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Contributed Paper

5α-Reductase Inhibition and Melanogenesis Activity of Sesamin from Sesame Seeds for Hair Cosmetics

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ABSTRACT

Sesamin is the primary compound in sesame seed oil which has been used in ayurvedic treatment for hair loss and canities. Sesamin was isolated, identified and investigated for antioxidative activities (including DPPH radical scavenging, lipid peroxidation inhibition and metal ion chelating activities), 5α-reductase inhibition in DU-145 and melanogenesis activities on B16F10 melanoma. Sesamin at the concentration of 0.001-10 mg/ml showed weak DPPH scavenging activity (the SC_{50} value of 109.90 ± 6.16 mg/ml), but quite high lipid peroxidation inhibition and metal ion chelating activities (the IPC_{50} value of 0.83 ± 0.54 and the CC_{50} value of 1.83 ± 0.90 mg/ml, respectively). At 0.1 mg/ml of sesamin demonstrated high 5α-reductase inhibition activity at 37.38±4.26% of the control, which was higher than the standard finasteride of 1.54 times, but lower than the standard dutasteride of 1.27 times. It gave high melanin and tyrosinase contents of 26.63±3.21 and 39.42±8.30%, respectively, but lower than theophylline of about 1.04 and 1.05 times, respectively. Although sesamin exhibited all investigated activities lower than the standards, the traditional use of sesame seeds containing sesamin for hair cosmetics has been confirmed, which will be beneficial for the further development as anti-hair loss and anti-canities products.

Keywords: sesamin, melanogenesis stimulation, 5α-reductase inhibition, antioxidative activities, hair cosmetics

1. INTRODUCTION

Nowadays, human has longer lifespan because of the increase of medical treatment technologies. Thus, the aged people population of both men and women are increasing. Hair loss and grey hair are the two personality problems that these people are suffering. With aging, the production of free radicals increases, while the endogenous defence mechanisms decrease, thereby causing the imbalance which leads to the progressive damage of cellular structures in

the body including hair. The aging phenotype of hair manifests as the decrease of melanocyte function or greying hair production or alopecia. Hair greying is a progressive loss of pigment from growing hair shafts caused by one or more impairments in melanocyte-keratinocyte functions such as the reduction of tyrosinase activity in the melanocytes. Therefore, melanogenesis stimulation can increase hair darkening [1,2]. For hair loss, dihydrotestosterone (DHT) which is a derivative of testosterone by reaction of the 5α-reductase can cause female pattern hair loss and male pattern baldness. The inhibition of this biologically active 5α-reductase enzyme can decrease hair loss especially in the aged androgenic alopecia patients. The type 1 5α-reductase is related to the genetic male pattern baldness which is expressed predominantly in the skin, scalp, sebaceous gland, liver and brain, while the type 2 5α-reductase is found predominantly in androgen target organs such as prostate, genital skin, and seminal vesicles [3,4].

Because of the benefits of the back to nature trends, natural products from several plants have been used for anti-hair loss and canities treatment. *Asiasari radix* (Aristolochiaceae), *Eclipta alba* (Asteraceae), essential oil of *Chamaecyparis obtuse* (Cupressaceae), *Zizyphus jujube* (Rhamnaceae) and *Sophora flavescens* (Leguminosae) have been investigated for hair growth promotion [5,6]. The extract from human placenta, *Astragalus mongholicus* (Leguminosae), *Lycium barbarum* (Solanaceae), *Angelica sinensis* (Apiaceae) and *Morus alba* (Moraceae) have been used for hair graying [7]. In the ancient ayurvedic Thai and Chinese medicine, it has implied that sesame seed oil could be used for hair treatment in order to nourish dry scalp, protect hair from the damaging effects of sun light and pollution, strengthen hair shafts and roots, darken hair and prevent hair loss [8]. Sesamin is a natural product extracted from sesame (*Sesamum indicum*; Family Pedaliaceae) especially its seeds and oil.

Sesamin is the primary compound in the sesame seeds, which is likely responsible for the increased stability of sesame oil against autooxidation and the development of rancidity caused by free radicals [9]. Sesamin has the protective effects against both hypoxia in rat adrenal pheochromocytoma (PC12) cells and hypoxiα-induced BV-2 microglia cell death through the suppression of reactive oxygen species generation [10,11]. Sesamin and its metabolites induced neuronal differentiation in PC12 cells through the activation of extracellular signal-regulated kinase (ERK1/2) signaling pathway [12]. In our previous study, the chondroprotective and anti-inflammatory effects of sesamin have been demonstrated [13]. However, other biological activities for hair cosmetics including the *in vitro* effects of sesamin on hair melanin promoting and anti-hair loss effect have never been performed.

In this present study, sesamin was investigated for antioxidative activities (including DPPH radical scavenging, lipid peroxidation inhibition and metal ion chelating activities) as well as 5α-reductase inhibition on DU-145 cell line and melanogenesis on B16F10 mouse melanoma for hair loss and canities treatment, respectively.

2. MATERIALS AND METHODS

2.1 Materials

L-(+)-Ascorbic acid, α-tocopherol, 2,2-diphenyl-1-picryhydrazyl radical (DPPH), ethylenediaminetetraacetic acid (EDTA), sulforhodamine B (SRB), dimethyl sulfoxide (DMSO), kojic acid, ammonium thiocyanate, ferrozine and ferric chloride ($FeCl_2$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tyrosinase from mushroom (4276 U/mg) and L-tyrosine were purchased from Fluka (Switzerland). Linoleic acid from Wako Pure Chemical Industrial Ltd. (Osaka, Japan) was used.

2.2 Isolation of Sesamin

Seeds of *Sesamun indicum* Linn. were collected from Lampang Province in Thailand and the voucher specimens (BKF No. 138181) were deposited at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment in Bangkok, Thailand. Sesamin was isolated as previously described [13]. Briefly, the finely powdered seed (460 g) of *S. indicum* was percolated with hexane. The liquid extract was evaporated under the reduced pressure to get the crude hexane extract then fractionated by a silica gel column chromatography. The fractions containing mainly the colorless crystals were re-applied to the silica gel column. The specific subfraction was further purified by crystallization with ethanol to yield the colorless needle crystal. The collected crystal was identified as sesamin (430 mg, 0.11% yield) using a nuclear magnetic resonance spectroscopy and mass a spectrometry in comparing to the standard sesamin (Sigmα-Aldrich®). The chemical structure of sesamin was shown in Figure 1.

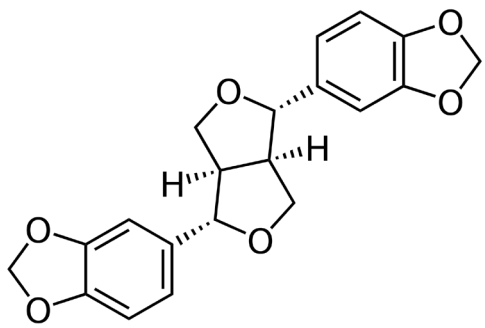


Figure 1. The chemical structure of sesamin.

2.3 Antioxidative Activity of Sesamin

2.3.1 2,2-Diphenyl-1-picryhydrazyl radical (DPPH) radical scavenging assay

Free radical scavenging activities of sesamin, standard antioxidants (ascorbic acid) was determined by a modified DPPH assay [14]. Briefly, 50 µl of five serial concentrations of sesamin at the concentration of 0.001-10 mg/ml dissolved in DMSO and 50 µl of ethanol solution of DPPH were put into each well of a 96-well microplate (Nalge Nunc International, NY, USA). The reaction mixtures were allowed to stand for 30 min at 27±2°C and the absorbance was measured at 515 nm by a well reader (Bio-Rad, model 680 microplate reader, USA) against the blank (DMSO). Ascorbic acid (0.001–10 mg/ml) was used as a positive control. The experiments were done in triplicate. The percentages of radical scavenging activity were calculated as follows: scavenging (%) = $[(A-B)/A]\times 100$, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% of scavenging (SC_{50}) were calculated from the graph plotted between the percentages of scavenging and the sample concentrations.

2.3.2 Lipid peroxidation inhibition activity

The antioxidative activity of sesamin was assayed by the modified Ferric-thiocyanate method [15]. An amount of 50 µl of five serial concentrations of sesamin at 0.001-10 mg/ml dissolved in DMSO was added to 50 µl of linoleic acid in 50% (v/v) DMSO. The reaction was initiated by the addition of 50 µl of NH_4SCN (5mM) and 50 µl of $FeCl_2$ (2mM). The mixture was incubated at 37±2°C in a 96-well microplate for 1 h. During the oxidation of linoleic acid, peroxides were formed leading to the oxidation of Fe^{2+} to Fe^{3+}. The latter ions forming a complex with thiocyanate can be detected at 490 nm. The solution without the sample was used as a negative control. α-Tocopherol (0.001-10 mg/ml) was used as a positive control. All determinations were performed in triplicate. The inhibition percentages of lipid peroxidation of linoleic acid were calculated by the following equation: Inhibition of lipid peroxidation (%) = $[(A-B)/A]\times 100$, where A was the absorbance of the control and B was the absorbance of the sample. The sample

concentrations providing 50% inhibition of lipid peroxidation (IPC_{50}) were calculated from the graph plotted between the percentages of lipid peroxidation inhibition and the sample concentrations.

2.3.3 Metal ion chelating assay

The metal ion chelating activity of sesamin was assayed by the modified ferrous ion chelating method [16]. Briefly, 100 µl of five serial concentrations of sesamin at 0.001-10 mg/ml dissolved in DMSO were added to the solution of 2mM $FeCl_2$ (50 µl) in distilled water. The reaction was initiated by the addition of 5mM ferrozine (50 µl) and the total volume was adjusted to 300 µl by distilled water. Then, the mixture was left at 27±2°C for 15 min. Absorbance of the resulting solution was then measured at 570 nm by a microplate reader. EDTA (0.001-10 mg/ml) was used as a positive control. The negative control contains $FeCl_2$ and ferrozine, which were complex formation molecules. All experiments were performed in triplicate. The inhibition percentages of ferrozine–Fe^{2+} complex formation were calculated by the following equation: Metal chelating activity (%) = $[(A-B)/A]\times100$, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% metal chelating activity (CC_{50}) were calculated from the graph plotted between the percentages of metal chelating activity and the sample concentrations.

2.4 5α-Reductase Inhibition

2.4.1 Cytotoxicity of sesamin on DU-145 cell line

2.4.1.1 Cell culture

The human prostate carcinoma cell line (DU-145) was provided by Prof. Dr. Toshihiro Akihisa at the College of Science and Technology, Nihon University in Tokyo, Japan. Cells were cultured under the standard conditions.

2.4.1.2 Cytotoxicity by the sulforhodamine B (SRB) assay

The sesamin was tested for cytotoxicity on DU-145 cells by the SRB assay as previous described [17]. The standard finasteride and dutasteride at 0.001-10 mg/ml were used as positive controls. The cells were plated at the density of 1.0×10^4 cells/well in 96-well plates and left overnight for cell attachment on the plate in 5% CO_2 at 37°C. Cells were then exposed to five serial concentrations of sesamin (0.001-10 mg/ml) for 24 h. After incubation, the adherent cells were fixed *in situ*, washed and dyed with SRB. The bound dye was solubilized and the absorbance was measured at 540 nm by a well reader. The experiments were done in triplicate. The percentages of cell proliferation were calculated according to the following equation: Cell viability (%) = $(Absorbance_{sample}/Absorbance_{control}) \times 100$. The concentrations of the samples which gave % cell viability of more than 90% were used in the 5α-reductase inhibition experiment.

2.4.2 Inhibition of 5α-reductase

2.4.2.1 Cell culture

The pellets of human DU-145 cells were plated onto the 6-well plates separately at the density of 2.5×10^5 cells/well, incubated with 10% (v/v) FBS-DMEM medium containing penicillin (100 U/ml) and streptomycin (100 mg/ml) in a 5% CO_2 incubator (Shel Lab, model 2123TC, USA) at 37°C. Cells were then exposed to sesamin, the standard finasteride and dutasteride at the final concentration of 0.1 mg/ml for 24 h. The medium were removed, and the cells were washed with PBS, trypsinized with 0.25% trypsin solution for 2 min and suspended in PBS.

2.4.2.2 Total RNA extraction

The total RNA from the cell pellets was extracted by the RNA extraction kit (NucleoSpin®, Macherey-Nagel, CA, USA) according to

the instructions of the manufacturer. The concentration of the total RNA was quantified by the Qubit Fluorometer and the Quant-iT™ RNA BR assay kit (Invitrogen, CA, USA). The total RNA solution was kept at -20°C until use.

2.4.2.3 Reverse transcription-polymerase chain reaction (RT-PCR)

The 5α-reductase type 1 and 2 genes were amplified from the extracted RNA by SuperScrip™ One-Step RT-PCR with Platinum® Taq kit (Invitrogen, CA, USA) according to the manufacturer's protocol. Briefly, five micrograms of the total RNAs were reverse transcribed with RT/ Platinum Taq® mix and subjected to PCR cycles with the primers for human 5α-reductase type 1 and 2 (SRD5A1 and 2) as follows: 94ºC for 15 s, 55ºC for 30 s, 72ºC for 45 sec for 35 cycles. The human 5α-reductase type 1 primers were designed based on GenBank accession no. NM_001047.2 and NM_000348, respectively, with a forward (5'- CCA TGT TCC TCG TCC ACT AC-3') and reverse (5'- TTC AAC CTC CAT TTC AGC GT -3'), produced 707 bp amplicon and human 5α-reductase type 2 (SRD5A2) forward (5'- GGG TGG TAC ACA GAC ATA CG-3') and reverse (5'- TCA CGA CTA TGA GGA GAG GG-3'), produced 938 bp amplicon [18]. The RT-PCR products were loaded on 1% agarose gel in the 1X tris-acetate-EDTA (TAE) buffer chamber at 100 V for 30 min. The human 5α-reductase type 1 and 2 dsDNA samples were quantified by Qubit fluorometer and the Quant-iT™ dsDNA assay kit (Invitrogen, CA, U.S.A.).

2.5 Induction of Melanin Production on B16F10 Murine Melanoma

2.5.1 Cell culture

B16F10 murine melanoma cells, purchased from the American Type Culture Collection (ATCC, VA, USA), were cultured in a 75-cm^2 flask (Nunc, Denmark) in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin antibiotic at 37°C in a humidified incubator with 5% CO_2. Cells were grown to semiconfluence and harvested by treating with 0.25%, w/v trypsin and 0.06 mM EDTA in phosphate buffer saline solution. Cells were resuspended in complete DMEM and counted in a hemacytometer for the following experiments. All experiments were performed individually triplicate.

2.5.2 Melanin content

The melanin content was measured as previously described (Oka et al., 1996) with some modifications. Briefly, cells at the density of 1.0×10^5 cells/well were plated in 6-well plates and incubated overnight for cell adhesion. The samples were then added and incubated for 72 h. The cells were then washed with phosphate-buffer saline (PBS) and dissolved in 500 μl of 2 N NaOH at 60°C for 1 h. The absorbance was measured at 450 nm using a microplate reader and the melanin amount was determined in comparing to the standard melanin, which demonstrated linear with high correlation ($r^2 = 0.9993$). The following regression equation was obtained: $y = 2.6875x + 0.0005$, where y was the absorbance at 450 nm and x was the quantity of melanin (μg/ml). For total protein content, cells were washed with PBS, then lysis buffer (300 μl) containing 5% mercaptoethanol was added and centrifuged at 15,000 g for 10 min at 4°C. Supernatants were transferred to new tube and Bradford reagent was added. Bovine serum albumin was used as a standard, which demonstrated linear with high correlation ($r^2 = 0.9987$). The following regression equation was obtained: $y = 0.001x + 0.0163$, where y was the absorbance at 595 nm and x was the quantity of protein (μg/ml). For the determination of the actual melanin formation from the same cell numbers, the melanin content of each treatment was divided by the total protein content. The percentages of the relative ratio of melanin content were

calculated as the following: *% relative ratio of the melanin content = (Mt/Mc) × 100*, where Mt was the melanin content of the sample divided by the total protein content of the sample and Mc was the melanin content of the control divided by the total protein content of the control.

2.5.3 Tyrosinase activity

Tyrosinase activity was analyzed by the method described previously [19] with some modifications. Briefly, the cells treated with sesamin were washed with ice-cold PBS and then lysed by incubation at 4°C for 30 min in the lysis reagent containing protease inhibitors (Roche, Germany). The lysates were centrifuged at 15,000 rpm for 10 min. The obtained supernatants were collected, mixed with the mixture containing 50 mM phosphate buffer (pH 6.8) and 0.05% *l*-dopa, and then incubated at 37°C for 2 h. After incubation, the dopachrome formation was measured at 450 nm using a microplate reader. Tyrosinase contents were determined from the standard curve of the standard mushroom tyrosinase, which demonstrated linear with high correlation ($r^2 = 0.9948$). The following regression equation was obtained: $y = 0.003x + 0.0071$, where y was the absorbance at 450 nm and x was the quantity of tyrosinase (μg/ml). Enzyme activity of the sample was compared to the control and calculated as the percentages of the relative ratio of the tyrosinase activity as the following equation: *% relative ratio of tyrosinase activity = (Tt/Tc) × 100*, where Tt was the tyrosinase content of the sample divided by the total protein content of the sample and Tc was the tyrosinase content of the control divided by the total protein content of the control.

2.6 Statistical Analysis

Data were expressed as the mean ± S.E. of three independent experiments. The Student paired *t*-test and Kruskall-Wallis test were used to evaluate the significance of differences at the significant level of p-value < 0.05. Statistical analysis was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Antioxidative Activities of Sesamin

The antioxidative activities of sesamin including DPPH radical scavenging, lipid peroxidation inhibition and metal ion chelating assays were shown in Table 1. Sesamin indicated weak scavenging activity against DPPH with the SC_{50} value of 109.90 ± 6.16 mg/ml which was lower than the standard ascorbic acid (SC_{50} value of 0.07 ± 0.01 mg/ml) of about 1500 times. For lipid peroxidation inhibition and metal ion chelating activities, sesamin exhibited quite high activities with the IPC_{50} value of 0.83 ± 0.54 and the CC_{50} value of 1.83 ± 0.90 mg/ml, respectively, but lower than the standard α-tocopherol and EDTA which gave the IPC_{50} value of 0.09 ± 0.01 and the CC_{50} value of 0.79 ± 0.01 mg/ml, respectively. As known, flavonoids such as catechin, epicatechin, quercetin, kaemferol, and cyanidin generally have antioxidative effects from their catechol or dihydroxyphenyl moieties which are necessary for the high radical scavenging activity [20]. The lower antioxidative activity of sesamin than the standards may be due to the absence of the hydroxyphenyl moieties. However, when in body, sesamin could be metabolized by demethylation of the methylenedioxyphenyl moieties to biologically active compounds containing the hydroxyphenyl moieties [21]. The strong antioxidative activities of these metabolites in comparing to sesamin owing to the radical scavenging activities toward superoxide anion radical in mono- and the di-catechol metabolite have been reported. Moreover, di-catechol metabolite can scavenge not only the superoxide anion radicals, but also the hydroxyl radicals [22]. Thus, sesamin itself has weak antioxidative properties, but can convert to the prominently antioxidative metabolites

Table 1. Antioxidative activities of sesamin (DPPH radical scavenging, lipid peroxidation inhibition and metal ion chelating assays).

Sample	Antioxidative activities		
	SC_{50} (mg/ml)	IPC_{50} (mg/ml)	CC_{50} (mg/ml)
Sesamin	109.90 ± 6.16	0.83 ± 0.54	1.83 ± 0.90
Standards			
ascorbic acid	0.07 ± 0.01*	NA	NA
α-tocopherol	NA	0.009 ± 0.010*	NA
EDTA	NA	NA	0.79 ± 0.01*

Note: NA = not appreciable. SC_{50} value (mg/ml) was the concentration of the sample that scavenged 50% of the DPPH radicals. IPC_{50} value (mg/ml) was the concentration of the sample that inhibited 50% of the lipid peroxidation and CC_{50} value (mg/ml) was the concentration of the sample that chelated 50% of the metal ion. Each value expressed as mean ± SD. Student paired t-test test was used to calculate the significant differences (n=3). *$p < 0.05$ compared to each positive control.

Table 2. Cytotoxicity of sesamin at 0.001-10 mg/ml on DU-145 and B16F10 cells.

Sesamin concentration (mg/ml)	% Cell viability	
	DU-145	B16F10
0.001	128.01±22.23	106.46±9.64
0.01	111.67±17.87	105.03±8.15
0.1	102.66±8.05	105.08±6.10
1	78.01±5.11	74.23±3.26
10	75.88±27.67	69.21±4.17

Note: Each value expressed as mean ± SD. Student paired t-test test was used to calculate the significant differences (n=3).

especially in the liver when administered into the body. These antioxidative metabolites of sesamin may be also responsible for the protective effects and the synergistic actions for anti-hair loss and anti-canities.

3.2 Effects on 5α-reductase Activity

3.2.1 Cytotoxicity assay of sesamin in DU-145

Table 2 showed the effects of sesamin on viability of DU-145. At higher concentrations, sesamin gave a slight decrease of %cell viability. When treated with sesamin at 1 and 10 mg/ml, the cell viabilities were 78.01±5.11 and 75.88±27.67%, respectively. However, sesamin at 0.1 mg/ml indicated no cytotoxicity on the DU-145 with the cell viability of 102.66±8.05. This sesamin concentration was selected for the 5α-reductase inhibition experiment.

3.2.2 The 5α-reductase inhibition assay

The human homogenates of BPH tissue can also be used for investigating the 5α-reductase inhibition activity. But, to obtain the human homogenates of BPH tissue was

too difficult and complicate which may have several interferences of the assay from other components in the tissue. Also, to use the BPH tissue needs human ethics to get the tissue from the patients in the hospital. Thus, the type 2 enzyme extract processes from the tissue have more troublesome than using the DU-145 cell line as the source of the enzyme. The type1 5α-reductase, the main enzyme causing hair loss, predominated in the human scalp skin especially in the dermal papilla [23]. DU-145 human androgen insensitive prostate adenocarcinoma cell line has been used for the 5α-reductase type 1 inhibition assay in several studies [24,25]. In fact, many studies have reported on the biological activities of sesamin [10,11]. However, there was no report on the effects of sesamin on 5α-reductase inhibition in DU-145. Sesamin gave the 5α-reductase type 1 inhibition activity on DU-145 cells at 37.38±4.26% of the control, which was higher than the standard finasteride, a 5α-reductase type 2 inhibitor (24.20±5.02%) of 1.54 times but, lower than the standard dutasteride, a 5α-reductase type 1 and 2 inhibitor (47.49±4.11%) of 1.27 times (Figure 2). The 5α-reductase inhibition activity of sesamin appeared to be related to its antioxidant activities. As reported, the inadequate antioxidant protection or excess production of reactive oxygen species creates a condition known as oxidative stress, which can cause hair loss [26]. Also, lipid peroxidation can be caused by free radicals which can initiate the free radical chain reaction on the cell membrane in hair dermal papilla. Thus, sesamin may inhibit free radicals which initiated lipid peroxidation reaction in the cell membrane involved hair dermal papilla, resulting in cell damage and hair shedding [27]. Moreover, the metal ion chelating activity may reduce the excess Cu level in the occipital area and also in the blood circulation thereby being able to control the balance of hair growth and hair loss in the androgenic alopecia patients [28].

3.3 Effect on Melanin Induction

3.3.1 Cytotoxicity assay in B16F10 mouse melanoma

The cytotoxicity assay of sesamin was performed in B16F10 mouse melanoma in order

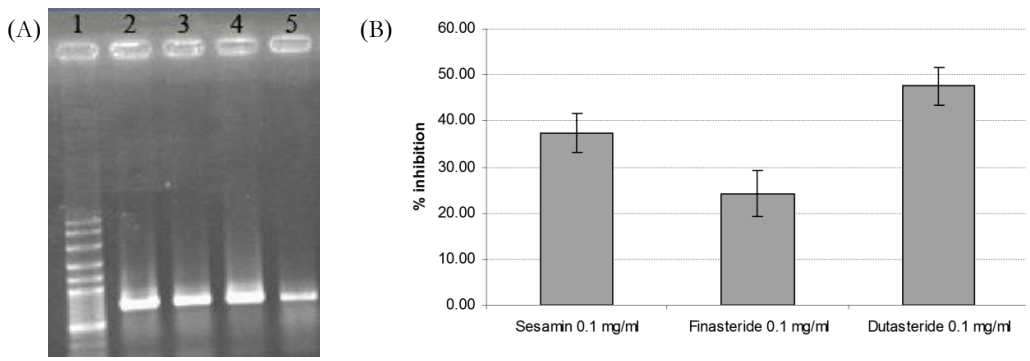


Figure 2. The 5α-reductase inhibition on DU-145 cells at 0.1 mg/ml of sesamin (3) in comparing to the standards: finasteride (4) and dutasteride (5), DNA ladder 100 bp (1) and the control 5α-reductase DNA (2). (A) Agarose gel electrophoresis of ds DNA of 5α-reductase enzyme and (B) the percentages of 5α-reductase inhibition; %Inhibition = [(control − sample)/ control] × 100. Each value expressed as mean ± SD. Kruskall-Wallis test was used to calculate the significant differences. $^{*}p < 0.05$ compared to each positive control.

to evaluate the appropriate concentration for the melanogenesis assay. Table 2 demonstrated the cytotoxic effects on B16F10 of sesamin at 0.001-10 mg/ml. At 10 and 1 mg/ml, sesamin exhibited cytotoxic effect with the cell viability of 69.21±4.17 and 74.23±3.26 %, respectively, whereas lower concentrations (0.001-0.1 mg/ml) demonstrated no cytotoxic effect. These concentrations of sesamin were selected for the melanin content assays.

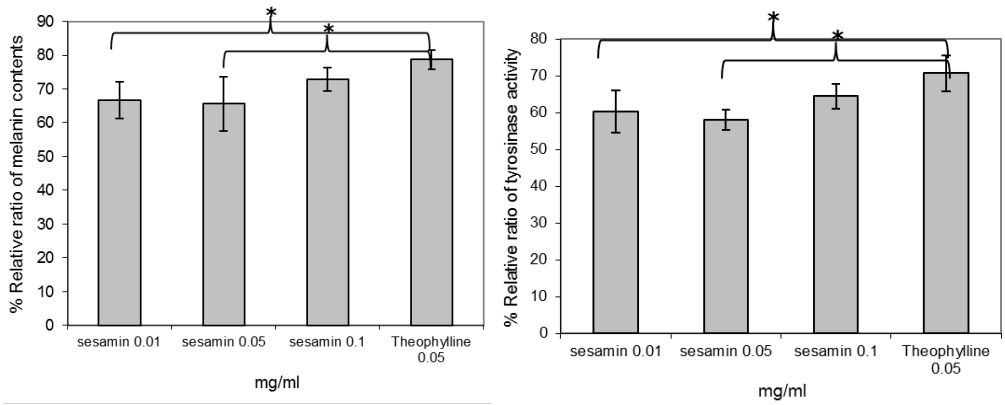
3.3.2 Induction of melanin content and tyrosinase activity

Melanin content and tyrosinase activity of sesamin at 0.01-0.1 mg/ml were investigated in comparing to the positive control (theophylline at 0.05 mg/ml) in B16F10 melanoma. Theophylline significantly demonstrated the highest melanin stimulation and tyrosinase contents of 27.76±8.84 and 41.45±9.98%, respectively. It has been reported to mediate the pigment biosynthesis through the cAMP pathway as well as increases the gammα-glutamyl transpeptidase- and tyrosinase-reactive cells, resulting in the induction of melanin production with the increased level of tyrosinase activity [29,30]. The induction of melanin and tyrosinase contents of sesamin at 0.01-0.1 mg/ml was shown in Table 3. Sesamin at 0.1 mg/ml indicated high melanin and tyrosinase contents of 26.63±3.21 and 39.42±8.30%, respectively, which were lower than theophylline of only 1.04 and 1.05 times. The melanin and tyrosinase contents were corrected as the percentages of relative ratio of melanin content and tyrosinase activity as shown in Figure 3. Sesamin at 0.1 mg/ml indicated the percentages of relative ratio of melanin content and tyrosinase activity of 72.93±3.49 and 64.45±3.44%, respectively which were lower than theophylline of only 1.08 and 1.10 times. Sesamin may be a potent melanogenic stimulator that could induce the melanin synthesis and dendritogenesis by the cAMP- protein kinase A (PKA) signaling pathway in B16 melanoma cells [31]. The dendric morphology of the melanoma cells treated with sesamin may become more pronounced when compared with the untreated cells, suggesting the induction of melanoma cell differentiation [31]. Melanin content and tyrosinase activity were increased with increased sesamin concentrations which no significant different when compare the melanin content and tyrosinase activity at 0.1 mg/ml sesamin with 0.05 mg/ml standard Theophylline (p>0.05). These changes were also accompanied by an

Table 3. The induction of melanin, tyrosinase and total protein contents of sesamin at various concentrations.

Sesamin concentration (mg/ml)	Melanin content (µg/ml)	Tyrosinase content (µg/ml)	Total protein content (µg/ml)
0 (control)	17.65±5.76**	29.14±3.44**	1044.23±48.55**
0.01	18.91±5.50*	31.49±8.56*	1630.25±45.33*
0.05	21.34±7.34*	31.66±11.24*	1955.56±11.78*
0.1	26.63±3.21	39.42±8.30	2177.39±66.50
Standard Theophylline			
0.05	27.76±8.84	41.45±9.98	2091.55±20.23

Note: Each value expressed as mean ± SD. Kruskall-Wallis test was used to calculate the significant differences. *p < 0.05 and **p < 0.01 compared to theophylline.



A **B**

Figure 3. The % relative ratio of melanin contents (A) and tyrosinase activity (B) of B16 F10 melanoma treated with sesamin at various concentrations. Each value expressed as mean ± SD. Kruskall-Wallis test was used to calculate the significant differences. *$p < 0.05$ compared to theophylline.

increase in the mRNA and protein levels of tyrosinase expression. The mechanisms of the sesamin-induced melanogenesis might attribute to the tyrosinase activation upon the signaling pathway regulating tyrosinase levels [31]. In our previous study, the standard sesamin at 1 mg/ml slightly increased the melanin content and tyrosinase activity of 1.03 and 1.10 folds of the control, respectively, while the activity on tyrosinase-related protein-2 (TRP-2) were not changed [32].

4. Conclusion

Sesame seeds, especially its oils have been traditionally used for hair treatment. Sesamin were isolated and investigated for antioxidative activities, 5α-reductase inhibition in DU-145 as well as the induction of melanin content and tyrosinase activity in B16F10 melanoma. Sesamin showed weak scavenging activity against DPPH which gave the SC_{50} value of 109.90 ± 6.16 mg/ml, but the quite high lipid peroxidation inhibition and metal ion chelating activities with the IPC_{50} value of 0.83 ± 0.54 and the CC_{50} value of 1.83 ± 0.90 mg/ml, respectively. Sesamin demonstrated high 5α-reductase inhibition activity at 37.38±4.26% of the control, which was significant higher than the standard finasteride of 1.54 times ($p<0.05$). The 5α-reductase inhibition activity of sesamin was related to its antioxidant activities especially the lipid peroxidation inhibition and metal ion chelating activities. Sesamin, at 0.1 mg/ml, gave high melanin and tyrosinase contents of 26.63±3.21 and 39.42±8.30%, respectively which gave no significant difference when compared with 0.05 mg/ml of the standard, theophylline. This study has confirmed the traditionally use of sesame seed for hair loss and hair grey treatment. It has also demonstrated the application potential of sesamin extracted from the sesame seeds for genetically hair loss and canities treatments. For the further possible study, the *in vivo* melanin induction should be investigated. In this case, because of the low aqueous solubility of sesamin, the development of sesamin in a proper form such as by entrapping in nanovesicular delivery systems should be performed before incorporating in a topical formulation. This will not only enhance the aqueous solubility of sesamin, but also increase the transfollicular of sesamin *in vivo* as well.

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