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Clitoria ternatea Flower Extract Inhibits Matrix Mettaloproteinase-3 leading to the promotion of α-SMA Gene Expression in UVB-Induced Rat Model

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ABSTRACT

Background: UVB radiation is known to induce direct DNA damage, leading to mutations, oxidative stress, and inflammatory responses. One of the critical processes involved in photoaging is the upregulation of matrix metalloproteinases (MMPs), especially MMP-3. Clitoria ternatea, commonly known as butterfly pea, is a plant with significant medicinal properties. **Objective:** This study aimed to determine the effect administration of *Clitorea ternatea* flower extract (*C. ternatea*) in the form of a gel on the expression of MMP-3 and α-SMA in the skin of rat models induced by UVB light exposure The design of this research is experimental research with post-test control group design. **Material and Methods:** This study was divided into four treatment groups consist of a healthy group, a UVB negative control, a 5% *C. ternatea* gel treatment group (T1) and a 10% *C. ternatea* gel treatment group (T2). Negative control, T1, and T2 groups were given UVB radiation with 302 nm energy of 160 mJ/cm2 for 5 days. In the treatment groups T1 and T2 were given the *C. ternatea* gel on the 6th day until the 14th day. On day 15, all treatment groups were terminated and the expression of MMP-3 and α-SMA genes were analyzed using qRT-PCR. **Result:** In the 10% *C. ternatea* gel treatment group, there was a decrease in MMP-3 gene expression (0.73±0.26) and an increase in α-SMA gene expression (16.4±8.3) compared to the UVB control group. **Conclusion:** The administration of *C. ternatea* has the potential to prevent UVB-induced hyperpigmentation.

Keywords: α-SMA, *Clitoria ternatea*, MMP-3, UVB irradiation

INTRODUCTION

Matrix metalloproteinase-3 (MMP-3), also known as stromelysin-1, is an enzyme that plays a crucial role in the degradation of extracellular matrix components, including proteoglycans, laminin, fibronectin, and collagen. This enzyme is typically involved in normal physiological processes such as tissue remodeling and wound healing. However, its overexpression can contribute to pathological conditions such as inflammation, arthritis, and photoaging of the skin. In the context of UVB-induced damage, MMP-3 is often upregulated, leading to the breakdown of the extracellular matrix and subsequent skin damage. Inhibiting MMP-3 can therefore be a therapeutic target to mitigate UVB-induced skin damage and promote skin repair mechanisms, such as the upregulation of α-SMA (alpha-smooth muscle actin) gene expression, which is associated with tissue repair and regeneration.

UVB exposure to the skin causes an increase in reactive oxygen species (ROS), which activates inflammation and inactivation of fibroblast cell proliferation and decreases skin collagen [1,2]. Inactivation of fibroblast cell proliferation is characterized by decreased expression of alpha smooth

actin (α-SMA) genes and increased expression of matrix metalloproteinase (MMP) genes such as MMP-3 [3-4] . Excessive UV exposure can alter the regulation of collagen homeostasis by fibroblasts, resulting in an imbalance between collagen synthesis and degradation [5,6]. Therapies commonly used to reduce collagen loss are still limited to chemicals such as tretinoin, which is reported to have side effects such as irritation [7].

α-SMA is a protein that plays a role in fibroblast contractile activity and collagen reorganisation [8,9]. α-SMA is expressed by myofibroblasts, activated form of fibroblasts[10]. Activated myofibroblasts stop proliferating and begin synthesizing extracellular matrix (ECM) protein components, including large amounts of collagen[10]. α-SMA expressed by myofibroblasts is the main source of fibrillar collagen, such as collagen types I, II, III, V and XI which are essential for tensile strength and mechanical strength. α-SMA is crucial for maintaining tensile strength and mechanical properties of the skin, preventing premature ageing [11].

C. ternatea is a plant widely used for ots strong antioxidant strong antioxidant compounds, such as anthocyanins, which can reduce ROS levels. The application of *C. ternatea* flower extract on the skin is still rare even though this plant's anthocyanins are beneficial is protecting the skin from due to UVB exposure. These compounds can suppress the production of MMP-3, leading to the inhibition of collagen degradation [12]. Additionally, reducing ROS levels candecrease the production of inflammatory molecules and trigger the production of TGF-β1. TGF-β1 plays a role in collagen production by activating the Smad2/3 complex and myofibroblasts, leading to the expression of α-SMA [13]. Previous study that topical or oral administration of anthocyanin-containing *C. ternatea* can reduce ROS [14–16]. This study aims to examine the effect of *C. ternatea* on MMP-3 and α-SMA gene expression in a UVB-induced rat model.

MATERIAL AND METHODS

Material and study design

This post-test only control group study was conducted in Stem Cell and Cancer Research (SCCR) Laboratory, Semarang from June to August 2022. The study was approved by the Ethics Committee of Sultan Agung Islamic University (No. 305/VIII/2022/Komisi Bioetik).

Preparation of Clitorea ternatea flower extract

The dried C. ternatea flower was macerated in methanol for 72 h. The filtrate was then evaporated using a rotary vacuum evaporator (IKA) and the crude extract west kept in a refrigerator at 4 ℃ until futher analysis.

Qualitative phytochemical screening

The phytochemical screening of the crude extract was performed to ascertain the presence of its secondary metabolites. The methids used included, saponins using the check method, alkaloids using the usage of Wagner approach, flavonoids using the Wilstater test, tannins using 1% FeCl3, and teriterpenoid using the Lieberman Burcham test 17–20.

Preparation of UVB-induced rats

Twenty-four male Wistar rats were isolated for three days and assessed for weight changes and any signs of injury. The rats were housed in plastic cages with sawdust, with six animals in each cage. They were provided with a standard diet and unlimited access to water. The study involved three-months-old male white Wistar rats (*Rattus norvegicus*), weighing approximately 200 g, divided into four groups. The negative control group consisted of rats with collagen loss exposed to UV radiation. The T1 group consisted of rats with collagen exposed to UVB radiation and treated with a topical gel

containing 5% C. ternatea. The T2 group consisted of rats with collagen exposed to UVB radiation and treated with a topical gel containing 10% C. ternatea. The healthy group was not exposed to UVB radiation. Each group consisted of six rats according to fedderer sample size calculation method. The hair on the left dorsal side of the rats was shaved to enhance the effects of UVB exposure on the skin. UVB exposure was administered to each group 5 times, with an intensity of 160 mJ/cm2 per day, resulting in a total dose of 800 mJ/cm2.

Preparation of topical gel

For the 5% dose, 5 g of *C. ternatea* extract was mixed with 95 g of a water-based gel. Similarly, for the 10% dose, 10 g of C. ternatea extract was mixed with 90 g of a water-based gel in a 100 mL beaker glass. The mixtures was then transferred and stored in a falcon tube throughout the treatment process. The topical gel extract was applied once a day for two weeks, starting on day 6 after UVB induction. The doses that used in this study according to our previous study. 16

MMP-3 and α-SMA gene expression under qRT-PCR

RNA was extracted from 50 mg of dorsal skin tissue, and the expression levels of α-SMA and MMP-3 were measured using real-time PCR. The skin tissue sample was homogenized, and total RNA was extracted using the FAVORGEN RNA isolation kit. From each skin sample, 25 µg of total RNA was used to prepare cDNA using Rever Tra Ace™ qPCR RT Master Mix containing gDNA Remover. The cDNA synthesis was carried out in a 50 µl PCR buffer (provided with the kit), which contained 2 µl of reverse transcriptase, 2 µl of oligo d(T) primer, and gDNA remover. For the PCR reaction, 50 ng of cDNA, 1 µM primers of TNF-α and caspase-3, and 10 µl of SYBR Green DNA polymerase were included in a 50 µl PCR buffer. The PCR reaction consisted of 30 cycles with the following conditions : denaturation at: at 95 °C for 30 min, annealing at 60 °C for 1 min, and extension at 74 °C for 15 min. The primer sequences used were: MMP-3 Forward: 5'-CACTCACAGACCTGACTCGGTT-3', Reverse: 5'-AAGCAGGATCACAGTTGGCTGG-3', and α-SMA Forward: 5'-CCGACCGAATGCAGAAGGA-3', Reverse: 5'-ACAGAGTATTTGCGCTCCGAA-3'.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± standard deviation (SD). Data analysis involved one-way ANOVA and followed by the Least Significant Difference (LSD) test, with a significant level sel at $p<0.05$.

RESULTS

Phytochemical screening of C. ternatea

Phytochemical screening is a qualitative testing method performed through colorimetric assays to examine secondary metabolites, which are chemical compounds found in plants. This qualitative analysis determined the presence of various compounds including flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids in *C. ternatea*. The results indicated the presence of flavonoids, which may contribute to the antioxidant activity of *C. ternatea*. The total flavonoid content of *C. ternatea* was 682.02 mg per gram of extract (Table 1).

Table. 1 Total Flavonoid of *C. ternatea* extract

Extract Concentration	TPC (mg/gram extract)
1000 ppm	690,21
1000 ppm	671,64
1000 ppm	684,21
Average	682,02

C. ternatea decreased MMP-3 gene expression

In the present study, we found that *C. ternatea* significantly decreased MMP-3 gene expression in a dose-dependent manner (Figure 1). In negative control group, MMP-3 gene expression was significantly elevated, 3.24±1.05-fold greater than healthy group. Interestingly, the 5% (T1) and 10% (T2) of *C. ternatea* groups significantly decreased MMP-3 gene expression, with 1.11±0.15-fold and 0.73±0.26- fold compared to healthy group.

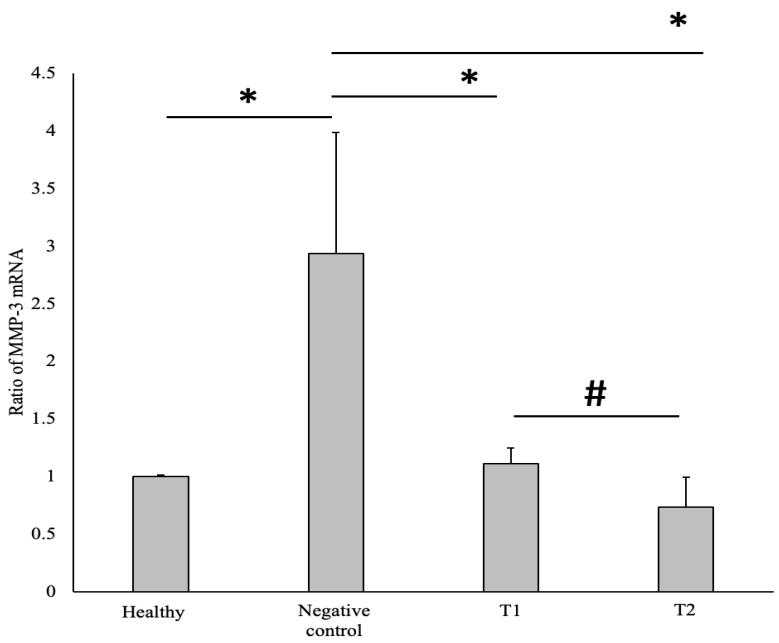

Figure 1. The effect of *C. ternatea* on MMP-3 gene expression on UVB irradiation rat models. RNA was extracted from the rat skin and analyzed for mRNA expression by qRT-PCR (n=5 ±SD). Data are presented as fold change in gene expression relative to UVB healthy group. *Differences were considered statistically significant at $p < 0.05$ T1 : treated with *C. ternatea* 5% and T2: treated with *C. ternatea* 10%.

C. ternatea increased α-SMA gene expression

In the present study, we also found that *C. ternatea* gels significantly induced α-SMA gene expression in a dose-dependent manner (Figure 2). In negative control group, α-SMA gene expression was significantly suppressed, 0.10±0.01-fold greater of healthy group. Interestingly, the 5% (T1) and 10% (T2) of *C. ternatea* groups significantly increased the α-SMA gene expression, with 3.40±1.30-fold and 16.40±8.30-fold compared to healthy group.

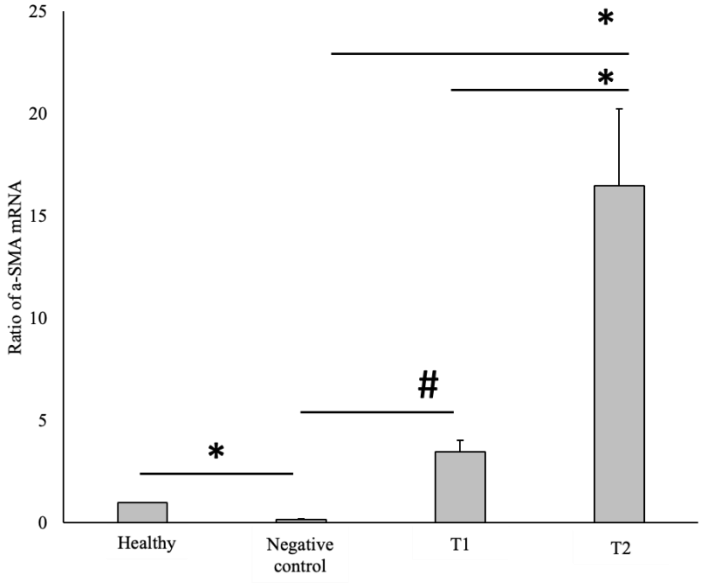

Figure 2. The effect of *C. ternatea* on α-SMA gene expression on UVB irradiation rat models. RNA was extracted from the rat skin and analyzed for mRNA expression by qRT-PCR (n=5 ±SD). Data are presented as fold change in gene expression relative to UVB healthy group. *Differences were considered statistically significant at $p < 0.05$ T1 : treated with *C. ternatea* 5% and T2: treated with *C. ternatea*.

DISCUSSION

The decrease in collagen is one of the negative effect of prolonged UVB irradiation, which can lead to wrinkles in certain areas of the skin [21]. UVB irradiation damages DNA structures, resulting in the death of various cell types, including fibroblasts, which are responsible for collagen production[22]. UVB exposure is known to increase ROS level triggering the activation of the intracellular MAP kinase pathway. This pathway, in turn, stimulates the transcription factor AP-1, comprising two subunits c-jun and c-fos. AP-1 plays a crucial role in regulating the transcription of MMP-3, a key exzyme involved in collagen degradation[23–25]. In addition, UVB exposure activates the NF-κB pathway, leading to the synthesis of inflammatory factors such as IL-1, IL-6, and TNF-α, which inhibit the activation of fibroblast [23,26].

Previous studies have shown that the polyphenols in *C. ternatea* posses anti-inflammatory properties, particularly in lipopolysaccharide-induced inflammation in macrophage cells [27–29]. In addition, the flavanoid content in *C. ternatea* is known to strongly inhibit COX-2 activity and inhibit partial ROS production [30,31]. On the other hand, flavonoids also inhibit NF-κB translocation, iNOS protein expression, and NO production [32]. The inhibition of ROS production is known to suppres the MAPK and AP-1 pathways, leading to decreased MMP-3 enzyme expression [33].

The results in this study align with previous research, which demonstrated that *C. ternatea* was proven positive for containing alkaloids, saponins, flavonoids, tannins, and terpenoids [34,35]. In this study, it was found that 60% of the extracts contained flavonoid compounds, with an average total flavonoid content of 682,04 mg per gram of *C. ternatea*. This suggest that flavonoid compounds play a significant role in the biological activity of *C. ternatea*. The reduction of MMP-3 gene expression to nearly normal levels following the administration of 5% and 10% *C. ternatea* indicates a protective effect against collagen degradation. MMP-3 enzymes are naturally involved in the breakdown of collagen in the body,

thus, a decrease in MMP-3 expression corresponds to reduced collagen degradation, which helps preserve skin elasticity [25,36,37].

The results of this study also demonstrated that a 10% *C. ternatea* treatment help maintain skin elasticity by increasing α-SMA gene expression. α-SMA, a marker of myofibroblast activation, is indicative of fibroblast activation and collagen. The increase in α-SMA gene expression observed with *C. ternatea* treatment may be attributed to its antioxidant-active compounds. Previous studies have highlighted the role of flavonoid-active substances in stimulating collagen production by fibroblast [38,39].

The role of *C. ternatea* in activating fibroblasts into myofibroblasts, as characterized by an increase in α-SMA expression, may be due to its antioxidants properties that reduce ROS levels. According to previous studies, ROS contributed to the initiation of inflammation by activating the NF-κB pathway, which leads to the secretion of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α [40,41]. M2 which can secrete pro-collagen factors such as PDGF and TGF-β[42]. The decrease in ROS levels due to antioxidants administration is correlated to the polarization of M1 to M2, which subsequently triggers the secretion of PDGF activating fibroblasts to become myofibroblasts, as evidenced by the increase α-SMA expression [43,44].

CONCLUSIONS

Topical application of 5% and 10% *C. ternatea* gel effectively reduced MMP-3 gene expression and increaced α-SMA gene expression in UVB-induced rat skin. Overall, *C. ternatea* has the potential to prevent UVB-induced hyperpigmentation.

CONFLICT OF INTEREST

Competing interests: No relevant disclosures.

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CONTRIBUTORS

NIM and NDA were involved in concepting and designing the study. NIM and SAH was involved in the experiment. NDA provided the data analysis. SAH and NDA performed the statistical analysis. NIM and NDA wrote and edited the manuscript. Meanwhile SAH helped in reviewing the manuscript.

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