**Original Research Article**

**THE EFFECT OF TAMARILLO FRUIT (*Solanum betaceum Cav.*) ETHANOL EXTRACT CREAM ON THE LEVELS OF MALONDIALDEHYDE (MDA), TYROSINASE ENZYME AND MELANIN IN THE SKIN OF MALE GUINEA PIGS (*Cavia porcellus*) EXPOSED TO ULTRAVIOLET B RADIATION**

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ABSTRACT

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| **Aims:** To evaluate the effect of tamarillo fruit ethanol extract cream on the levels malondialdehyde (MDA), tyrosinase enzyme and melanin in the skin of male guinea pigs (Cavia porcellus) exposed to ultraviolet B (UVB) radiation.  **Study design:** Randomized post-test only control group design.  **Place and Duration of Study:** Food Analysis Laboratory, Faculty of Agricultural Technology; Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University; between September 2024 and December 2024.  **Methodology:** We included 25 locally bred male guinea pigs, aged three to four months, weighing between 300 and 350 grams, in healthy condition. They were divided into five groups: normal control group (K0), negative control group (K-), and treatment groups administered with tamarillo fruit ethanol extract cream at concentrations of 7,5% (P1); 15% (P2) and 30% (P3). K-, P1, P2 and P3 groups were exposed to UVB radiation at a total dose of 390 mJ/cm2 for two weeks. The levels of MDA and tyrosinase enzyme were measured using the ELISA method, while melanin levels were measured by the Fontana-Masson staining method. Data were analyzed using one way ANOVA test followed by a post-hoc test.  **Results:** The levels of MDA, tyrosinase enzyme and melanin in K0, P1, P2, and P3 groups were significantly lower compared to group K-. Tyrosinase levels in the 30% treatment group were not significantly different from the 15% group, but were lower than the 7.5% group. All three concentrations of tamarillo extract cream (7.5%; 15%; 30%) had the same effectiveness in reducing melanin levels in UVB exposed guinea pigs.  **Conclusion:** Tamarillo fruit ethanol extract cream effectively reduced MDA, tyrosinase and melanin levels in guinea pigs exposed to UVB radiation. The 15% concentration showed potential in preventing hyperpigmentation and could serve as a natural sunscreen. Futher research is required to confirm this finding in human used. |

*Keywords: Tamarillo, UVB, MDA, Tyrosinase, Melanin, hyperpigmentation*

1. INTRODUCTION

Exposure to ultraviolet (UV) radiation triggers the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress in the skin(Alcantara et al., 2020; Gery Umami et al., 2022; Hart et al., 2019; Reddy, 2023)

This result in lipid peroxidation with malondialdehyde (MDA) as an end product and increases melanogenesis through the activation of the key enzyme tyrosinase(Harlisa et al., 2021; Lee et al., 2014; Marza et al., 2023). A number of tyrosinase inhibitors have been identified, both from natural and synthetic sources(Obaid et al., 2021). Phytochemical screening showed that tamarillo fruit extract contains flavonoids, phenolics, terpenoids, steroids, saponins, alkaloids, and tannins. Flavonoids have the potential as free radical inhibitors(Haerani et al., 2018; Jeane et al., 2018; Rito et al., 2023; Siti et al., 2024; Widayanti et al., 2016). Tannins are able to bind metal ions such as copper (Cu) which is an important cofactor for the tyrosinase enzyme. By attaching to these metal ions, tannins hinder the function of the tyrosinase enzyme, leading to a decrease in melanin production(Guo et al., 2023; Molino, 2022; Pizzi, 2021).

Flavonoids are a type of secondary metabolite classified as natural polyphenols founds in plants. These compounds has a core structure in the form of a C6-C3-C6 carbon skeleton, which consists of 15 carbon atoms(Haerani et al., 2018; Obaid et al., 2021; Siti et al., 2024; Widayanti et al., 2016).

The tyrosinase enzyme plays an important role in melanin synthesis. Decreased activity of this enzyme leads to reduced melanin production. Melanin production starts with the tyrosinase enzyme converting L-tyrosine to L-DOPA, which is then oxidized by the same enzyme into L-dopaquinone. L-dopaquinone is the key building block for melanin(Choi, 2016; D’Mello et al., 2016; Ruchi, 2020).

Tamarillo fruit contains high nutrients such as carotenoids, anthocyanins, phenolic compounds, vitamin and flavonols. Consumption of tamarillo fruit has been shown to have antioxidant, anti-inflammatory, anti-obesogenic and chemopreventif effects on cancer cell models in rodents(Diep et al., 2022; Tanjung, 2023).

The study conducted by Isla (2022) have shown that polyphenolic extracts from tamarillo peel and seed inhibit the activity of hydrolytic enzymes sush as tyrosinase, elastase, collagenase and hyaluronidase, especially elastase and tyrosinase. These finding suggest that tamarillo fruit subproducts are a promising source of bioactive phenols for application in the cosmetic industry. Formulations such as hydrogels, lotions, or creams using this extract offer a more environmentally friendly, affordable and effective solution compared to other plant extract commonly used in cosmetic products (Isla et al., 2022).

2. material and methods

Tamarillo fruit extract was obtained through the maceration method using 96% ethanol at the Food Analysis Laboratory, Faculty of Agricultural Technology, Udayana University. Cream formulations containing tamarillo fruit ethanol extract at concentrations of 7,5%, 15% and 30% were developed at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University, following established protocols.

This study used a randomized post-test only control group experimental design and was conducted at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University from September to December 2024. The samples included 25 locally bred male guinea pigs (Cavia porcellus), aged between three to four months, weighing between 300 and 350 grams, with brown fur, in healthy condition, exhibiting normal feeding and drinking behavior. The subjects were categorized into five groups, including a normal control group, that did not receive any treatment (K0); a negative control group that received a basic cream and was exposed to UVB radiation (K-), P1 group that received 7,5% tamarillo fruit ethanol extract cream and was exposed to UVB radiation, P2 group that received 15% % tamarillo fruit ethanol extract cream and was exposed to UVB radiation; and P3 group that received 30% tamarillo fruit extract cream and was exposed to UVB radiation. The topical formulation were applied once daily at 10.00 AM Central Indonesia Time on non-UVB irradiation days. On days of UVB exposure, the topical formulation was applied at 10.00 AM Central Indonesia Time (20 minutes before UVB exposure) and again at 2:20 PM Central Indonesia Time (four hours after each UVB exposure), 0,4 mg of cream was applied to the shaved backs of the guinea pigs. UVB exposure (65 mJ/cm2 for 65 seconds) was administered three times a week (Monday, Wednesday, and Friday) at 10:20 AM Central Indonesia Time for a total of 14 days. MDA and tyrosinase enzyme levels were then measured using ELISA, while melanin levels measured using the Fontana-Masson staining method following previously established protocols.

**2.1 statistical analysis**

All data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS software, version 26 (SPSS Inc., Chicago, IL, USA). The normality test of MDA levels, tyrosinase enzyme activity, and melanin content was assessed using the Shapiro-Wilk test, while homogeneity was evaluated with Levene’test. One-way ANOVA was used to compare the groups, with post hoc test performed to determine significant differences between specific groups.

3. results and discussion

The findings of this study revealed that the total phenol content of tamarillo fruit ethanol extract was 3594.847 mg/100 gr GAE. The total flavonoids content was 104.907 mg/100 gr QE, while the tannin content was 3857.756 mg/100g TAE. The IC50 value of the extract was found to be 418.828 ppm.

The average MDA levels in the K- group was 25.25 ± 1,74 nmol/mL, higher than in the groups K0 (18.10 ± 0,17 nmol/mL), P1 (22.19 ± 0,32 nmol/mL), P2 (21.45 ± 0,49 nmol/mL), P3 (20.06 ± 0,29 nmol/mL) (P<0.05) indicating that 7.5%, 15% and 30% tamarillo fruit ethanol extract creams were equally effective in reducing MDA levels in the skin of guinea pigs after UVB exposure were compared to those in the negative control group. Among the three treatment groups, the P3 group exhibited the lowest average MDA levels.

The average tyrosinase levels in the K- group was 39.74 ± 4.40 ng/mL higher than that in the groups K0 (19.11 ± 1.92 ng/mL), P1 (31.36 ±1.65 ng/mL) (P<0.05), indicating that three doses of tamarillo fruit ethanol extract cream was effective in reducing tyrosinase levels in the skin of guinea pigs after UVB exposure compared to the negative control group, no significant difference was observed (P>0.05) between the effects of 15% and 30% tamarillo fruit ethanol extract creams.

The average melanin level in the K0 group was 3.64 ± 1.99%, which did not differ significantly from that in the P1, P2, P3 groups. However, the average melanin level in the K- group was 11.37 ± 3.37% higher than that in the K0 (3.64 ± 1.99%), P1 (2.94 ± 1.45%), P2 (2.16 ± 1.45%) and P3 (0.47 ± 0.38%) groups with a P value of less than 0.05. Likewise, in terms of melanin levels between groups P1, P2 and P3 was not different significantly, indicating that the three doses of tamarillo fruit ethanol extract cream were equally effective in reducing the levels of melanin in the skin of guinea pigs after UVB exposure.

Table 1. Comparison the level of MDA, tyrosinase enzyme and melanin among treatment groups and control

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| **Group** | **MDA (nmol/mL)** | **Tyrosinase (ng/mL)** | **Melanin (%)** |  |
| K0 | 18.10 ± 0.17abf | 19.11 ± 1.92abh | 3.64 ± 1.99ab |  |
| K- | 25.25 ± 1.74b | 39.74 ± 4.41b | 11.37 ± 3.37b |
| P1 | 22.19 ± 0.32bcdf | 31.36 ± 1.65bdfh | 2.95 ± 1.45ab |
| P2 | 21.46 ± 0.49bcdf | 25.36 ± 0.73bcdh | 2.17 ± 1.45ab |
| P3 | 20.06 ± 0.28bdf | 22.59 ± 0.99bcfh | 0.48 ± 0.38ab |
| p-values | < 0.05 | < 0.05 | < 0.05 |  |

*Note: The superscripts in the same column”a, c” do not vary significantly from the negative control and the various groups, while superscripts “b, d, f, h” vary significantly from the negative control and various groups*

One of the characteristics of skin aging due to UVB radiation is skin hyperpigmentation, can be treated with oral or topical therapies. Previous studies have identified that inhibition of tyrosinase activity is an important for development of depigmentation therapy (Siahaan et al., 2017).

The effectiveness of tamarillo fruit extract in decrease in the levels of tyrosinase and melanin can be attributed to its phytochemical content. Flavonoids can lighten skin by directly reducing the activity of the tyrosinase enzyme, which is involved in melanin production (Harlisa et al., 2021). Additionally, flavonoid can inhibit the tyrosinase enzyme by inhibiting ROS (Friama et al., 2021; Z.Sagala, 2020).

Tannin can also inhibit the tyrosinase enzyme by binding copper (Cu) ions which are important cofactors for the enzyme. As a result, following exposure to UVB radiation, melanin formation prevented because the melanin biosynthesis process has been inhibited (Molino, 2022).

This study aims to demonstrated that tamarillo fruit ethanol extract cream can decrease in the levels of MDA, tyrosinase enzymes and melanin production in the skin of guinea pigs after UVB exposure. It was found that average of MDA, tyrosinase enzyme and melanin between groups differed significantly with lower values in tamarillo fruit ethanol extract cream compared to the negative control group. These findings suggest that the three doses of tamarillo fruit ethanol extract cream effectively decrease in the levels of MDA (K0<P3<P2=P1<K-), tyrosinase enzyme (K0<P3=P2<P1<K-) and melanin (K0=P3=P2=P1<K-). Tamarillo fruit ethanol extract cream has been shown to inhibit UVB-induced hyperpigmentation and has the potential as an anti-hyperpigmentation agent with a mechanism of action involving the suppression of ROS, leading to the decreased of tyrosinase enzyme production and a reduction in melanin density due to exposure to UVB radiation.

4. Conclusion

Tamarillo fruit ethanol extract cream has been demonstrated to be more effective in decrease in the levels of MDA, tyrosinase enzyme activity and melanin levels in the skin of guinea pigs after UVB exposure compared to negative control group. The 15% concentration showed potential in preventing hyperpigmentation and could serve as a natural sunscreen. However, further research is needed to evaluate the potential toxicity of this cream to the skin, including potential adverse reaction, such as skin irritation, may occur with both short-term and long-term use. In addition, clinical trials are needed as an important step before this cream may offer a different treatment option for hyperpigmentation in humans.

Consent

"All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

Ethical approval (where ever applicable)

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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