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The Administration of 2% Plum (*Prunus domestica* L.) Extract Cream Inhibited the Increase of Tyrosinase Enzyme Expression and the Amount of Skin Melanin in Male Guinea Pigs (*Cavia porcellus*) Skin Exposed to UV B Light

Mila Damayanti Wahyuningsih, Wimpie Pangkahila, and Ni Wayan Winarti

ABSTRACT

Medical science has progressed a lot and Anti Aging Medicine (AAM) is one of the sciences in the medical world that brings a new paradigm. Hyperpigmentation is one of the most common skin problems caused by excessive pigment melanin production. Plums are nutrient-rich and can be eaten raw, dried, or processed. This research aimed to evaluate the administration of 2% plum extract in inhibiting the increase of tyrosinase enzyme expression and the amount of skin melanin in male guinea pigs' skin exposed to UV B light. This was a randomized post-test-only control group design using 30 male guinea pigs (*Cavia porcellus*), aged 12-16 weeks, weight 300-350 grams, and divided into three groups, the control group did not get any cream, treatment group 1 received cream base, and treatment group 2 got 2% plum extract cream. Creams were applied daily 0.1 mg/cm^2 at 09.40 and 14.00 WITA. All guinea pigs received UV-B irradiation, three times a week with a total dose of 390 mJ/cm^2. Tyrosinase enzyme expression was examined by calculating the percentage of cells expressing the enzyme using immunohistochemical methods. On the other hand, the amount of melanin was obtained by calculating the melanin percentage that appeared on histopathological preparations using Masson-Fontana staining. The data obtained were analyzed using the One-way ANOVA test with a significance value of p<0.05. The expression of the tyrosinase enzyme in the control group and group with base cream was not significantly different (22.75 ± 5.50% vs. 21.30 ± 6.50%, p = 0.601). Similarly, the mean amount of melanin (4.42 ± 1.57% vs 4.27 ± 1.63%, p = 0.775). On the other hand, the results of the mean tyrosinase expression and the mean amount of melanin in the group receiving 2% plum extract were significantly lower than the treatment group receiving base cream (7.06 ± 5.18% vs 21.30 ± 6.50% and 0.42 ± 0.19% vs. 4.27 ± 1.63%) with p-value < 0.000. It can be concluded that the administration of 2% plum (*Prunus domestica* L.) extract cream inhibited the increase in the expression of the tyrosinase enzyme and the amount of melanin in the skin of male guinea pigs (*C. porcellus*) exposed to UVB light.

Keywords: Plum extract, tyrosinase, melanin, UVB.

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I. Introduction

Medical science has progressed a lot and anti-aging science or AAM is one of the sciences in the medical world that brings a new paradigm. Various efforts can be made to inhibit, prevent, and restore the aging process that occurs so that a person becomes a person who is youthful and has a good quality of life.

The aging process occurs in every living thing, and increasing age and hyperpigmentation are one of the common skin problems associated with aging. This condition is caused by the overproduction of the pigment melanin. The prevalence of hyperpigmentation in Indonesia is quite high. this is because Indonesian skin types are type 4 and 5 in the Fitzpatrick skin phototypes. Besides that, Indonesia's tropical climate and high intensity of sun exposure. Nowadays, hyperpigmentation cases in Indonesia are increasing [1]. In the skin aging process, the most important extrinsic factor comes from exposure to UV rays and is also known as photoaging [2].

Treatment of hyperpigmentation generally begins with the prevention of risk factors, protection against ultraviolet rays, and reduction of lesions by using therapies that have minimal side effects. The therapy principle aims to inhibit the melanin synthesis pathway, reduce the transfer of melanosomes from

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melanocytes to keratinocytes, and accelerate the melanin removal pathway [3].

Plums are nutrient-rich food and can be eaten raw, dried, or processed. This fruit has many bioactive compounds such as phenolic acids, anthocyanins, carotenoids, flavanols, organic acids (e.g., citric and malic acids), fiber (pectin), tannins, aromatic substances, enzymes, minerals (e.g., potassium, phosphorus, calcium, and magnesium) and vitamins A, B, C, and K [4]. Plums also have various health benefits, namely as an antioxidant, antibacterial, antihemolytic, anti-inflammatory, and anticancer [5].

Plums contain high amounts of antioxidant compounds [6]. The results of the phytochemical analysis test conducted at the Laboratory Unit of the Faculty of Agricultural Technology, Udayana University obtained an IC50 value of 375.58 ppm, an antioxidant capacity of 1877.03 mg/L GAEAC, phenol 252.09 mg/100 g, flavonoids 2176.57 mg/100 g, and tannins 423.92 mg/100 g. This plum extract has also been used in preliminary research and can inhibit the increase in tyrosinase expression and the amount of melanin in guinea pig skin. The concentrations used were 2%, 4%, 5%, and 10%. Based on the preliminary research, the extract that gave the most optimal results was the plum extract with a concentration of 2%.

II. Methods

This research was an experimental post-test-only control group research conducted at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University, and Sentra Pathology Diagnostic, Bali. This research received a certificate of animal ethics from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University (Number: B/13/UN14.2.9/PT.01.04/2022).

A. Animal Experiment

This study used 30 male guinea pigs (*Cavia porcellus*) with inclusion criteria of 12-16 weeks of age, 300-350 grams of weight, brown-dominant fur, healthy, willing to eat and drink, and had no skin lesions. No guinea pigs died during the study.

Random sampling was carried out on 30 guinea pigs and divided into three groups, namely the control group (K), treatment 1 (P_1), and treatment 2 (P_2). All guinea pigs were exposed to UV B rays three times a week for two weeks, with a total dose of 390 mJ/cm^2. The guinea pigs in groups P_1 and P_2 received 0.1 mg/cm^2 of cream before and after exposure to UV light (at 09.40 and 14.00 WITA). The P_1 group got the base cream and the P_2 the 2% plum extract cream. The cream was applied before and after UV irradiation and still carried out on days without irradiation.

B. Tyrosinase Level Examination

The expression of the tyrosinase enzyme was examined by counting the melanocyte cells in three large fields of view with 400 times magnification from left to right, then each sample was summed and averaged using immunohistochemical methods.

C. Melanin Quantification

The calculation of the amount of melanin is carried out by calculating the percentage of melanin pixels that appear in histopathological preparations with Masson-Fontana staining.

D. Data Analysis

The data obtained were analyzed statistically using the One-way ANOVA test with a significance value of $p<0.05$.

III. Result

After two weeks of treatment, the guinea pig's back was shaved. Macroscopically, the skins of guinea pigs from groups K and P_1 appeared to have quite a prominent pigmentation when compared to P_2 which only experienced minimal pigmentation (Figure 1).

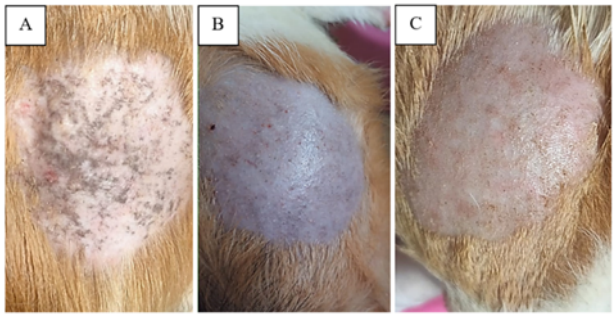

Fig. 1. Clinical photos of guinea pig skin before biopsy sampling. Photo (A) is a sample of the control group, (B) is a sample of treatment 1, and (C) is a sample of treatment 2

The expression of the tyrosinase enzyme in the control and treatment groups 1 was not significantly different (22.75±5.50% vs. 21.30±6.50%, p = 0.601). Similarly, the mean amount of melanin (4.42±1.57% vs 4.27±1.63%, p = 0.775). On the other hand, the results of the mean tyrosinase expression and the mean amount of melanin in the group receiving 2% plum extract were significantly lower than the treatment group receiving base cream (7.06±5.18% vs. 21.30±6.50% and 0). 0.42±0.19% vs. 4.27±1.63%) with p-value = 0.000.

TABLE I: TYROSINASE AND MELANIN LEVELS OF THREE EXPERIMENTAL GROUPS

Group	n	Tyrosinase		Melanin	
		Mean±SD	*P*	Mean±SD	*P*
K	10	22.75 ± 5.50	0.601	4.42 ± 1.57	0.775
P_1	10	21.30 ± 6.50	<0.001	4.27 ± 1.63	<0.001
P_2	10	7.06 ± 5.18	<0.001	0.42 ± 0.19	<0.001

IV. Discussion

Lighter and whiter skin color tends to be associated with the criteria of beauty and youth, especially in Asia. However, many factors can affect skin color, both intrinsic and extrinsic factors. One of the extrinsic factors that influence it is sun exposure. Under physiological conditions, pigmentation will appear as a defense response and act as a natural sunscreen that can absorb UV radiation. UVB light tends to cause delayed tanning that occurs several days after sun exposure and involves neo-melanogenesis through activation of the melanin synthesis pathway. Although it is protective, excessive melanin formation will cause problems, especially in the field of aesthetics and anti-aging [7], [8].

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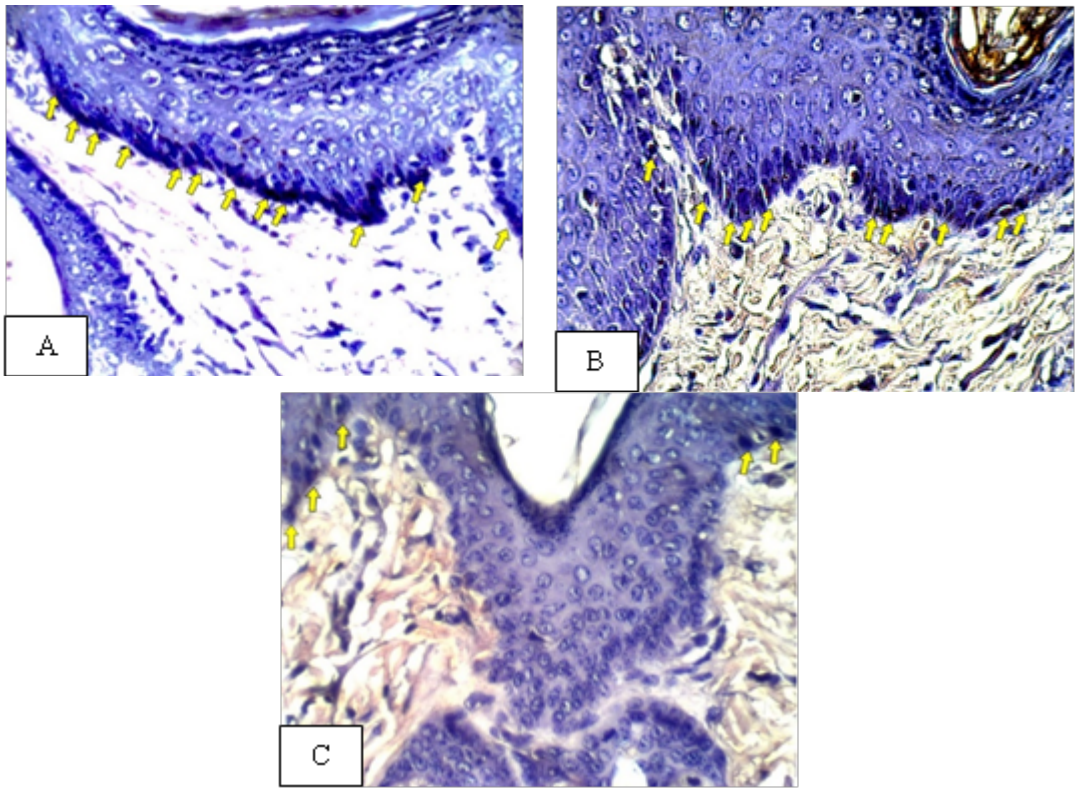

Fig. 2. Overview of tyrosinase enzyme expression in guinea pig skin. This examination uses Tyrosinase IHK staining with 400x magnification. Figures A, B, and C represent the network of samples from the control group, treatment 1, and treatment 2, respectively. Yellow arrows indicate brown keratinocyte cytoplasm which indicates the presence of tyrosinase enzyme expression. It appears that the expression of the tyrosinase enzyme in treatment group 2 was less when compared to the other two groups.

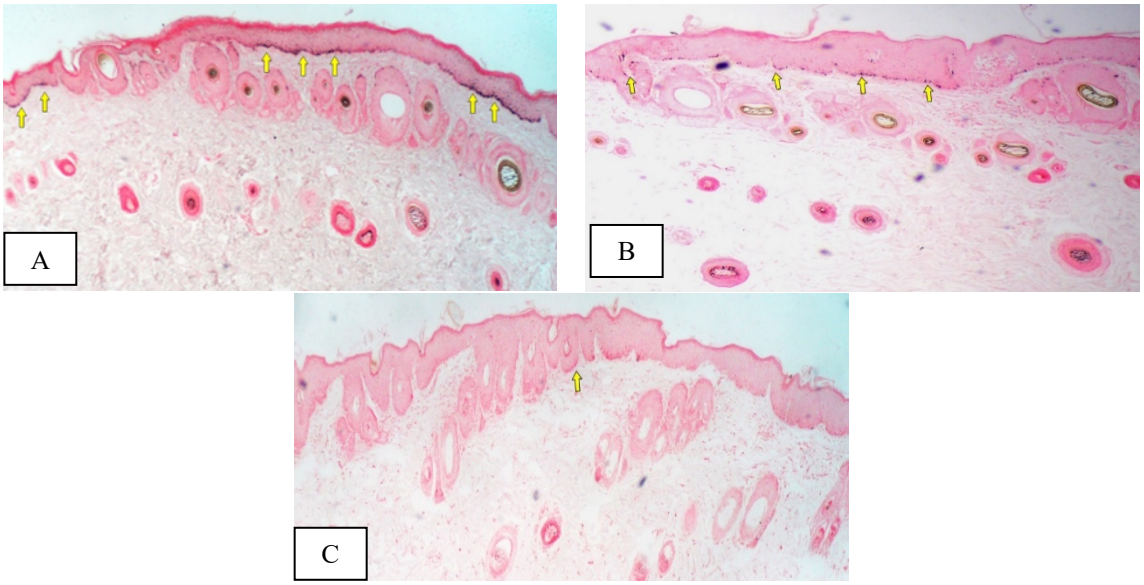

Fig. 3. Overview of melanin in guinea pig skin. This examination uses Masson-Fontana staining at 400x magnification. Figures A, B, and C represent the network of samples from the control group, treatment 1, and treatment 2, respectively. Red arrows indicate a black melanin pigmentation lesion

Several important proteins in melanogenesis increase after UV exposure, one of which is tyrosinase [9]. Tyrosinase inhibitors can be used topically for the treatment of localized hyperpigmentation and external antioxidants obtained from herbal extracts can act as inhibitors of tyrosinase activity and inhibit melanin production [10].

Plums are one of the fruits that have a high antioxidant content [11]. It is well known that ROS formed after exposure to UV light will stimulate melanin by melanocytes. Therefore, supplementation of exogenous antioxidants will neutralize the adverse effects of increased ROS and also suppress melanin production [12]. These antioxidants work as scavengers that prevent the formation of free radicals and also through metal ion chelator mechanisms [13].

Radish extract which has an IC50 of 781.17 g/ml has a tyrosinase inhibitor activity of 43.44% and has been used as a tyrosinase inhibitor in the form of facial toner preparations [14]. Plum extract in this study had a better IC50 value than radish extract which had been used as a tyrosinase inhibitor, which was 375.588 g/ml. This was supported by the significantly lower tyrosinase enzyme expression in the extract group than placebo and control (7.06% vs 21.30% vs 22.75%, respectively). When compared with tamarind leaf extract, plum fruit extract was able to give superior results

(7.06% vs 16.05%) [15].

Giving plum extract cream containing phenolic content of 252.09 mg/100g GAE to guinea pigs exposed to UV light had a lower mean amount of melanin than the placebo group or the control group which only received UV light (0.42% vs. 4.27% vs. 4.42%, respectively). Although the 2% plum extract cream contains less phenol than the 3% tamarind leaf extract cream (14.552 mg/100g GAE), the average amount of melanin in the plum extract is lower (0.42% vs 7.30%) [15].

Polyphenols are known to be able to act as inhibitors of ROS formation and also provide anti-melanogenic effects [16]. Phenolic components can inhibit melanogenesis by mediating MITF degradation associated with ERK signaling. On the other hand, anti-melanogenic effects are also exerted by suppressing the regulation of the cAMP/CREB signaling pathway and/or activating a cascade associated with triggering melanocyte cell apoptosis. MITF levels decreased with the administration of phenolic components, and this phenomenon was dose-dependent. When compared with the control, MITF expression decreased by 16.89 and 24.92% when given 60 and 100 g/mL of free phenolic extract, respectively. With decreased MITF expression, there is suppression of melanogenesis [17].

Plum fruit extract in this study contained flavonoids of 2176.57 mg/100g. The content of flavonoids acts as primary and secondary antioxidants. As a primary antioxidant, this component suppresses the adverse effects of free radicals directly by oxidizing and converting them into inactive radicals [18]. On the other hand, as secondary antioxidants, flavonoids serve to activate endogenous antioxidants, such as superoxide dismutase and glutathione peroxidase [19]. These endogenous antioxidants will be able to directly inhibit tyrosinase and eliminate oxidative stress on the skin [20].

Flavonoids are also able to provide a whitening effect by suppressing the regulation of PKA/CREB expression mediated by MITF. In addition, the tannins contained in the extract of plums (423.92 mg/100g) can react with most metal ions through chelation and form complexes. Tannins composed of epicatechin and catechol have competitive inhibitory properties against L-DOPA. Therefore, melanogenesis will be inhibited. Phytochemical components with copper chelating ability and antioxidant activity also have tyrosinase inhibitory activity. This is based on the oxidation state of copper ions embedded in the active region, which is required for the tyrosinase-catalyzed reaction. In addition, the conversion of L-DOPA to DOPA-quinone also occurs through an oxidation reaction [21].

Red fruit extract containing flavonoids (238.63 mg/100 g QE) and quite high tannins (600.71 mg/100g TAE) was able to suppress melanin production after UV exposure when compared to placebo, namely 1.25% vs 19.78% [22]. A similar effect was observed in this study with a higher content of flavonoids and tannins. Melanin obtained in this study was also lower, 0.42%.

Based on the above explanation, it is proven that plums have the effect of inhibiting the tyrosinase enzyme and increasing the amount of melanin. This effect is based on the various active substances in plums that act as antioxidants that can suppress and counteract the adverse effects of excessive UVB exposure. Not only in the process of melanogenesis but the phytochemical components contained in this plum also have the potential to provide other anti-aging effects, such as preventing melanoma and also the appearance of wrinkles [23].

As it is known that exposure to UV rays can also cause various clinical conditions. In addition to hyperpigmentation, another complaint that is often disturbing is the appearance of wrinkles. Antioxidants contained in various parts of the plant can inhibit the formation of wrinkles. One example is tamarind leaf extract cream which has been shown to inhibit the increase in matrix metalloproteinase-1 and decrease the amount of dermal collagen. Phytochemical components contained in tamarind leaves are also owned by plums, including phenols, flavonoids, and tannins. Therefore, the plum extract may also provide other anti-aging effects in the form of preventing the formation of wrinkles. However, this is still an assumption and needs to be proven further in research.

V. Conclusion

Hyperpigmentation is one of the common skin problems associated with aging and is caused by excessive production of the pigment melanin. Exogenous antioxidant supplementation can neutralize increased ROS's adverse effects and suppress melanin production. One source of exogenous antioxidants that can be utilized is plums. These antioxidants work as scavengers that prevent the formation of free radicals and also through metal ion chelator mechanisms. Based on this study, administration of 2% plum (*Prunus domestica* L.) extract cream was able to inhibit the increase in the expression of the tyrosinase enzyme and the amount of melanin in the skin of male guinea pigs (*Cavia porcellus*) exposed to UVB light.

Conflict of Interest

The authors declared that they do not have any conflict of interest.

References

[1] Lestari S. Cosmeceutical for hyperpigmentation. *J Cosmet Dermatology Updat Symp Proc*. 2011;3(1):1–10.

[2] Helfrich YR, Sachs DL, Voorhees JJ. Overview of skin aging and photoaging. *Dermatol Nurs.* 2008;20(3):177–83.

[3] Trivedi MK, Yang FC, Cho BK. A review of laser and light therapy in melasma. *Int J Women's Dermatology*. 2017;3(1):11–20. Available from: http://dx.doi.org/10.1016/j.ijwd.2017.01.004

[4] Birwal P, Deshmukh G, Saurabh S, Pragati S. Plums: A brief introduction. *J Food, Nutr Popul Heal.* 2017;1(1:8).

[5] Abdel-Rahim EA, El-Beltagi HS. Constituents of apple, parsley and lentil edible plants and their therapy treatments for blood picture as well as liver and kidneys functions against lipidemic disease. *Electron J Environ Agric Food Chem.* 2010;9(6):1117–27.

[6] Elena I, Nour V, Trandafir I. The influence of aero-ionized stream on the storage capacity of plums. *Acta Hortic*. 2012;968:205–10.

[7] Qian W, Liu W, Zhu D, Cao Y, Tang A, Gong G, et al. Natural skin-whitening compounds for the treatment of melanogenesis (Review). *Exp Ther Med.* 2020;20(1):173–85.

[8] Yang CY, Guo Y, Wu WJ, Man MQ, Tu Y, He L. UVB-Induced secretion of IL-1 β promotes melanogenesis by upregulating TYR/TRP-1 expression in vitro. *Biomed Res Int.* 2022.

[9] Wang Y, Zhao J, Jiang L, Mu Y. The application of skin care product in melasma treatment. *Clin Cosmet Investig Dermatol.* 2021;14:1165–71.

[10] Athipornchai A, Jullapo N. Tyrosinase inhibitory and antioxidant activities of orchid (*Dendrobium* spp.). *South African J Bot*. 2018;119:188–92. https://doi.org/10.1016/j.sajb.2018.09.003
[11] El-Beltagi HS, El-Ansary AE, Mostafa MA, Kamel TA, Safwat G. Evaluation of the phytochemical, antioxidant, antibacterial and anticancer activity of *Prunus domestica* fruit. *Not Bot Horti Agrobot Cluj-Napoca.* 2019;47(2):395–404.
[12] Kumar JP, Mandal BB. The inhibitory effect of silk sericin against ultraviolet-induced melanogenesis and its potential use in cosmeceutics as an anti-hyperpigmentation compound. *Photochem Photobiol Sci.* 2019;18(10):2497–508.
[13] Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. *J Cell Mol Med.* 2010;14(4):840–60.
[14] Sholikha M, Febriani A, Wahyuningrum A. Formulasi gel ekstrak lobak (*Raphanus sativus* L.) sebagai antioksidan dan inhibitor tirosinase. *J Ilmu Kefarmasian.* 2020;13(1):15–20.
[15] Prabowo AO, Pangkahila W, Sriwidyani NP. The administration 3% Tamarind (*Tamarindus indica*) leaves ethanol extract cream inhibited the increase of tyrosinase expression and the amount of skin melanin in male Guinea pigs (*Cavia porcellus*) skin exposed to UV B. *Neurol Spinale Med Chir.* 2021;4(3).
[16] Di Petrillo A, González-Paramás AM, Era B, Medda R, Pintus F, Santos-Buelga C, et al. Tyrosinase inhibition and antioxidant properties of Asphodelus microcarpus extracts. *BMC Complement Altern Med*. 2016;16(1):1–9. http://dx.doi.org/10.1186/s12906-016-1442-0
[17] Sun L, Guo Y, Zhang Y, Zhuang Y. Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by cAMP/MITF/TYR pathway in B16 mouse melanoma cells. *Front Pharmacol.* 2017;8(MAR):1–9.
[18] Gupta VK, Kumria R, Garg M, Gupta M. Recent updates on free radicals scavenging flavonoids: An overview. *Asian Journal of Plant Sciences.* 2010;9:108–17.
[19] Salehi B, Azzini E, Zucca P, Varoni EM, Kumar NVA, Dini L, et al. Plant-derived bioactives and oxidative stress-related disorders: A key trend towards healthy aging and longevity promotion. *Appl Sci.* 2020;10(3).
[20] Putra Wiraguna AAG, Hari ED, Praharsini IGAA. Correlation between glutathione plasma with degree severity of melasma in balinese women. *Clin Cosmet Investig Dermatol.* 2020;13:455–9.
[21] Liu J, Chen Y, Zhang X, Zheng J, Hu W, Teng B. Hop tannins as multifunctional tyrosinase inhibitor: Structure characterization, inhibition activity, and mechanism. *Antioxidants.* 2022;11(4):1–15.
[22] Dumaria CH, Wiraguna AA, Pangkahila W. Krim Ekstrak Buah Merah (*Pandanus conoideus*) 10% sama efektifnya dengan krim hidrokuinon 4% dalam mencegah peningkatan jumlah melanin kulit marmut (*Cavia porcellus*) yang dipapar sinar ultraviolet B. *J Biomedik*. 2018;10(2):85–91.
[23] Kaminski K, Kazimierczak U, Kolenda T. Oxidative stress in melanogenesis and melanoma development. *Wspolczesna Onkol.* 2022;26(1):1–7.

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