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# In Vitro Anti-melanogenesis and Collagen Biosynthesis Stimulating Activities of Star Grass (*Hypoxis aurea* Lour.) Extracts

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**ABSTRACT—** ***Star Grass (SG) or Hypoxis aurea Lour. is the Thai traditional plant from genus Hypoxidaceae, which has been used to treat acnes, dark spots and blemish. However, there is no scientific report for bioactivity of SG especially the bioactivity claiming for cosmeceutical uses. This present study was investigated the anti-melanogenesis and collagen biosynthesis stimulating of the SG extracts, in order to evaluate the possible use as a whitening or anti-aging agent for cosmeceuticals. It was found that the phytochemicals detected in these extracts were glycosides (deoxysugars) and tannins. The leave extract from Star grass prepared by the maceration with 95%(v/v) ethanol at ambient temperature (SGLE) demonstrated the highest free radical scavenging activity by DPPH assay, tyrosinase inhibition activity by modified dopachrome method and anti-melanogenesis on B16F10 cells which was comparable to vitamin C (p< 0.05) ; and stimulation of collagen biosynthesis on human skin fibroblasts, which was superior than vitamin C (p< 0.05). This study has suggested that the leaves from Star grass extracted by 95%(v/v) ethanol (SGLE) can be applied as whitening and anti-aging agents for cosmeceuticals.***

**Keywords—** Anti-melanogenesis, Collagen biosynthesis, Star Grass, *Hypoxis aurea* Lour.

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## 1.INTRODUCTION

The Star grass or *Hypoxis aurea* Lour. is the plant from genus Hypoxidaceae (**Figure 1**), which is widely distributed in Torrid Zone in the world especially China, Japan and Southeast of Asia countries including Thailand. For the traditional medicine evidences, Star Grass (SG) has been used to treat hernia and warm kidney in China [1], while it can be used for treatment of acnes, dark spots and blemish in Thailand. It has been previously reported that the SG extracted by ethanol contains quercetin-3-O-β-D-glucoside, kaemferol-3-O-β-D-glucoside, apigenin-5-O-β-D-glucopyranoside, a-spinasterol, 2,6-dimethoxy-benzoic acid, 1H-indole-3-carboxylic acid, (2S, 3R, 4E, 8E)-1-(β-D-ghicopyranosyloxy)-3-hydroxy-2 [((R)-2'-hydroxyeicosanoly)amino]-9-methy-4,8-octadeeadiene, n-dotriacontanol, 14,15-eicosenic acid, lignoceric acid, β-sitosterol, daucosterol, aureaside A, aureaside B, curculigoside I, orcinol glucoside, curcapital, cimifugin prim-O-β-D-glucopyranoside, 2-O-β-D-apiofuranosyl (1→6)-O-β-D-glucopyranoside, (2R,5S)-bornane-2,5-diol-2-O-β-D-glucopyranoside, and bornyl-7-O-β-D-apio-D-furanosyl(1→6)-O-β-D-glucopyranoside [1, 2]. However, there is no scientific report for investigation of bioactivities especially for anti-melanogenesis and collagen biosynthesis stimulating activities that use to evaluate for cosmeceuticals. Free radical scavenging activity and tyrosinase inhibition activity, is used to prevent aging by oxidants such as reactive oxygen species (ROS), and to inhibition melanin production, respectively. These two activities have been widely used to evaluate the biological functions of anti-aging and whitening agents in cosmeceuticals [3].

In melanogenesis, tyrosinase is an enzyme-copper containing and catalyzes the two reactions, hydroxylation and oxidation, which can change L-tyrosine to L-dopa and L-dopa to o-dopaquinone-H+, and then pass the intermediates finally to melanin [4]. In addition, ROS play a significant role in the regulation of the melanogenesis. Melanin is the polyphenolic pigment, which is responsible for eye, hair and skin in animals [5]. Melanin plays the important role in protection the skin from ultraviolet (UV) radiation damages and removing (ROS). However, the high melanin

accumulation and overproduction can cause the skin problems such as dark spots, melasma, freckles and several hyperpigmentation syndromes, which are the sign of aging [6]. Vitamin C and L-glutathione are a tyrosinase inhibitor and ROS scavenger, which can down-regulate melanogenesis [7]. As well, several a tyrosinase inhibitors and ROS scavengers from natural products can inhibit melanogenesis such as Artocarpus lakoocha heartwood extract which is the anti-tyrosinase and improve skin whitening [8].

**Figure 1** Star Grass or *Hypoxis aurea* Lour.

Collagen plays a role in maintaining skin structure, and Type I and type III collagen are about 70% of the weight of the skin. During ageing with age more 20 years, fibroblasts cannot produce collagen properly leading to decrease the collagen level, and the collagen fibers begin to cross-link resulting to lose skin elasticity [9]. Thus, synthesis of collagen fibers from fibroblasts is essential for healthy and firm skin. Vitamin C or vitamin C has been widely used to as an active ingredient in cosmetics for stimulation the proliferation of human fibroblasts and collagen synthesis. However, it is unstable and links with accelerated cell death. The natural extracts from several plants have been developed to be an anti-aging agent such as *Ampelopsis brevipedunculata* extract showed collagen stimulation on human dermal fibroblasts with high superoxide as an anti-oxidant [10]. This present study has investigated the in vitro anti-melanogenesis on murine melanoma cells and collagen biosynthesis stimulating on human skin fibroblasts of Star Grass extracts, in order to evaluate the possible use as a whitening and anti-aging agent for cosmeceutical application.

## 2.MATERIALS AND METHODS

### *2.1 Plant authentication and extraction*

*Authentication*: The leaves and tubers of Star Grass were collected from Kanchanaburi, Thailand during October to November, 2012. The specimen was authenticated by Mr. Tanongsak Jonganurak, a botanist at Forest Herbarium-BKF, Department of National Parks, Wildlife and Plant Conservation, Thailand. The voucher specimens were kept at Thai Traditional Medicine College (TMC), Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand. Parts of the plant were collected, washed, and cut with a sharp knife or machete. The plant materials were dried at 50°C in hot air oven and ground into powder using a stainless steel grinder

*Extraction*: 10 g of leave and tuberous powders of Star Grass (SGL and SGT) were extracted by a) maceration with 100 ml of distilled water at room temperature (25 ±2°C) for 24 hr (SGLW and SGTW); b) maceration with 100 ml of 95%(v/v) ethanol at room temperature for 24 hr (SGLE and SGTE); and c) boiling with 100 ml of distilled water for 2 hr (SGLWH and SGTWH). Then, the extracts were filtered through Whatman no.1 filter paper connected with a vacuum pump. The filtrates were collected, pooled and dried by a rotary evaporator and lyophilized by a freeze dryer . The lyophilized extract was kept in glass bottles and stored at 4°C until use. The percentage yields were calculated on the dry weight basis.

### *2.2 Phytochemical analysis and total phenolic compounds by Folin-Ciocalteu assay*

Phytochemicals contents in the Star Grass extracts such as alkaloids, saponins, flavonoids, anthocyanins, glycosides and tannins, were investigated. Phytochemical tests of the SG extracts were assayed as previously described [11]. The total phenolic compounds contents in the SG extracts were determined by Folin-Ciocalteu assay with slightly modification [12]. Briefly, 50 µl of the samples at 1 mg/ml and 75 µl of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) (0.5 mg/ml in ethanol) were put into each well of a 96-well microplate. Then 75 µl of 7.5% sodium carbonate solution was added into each well. The absorbances were measured by a microplate reader (Z-TEX 340r, Austria) at 725 nm after 90 min of the reaction at 25ºC. The distilled water was used as control group. The amounts of phenolic contents in the extracts were calculated from the standard curve of gallic acid (Sigma-Aldrich, USA) at various concentrations. The results were expressed as gGAE/100g extract.

### *2.3 Free radical scavenging activity by DPPH assay*

Free radical scavenging activity of the SG extracts was determined by DPPH assay as previously described [11]. Briefly, 50 µl of the extracts at the various concentrations and 50 µl of DPPH (Sigma-Aldrich, USA) solution (0.5 mg/ml in ethanol) were put into each well of a 96-well microplate. The absorbances were measured by a microplate reader at 515 nm after 30 min of the reaction at 25ºC. Vitamin C (Sigma-Aldrich, USA) was used as a standard. The percentages of the DPPH radical scavenging activity were calculated as the following:

$$\% \text{ Scavenging} = [(A0 - A1)/A0] \times 100$$

Where, A0 was the absorbance of the control and A1 was the absorbance of the treated sample. The concentrations providing 50% scavenging ($SC_{50}$) were calculated from the graph plotted between the free radical scavenging percentages and the sample concentrations.

### *2.4 Tyrosinase inhibition activity by the modified dopachrome method*

Tyrosinase inhibition activity was assayed by the modified dopachrome method using tyrosine as a substrate as previously described [3]. Briefly, 50 µl of the samples at various concentrations, 50 µl of 0.1 mg/ml L-tyrosine (Sigma-Aldrich, USA), 50 µl of 0.1 mg/ml mushroom tyrosinase (Sigma-Aldrich, USA), and 50 µl of 0.1mM phosphate buffer were added in 96-well microplates. Vitamin C was used as a standard. The mixture was incubated at 37°C for 60 min. Before and after incubations, the absorbances were measured at 450 nm by a microplate reader. The percentages of tyrosinase inhibition were calculated according to the following equation:

$$\% \text{ Inhibition activity} = [(A-B) - (C-D)] / (A-B) \times 100$$

Where, A was the absorbance of the blank after incubation, B was the absorbance of the blank before incubation, C was the absorbance of the samples after incubation, and D was the absorbance of the samples before incubation. The concentrations providing 50% inhibition (IC50) was calculated from the graph plotted between % inhibition activity and the concentrations.

### *2.5 Cell cultures*

The $4^{th}$ passaged of murine melanoma ($B_{16}F_{10}$) cells and human skin fibroblasts were obtained from American Type Culture Collection (ATCC), Virginia, USA. The cells were cultured under the standard conditions in the DMEM (Sigma-Aldrich Biotechnology, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich Corporation, St. Louis, MO, USA), 100 IU/ml of penicillin and 100 mg/ml of streptomycin (Gibco BRL, Gaithersburg, USA). The cells were incubated at 37°C under 5% $CO_2$ for 24 hr.

### *2.6 In vitro biological activities of the selected Star Grass extracts*

#### *2.6.1 Cytotoxicity test by MTT assays*

The selected Star Grass extracts at various concentrations (0.001-10 mg/ml) were tested for cytotoxicity on $B_{16}F_{10}$ cells and human skin fibroblasts by the MTT assay as previous described [13], to evaluate for the appropriate concentration that gave more than 90% cell viability on the cells, in order to use for anti-melanogenesis and collagen biosynthesis assays. Briefly, amount of $1 \times 10^4$ cells of cells were seeded into each well of 96-well plates, adjusted to 180 µl with DMEM, and incubated at 37°C under 5% $CO_2$ atmosphere for 24 hr. Then, the cells were treated with 20 µl of the samples and incubated at 37°C under 5% $CO_2$ atmosphere for 24 hr. After incubation, the medium was removed and the cells were washed with phosphate buffer saline for 3 times. Then, 200 µl of 0.5 mg/ml MTT solution was added into each well and further incubated for 3 hr. After incubation, the MTT solution was removed and 100 µl of dimethyl sulfoxide were added to dissolve the blue-violet crytals. The plates were shaken at 200 rpm for 15 min and measured the absorbance at 570 nm by a microplate reader. The percentage of cell viability was calculated by comparison to 100% viability of untreated cells.

#### *2.6.2 Anti-melanogenesis activity on murine melanoma ($B_{16}F_{10}$) cell line*

The melanin content was measured according to the previously described method with some slight modifications [14]. Briefly, the cells at the density of $10^5$ cells/well were plated in 6-well plates and incubated overnight. The extracts at the proper concentration were then added and incubated for 24 hr. Vitamin C was used as a positive control. The cells were then washed with 1X PBS, dissolved in 500 µl of 10% NaOH, and incubated at 60°C for 1 h. The absorbance was measured at 450 nm using a microplate reader and the melanin amount was determined in compared to the standard melanin. The percentages of the melanin content were calculated as the following:

$$\% \text{ Melanin content} = (Mt/Mc) \times 100$$

Where, Mt was the melanin content of the treated samples, and Mc was the melanin content of the control.

#### *2.6.3 Collagen biosynthesis stimulation on human skin fibroblasts*

The collagen content was measured according to the previously described method with some slight modifications [15]. Briefly, 1 x $10^5$ of human skin fibroblasts were seeded into 6-well plates with the final volume of 1.8 ml, and incubated in $CO_2$ incubator at 37°C for 24 hr. Then, 200 µl of the extract at the proper concentration was added and incubated for 24 hr. The standard whitening agent, vitamin C was used as a positive control. Then, 1 ml of 0.1%(w/v) Sirius red solution in saturated picric acid was added in the plate, and incubated at room temperature for 1 hr. The dye was removed, and the plate was washed with 1 ml of 10 mM HCl for 5 times. Then, 1 ml of 0.1 M NaOH was added to dissolve the dye. The clear solution was read the absorbance at 540 nm by a microplate reader. The collagen amount was determined in compared to the standard collagen type I. The percentage of the collagen content was calculated as the following:

$$\% \text{ Collagen content} = (Ct/Cc) \times 100$$

Where, Ct was the collagen content of the treated samples, and Cc was the collage content of the control.

### *2.7 Statistical analysis*

The results were presented as the mean ± SD of three independent experiments (n = 3). ANOVA was used for the analysis of the test results (LSD test) at the significance level of $p$-value < 0.05.

## 3.RESULTS AND DISCUSSION

### *3.1 The yields and characteristics of the Star Grass extracts*

The plant, Star Grass has been known as a whitening and peeling agent in Thailand long times ago. The extraction yields and characteristics of the leave and tuberous extracts from Star Grass were showed in **Table 1**. The yield of the leave extract by maceration with distilled water (SGLW) at room temperature (25 ±2°C) for 24 hr showed the highest extraction yield (25.47%).

**Table 1** The extraction yields and characteristics of the leave and tuberous extracts from Star Grass

| Extracts | Extraction Yields (%) | Characteristics |
|---|---|---|
| SGLW | 25.47 | Deeply green-Black, Viscous-oily, Slight odor |
| SGTW | 11.84 | Brown, Powder, Strong odor |
| SGLE | 4.83 | Deeply green, Viscous, Odorless |
| SGTE | 6.00 | Deeply brown, Viscous, Slight odor |
| SGLB | 10.54 | Deeply green, Viscous, Odorless |
| SGTB | 9.86 | Brown, Powder, Strong odor |

**Note:** SGLW was the leave extract from Star Grass prepared by maceration with distilled water at room temperature (25 ±2°C) for 24 hr; SGTW was the tuberous extract from Star Grass prepared by maceration with distilled water at room temperature for 24 hr; SGLE was the leave extract from Star Grass prepared by maceration with 95%(v/v) ethanol at room temperature for 24 hr; SGTE was the tuberous extract from Star Grass prepared by maceration with 95%(v/v) ethanol at room temperature for 24 hr; SGLB was the leave extract from Star Grass prepared by boilin g with distilled water for 2 hr; and SGTB was the tuberous extract from Star Grass prepared by boiling with distilled water for 2 hr

### *3.2 Phytochemical analysis and total phenolic compounds of the Star Grass extracts*

Phytochemicals of the Star Grass extracts were presented in **Table 2**. Phytochemicals detected in these extracts were glycosides (deoxysugars) and tannins, while alkaloids, saponins, flavonoids and anthrocyanins were not found. Only the leave (SGLB) and tuberous (SGTB) extracts from Star Grass prepared by boiling with distilled water for 2 hr, showed only glycoside contents. In addition, the SGLE extract demonstrated the highest amount of 2.979 ± 0.008 gGAE/100g Extract ($p$ < 0.05).

**Table 2** Phytochemical contents and total phenolic compounds of the Star Grass extracts

| Extracts | Phytochemical contents | | | | | | Total Phenolic Compounds (gGAE/100g Extract) |
|---|---|---|---|---|---|---|---|
| | Alkaloids | Saponins | Flavonoids | Anthocyanins | Glycosides | Tannins | |
| SGLW | - | - | - | - | + | + | $2.188 \pm 0.013^{a}$ |
| SGTW | - | - | - | - | + | + | $2.304 \pm 0.044^{b}$ |
| SGLE | - | - | - | - | + | + | $2.979 \pm 0.008^{c}$ |
| SGTE | - | - | - | - | + | + | $1.863 \pm 0.145^{d}$ |
| SGLB | - | - | - | - | + | - | $2.142 \pm 0.018^{a,d}$ |
| SGTB | - | - | - | - | + | - | $1.279 \pm 0.005^{e}$ |

**Note:** The symbols -, + were negative and positive test, respectively; Superscript letters (a-e) in the same column indicate significant differences at $p$ < 0.05.

Phytochemicals found in the extracts depend on kinds of medicinal plants, solvent and temperature used in the

extraction process. The heat labile phytochemicals might be degraded by high temperature and long extraction time [16]. Thus, absence of tannins a class of a heat-labile polyphenol [17], in the SGLB and SGTB extracts might be from boiling at high temperature (80-95ºC) for 2 hr, whereas the glycoside content was still presence. The absence of tannins in these extracts might affect with their bioactivities such as free radical scavenging and tyrosinase inhibition activities. Phenolic compounds are considered to be the most important anti-oxidants and tyrosinase inhibitors of natural extracts. All extracts showed total phenolic contents determined by Folin-Ciocalteu colorimetric method. This might be due to the phytochemical contents (tannins and glycosides) in the Star Grass extracts. It has been reported that phenolic compounds showed several bioactivities such as anti-oxidation [18], anti-tyrosinase activity [19], anti-inflammatory [20] and anti-bacterial activity [21]. Thus, the presence of total phenolic compounds in the Star grass extracts might have some bioactivities.

### *3.3 Free radical scavenging and tyrosinase inhibition activities of the Star Grass extracts*

This present study showed that all Star Grass extracts gave both the free radical scavenging activity by DPPH assay and the tyrosinase inhibition activity by the modified dopachrome method (**Table 3**). In addition, the SGLE extract exhibited the highest free radical scavenging activity, but it was lower than that of vitamin C ($SC_{50}$ value of 0.05 ± 0.01 mg/ml) ($p < 0.05$). Whereas, the highest tyrosinase inhibition activity was also found in the SGLE, SGTW and SGTE extracts, which was comparable to vitamin C ($IC_{50}$ value of 0.13 ± 0.02 mg/ml) ($p < 0.05$). As known, cellular oxidation generates many reactive oxygen species (ROS), which can damage DNAs, chromosomes and healthy cells leading to the development of degenerative diseases such as cancers, diabetes, alzheimer, as well as skin aging such as increased protein degradation, the decreased amount of fibroblasts as a result of the suppression of protein synthesis, including type 1 and 3 collagen [22]. ROS play also a significant role in the regulation of the melanogenesis which can increase signs of aging such as dark spots, melasma, freckles and several hyperpigmentation syndromes [6]. Antioxidants interact with the stabilize free radicals (ROS) and prevent the damage of the DNA and cells leading to improve skin aging and melanogenesis related with tyrosinase activity. These activities of the Star grass extracts might be due to their phytochemical contents. It has been reported that phytochemicals such as tannins and glycosides, are an antioxidant and tyrosinase inhibitor, which is the same with vitamin C [18], [23]. The different free radical scavenging and tyrosinase inhibition activities of the SG extracts might be due to different tannin contents. It has been demonstrated that the tannins of Aruncus silvester Kostel. ex Maxim and Potentilla alba L. extracts showed strong anti-oxidative activity [24]. It has been reported that tannins inhibit some enzyme activities such as trypsin, amylase and lipase by forming insoluble complexes, and divalent ions such as $Fe^{2+}$ and $Zn^{2+}$ ions. Thus, the SGLW, SGTW and SGTE extracts that showed tannin contents, might inhibit the tyrosinase activity by divalent ions because tyrosinase is an enzyme-Cu2+ containing [17].

**Table 3** Free radical scavenging activity by DPPH assay and tyrosinase inhibition activity by the modified dopachrome method of the Star Grass extracts

| Extracts | Free Radical Scavenging Activity ($SC_{50}$ mg/ml) | Tyrosinase Inhibition Activity ($IC_{50}$ mg/ml) |
|---|---|---|
| SGLW | $1.19 \pm 0.29^{a}$ | $1.34 \pm 0.10^{a}$ |
| SGTW | $0.65 \pm 0.06^{b}$ | $0.12 \pm 0.01^{b}$ |
| SGLE | $0.44 \pm 0.02^{c}$ | $0.11 \pm 0.03^{b}$ |
| SGTE | $3.21 \pm 0.21^{d}$ | $0.15 \pm 0.02^{b}$ |
| SGLB | $1.50 \pm 0.04^{a}$ | $1.54 \pm 0.15^{c}$ |
| SGTB | $8.60 \pm 0.83^{e}$ | $1.66 \pm 0.19^{c}$ |
| Vitamin C | $0.05 \pm 0.01^{f}$ | $0.13 \pm 0.02^{b}$ |

**Note:** Superscript letters (a-e) in the same column indicate significant differences at $p < 0.05$.

**Table 4** exhibited the correlation (R) between the total phenolic compounds, the free radical scavenging and tyrosinase inhibition activities of the Star Grass extracts. There was excellent positive correlation between the total phenolic compound (*PC*) and the free radical scavenging (*RS*) with the *R* of 0.9281, whereas, the correlation between total phenolic compound (*PC*) and tyrosinase inhibition activities (*TI*) (*R* of 0.6150) was fairly, which indicating that only free radical scavenging activity of the SG extracts might be from their total phenolic compounds.

**Table 4** Correlation (*R*) between the total phenolic compounds, the free radical scavenging and tyrosinase inhibition activities of the Star Grass extracts.

| Biological activities | Correlation (*R*) | | |
|---|---|---|---|
| | Total Phenolic Compound (*PC*) | Free Radical Scavenging (*RS*) | Tyrosinase Inhibition (*TI*) |
| Total Phenolic Compound (*PC*) | 1 | 0.9281 | 0.6150 |
| Free Radical Scavenging (*RS*) | 0.9281 | 1 | 0.7022 |
| Tyrosinase Inhibition (*TI*) | 0.6150 | 0.7022 | 1 |

**Note:** total phenolic compound (*PC*) was phenolic content (mg/g.Extract). Free cavenging activity (*RS*), and tyrosinase inhibition (*TI*) were calculated

from $1/SC_{50}$ (ml/mg) and $1/IC_{50}$ (ml/mg), respectively.

On the other hand, the correlation between the free radical scavenging (*RS*) and tyrosinase inhibition activities (*TI*) was good with the positive *R* of 0.7022, which meant if the free radical scavenging of the tuberous extracts was increased, the tyrosinase inhibition activity would be increased as well. In addition, the free radical scavenging (RS) and tyrosinase inhibition activities (TI) exhibited the positively good correlation, the decreasing of tyrosinase activity as well as the presence of radical scavenging activity of the Star Grass extracts should directly to down-regulate on the melanogenesis [25]. Following the results, the tuberous extract from Star Grass prepared by maceration with distilled water at room temperature for 24 hr (SGTW), and the leave extract from Star Grass prepared by maceration with 95%(v/v) ethanol at room temperature for 24 hr (SGLE) that showed the highest both free radical scavenging and tyrosinase inhibition activities, were selected for investigation of cytotoxicity, anti-melanogenesis and collagen biosynthesis.

### *3.4 Cytotoxicity of the Star Grass extracts*

The % cell growth of the treated cells has been compared to the control (%100 viability). **Figure 2** demonstrated that the selected Star Grass extracts, SGTW and SGLE at various concentrations, exhibited no cytotoxicity on the 4$^{th}$ passaged murine melanoma cells and human skin fibroblasts when compared with the control ($p < 0.05$), indicating possible to use these cells as a model for investigation of anti-melanogenesis and collagen biosynthesis of the selected extracts. At the concentration at 0.1 mg/ml of the extracts showed the highest growth on human skin fibroblasts, whereas similar to the control group on $B_{16}F_{10}$ cells ($p < 0.05$). The difference cytotoxic effect of these cells might depend on the metabolic activity type of cells [26]. Therefore, the SGTW and SGLE extracts at concentration of 0.1 mg/ml was the proper concentration for investigation the anti-melanogenesis on $B_{16}F_{10}$ cells and collagen biosynthesis on human skin fibroblasts. Following to this result, the SGTW and SGLE extracts showed no cytotoxicity on human skin fibroblasts as a normal cell leading to no toxicity when apply as a skin product as well. This indicated that the reduction of the melanin content and stimulation of collagen was not due to cell death. In addition, the SGLE extracts at 0.1 mg/mL indicated significant the highest stimulation index (SI), which was the ratio between the percentages of cell growth treated with the extracts and the control, on human skin fibroblast proliferation of 1.727 which was more active than vitamin C (SI of 1.569) ($p < 0.05$). This results might be from the biphasic dose response phenomenon hich is graphically represented by either an inverted U-shaped dose response or by a J- or U-shaped dose response that describes the maximum activity induced by low concentrations of natural compounds [27]. This result can anticipate the effect of the extract on collagen biosynthesis from the stimulation of human skin fibroblast proliferation.

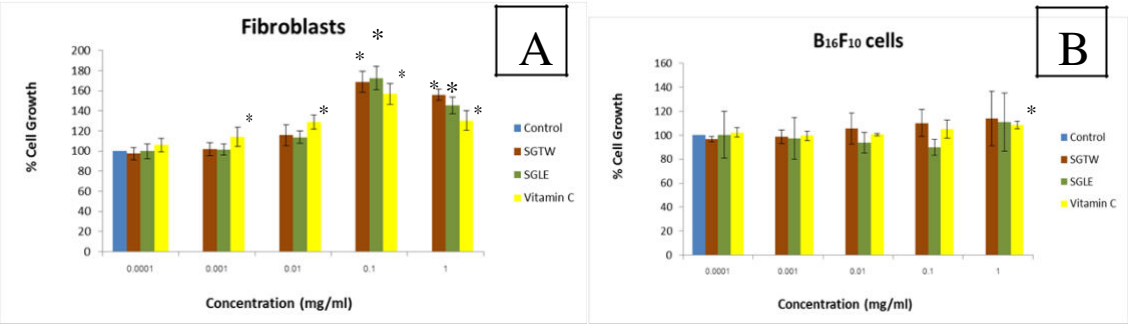


**Figure 2** Cytotoxicity of the SGTW and SGLE extracts at various concentrations on the 4$^{th}$ passaged human skin fibroblasts (A) and murine melanoma ($B_{16}F_{10}$) cells (B). Superscript asterisks (*****) in the column indicate significant differences compared with untreated group (control) ($p < 0.05$).

### *3.5 Anti-melanogenesis and stimulation of collagen biosynthesis of the Star Grass extracts*

**Figure 3** exhibited the anti-melanogenesis by the Star Grass extracts on $B_{16}F_{10}$ cells (4$^{th}$ passaged) compared with the vitamin C. The SGTW and SGLE extracts at 0.1 mg/ml showed the anti-melanogenesis on the $B_{16}F_{10}$ cells at 24 hr, which was comparable with vitamin C ($p < 0.05$). The anti-melanogenesis activity of the SG extracts might be due to their phytochemical contents such as glycosides and tannins, and phenolic compounds. Several reports have been reported that phytochemical containing in medicinal plant extracts such as tannins, glycosides, phenolic compounds showed anti-melanogenesis on $B_{16}F_{10}$ cell line [19], [28], [29]. The ethyl acetate extract of tamarind seed coat, which contents phenolic compounds, exhibited anti-melanogenesis stimulated by α-MSH and tyrosinase inhibition activity [30]. Moreover, the decrease of tyrosinase activity as well as the presence of radical scavenging activity directly relates to down-regulate on the melanogenesis [25]. **Figure 4** showed the stimulation of collagen biosynthesis on human skin

fibroblasts (4th passaged) of the Star grass extracts. The SGTW and SGLE extracts at 0.1 mg/ml exhibited significant stimulated the collagen biosynthesis on human skin fibroblasts when compared to control (no treated) ($p < 0.05$). As well, it have been also reported that phytochemical containing in several plant extracts such as tannins, can stimulate the collagen synthesis on human skin fibroblasts. The aqueous extract from *Phyllanthus muellerianus* (Kuntze) Exell., which contains of tannins such as geraniin, showed stimulation of collagen biosynthesis on human skin fibroblasts and keratinocytes [31]. In addition, the collagen stimulation of the SGTW extract was comparable to vitamin C, whereas the SGLE extract gave superior the stimulation than vitamin C about 26% ($p < 0.05$). The free radical scavenging activity and the amount of total phenolic compounds might response for the higher stimulation activity of the SGLE extract.

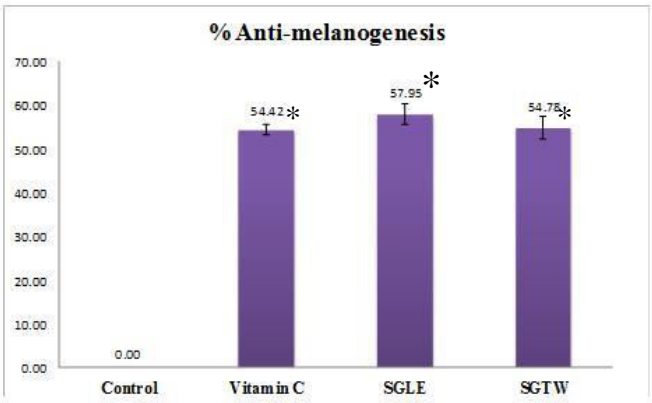


**Figure 3** Anti-melanogenesis of the SGTW and SGLE extracts on $B_{16}F_{10}$ cells. The concentration of the extracts and the vitamin C was 0.1 mg/ml. Each value is expressed in Mean ± SD (n = 3). Superscript asterisks (*) in the columns indicate significant differences compared with the control ($p < 0.05$).

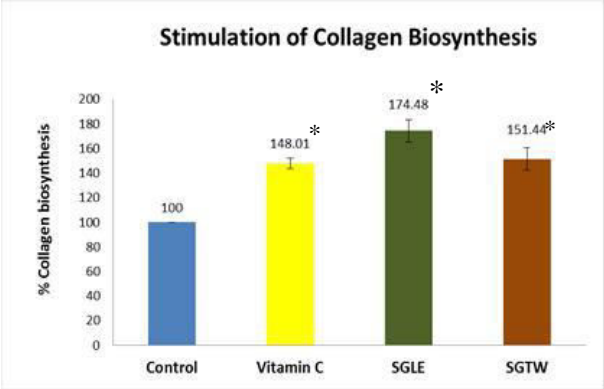


**Figure 4** Stimulation of collagen biosynthesis on human skin fibroblasts of the SGTW and SGLE extracts on $B_{16}F_{10}$ cells. The concentration of the extracts and the vitamin C was 0.1 mg/ml. Superscript asterisks (*) in the columns indicate significant differences from the control ($p < 0.05$).

## 4. CONCLUSION

Consequently, the present study showed that the Star grass extract is a promising candidate for the development of a safe whitening or anti-ageing agent, which has potent inhibitory effects on tyrosinase activity and melanogenesis; and stimulation of collagen biosynthesis. It has suggested that the leave extract from Star grass prepared by the maceration with 95%(v/v) ethanol at ambient temperature (25 ± 2°C) which demonstrated the highest anti-melanogenesis on murine malanoma ($B_{16}F_{10}$) cells; stimulation of collagen biosynthesis as well as the free radical scavenging activity, inhibition of tyrosinase activity, can be further developed as whitening and anti-aging agents for cosmeceuticals.

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