clinical science, 249-280.

Brand, R. M., Wipf, P., Durham, A., Epperly, M. W., Greenberger, J. S., & Falo Jr, L. D. (2018). Targeting mitochondrial oxidative stress to mitigate UV-induced skin damage. Frontiers in Pharmacology, 9, 920.

Bravo, B., Correia, P., Gonçalves Junior, J. E., Sant'Anna, B., & Kerob, D. (2022). Benefits of topical hyaluronic acid for skin quality and signs of skin aging: From literature review to clinical evidence. Dermatologic therapy, 35(12), e15903.

Buckley, D. (2021). The Ageing Skin. Textbook of Primary Care Dermatology, 183-189.

Cecon, E., Oishi, A., & Jockers, R. (2018). Melatonin receptors: molecular pharmacology and signalling in the context of system bias. British journal of pharmacology, 175(16), 3263-3280. https://doi.org/10.1111/bph.13950

Chaudhary M, Khan A, Gupta M. Skin Ageing: Pathophysiology and Current Market Treatment Approaches. Curr Aging Sci. 2020;13(1):22-30. doi: 10.2174/1567205016666190809161115.

Chermahini, S. H., Majid, F. A., & Sarmidi, M. R. (2011). Cosmeceutical value of herbal extracts as natural ingredients and novel technologies in anti-aging. J. Med. Plants Res, 5(14), 3074-3077.

Chrustek, A., & Olszewska-Słonina, D. (2021). Melatonin as a powerful antioxidant. Acta Pharmaceutica, 71(3), 335-354.

Costa, E. F., Magalhães, W. V., & Di Stasi, L. C. (2022). Recent advances in herbal-derived products with skin anti-aging properties and cosmetic applications. Molecules, 27(21), 7518

de Paula Corrêa, M., Germano Marciano, A., Silveira Barreto Carvalho, V., Bernardo de Souza, P. M., da Silveira Carvalho Ripper, J., Roy, D., Breton, L., & De Vecchi, R. (2021). Exposome extrinsic factors in the tropics: The need for skin protection beyond solar UV radiation. Science of the Total Environment, 782, 146921.

Dewiastuti, M., & Hasanah, I. F. (2016). Pengaruh Faktor - Faktor Risiko Penuaan Dini Di Kulit Pada Remaja Wanita Usia 18-21 tahun. Jurnal Profesi Medika, 10(1), 21- 25. https://doi.org/10.33533/jpm.v10i1.10.

Ding, A. J., Zheng, S. Q., Huang, X. B., Xing, T. K., Wu, G. S., Sun, H. Y., & Luo, H. R. (2017). Current perspective in the discovery of anti-aging agents from natural products. Natural products and bioprospecting, 7, 335-404.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. International journal of molecular sciences, 14(6), 12222-12248.

Erpina, E., Rafi, M., Darusman, L. K., Vitasari, A., Putra, B. R., & Rohaeti, E. (2017). Simultaneous quantification of curcuminoids and xanthorrhizol in Curcuma xanthorrhiza by high-performance liquid chromatography. Journal of Liquid Chromatography & Related Technologies, 40(12), 635-639.

Farida, Y., Rahmat, D., & Amanda, A. W. (2018). Anti-Infl ammation Activity Test of Nanoparticles Ethanol Extract of Temulawak Rhizome (Curcuma xanthorrhiza Roxb.) with Protein Denaturation Inhibition Method. Jurnal Ilmu Kefarmasian Indonesia, 16(2), 225 - 230. https://doi.org/10.35814/jifi.v16i2.569

Fischer, T. W., Slominski, A., Zmijewski, M. A., Reiter, R. J., & Paus, R. (2008). Melatonin as a major skin protectant: from free radical scavenging to DNA damage repair. Experimental dermatology, 17(9), 713-730

Fitraneti, E., Rizal, Y., Nafiah, S. R., Primawati, I., & Hamama, D. A. (2024). Pengaruh Paparan Sinar Ultraviolet terhadap Kesehatan Kulit dan Upaya Pencegahannya: Tinjauan Literatur. Scientific Journal, 3(3), 185-194.

Girsang, E., Ginting, C. N., Lister, I. N. E., yashfa Gunawan, K., & Widowati, W. (2021). Anti-inflammatory and antiaging properties of chlorogenic acid on UV-induced fibroblast cell. PeerJ, 9, e11419.

Girsang, E., Lister, I. N. E., Ginting, C. N., Nasution, S. L., Suhartina, S., Munshy, U. Z., & Widowati, W. (2019). Antioxidant and anti-inflammatory activity of chlorogenic acid on lead-induced fibroblast cells. In Journal of Physics: Conference Series (Vol. 1374, p. 012006). IOP Publishing.

Girsang, E., Lister, I. N. E., Ginting, C. N., Widowati, W., Arumwardana, S., Marthania, M., & Rizal, R. (2023). Chlorogenic acid in preventing and curing ultraviolet-induced damage in human skin fibroblast as an antiaging cell model.

Gunarti, N., Yuniarsih, N., Toni. S, R.M., Khoerunnisa, R., Allahuddin, A., et al., 2022. Artikel Review: Kandungan Senyawa Aktif Tanaman untuk Kesehatan Kulit. Jurnal Farmasi Indonesia 14, 190–195. https://doi.org/10.35617/jfionline.v14i2.86

Hadi Kartiko, B., & Milas Siswanto, F. (2016). Hormon Dalam Konsep Anti Aging Medicine. Virgin: Jurnal Ilmiah Kesehatan Dan Sains, 1(2), 108 - 122. https://jurnal.undhirabali.ac.id/index.php/virgin/article/view/59

Hadinata, E. A., Monica, E., & Hendra, G. A. (2022). Eksplorasi Bahan Alam Sebagai Kosmetik Guna Pencegahan Stress Oksidatif Pada Kulit Manusia . SAINSBERTEK Jurnal Ilmiah Sains & Teknologi, 2(2), 1-16. https://doi.org/10.33479/sb.v2i2.120

Haerani, A., Chaerunisa, A. Y., & Subarnas, A. (2018). Antioksidan Untuk Kulit . Farmaka, 135 - 151.

Hughes, L. (2019). Melatonin: The Natural Supplement for Better Sleep. Union Square & Co..

Jadoon, S., Karim, S., Asad, M. H. H. B., Akram, M. R., Kalsoom Khan, A., Malik, A., & Murtaza, G. (2015). Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity. Oxidative medicine and cellular longevity, 2015(1), 709628.

Jiratchayamaethasakul, C., Ding, Y., Hwang, O., Im, S. T., Jang, Y., Myung, S. W., & Lee, S. H. (2020). In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. Fisheries and Aquatic Sciences, 23, 1-9.

Jusri, R., Widodo, W.S., Widowati, W., Armansyah, A., Sormin, D.E., Fachrial, E., & Lister, I.N. (2019). Comparison of Antioxidant and Anti-hyaluronidase Potentials of Pineapple Core Extract (Ananas comosus (L.) Merr.) and Luteolin. Majalah Kedokteran Bandung, 51(2).

Khamidah, A., Antarlina, S. S., & Sudaryono, T. (2017). Ragam produk olahan temulawak untuk mendukung keanekaragaman pangan. Jurnal Litbang Pertanian, 36(1), 1-12.

Khojah, H., Ahmed, S. R., Alharbi, S. Y., AlSabeelah, K. K., Alrayyes, H. Y., Almusayyab, K. B., ... & Qasim, S. (2024). Skin anti-aging potential of Launaea procumbens extract: Antioxidant and enzyme inhibition activities supported by ADMET and molecular docking studies. Saudi Pharmaceutical Journal, 102107.

Kim, M., & Park, H. J. (2016). Molecular Mechanism of skin Aging and Rejuvenation. INTECH open science, 57 - 68. http://dx.doi.org/10.5772/62893.

Kusumawulan, C. K., Rustiwi, N. S., Sriwidodo, S., & Bratadiredja, M. A. (2022). Review: Efektivitas Sari Kedelai sebagai Anti-aging dalam Kosmetik. Majalah Farmasetika, 8 (1), 1-12.

Laksmitawati, D. R., Pratami, D. K., Widowati, W., Kusuma, H. S. W., Wijayanti, C. R., Wahyuni, C. D., & Rizal, R. (2022). Antioxidant Properties of Curcuma longa L. and Curcuma xanthorriza Rhizomes. In Proceedings of the 1st International Conference on Emerging Issues in Technology, Engineering and Science (ICE-TES 2021),104-111

Liu, R., Fu, A., Hoffman, A.E. et al. Melatonin enhances DNA repair capacity possibly by affecting genes involved in DNA damage responsive pathways. BMC Cell Biol 14, 1 (2013). https://doi.org/10.1186/1471-2121-14-1

Madkour, L. H. (2019). Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. Pharm. Sci. Anal. Res. J, 2, 180023.

Malau, R. C., Nasution, S. W., Nasution, A. N., Widowati, W., Nindya, F. S., & Kusuma, H. S. W. (2024). Antioxidant Potential of Temulawak (Curcuma xanthorrhiza) Extract Gel as A Candidate for Wound Healing. Journal of Vocational Health Studies, 7(3), 166-174.

Marinho, A., Nunes, C., & Reis, S. (2021). Hyaluronic acid: A key ingredient in the therapy of inflammation. Biomolecules, 11(10), 1518.

Marta, L., Agustinus, R., Rizaldy, T. (2016). Proses Menua, Stres Oksidatif, dan Peran Antioksidan. Kalbemed. 43(10): 733-736.

Mazuka, M. O., Danimayostu, A. A., & Iswarin , S. J. (2018). Uji Antioksidan Etosom Ekstrak Daun Jeruk Purut (Citrus hystrix D.C.) sebagai Anti Penuaan Kulit dengan Metode DPPH. PHARMACEUTICAL JOURNAL OF INDONESIA , 3(2), 33 - 38. http://.pji.ub.ac.id

Meri, R., & Perdana , R. W. (2022). Jaringan Syaraf Tiruan Menggunakan Algoritma Hebb Rule Untuk Diagnosa penyakit Kulit Manusia. JOISIE Journal Of Information System And Informatics Engineering, 6(2), 78-87. https://doi.org/10.35145/joisie.v6i2.2663

Mir, S. M., Aliarab, A., Goodarzi, G., Shirzad, M., Jafari, S. M., Qujeq, D., Samavarchi Tehrani, S., & Asadi, J. (2022). Melatonin: A smart molecule in the DNA repair system. Cell biochemistry and function, 40(1), 4–16. https://doi.org/10.1002/cbf.3672

Mulianto, N., 2020. Malondialdehid sebagai Penanda Stres Oksidatif pada Berbagai Penyakit Kulit. Cermin Dunia Kedokt. 47, 39–44.

Muslikh, F., Aryantini, D., Sari, F., Hesturini, R. J., Winartiana, W., & Prasetyawan, F. (2024). Skrining Fitokimia dan Formulasi Sediaan Masker Gel Peel-Off Ekstrak Etanol Temulawak (Curcuma xanthorrhiza Roxb.). Borneo Journal of Pharmascientech, 8(1), 34-40.

Ng, K. W., & Lau, W. M. (2015). Skin Deep: The Basics of Human Skin Structure and Drug Penetration. Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle Effects, 3-11. DOI 10.1007/978-3-662-45013-0_1.

Novianto, F., Zulkarnain, Z., Triyono, A., Ardiyanto, D., & Fitriani, U. (2020). Pengaruh formula jamu

temulawak, kunyit, dan meniran terhadap kebugaran jasmani: suatu studi klinik. Media Penelitian dan Pengembangan Kesehatan, 30(1), 37-44.

Nugroho, V. A. (2021). Hubungan Metabolisme, Saraf Simpatik dan Sirkadian Setelah Melakukan Olahraga. FiK care, 1 - 5.

Pandel, R., Poljšak, B., Godic, A., & Dahmane, R. (2013). Skin photoaging and the role of antioxidants in its prevention. International Scholarly Research Notices, 2013(1), 930164.

Panich, U., Sittithumcharee, G., Rathviboon, N., & Jirawatnotai, S. (2016). Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. Stem cells international, 2016.

Panjaitan, J., Girsang, E., & Chiuman, L. (2022). Effectiveness of temulawak (Curcuma xanthorrhiza) ointments as wound healing agents in wistar rats. Jurnal Teknologi Laboratorium, 11(2), 95-104.

Panjaitan, J., Girsang, E., & Chiuman, L. (2022). Effectiveness of temulawak (Curcuma xanthorrhiza) ointments as wound healing agents in wistar rats. Jurnal Teknologi Laboratorium, 11(2), 95-104.

Papaemmanouil, C. D., Peña-García, J., Banegas-Luna, A. J., Kostagianni, A. D., Gerothanassis, I. P., Pérez-Sánchez, H., & Tzakos, A. G. (2022). ANTIAGE-DB: A database and server for the prediction of anti-aging compounds targeting elastase, hyaluronidase, and tyrosinase. Antioxidants, 11(11), 2268.

Prayudo, A. N., Novian, O., Setyadi, & Antaresti. (2015). Koefisien Transfer Massa Kurkumin Dari temulawak . Jurnal Ilmiah Widya Teknik, 14(1), 26 -31. https://doi.org/10.33508/wt.v14i1.1739.

PS-Statistics Indonesia, Statistics of Medicinal PlantsIndonesia, BPS-statistics Indonesia, Jakarta, Indonesia, 2019

Pujimulyani, D., Yulianto, W. A., Setyowati, A., Arumwardana, S., Sari Widya Kusuma, H., Adhani Sholihah, I., & Maruf, A. (2020). Hypoglycemic activity of Curcuma mangga val. extract via modulation of GLUT4 and PPAR-γ mRNA expression in 3T3-L1 Adipocytes. Journal of Experimental Pharmacology, 363-369.

Putri, A. R., & Suhartinah, S. (2023). Uji Aktivitas Krim Anti-Aging Ekstrak Etanol Daun Bayam Merah (Amaranthus tricolor L.) pada Kulit Punggung Kelinci New Zealand yang dipapar Sinar UV-A. Indonesian Journal of Pharmaceutical Education, 3(1).

Rahmat, E., Lee, J., & Kang, Y. (2021). Javanese turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evidence-Based Complementary and Alternative Medicine, 2021(1), 9960813.

Rahmat, E., Lee, J., & Kang, Y. (2021). Javanese turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evidence-Based Complementary and Alternative Medicine, 2021(1), 9960813.

Rahmat, E., Lee, J., & Kang, Y. (2021). Javanese Turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, Phytochemistry, Biotechnology, and Pharmacological Activities. Evidence-based complementary and alternative medicine : eCAM, 2021, 9960813. https://doi.org/10.1155/2021/9960813

Ramalhete, C., Mulhovo, S., Lage, H., & Ferreira, M. J. U. (2018). Triterpenoids from Momordica balsamina with a collateral sensitivity effect for tackling multidrug resistance in cancer cells. Planta Medica, 84(18), 1372-1379.

Roy, A., Sahu, R. K., Matlam, M., Deshmukh, V. K., Dwivedi, J., Jha, A. K. (2013). Pharmacogn. Rev., 7, 97-106.

Rusanova, I., Martínez-Ruiz, L., Florido, J., Rodríguez-Santana, C., Guerra-Librero, A., Acuña-Castroviejo, D., & Escames, G. (2019). Protective effects of melatonin on the skin: future perspectives. International journal of molecular sciences, 20(19), 4948.

Rusanova, I., Martínez-Ruiz, L., Florido, J., Rodríguez-Santana, C., Guerra-Librero, A., Acuña-Castroviejo, D., & Escames, G. (2019). Protective effects of melatonin on the skin: future perspectives. International journal of molecular sciences, 20(19), 4948.

Sadeghi, A., Rostamirad, A., Seyyedebrahimi, S., & Meshkani, R. (2018). Curcumin ameliorates palmitate-induced inflammation in skeletal muscle cells by regulating JNK/NF-kB pathway and ROS production. Inflammopharmacology, 26, 1265-1272.

Salsabilla, N. D., Yulia, E., & Jubaedah, L. (2024). Hubungan Perilaku Konsumen Dalam Melakukan Suntik Botox Untuk Mengatasi Kerutan Wajah Dengan Aspek Kebutuhan dan Lingkungan Sosial. Jurnal Adijaya Multidisplin, 2(03), 427-445.

Shanbhag, S., Nayak, A., Narayan, R., & Nayak, U. Y. (2019). Anti-aging and sunscreens: paradigm shift in cosmetics. Advanced pharmaceutical bulletin, 9(3), 348.

Shin, J. W., Kwon, S. H., Choi, J. Y., Na, J. I., Huh, C. H., Choi, H. R., & Park, K. C. (2019). Molecular mechanisms of dermal aging and antiaging approaches. International journal of molecular sciences, 20(9), 2126.

Sholikhah, N. I., Alfian, M., & Fatimah, F. A. (2023). Uji Aktivitas Antioksidan Minuman Serbuk Instan Temulawak (Curcuma Xanthorriza Roxb) Produksi Mitra Sehat Kiringan Bantul. Jurnal Kefarmasian Akfarindo, 50-55.

Suyanto, M. V. P., & Widianti, I. G. A. (2023). Protective role of melatonin in ultraviolet radiation-induced oxidative stress in human skin photoaging. Universa Medicina, 42(3), 346-359.

Syamsudin, R. M., Perdana, F., Mutiaz, F. S., Rina, A. P., Cahyani, N. D., Aprilya, S., Khendri, F. (2019). Temulawak Plant (Curcuma Xanthorrhiza Roxb) as a Traditional medicine. Jurnal Ilmiah Farmako Bahari , 10(1), 51 - 65. http://dx.doi.org/10.52434/jfb.v10i1.648

Tanha, A., Rabiee, M., Rostami, A., & Ahmadi, S. (2023). A green-based approach for noninvasive skin rejuvenation: Potential application of hyaluronic acid. Environmental Research, 234, 116467.

Verheyden, J., & Blitzer, A. (2002). Other noncosmetic uses of BOTOX. Disease-a-Month, 48(5), 357-366.

Verma, A., Zanoletti, A., Kareem, K. Y., Adelodun, B., Kumar, P., Ajibade, F. O., Silva, L. F. O., Phillips, A. J., Kartheeswaran, T., Bontempi, E., & Dwivedi, A. (2024). Skin protection from solar ultraviolet radiation using natural compounds: a review. Environmental Chemistry Letters, 22(1), 273–295.

Walsh, S., & Kayser, M. (2016). Predicting human appearance from DNA for forensic investigations. Handbook of forensic genetics: Biodiversity and heredity in civil and criminal investigation, 2016, 415-448.

Wargasetia, T. L., Widowati, W., & Muthmainnah, A. S. (2023). An In Vitro Assay Reveals The Anti-aging Properties of Temulawak Extract (Curcuma xanthorrhiza L.). Folia Medica Indonesiana, 59(4):2355-8393

Weber, G. C., Buhren, B. A., Schrumpf, H., Wohlrab, J., & Gerber, P. A. (2019). Clinical applications of hyaluronidase. Therapeutic Enzymes: Function and Clinical Implications, 255-277.

Widowati, W., Faried, A., Susanah, S., Priyandoko, D., Sugiaman, V. K., Nainggolan, I. M., & Wargasetia, T. L. (2024). Isolation and Characterization of Fibroblast from Normal and Thalassemia Foreskin. Trends in Sciences, 21(6), 7672-7672.

Widowati, W., Noverina, R., Ayuningtyas, W., Kurniawan, D., Arumwardana, S., Kusuma, H. S. W., ... & Faried, A. (2022). Potential of conditioned medium of hATMSCs in aging cells model. HAYATI Journal of Biosciences, 29(3), 378-388.

Widyastuti, I., Luthfah, H.Z., Hartono, Y.I., Islamadina, R., Can, A.T., Rohman, A. (2021). Aktivitas Antioksidan Temulawak (Curcuma xanthorrhiza Roxb.) dan Profil Pengelompokannya dengan Kemometrik. Journal of Pharmaceutical Analysis, 1(1):29–42.

Yanti, D. E., & Desiani, A. (2023). Sistem Pakar Diagnosa Penyakit Kulit Manusia Menggunakan Metode Certainty Factor. Kumpulan jurnaL Ilmu Komputer (KLIK), 10(2), 214-227. http://dx.doi.org/10.20527/klik.v10i2.634

Yoshimura, T., Manabe, C., Nagumo, J., Nagahama, T., Sato, T., & Murakami, S. (2023). Taurine accelerates the synthesis of ceramides and hyaluronic acid in cultured epidermis and dermal fibroblasts. Experimental and Therapeutic Medicine, 26, 512. https://doi.org/10.3892/etm.2023.12211

Yuksel Egrilmez, M., Kocturk, S., Aktan, S., Oktay, G., Resmi, H., Simsek Keskin, H., ... & Ozkan, S. (2022). Melatonin prevents UVB-induced skin photoaging by inhibiting oxidative damage and MMP expression through JNK/AP-1 signaling pathway in human dermal fibroblasts. Life, 12(7), 950.

Yusharyahya, S. (2021). Mekanisme Penuaan Kulit sebagai Dasar Pencegahan dan Pengobatan Kulit Menua: Mechanism of Skin Aging. EJournal Kedokteran Indonesia, 9(2), 150-159. https://doi.org/10.23886/ejki.9.49.150

Yusharyahya, S. N. (2021). Mekanisme Penuaan Kulit sebagai Dasar Pencegahan dan Pengobatan Kulit Menua: Mechanism of Skin Aging. eJournal Kedokteran Indonesia, 150-150.

Zhang, S., Duan, E. (2018). Fighting against skin aging : the way from bench to bedside. Cell Transplantation. 27(5): 729-738.