Trop J Nat Prod Res, January 2025; 9(1): 235 - 239 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Unlocking the Potential of Stink Bean Peel Extract: A Natural Solution for UVB-Induced Skin Hyperpigmentation

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ARTICLE INFO

Article history:
Received 09 May 2024
Revised 15 May 2024
Accepted 13 December 2024
Published online 01 February 2025

ABSTRACT

Prolonged exposure to high-intensity UVB radiation can induce the formation of Reactive Oxygen Species (ROS), inhibit alpha-smooth muscle actin (*α*-SMA), and collagen type 1 expression, leading to skin hyperpigmentation. This study aimed to investigate the effect of topical application of stink bean peel extract gel on the expression of *α*-SMA and collagen type 1 genes in the skin tissue of hyperpigmentation rats. Stink bean peel extract gel was prepared by mixing ethanol extract of stink bean peel with a gel base (Catechu) to make 10% and 20% formulations. Hyperpigmentation was induced in rats by exposure to UVB radiation (λ302 nm, 390 mj/cm^2/day) three times a week for two weeks. The animals were divided into four groups: K1 (healthy rats), K2 (negative control), K3 (10% extract gel, and K4 (20% extract gel). The effect of stink bean peel extract gel treatment on the expression of *α*-SMA and collagen type 1 genes was analyzed using *Real-Time* Quantitative Reverse Transcription polymerase chain reaction (qRT-PCR). qRT-PCR analysis indicated a significant ($p < 0.05$) increase in the expression of *α*-SMA gene in group K4 with a mean expression ratio of 3.11±1.08 compared to the negative control (0.67±0.17). Collagen type 1 gene expression also increased significantly in K3 and K4 groups with expression ratios of 2.21±0.68, and 2.12±0.73, respectively compared to the negative control (0.51±0.14). Findings from this study has shown that stink bean peel extract has the potential to be used as a protective and therapeutic agent against UVB-induced skin damage.

Keywords: Ultraviolet B exposure, Stink bean peel extract, Alpha-smooth muscle actin, Collagen type 1.

Introduction

The exposure to ultraviolet B (UVB) radiation from the sun can trigger various skin problems such as hyperpigmentation.[1] Prolonged exposure to UVB radiation can induce the formation of reactive oxygen species (ROS) in the skin, which activates pro-inflammatory cytokines, leading to increased melanin production.[2] The increased ROS also inhibits the proliferation of fibroblast cells, characterized by a decrease in the expression of alpha-smooth muscle actin (*α*-SMA) and an increase in the degradation of type 1 collagen.[3–5] The decrease in *α*-SMA expression and inhibition of type 1 collagen gene expression result in reduced collagen density and increased melanin production, leading to hyperpigmentation. [6,7] The administration of antioxidants has been shown to reduce ROS levels, thus preventing inflammation, and stimulating type 1 collagen synthesis. [8] *Parkia speciosa* (stink bean) skin is known to contain high levels of antioxidant compounds such as flavonoids (quercetin, and luteolin), alkaloids, tannins, and saponins, which can reduce ROS levels. [9,10]

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Citation: Ustika CR, Priyantini S, Putra A, Subchan P, Sumarawati T, Amalina ND. Unlocking the Potential of Stink Bean Peel Extract: A Natural Solution for UVB-Induced Skin Hyperpigmentation. Trop J Nat Prod Res. 2025; 9(1): 235 – 239 https://doi.org/10.26538/tjnpr/v9i1.35

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The use of antioxidant compounds can reduce the production of inflammatory molecules and trigger *α*-SMA production through the activation of transforming growth factor beta 1 (TGF-*β*1), which plays a role in collagen production by activating the SMAD family member 2/3 complex. [11,12] This activation induces fibroblast transformation into myofibroblasts, leading to *α*–SMA expression.[13] Polyphenolic compounds in stink bean peel, such as cinnamic acid, isoferulic acid, caffeic acid, and ferulic acid, have antioxidant activity by inhibiting melanocyte degradation, thus preventing hypermelanogenesis. Some standard hyperpigmentation therapies such as retinol, hydroquinone, and tranexamic acid can cause cancer on long-term use.[14] Standard skin hyperpigmentation treatment focuses on physical and chemical protection against UVB exposure without affecting the melanin production pathway. [15] Therefore, a safe and effective therapeutic approach is needed to prevent UVB-induced hyperpigmentation, one of which is the use of natural antioxidant compounds.

In 2015, 4.2% of 142 subjects tested positive for hyperpigmentation after being exposed to UVB three times with a minimal erythema dose (MED) of 390 mj/cm^2. The number of hyperpigmentation cases increased to ~100,350 new cases in 2020.[16] Primary fibroblast cell cultures exposed to UVB showed a 40-60% increase in pro-inflammatory cytokines, decreased proliferation, decreased *α*-SMA expression, and decreased collagen synthesis, both type 1 and type 3, leading to increased melanin production. [17,18] However, no study has examined the effect of stink bean peel extract on the expression of *α*-SMA and type 1 collagen in UVB-induced hyperpigmented skin. Previous studies have stated that the topical or oral administration of extracts containing flavonoids and polyphenols can reduce ROS, thus potentially proving the effect of stink bean peel extract on the expression of *α*-SMA and type 1 collagen in UVB-induced hyperpigmented rat skin. Based on this background, this study aims to determine the effect of stink bean peel extract on the expression of *α*-

SMA and type 1 collagen genes in UVB-induced hyperpigmented Wistar rat skin.

Materials and Methods

Plant collection and identification
Parkia speciosa were collected from Tawangmangu in Central Java Indonesia (Latitude -7.665158 and Longitude 111.129500) in May, 2022. A voucher specimen (UDP-23B-SBPE) was deposited in the herbal laboratory of stem cell and cancer research Indonesia. They were rinsed with tap water followed by distilled water to remove the dirt on the surface.

Plant extraction
Dried stink bean skin (50 g) was extracted by maceration in 500 mL of 98% ethanol for 24 h. The extract was filtered, and the filtrate was evaporated in vacuo using a rotary evaporator. The crude extract obtained was kept in the refrigerator at 4℃.[19,20] The stink bean peel extract was dissolved in a gel base (Catechu, USA) to make 10% and 20% formulations, and then stored at 4℃ until further analysis.

Phytochemical screening
The crude stink bean peel extract was tested for the presence of flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins following standard procedures as previously reported.[21–25].

Determination of total flavonoid content of Parkia speciosa extract
Total flavonoid content was determined using the aluminum chloride colorimetric method [26] with some modifications. Gallic acid was used as the standard. A calibration curve of gallic acid was prepared in several concentrations; 200, 300, 400, 500, 600, and 700 µg/mL. Briefly, extract (0.5 mL) and standard (0.5 mL) were placed in different test tubes and to each solution, 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL) and distilled water (2.8 mL) were added and mixed. A blank was prepared in the same manner where 0.5 mL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm using UV/Vis spectrophotometer (Shimadzu Corporation, Japan). The concentration of flavonoid was expressed as mg gallic acid equivalent (GAE) per gram of extract.
Induction of hyperpigmentation and treatment with stink bean peel extract gel
Twenty healthy male Wistar rats (250 ± 25 g) CV=10% were fed *ad libitum* and reared at 28ºC and a photoperiod of 12 hours. After a week of acclimatization, the rats were randomly divided into four groups of five rats each: Sham/Untreated (K1), UVB irradiation (K2), UVB irradiation + 10% stink bean peel extract gel (K3), and UVB irradiation + 20% stink bean peel extract gel (K4). This study used UVB light (broadband with peak emission at 302 nm CL-100M, UVP, USA). Rats were exposed to UVB light of 390 millijoules/cm^2 (mJ/cm^2) for 30 minutes for 5 consecutive days according to a previous study with slight modification.[27] The stink bean peel extract gels (200 mg) were administered topically on the dorsal surface of the rats' skin daily for 14 days. UVB group rats did not receive any treatment. On day 15, all rats were euthanized, and their skin tissues were collected for further analysis.

Melanin analysis
The tissue paraffin block was cut using a microtome to a thickness of 5 μm then stained with Fontana Masson and observed under the light microscope (Olympus CX21, Tokyo, Japan). The percentage of melanin was calculated from the area of epidermis tissue formed on each slide using ImageJ version 1.54 (National Institutes of Health-2024).

α-SMA and collagen type 1 gene expression by qRT-PCR
Total RNA from rat skin tissue was extracted with TRIzol (Invitrogen, Shanghai, China) according to the manufacturer's protocol. Briefly, first-stranded cDNA was synthesized with 1 g of total RNA using Super-Script II (Invitrogen, Massachusetts, USA). SYBR No ROX Green I dye (SMOBIO Technology Inc, Hsinchu, Taiwan) was used for reverse-transcription in a real-time PCR instrument (PCR max Eco 48), and mRNA levels of the a-SMA and collagen type 1 genes were measured using the respective primers. The thermocycler conditions used were as follows: initial step at 95℃ for 10 minutes, followed by 50 cycles at 95℃ for 15 seconds, and 60℃ for 1 minute. The gene expression was recorded as the Cycles threshold (Ct). Data were obtained using Eco Software v5.0 (Illumina Inc, San Diego, CA, USA). All reactions were performed in triplicate, and data analysis was done using the $2^{-\Delta\Delta}$ Ct method (Livak method).[28]

Statistical analysis
All data were presented as mean ± standard deviation (SD). Data analysis was performed using SPSS software, version 28.0, developed by IBM Corporation, USA. One-way ANOVA was used, followed by the Least Significant Difference (LSD) test with significance level at $p < 0.05$.

Results and Discussion

The exposure to UVB radiation is the primary cause of skin pigmentation, leading to an increase in melanin production. This increase is attributed to a combination of intrinsic and extrinsic factors, including UVB radiation. UVB-exposed skin generates an elevated level of reactive oxygen species (ROS), which react with DNA, proteins, and fatty acids, resulting in oxidative damage.[29] Oxidative stress triggers a signaling cascade, inducing activator protein 1 (AP-1) and down-regulating TGF-β, subsequently inhibiting α-SMA expression and collagen synthesis. [30] Excessive ROS also activates the protein kinase pathway, leading to increased matrix metalloproteinase (MMP) production, which degrades collagen.[31] Additionally, increased ROS impacts the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), leading to cell apoptosis. Continuous pro-inflammatory conditions inhibit the production of pro-collagen growth factors, such as TGF-β, suppressing α-SMA production and inhibiting type 1 collagen synthesis.[32] Stink bean peel contains active compounds, including flavonoids, alkaloids, tannins, and saponins, which act as antioxidants and anti-inflammatories, suppressing oxidative stress and preventing melanin production.[33,34] In this study, the extract of stink bean peel was obtained using the maceration method with ethanol as the solvent, resulting in an extract yield of 8.00%. The phytochemical screening of the stink bean peel extract showed the presence of phenolic compounds, tannins, flavonoids, terpenoids, and saponins (Table 1).

Table 1: Phytochemical constituents of Stink bean peel extract

Constituent	Test method	Inference
Alkaloids	Wagner test	-
Flavonoids	Wilstater test	+
Tannins	Braemer's test and Keller-Killiani test	+
Saponins		+

+ presence of component
- abscence of component

The effect of stink bean peel extract on the α-SMA gene expression
The stink bean peel extract was able to significantly increase the expression of α-SMA and type 1 collagen genes in a dose-dependent manner in a hyperpigmentation rat model. Based on the results as shown in Figure 1, the expression of the α-SMA gene was highest in the group that received 20% stink bean peel extract gel (K4) with a mean ratio of 3.11 ± 1.08, followed by the K3 group with a mean expression ratio of α-SMA gene of 2.93 ± 0.85. The lowest α-SMA gene expression was observed in the negative control group (K2) with an expression ratio of 0.67 ± 0.17. The study demonstrated that the gel extract of stink bean peel has a dose-dependent effect on the expression of the α-SMA gene

in a hyperpigmentation rat model.

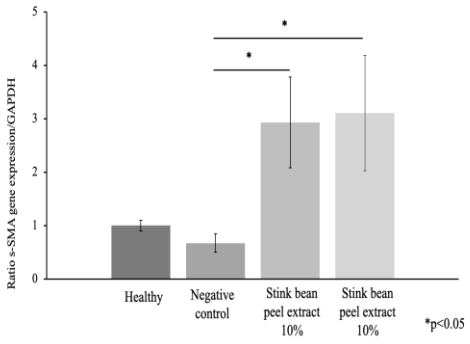

Figure 1: The effect of *Parkia speciosa* extract gel on α-SMA expression in UVB irradiation-induced hyperpigmentation rat model. Data are presented as fold change in gene expression relative to UVB unexposed group. * Indicates significant difference at $p < 0.05$.

This phenomenon indicates that increased *α*-SMA gene expression can prevent hyperpigmentation and increase collagen formation through increased type 1 collagen gene expression, which is an important component of the extracellular matrix. Flavonoid compounds from stink bean peel extract may activate the PI3K pathway through the TGF-*β* pathway involving TGF-*β* receptors I and II. PI3K activation in turn leads to the activation of Akt, which impacts mTOR activation. Activated mTOR induces SMAD2/3 expression, leading to SMAD2/3 activation. Released SMAD2/3 triggers *α*-SMA release, leading to fibroblast transformation into myofibroblasts, ultimately inducing type 1 collagen synthesis.[35] Compounds in stink bean peel extract, such as flavonoids and phenols, also act as metal ion chelators, and stabilize hydrogen atoms from hydroxyl groups, reducing ROS formation and inhibiting photoaging.[10]

The effect of stink bean peel extract on the collagen type 1 gene expression

In this study, it was found that the gel extract of stink bean peel significantly increased the expression of the type 1 collagen gene in a dose-dependent manner in a hyperpigmentation rat model. The mean expression of the type 1 collagen gene was highest in group K3 (2.22 ± 0.68), closely followed by group K4 with a mean expression of type 1 collagen gene of 2.21 ± 0.73. The lowest expression of type 1 collagen gene was observed in the negative control group with a mean expression of 0.52 ± 0.14 (Figure 2). This demonstrates that the stink bean peel extract gel has a dose-dependent effect on the expression of the type 1 collagen gene in a hyperpigmentation rat model.

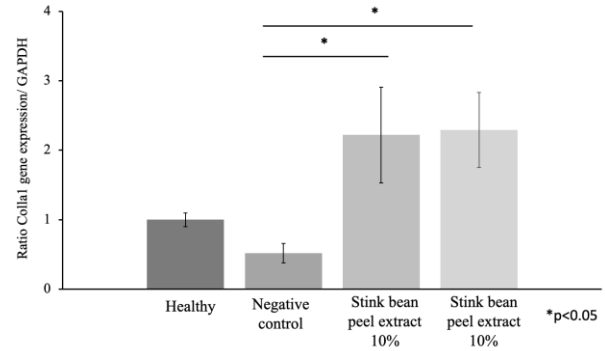

Figure 2: The effect of *Parkia speciosa* extract gel on collagen type 1 expression in UVB irradiation-induced hyperpigmentation rat model. Data are presented as fold change in gene expression relative to UVB unexposed group. * Indicates significant difference at $p < 0.05$.

In this study, the increase in *α*-SMA expression is in line with the increase in type 1 collagen gene expression. Overexpression of ROS can also activate NF-kB, which then translocate into the nucleus to induce transcription activation and MMP-1 transcriptional regulation, the main factor in type 1 collagen degradation. [1,36–38] This is consistent with the results of the current study, which showed that UVB radiation can decrease *α*-SMA gene expression and type 1 collagen gene expression. The increase in collagen expression in UVB-exposed rat skin treated with 10% and 20% stink bean peel extract gel also correlates with the increase in *α*-SMA expression. The results therefore shows that 10% and 20% stink bean peel gel significantly increased the relative expression of the type 1 collagen gene compared to the negative control.

α-SMA is a prototype fibrogenic cytokine that can increase extracellular matrix (ECM) gene expression in activated fibroblast cells and regulate the decrease of matrix-degrading enzymes through the SMAD pathway, especially related to collagen synthesis.[38] To interact with collagen fibers, fibroblasts express surface adhesion receptors that promote connections between extracellular adhesion molecules such as fibronectin and intracellular cytoskeletal proteins. Myofibroblasts are regulated by TGF-*β*1, which binds to the TGF-*β* receptor (TGF-*β*R) complex and stimulates intracellular signaling that drives *α*-SMA production to activate the SMAD 2/3 pathway, impacting type 1 procollagen synthesis and ultimately collagen formation. Stink bean peel in this study played a role in increasing *α*-SMA expression, activating the SMAD and ERK1/2 complexes, leading to fibroblast activation into myofibroblasts and increased type 1 collagen in UVB-exposed skin.[39–41] A limitation of this study was the lack of evaluation of ROS and TGF-*β* levels after stink bean peel extract gel treatment, so the direct molecular mechanism of stink bean peel extract on melanin production is not known.

Conclusion

Overall, the findings from this study have shown that stink bean peel extract increase the expression of *α*-SMA gene, leading to enhanced collagen formation and a reduction in melanin production. This may be attributed to the ability of the extract to activate key pathways involved in collagen synthesis and fibroblast transformation. The findings underscore the potential of stink bean peel extract to be used as a protective and therapeutic agent against UVB-induced skin damage.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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