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Original Article

Antioxidant and Tyrosinase Inhibitory Properties of an Aqueous Extract of *Garcinia atroviridis* Griff. ex. T. Anderson Fruit Pericarps

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ABSTRACT

Background: Ultraviolet radiation (UVR) is the major cause for hyperpigmentation, and to prevent this natural products are increasingly being explored as potential skin whitening agents. The aim of this study was to determine the total phenolic and flavonoid content, free radical scavenging activity, anti-tyrosinase activity and the inhibition of melanin content in α-melanocyte stimulating hormone-induced B16F10 melanoma cells of an aqueous extract of *Garcinia atroviridis* Griff. ex. T. Anderson fruit pericarps. **Methods:** The aqueous extract was prepared by extraction with distilled water at 105°C for 60 min. Total phenolic and flavonoid content were determined using the Folin-Ciocalteau and aluminium chloride methods, respectively. Scavenging activity was assessed using 2,2-Diphennyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). Tyrosinase activity and melanin content were determined spectrophotometrically. **Results:** The results showed that the aqueous extract of *Garcinia atroviridis* fruit pericarps had a phenolic (26.33 ± 0.77 mg GAE/g plant extract) and flavonoid content (9.31 ± 0.40 mg QE/g plant extract). The aqueous extract of *Garcinia atroviridis* significantly inhibited mushroom tyrosinase activity (IC_{50} of 40.72 ± 1.83 µg/mL) and cellular tyrosinase activity (at a concentration of 125 µg/mL) in α-melanocyte stimulating hormone-induced B16F10 melanoma cells. The *Garcinia atroviridis* extract also suppressed melanin content at concentrations of 31.25-125 µg/mL. Correlations of mushroom tyrosinase inhibition with DPPH and ABTS scavenging activities were 0.8673 and 0.9468, respectively. **Conclusion:** Our findings show that an aqueous extract of *Garcinia atroviridis* fruit pericarps is a source of natural compounds and antioxidant capacity which can inhibit tyrosinase activity and melanin content. Thus, aqueous extracts of *Garcinia atroviridis* may be a potential source of skin whitening agents for hyperpigmentation.

Key words: *Garcinia atroviridis*, Tyrosinase activity, Melanin, α-MSH, B16F10 cells.

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INTRODUCTION

Ultraviolet radiation (UVR) is an external factor for skin disorders including tanning, hyperpigmentation, cancer and immunomodulation.[1] Repeated exposure to suberythemal UV irradiation activates melanin synthesis which results in increased melanin content in human skin.[2] UV also induces oxidative stress through the production of reactive oxygen species (ROS), which induces DNA damage in keratinocytes and activates p53.[3] Then, p53 stimulates opiomelanocortin (POMC) gene expression, the product of which is cleaved to produce α-melanocyte stimulating hormone (α-MSH), adrenocorticotropic hormone and β-endorphin.[4] In turn α-MSH induces the upregulation of tyrosinase enzyme which is the rate limiting enzyme for melanin synthesis in melanocytes.[5] Thus, tyrosinase is a target for melanogenesis inhibition. In addition, free radical scavenging activity is also a desirable property for skin whitening agents.[6]

Phenolic and flavonoid compounds have the capacity to protect against UV induced DNA damage in keratinocytes. Moreover, as phenolic and flavonoid compounds have a structure similar to tyrosine, which is a substrate for melanin, they can directly inhibit tyrosinase activity.[7] Nowadays, there are many well characterized tyrosinase inhibitors such as hydroquinone, arbutin, kojic acid, azelaic acid and L-ascorbic acid.[8] While hydroquinone is the most popular agent for treating hyperpigmentation as it can inhibit tyrosinase activity, it has many adverse effects including skin irritation, toxicity, mutagenicity, carcinogenicity and exogenous ochronosis.[9,10] Thus, many researchers have focused on botanical and natural ingredients as alternative depigmenting agents which are safe and have efficacy.[11]

Garcinia atroviridis Griff. ex. T. Anders (common name asam gelugur), belonging to the family *Clusiaceae* is commonly grown in Asian countries including Thailand, Malaysia and Indonesia. The fruits are yellowish green to yellow and globular in shape, and are commonly used as a flavouring agent in food. In Thailand, dried fruits are mixed in the popular local dish, Tom-Yum soup. In folk medicine, fruits of *Garcinia atroviridis* are used as pre- and postpartum medication for treating stomachache during pregnancy and as a lotion for rubbing on women's abdomen after confinement.[12]

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Methanol crude extracts of leaves, fruits, roots, stem and trunk barks of *Garcinia atroviridis* have antioxidant, antibacterial, antifungal and antitumor activities.[13] Ethyl acetate and ethanol crude extracts of fruits also have antibacterial activity.[14] Aqueous extracts of fruits have non-cytotoxic effects on human skin fibroblasts and have antifungal[15], and antihyperlipidemic activity[16,17] and inhibit acetylcholinesterase.[18]

However, there are no reports on the inhibition of tyrosinase activity. In this study, we aimed to determine the total phenolic and flavonoid content, free radical scavenging activity, anti-tysoinase activity and the ability to inhibit melanin content in α-MSH induced B16F10 melanoma cells of aqueous extract of *Garcinia atroviridis* Griff. ex. T. Anders fruit pericarps. The findings from this study will be useful for application and development of skin whitening agents.

MATERIALS AND METHODS

Materials

2,2-Diphennyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), aluminium chloride ($AlCl_3$), Folin-Ciocalteu reagent, gallic acid, kojic acid, L-DOPA, sodium carbonate (Na_2CO_3), NaOH, quercetin, Triton X-100 and mushroom tyrosinase were purchased from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), phosphate buffer saline (PBS) and trypsin/EDTA were obtained from Thermo Scientific Hyclone (Logan, Utah). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin/streptomycin and fetal bovine serum were purchased from Merck (Darmstadt, Germany). Melanocyte stimulating hormone was purchased from Abcam (Cambridge, UK).

Plant materials

Fruit pericarps of *Garcinia atroviridis* Griff. ex. T. Anderson were collected from Thasala, Nakhon Si Thammarat, Thailand. A voucher number was identified and collected at Department of Botany, Faculty of Science, Chulalongkorn University, Thailand with a herbarium number 016443.

Plant extraction

Fruit pericarps of dried plant material (125 g) were blended and mixed with water (12.5 L). The mixture was aliquoted into duran bottles (1 L/bottle). Extraction was performed at 105°C, 15 psi for 60 min in autoclave. After that, the bottle was cooled down to room temperature. The mixture was filtered through Whatman No.1 filter paper (Whatman International Ltd., Kent, UK), and the filtrates were then lyophilized. The extracts were dissolved in dimethyl sulfoxide at a stock concentration of 100 mg/mL and stored at -20°C until analyses. The extract yield of *Garcinia atroviridis* was 7.39 ± 1.69% (w/w).

Total phenolic content

The Folin-Ciocalteu method was chosen to determine total phenolic content as described previously.[19] Briefly, a stock concentration of *Garcinia atroviridis* Griff. Ex. T. Anderson aqueous extract (GA) was diluted in distilled water to give a concentration of 1 mg/mL. Then 100 µL of 0.1 M Na_2CO_3 solution and 100 µL of 10%Folin-Ciocalteu reagent were mixed in a well of a 96-well plate. The reaction mixture was incubated for 1 h at room temperature. After incubation, the absorbance was measured at 750 nm. A standard curve was plotted using gallic acid (concentration range from 1.569-200 µg/mL. Total phenolic content was determined as gallic acid equivalents (GAE) in mg per 1 g of dry plant extract.

Total flavoniod content

Total flavonoid content of the GA extract was performed as described by others.[20] Briefly, 100 µL of extract1 (mg/mL) or quercetin (1.56-100 µg/mL) was incubated with 100 µL of 2%$AlCl_3$ solution in methanol for 30 min at room temperature. The absorbance was measured at 415 nm against a blank. The resultant values were determined as mg quercetin equivalents (QE) per 1 g of dry plant extract.

2,2-Diphennyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity of the GA extract was determined according to the procedure previously described by others.[21] Briefly, 20 µL of GA extract or ascorbic acid in ethanol was added to 180 µL of DPPH solution which was prepared daily. The mixture was shaken and left to stand at room temperature in the dark. After 30 min, the absorbance was measured at 517 nm against a blank. Assays were done in triplicate. The DPPH scavenging activity was estimated using the equation: %Scavenging activity = 100 x (Abs of control - (Abs of sample - Abs of blank))/Abs of control. IC_{50}, the concentration giving 50% inhibition of DPPH, was determined from a graph of free radical scavenging activity.

$ABTS^{\cdot+}$ radical scavenging activity

Free radical scavenging activity of extract was estimated by ABTS decolorization assay.[22] $ABTS^{\cdot+}$ was produced by mixing 7 mM $ABTS^{\cdot}$ and 2.45 mM potassium sulfate at a ratio of 2:3 v/v, and storage in the dark at room temperature for 12-16 h until analysis. The $ABTS^{\cdot+}$ reagent was further diluted with methanol to reach an absorbance between 0.70 ± 0.02. After adding 20 µL of extract or ascorbic acid and 180 µL of $ABTS^{\cdot+}$ reagent, the mixture was measured at 45 min after mixing. Analysis was carried out at least three times. The percent inhibition of absorbance at 734 nm was calculated using the formula: %Scavenging activity = 100 x (Abs of control - (Abs of sample - Abs of blank))/ Abs of control. IC_{50} was determined as the concentration giving 50% inhibition of $ABTS^{\cdot+}$.

Mushroom tyrosinase activity

Dopachrome formation in the oxidation of L-DOPA by mushroom tyrosinase activity was measured spectrophotometrically as described previously.[19] The reaction mixture contained 20 µL of extract or kojic acid, 20 µl of mushroom tyrosinase (203 U/mL) and 160 µl of 20 mM phosphate buffer. The mixture was pre-incubated at room temperature for 10 min. After 10 min, 20 µL of 2.5 mM L-DOPA was added into the mixture and the reaction was incubated for 60 min. Kojic acid served as a positive control. Each experiment was performed in triplicate. The percent of mushroom tyrosinase activity at 475 nm was estimated using the formula: 100 x [(Abs of sample - Abs of sample blank)]/[(Abs of control - Abs of control blank)]. The results were compared with the control group. IC_{50} is the concentration giving 50% of inhibition of mushroom tyrosinase activity.

Cell culture

B16F10 mouse melanoma cells (CRL-6475) were obtained from The American Type culture collection (ATCC, Rockville, MD). The cells were grown in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum and 1% penicillin (100 U/mL)/streptomycin (100 µg/mL) at 37°C in a humidified atmosphere with 5% CO_2.

Cell viability

Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In brief, 5 x 10^3 cells/well were seeded into a 96-well plate. After 24 h, the cells were incubated in the presence of 1 µM α-MSH and treated with aqueous GA extract (7.8-1000 µg/mL) or kojic acid (500 µg/mL). After 48 h, the MTT solution was added into each well. The absorbance of wells was read at 550 nm

using microplate reader (Biotek, Winooski, USA). Each experiment was performed in triplicate. The percentage of cell viability was determined relative to the control.

Cell tyrosinase activity assay

The oxidation of L-DOPA to dopachrome was determined by spectrophotometry as an indicator of cellular tyrosinase activity.[23] B16F10 cells ($1x10^5$ cells/well) were plated into each well of a 6-well plate for 24 h. After treatment with 1 μM α-MSH and GA extract or kojic acid, the cells were washed twice with 100 mM sodium phosphate buffer saline and lysed cells with 1%Triton X-100 in PBS. A total of 100 μL of each lysate (40 μg) was mixed with 100 μL of 5 mM L-DOPA solution in a 96-well plate and incubated at 37 °C for 1 h. The absorbance at 475 nm reflected the production of dopachrome. The cellular tyrosinase activity of each lysate was compared with α-MSH treated cells.

Determination of melanin content

B16F10 cells were cultured at a density of $1x10^5$ cells/well in a 6-well plate. After 24 h, the cells were treated with 1 μM α-MSH and GA extract or kojic acid for 48 h. After incubation, the cells were washed with phosphate buffer saline twice and collected using trypsin. The cell pellets were solubilized in 1 M NaOH at 80°C for 1 h. The melanin content was determined at by absorbance at 475 nm and calculated from a standard curve of melanin. The percentage of melanin was calculated relative to control cells (treated with only α-MSH).

Statistical analysis

All results of experiments are presented as the mean ± standard error of the means (SEM). Statistical analyses were performed using one-way ANOVA with Dunnett's post-hoc test. *P*-values less than ($P < 0.05$) were considered statistically significant. Correlations between free radical scavenging activity and tyrosinase inhibition were analyzed using the Pearson's method.

RESULTS

Total phenolic and flavonoid contents

Total phenolic content of an aqueous extract of fruit pericarp of *Garcinia atroviridis* (GA) was determined using a calibration curve of gallic acid, where y = 0.1023x – 0.0691, $R^2 = 0.9954$, where Y is an absorbance and X is amount of gallic acid in μg. The Folin-Ciocalteu method showed that the total phenolic content in GA was 26.33 ± 0.77 mg GAE/g dry plant extract.

The total flavonoid content of GA was determined from a calibration curve of quercetin (y = 0.1739x, R = 0.9997) (1.56-100 μg/mL) where Y is an absorbance and X is amount of quercetin in μg. Values were determined in mg quercetin equivalents (QE) per g dry plant extract. The total flavonoid content of GA was 9.31 ± 0.40 mg QE/g dry plant extract.

DPPH radical scavenging activity

Free radical scavenging activity of an aqueous extract GA was determined using DPPH. The activity was determined over a range of concentrations (62.5 μg/mL to 1000 μ g/mL), and results (Figure 1) showed that the free radical scavenging activity was dose dependent, and at the highest concentration investigated the free radical scavenging activity was about 72.29%. The IC_{50} values, the concentrations giving 50% inhibition of DPPH for GA and ascorbic acid were 628.85 ± 32.67 μg/mL and 62.22 ± 0.67 μg/mL, respectively.

ABTS.+ radical scavenging activity

Free radical scavenging activity of an aqueous extract GA was additionally determined using $ABTS^{+}$, using the same range of concentrations (62.5 μ g/mL to 1000 μ g/mL). The results (Figure 2) again showed a dose dependent inhibition of free radicals, with maximal inhibition (97.00%) at 1000 μ g/mL, compared to 93.75% for ascorbic acid (12.5 μg/mL). The IC_{50} of ascorbic acid was 6.27 ± 0.19 μg/mL while that of GA was 321.41 ± 12.76 μg/mL.

Effect of GA on mushroom tyrosinase activity

To determine the anti-tyrosinase activity of the GA extract, a mushroom tyrosinase activity assay was performed using L-DOPA as a substrate. The results (Figure 3) showed that GA extract at concentrations of 7.81-1000 μg/mL significantly inhibited mushroom tyrosinase activity in a dose-dependent manner ($P < 0.001$) when compared with the negative (untreated with GA) control. Kojic acid (50 μg/mL) as a positive control significantly ($P < 0.001$) decreased mushroom tyrosinase activity. The IC_{50} of GA extract was 40.72 ± 1.83 μg/mL, while that of kojic acid was 8.00 ± 0.47 μg/mL.

Effect of GA extract on cell viability

To determine the effect of GA on cell viability, B16F10 were seeded and after 24 h, the cells were untreated or treated with 1 μM α-MSH alone, or with 1 μM α-MSH and GA extract or kojic acid for 48 h, after which viability was determined using the MTT assay. Results (Figure 4) showed that the cell viability was not significantly affected by treatment with 1 μM α-MSH when compared to control group (untreated cells). Markedly however, significant cytotoxicity was observed in cells treated with GA at concentrations of 250 μg/mL and above. Therefore, three

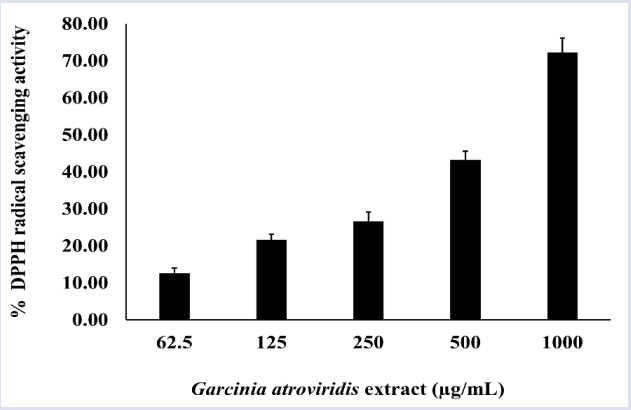

Figure 1: Scavenging activity of an aqueous extract of GA as determined by DPPH assay. Each bar represents the mean of three replicates with the standard error of the means.

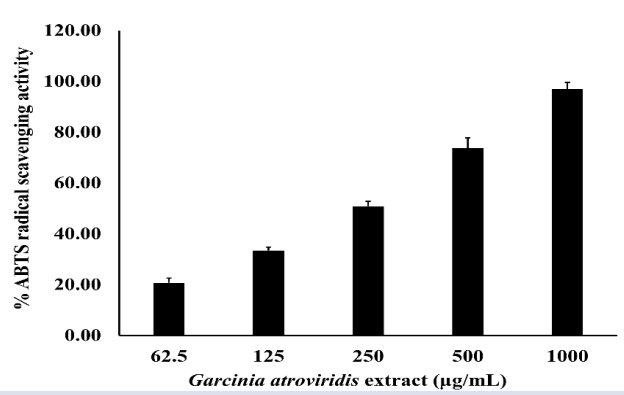

Figure 2: ABTS radical scavenging activity of GA extract. Values are mean ± SEM of three replicates.

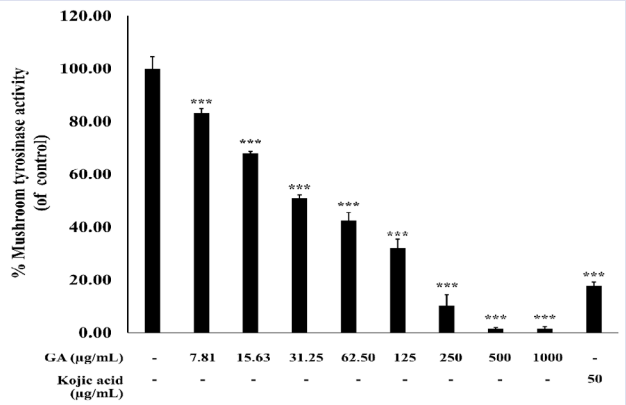

Figure 3: Effect of GA extract on mushroom tyrosinase activity. Data are mean ± SEM of three independent replicates. ***P < 0.01 as compared to control (untreated) group.

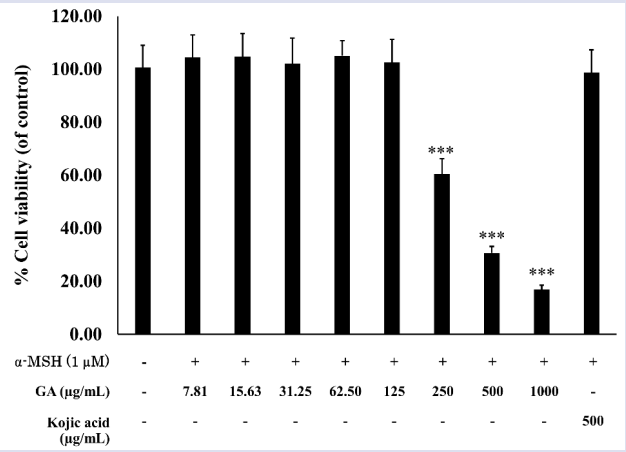

Figure 4: Effect of GA extract on cell viability. Data are presented as mean ± SEM (n = 3) and expressed as % cell viability compared to the control group. ***P < 0.001 as compared to control group.

non-cytotoxic concentrations (31.25-125 µg/mL) were selected for further experiments.

Effect of GA on cellular tyrosinase activity in α-MSH-stimulated B16F10 cells

To determine the effect of GA on cellular tyrosinase activity, B16F10 were seeded and after 24 h, the cells were untreated or treated with 1 µM α-MSH alone, or with 1 µM α-MSH and GA at three different non-cytotoxic concentrations or with kojic acid for 48 h, after which cellular tyrosinase activity was determined by evaluating the oxidation of L-DOPA to dopachrome. The results (Figure 5a) showed that α-MSH significantly increased the cellular tyrosinase activity when compared to untreated cells (without treatment). Kojic acid as a positive control significantly inhibited the cellular tyrosinase activity (P < 0.001) as compared to cells treated with α-MSH only. GA extract at a concentration of 125 µg/mL significantly inhibited cellular tyrosinase, albeit it less effectively than kojic acid. These results suggest that GA extract can inhibit the cellular tyrosinase activity which may lead to suppress cellular melanin content in B16F10 cells.

Effect of GA extract on cellular melanin content in α-MSH-stimulated B16F10 cells

To evaluate the inhibitory effect of GA extract on melanin synthesis, we determined the melanin content of α-MSH-stimulated B16F10 cells at 48 h after treatment with various concentrations of the extract (31.25-125 µg/mL), or with kojic acid (500 µg/mL). As shown in Figure 5b, α-MSH-stimulated B16F10 cells significantly (P < 0.001) increased melanin content when compared to untreated cells. The melanin content of α-MSH-stimulated B16F10 cells treated with GA was significantly reduced in a concentration dependent manner. Compared to control cells (treated with α-MSH only), the melanin content of cells treated with kojic acid or the maximum concentration of GA tested (125 µg/mL) was reduced to 46.94% and 46.96%, respectively. These results indicate that GA treatment significantly reduced melanin content in of α-MSH-stimulated B16F10 cells.

Correlation between mushroom tyrosinase inhibition and free radical scavenging activity

At concentrations of 62.5-500 µg/mL GA, there was a high correlation of mushroom tyrosinase inhibition with DPPH (correlation coefficient R^2 = 0.8673; Figure 6a). Similarly, a high correlation was also found between ABTS radical scavenging activity and mushroom tyrosinase inhibition (R^2 = 0.9468; Figure 6b). These findings suggest that DPPH and ABTS scavenging activity of GA extract enhanced the inhibition of mushroom tyrosinase activity.

DISCUSSION

Garcinia atroviridis Griff. Ex. T. Anderson is commonly found in India, Malaysia and Thailand, and a number of compounds including bioflavonoids, benzophenones and xanthones are found in members of the *Garcinia* genus.[24] In *Garcinia atroviridis* fruit, a number of organic acids including hydroxycitric acid (HCA), citric acid, malic acid and tartaric acid have been identified.[25] Generally, dietary plants contain large amount of polyphenols such as phenolic acid, flavonoids, tannin, curcuminoids, coumarins, quinones and other compounds.[26] Some phenolic and flavonoid compounds including hydroquinone, arbutin, vanillin, resorcinols, hydroxycinnamic acid, quercetin and kaemferol have been reported to be mushroom tyrosinase inhibitors and to inhibit melanogenesis.[27,28] In this study, we selected the fruit pericarp of *Garcinia atroviridis* fruits, which were extracted with distilled water at high temperature (105° C) for 1 h. We investigated the total phenolic and flavonoid contents in an aqueous extract of *Garcinia atroviridis* (GA) fruit pericarps. Our aqueous extract of GA contained phenolic (26.33 ± 0.77 mg GAE/g dry plant) and flavonoid (9.31 ± 0.40 mg QE/g dry plant) contents. In a previous study, the total phenolic content of an aqueous extract of *Garcinia atroviridis* fruit extracted with distilled water at 100°C was higher than an aqueous extract distilled at 40°C.[29]

Ultraviolet radiation (UV) is an environmental factor which stimulates the production of reactive oxygen species (ROS).[30] Accumulation of ROS in skin cells can induce skin injury, hyperpigmentation, skin aging and oxidative stress.[3] Antioxidants from plants might be an alternative strategy against ROS induced skin aging and hyperpigmentation.[31] Therefore, we investigated the free radical scavenging activity of GA by DPPH and ABTS assays. The results showed that the scavenging activities of DPPH and $ABTS^{\cdot+}$ radicals by GA were dose dependent, and that the scavenging activities of the GA extract at a concentration of 1000 µg/mL was 72.29% and 97.00%, respectively. Previous studies have shown that aqueous, methanol and ethanol extracts of *Garcinia atroviridis* had high antioxidant effect in scavenging DPPH and $ABTS^{\cdot+}$ radicals.[16,21,32] The increased phenolic contents of *Garcinia atroviridis* fruits were proportional to the high antioxidant activities by the DPPH assay.[29] These finding suggest that the phenolic or flavonoid content is the major compound for antioxidant activities which might defend against oxidants or ROS from ultraviolet radiation.

Skin pigmentation is caused by ultraviolet radiation which stimulates the secretion of α-melanocyte stimulating hormone (α-MSH) by keratinocytes.[33] With long exposure to UV, UV activates the increased

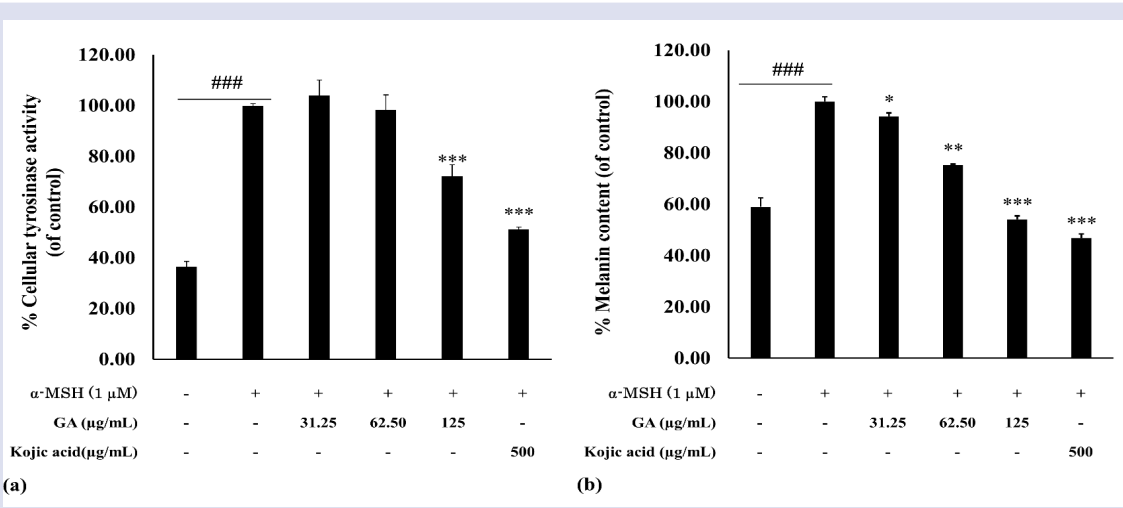

Figure 5: Effect of GA extract on (a) cellular tyrosinase activity and (b) melanin content in α-MSH-stimulated B16F10 cells. Values are expressed as mean ± SEM in triplicate experiments and shown as % cell tyrosinase activity of control group (with α-MSH only). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to control group.

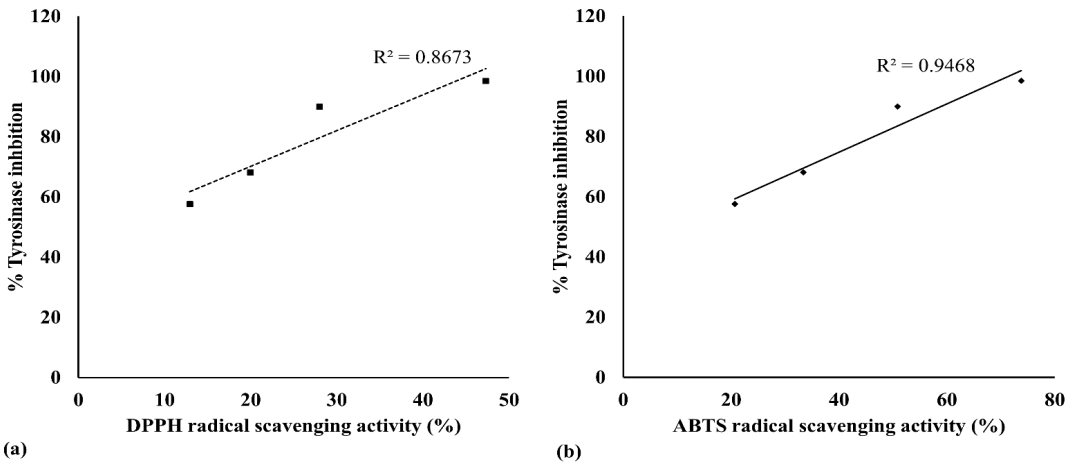

Figure 6: Correlations between mushroom tyrosinase inhibition and free radical scavenging activity including (a) DPPH radical scavenging activity and (b) ABTS radical scavenging activity.

production of α-MSH which increases melanin synthesis through upregulation of tyrosinase activity.[34] Many researchers are interested in natural products to find new potent tyrosinase inhibitor for cosmetics and hyperpigmentation disorders, and many compounds from plants are key sources for tyrosinase inhibitors. In this study, we found that an aqueous extract GA significantly inhibited mushroom tyrosinase activity and cellular tyrosinase activity in α-MSH-induced B16F10 cells. The GA extract also decreased melanin content in α-MSH-induced B16F10 cells. Moreover, there were positive correlations between mushroom tyrosinase inhibition and free radical scavenging activity. Previous studies have reported that seedcases of *Garcinia mangostana*[35], a hydroalcoholic leaf extract of *Garcinia gadnerina*[36] and seeds of *Garcinia kola*[37] significantly inhibited tyrosinase activity, but until now there was no report about tyrosinase inhibitors in extracts from *Garcinia atroviridis*. Our finding showed that an aqueous extract of *Garcinia atroviridis* fruit pericarps had significant phenolic and flavonoid contents, antioxidant activity, and antityrosinase activity, and that GA extract has the potential for development as an alternative skin whitening agent.

CONCLUSION

In this study, we have shown that an aqueous extract of *Garcinia atroviridis* fruit pericarps (GA) can inhibit melanin synthesis and tyrosinase activity through its antioxidant properties. GA extract contains phenolic and flavonoid compounds which are beneficial for health and disease treatment or prevention, and in cosmetic formulations. GA extract has the potential to be an alternative whitening agent for treating hyperpigmentation. Future studies aim to identify the major active compounds with tyrosinase inhibitory action.

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CONFLITCS OF INTEREST

The authors declare no conflicts of interest.

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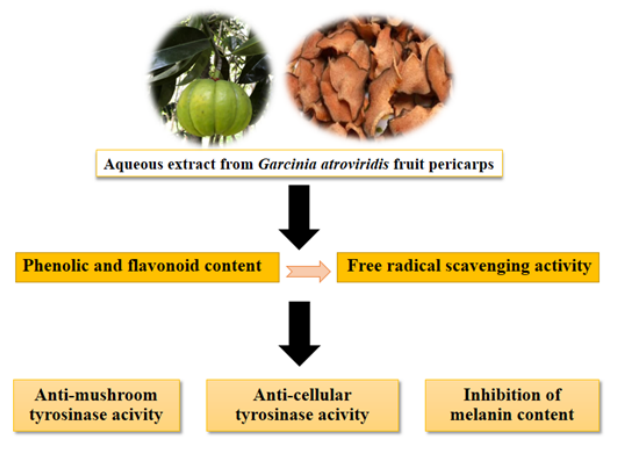
GRAPHICAL ABSTRACT



SUMMARY

- Aqueous extract of *Garcinia atroviridis* fruit pericarps had phenolic and flavonoid contents which scavenge DPPH and ABTS free radicals.
- The aqueous extract of *Garcinia atroviridis* significantly inhibited both mushroom tyrosinase activity and cellular tyrosinase activity in α-MSH-induced B16F10 cells.
- The aqueous extract of *Garcinia atroviridis* also suppressed melanin content in α-MSH-induced B16F10 cells.
- In a correlative study, there was high correlation between mushroom tyrosinase inhibition and free radical scavenging activity (DPPH and ABTS radicals).

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