



Effect of ethanol polarity and temperature on antioxidant activity, tyrosinase inhibition, and total phenol content of *Garcinia atroviridis* (Asam gelugor) fruits extract

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Abstract

In this study, the fruit of *G. atroviridis* was extracted and investigated their antioxidant activities and total phenolic content (TPC) using many extraction conditions, including temperature (10 °C, 25 °C, and 40 °C) and polarity of solvent (90%, 70%, and 50% w/w of ethanol). The extraction percentage yields were found in the range of 6.68 -18.97, depending on extraction temperatures and the polarity of solvents. The highest percentage yield of providing condition was at ethanol 40 °C and 50% (w/w). Because of a different mechanism in assessing antioxidant capacity, antioxidant activities were performed using DPPH and ABTS techniques and reported as the percent inhibition. According to both techniques, the percent inhibition of crude extract was different in the range of 24.61 – 41.01 and 12.90 – 27.74 of DPPH and ABTS assay, respectively, depending on extraction temperatures and polarity solvents as well. The extract condition at 40 °C and 50% (w/w) of ethanol provided the crude extract showing the highest antioxidant activity at percent inhibition of 41.01 ± 4.17 and 27.74 ± 0.84 for DPPH and ABTS assay, respectively. In addition, tyrosinase inhibition of crude extract displayed in the range of 42.88 – 56.13. The extracted temperature at 50 °C and 50% (w/w) of ethanol provided the crude extract showing the highest tyrosinase inhibition at a percent inhibition of 46.13 ± 0.63 . However, the extract temperatures used in this study did not affect the total phenolic content of crude extract, which was 13.39-17.41 mg GAE/g. The proper condition for extracting the crude *G. atroviridis* fruit was 50% ethanol as a solvent and 40 °C as an extraction temperature. The crude extract provided the highest yield, antioxidant capacity, tyrosinase inhibition, and total phenolic content from this condition.

Keywords: *Garcinia atroviridis*, antioxidant activities, total phenol content, solvent polarity, tyrosinase inhibition



Introduction

Som-kak (*Garcinia atroviridis* Griff.) is a medium-sized plant that is twenty meters in height and belongs to the Clusiaceae family. This plant was found in tropical rainforest countries such as Thailand, Malaysia, Myanmar, and India [1]. Its fruit is yellow-orange and popular for use as a seasoning ingredient in food making sour taste. *G. atroviridis* has been used as an agent to treat cough and throat irritation and as a laxative. It is believed that it also assisted in blood circulation, such as blood pressure [2]. Moreover, the fruit is a lotion blended with vinegar, which is used to relieve stomachache associated with pregnancy by rubbing upon a woman's abdomen in confinement [1, 3].

The fruit of *G. atroviridis* contains natural fruit acids such as citric, pentadecanoic, octadecanoic, nonadecanoic, and dodecanoic. In addition, an investigation of its chemical composition revealed that it contains garcinia acid, atroviridin, atroviridone, atrovirinone, and some flavonoids, which are a group of compounds that showed widely biochemical and medicinal activities [2]. For example, the biological activities of crude extracts and pure substances from different parts of *G. atroviridis* showed antimicrobial activity [4], anti-cancer cell lines [5-7], antioxidant activities (1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays) [8, 9], enzyme cholinesterase inhibition, reducing hyperlipidemia [8], and tyrosinase inhibition [10].

Although solvent extraction has been widely used to investigate antioxidant compounds from plants, some antioxidant compounds may not be soluble in a particular solvent [8, 11, 12]. Because of the safe for humans, ethanol has generally been

used to extract plants containing high polyphenol content. There have been reported that the different extract conditions, such as solvents, extraction time, and extraction methods, resulted in different extraction yields [8, 11, 12]. In addition, the determination of antioxidant potential has been performed using different techniques because each method has a different mechanism for assessing antioxidant capacity [13]. In this study, *G. atroviridis* fruits were extracted from varying conditions: the polarity of solvent and extraction temperatures. The crude extracts from different conditions were determined and compared the extraction yield, total phenolic content (TPC), tyrosinase inhibition, and *in vitro* antioxidant activities using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The results of this study could reveal the suitable extract condition of *G. atroviridis* fruits for applying in cosmetic ingredients.

Methods and Materials

Chemicals and Reagents

All analytical grade solvents, ethanol (EtOH) and dimethyl sulfoxide (DMSO), were purchased from RCI Labscan (Bangkok, Thailand). The 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), sodium nitroprusside (SNP), sulphanilamide, naphthylenediamine dihydrochloride, 2,4,6-tripyridyl-s-triazine, Folin-ciocalteau reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), mushroom tyrosinase enzyme, kojic acid, and phosphate buffer were purchased from Sigma (Singapore). All other reagents were of analytical grade.



Plant materials

The *G. atroviridis* were collected from Yala Province, Thailand, in February 2018. The voucher specimen was compared and stored at the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand (BKF.No. 190331). The fruits of *G. atroviridis* were cleaned with deionized water, cut into small pieces, air dried, and oven at 50°C in a hot air oven (Memmert, UM 400, Germany) until stable weight (moisture content < 5 %). Before extraction, the dried sample was pulverized mill to get a 40 to 60 mesh powder.

Sample Preparation and Extraction

The *G. atroviridis* fruit powder was extracted using three concentrations of aqueous ethanol: 90%, 70%, and 50% (w/w). In addition, 10 g of dry powder was extracted with 100 mL of solvent in a 250 mL Erlenmeyer flask for each condition. The mixtures were shaken at 10, 25, and 40°C for 24 hours at 200 rpm (JSR, JSSI-100, KOREA). After extraction, the solution was filtrated, evaporated under vacuum (IKA, RV10, Germany), and freeze-dry

(VIRTIS, Benchtop Freeze dryer, USA). The crude extract powder was dissolved in DMSO and used to investigate antioxidant activities and total phenolic content.

Crude extract yield

The yield of crude extract was calculated from $\{[W_{crude}/W_{plant}] \times 100\}$, where W_{crude} was the weight of dry crude extract and W_{plant} was the dry weight of the *G. atroviridis* fruit powders.

DPPH assay

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay was performed with a slight modification [14]. The 50 uL of crude extract (1.0 mg/mL in DMSO) or Trolox (10 ug/mL in EtOH) and 150 uL of DPPH solution (0.1 mM in 50% v/v EtOH) were mixed and incubated in the absent of light at 25°C for 30 minutes. The absorbance of the mixture was read at wavelength (λ) of 517 nm (Biochrome, EZ2000, UK). The percentage inhibition of DPPH radical of each extract was calculated as the following equation:

$$\text{Inhibition (\%)} = \{[A_c - (A_s - A_b)]/A_c\} \times 100\% \quad (1)$$

where

$$A_c = 150 \text{ uL DPPH} + 50 \text{ uL DMSO}$$

$$A_s = 150 \text{ uL DPPH} + 50 \text{ uL Sample/standard}$$

$$A_b = 150 \text{ uL 50\% (v/v) EtOH} + 50 \text{ uL Sample/standard}$$

ABTS assay

The 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging assay was investigated as previously reported with a slight modification [14]. The 50 uL of crude extract (1 mg/mL) and 150 uL of ABTS solution were transferred and mixed into a 96-well

microplate. The reaction mixture was incubated in the absence of light at 25°C for 10 minutes, and the absorbance was measured at λ of 734 nm (Biochrome, EZ2000, UK). The experiment data were analyzed as percent inhibition of the ABTS•+ radical as the following equation:



$$\text{Inhibition (\%)} = \frac{[A_c - (A_s - A_b)]}{A_c} \times 100\% \quad (2)$$

where A_c = the absorbance of control (150 μ L ABTS + 50 μ L DMSO)
 A_s = the absorbance of sample (150 μ L ABTS + 50 μ L sample)
 A_b = the absorbance of blank (150 μ L Water + 50 μ L sample)

Total phenolic content

The total phenolic contents (TPC) of *G. atroviridis* fruit extract were tested using Oliveira method with slight modification [14]. The 12.5 μ L of crude extract (1 mg/mL in DMSO), 200 μ L deionized water, and 12.5 μ L Folin-Ciocalteu reagent were mixed in a 96-well microplate and shaken for 3 minutes (Eppendorf, ThermoMixer C, Germany). Next, the 25 μ L of saturated Na_2CO_3 was added, shaken for 3 minutes, and stood in the absence of light at 25°C for 60 minutes. The absorbance of the mixture was measured with a microplate reader (Biochrome, EZ2000, UK) at λ of 720 nm. TPC was expressed as gallic acid (10 – 200 μ g/mL) equivalent.

$$\text{Inhibition (\%)} = \frac{[A_{\text{control}} - (A_{\text{test sample}} - A_{\text{blank}})]}{A_{\text{control}}} \times 100\% \quad (3)$$

where A_{control} = 80 μ L phosphate buffer +40 μ L 25 mM L-DOPA + 40 μ L Tyrosinase + 40 μ L 50% DMSO
 $A_{\text{test sample}}$ = 80 μ L phosphate buffer +40 μ L 25 mM L-DOPA + 40 μ L Tyrosinase + 40 μ L Sample
 A_{Blank} = 120 μ L phosphate buffer +40 μ L 25 mM L-DOPA + 40 μ L Sample

Statistical analysis

All data analysis was performed using SPSS version 28.0 (SPSS Inc.). Values were expressed as the mean \pm standard deviation (SD) of three replications. For comparison among all groups, one-way analysis of variance (one-way ANOVA) was used and followed by Duncan multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

Tyrosinase inhibition [15]

The *G. atroviridis* fruit extracts or kojic acid were dissolved in 50% (v/v) DMSO solution (2 mg/mL). The 80 μ L of phosphate buffer (pH 6.8), 40 μ L of 25 mM L-DOPA, and 40 μ L sample or kojic acid were mixed in a 96-well microplate and shaken for 3 minutes (Eppendorf, ThermoMixer C, Germany). Next, the 40 μ L of tyrosinase enzyme (500 U/mL) was added and shaken for 10 minutes without light at 25°C. The absorbance of the mixture was measured with a microplate reader (Biochrome, EZ2000, UK) at λ of 490 nm. The experiment data were analyzed as percent inhibition of the tyrosinase enzyme as the following equation:

Results and Discussion

The yields of *G. atroviridis* fruit extract was determined with varying polarity from higher to lower (50%, 70%, and 90% w/w ethanol) at different extraction temperatures (10°C, 25°C, and 40°C). At a fixed temperature, the extract yields of *G. atroviridis* fruit were significantly increased by the varying polarity of solvents (Figure 1). According to previous reports, the effect of solvent polarity on

the percentage yields of the crude extracts depended on various factors such as plant species, chemical constituents, solvent polarity, and extraction time [16, 17]. The *G. atroviridis* fruit mainly

contained polar group substances, highly soluble in polar solvents [18]. Therefore, the increase in solvent polarity of the *G. atroviridis* fruit extraction process provided a higher yield of crude extract.

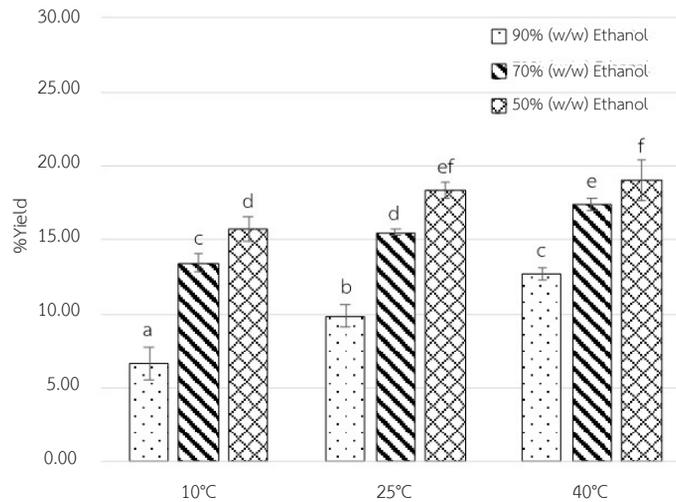


Figure 1 Effect of solvent polarity and temperature on *G. atroviridis* fruit extract yield. Data are expressed as mean \pm SD. Different letters indicate significant differences between groups using one-way ANOVA ($P < 0.05$) followed by Duncan multiple comparison test. $n = 3$ per group.

The DPPH antioxidant assay of *G. atroviridis* extracts and Trolox (20 $\mu\text{g/mL}$), a standard compound, were compared (Figure 2). The antioxidant activities of the extracts reported as percent inhibitions were higher when the extraction temperature and the polarity of solvent were increased. At 40°C, the extracts showed the highest antioxidant level at 34.48 ± 2.28 , 37.58 ± 2.13 , and 41.01 ± 4.17 , for 90%, 70%, and 50% (w/w) of ethanol, respectively. The crude extracted at 40°C and 50% (w/w) ethanol

exhibited the highest antioxidant activity, similar to the Trolox value (47.10 ± 0.46). While the crude extracted at room temperature (25°C) displayed lower antioxidant activity at the percent of inhibition of 28.74 ± 2.18 , 29.75 ± 1.57 , and 34.59 ± 2.40 , for 90%, 70%, and 50% (w/w) of ethanol, respectively. The crude extracted at the cool condition (10°C) showed a low percent of DPPH inhibition instead at 24.61 ± 1.77 , 30.72 ± 2.88 , and 33.28 ± 3.28 , for 90%, 70%, and 50% (w/w) of ethanol, respectively.

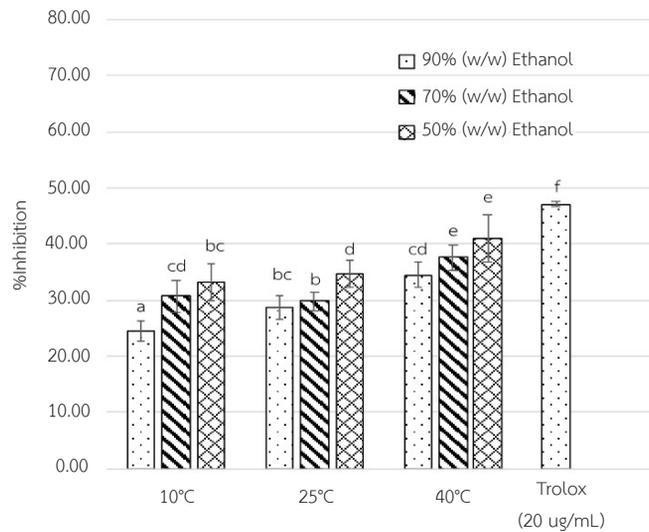


Figure 2 Effect of solvent polarity and temperature on radical scavenging activity (DPPH) of *G. atroviridis* fruit extract. Data are expressed as mean \pm SD. Different letters indicate significant differences between groups using one-way ANOVA ($P < 0.05$) followed by Duncan multiple comparison test. $n = 3$ per group.

The antioxidant capacity of *G. atroviridis* fruit extracts was also performed using ABTS assay. The ABTS \bullet + radical can be soluble in both water and polar organic solvents and can be used over a wide pH range. Whereas ABTS \bullet + reacts rapidly with antioxidants, the DPPH free radical was relatively stable compared to the free radicals from reactions born in the body [19]. From the previous reports, the chemical constituents of *G. atroviridis* fruits contained many organic acids high in polarity and can be sensitive to free radicals using various antioxidant assays [13]. The scavenging effect of the crudes extracted from various conditions to the ABTS \bullet + radical was shown in Figure 3 as percent inhibition. The *G. atroviridis* fruits extracted at 40°C and 50% (w/w) of ethanol displayed the highest antioxidant activity at 27.74 ± 0.84 . While Trolox, the standard compound (40 μ g/mL), presented a

percent inhibition value to the ABTS \bullet + radical at 22.79 ± 2.00 . The *G. atroviridis* fruits extracted at 40°C with the solvent having less polarity (90% and 70% (w/w) of ethanol) manifested lower antioxidant efficiency at 15.23 ± 2.00 and 19.29 ± 0.88 , respectively. The antioxidant activity of other *G. atroviridis* fruit extracts from other extraction conditions (10°C and 25°C) exhibited a similar trend as mentioned above. The crude extracted at room temperature (25°C) displayed lower antioxidant activity at the percent of inhibition of 12.90 ± 2.30 , 17.86 ± 1.43 , and 22.51 ± 1.15 for 90%, 70%, and 50% (w/w) of ethanol, respectively. While the crude extracted at the cool condition (10°C) showed a relatively low percent of ABTS \bullet + radical inhibition at 13.83 ± 1.60 , 16.09 ± 0.64 , and 19.62 ± 0.69 , for 90%, 70%, and 50% (w/w) of ethanol, respectively.

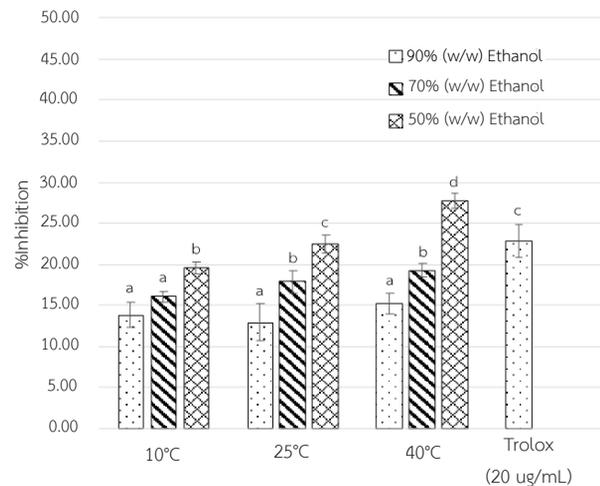


Figure 3 Effect of solvent polarity and temperature on radical scavenging activity (ABTS) of *G. atroviridis* fruit extract. Data are expressed as mean \pm SD. Different letters indicate significant differences between groups using one-way ANOVA ($P < 0.05$) followed by Duncan multiple comparison test. $n = 3$ per group.

The tyrosinase enzyme inhibition of *G. atroviridis* fruit extracts using different temperatures and ethanol ratios are shown in Figure 4. Their tyrosinase inhibition percentage of them was 42.88 – 56.13. The crude extracts temperature of 10 °C showed tyrosinase inhibition efficiency at 54.54 ± 0.55 , 53.10 ± 1.26 , and 52.27 ± 1.55 for the ethanol

ratio of 90, 70, and 50 (w/w), respectively. The tyrosinase inhibitory efficacy of crude extract at 25°C was 53.91 ± 0.77 , 52.74 ± 0.56 and 55.48 ± 0.33 , respectively. In addition, an extraction temperature of 40 °C showed an essential factor in the anti-tyrosinase efficacy of *G. atroviridis* fruit extracts.

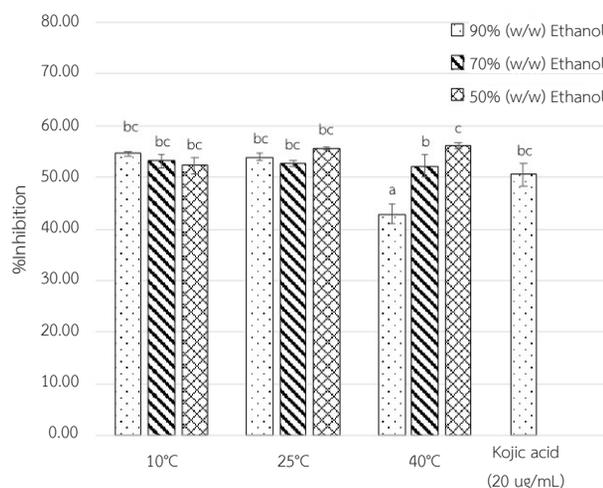


Figure 4 Effect of solvent polarity and temperature on tyrosinase inhibition of *G. atroviridis* fruit extract. Data are expressed as mean \pm SD. Different letters indicate significant differences between groups using one-way ANOVA ($P < 0.05$) followed by Duncan multiple comparison test. $n = 3$ per group.



Phenolic compounds, bioactive constituents present in many plants, exhibit antioxidant effects [18]. In this study, total phenolic contents (TPC) of *G. atroviridis* fruit extracts from different temperatures and the solvent polarity

were informed in Figure 5. It was found that the crude extracted at fixed temperature with lower to higher polar solvents (from 90%, 70%, and 50% w/w of ethanol) displayed consecutive increases in TPC.

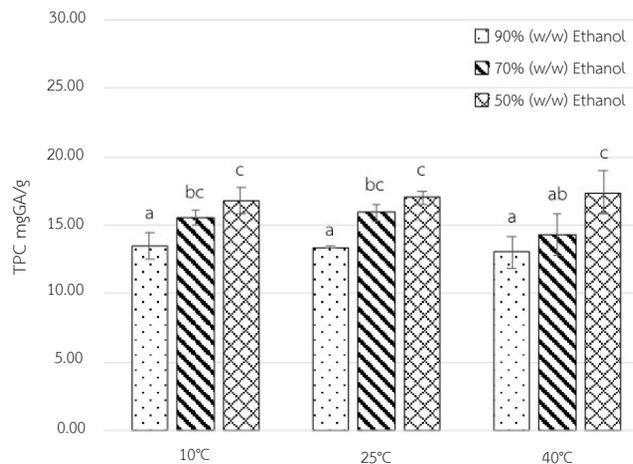


Figure 5 Effect of solvent polarity and temperature on total phenolic content (TPC) of *G. atroviridis* fruit extract. Data are expressed as mean ± SD. Different letters indicate significant differences between groups using one-way ANOVA ($P < 0.05$) followed by Duncan multiple comparison test. $n = 3$ per group.

Table 1 DPPH, ABTS, tyrosinase inhibitor and TPC of *G. atroviridis* fruit extract.

Sample		% Inhibition			TPC
Solvent	Temperature	DPPH	ABTS	Tyrosinase inhibitor	(mgGAE/g)
90% w/w ethanol	10°C	24.61 ± 1.77	13.83 ± 1.60	54.54 ± 0.55	13.47 ± 0.96
	25°C	28.74 ± 2.18	12.90 ± 2.30	53.91 ± 0.77	13.39 ± 0.05
	40°C	34.48 ± 2.28	15.23 ± 1.23	42.88 ± 1.92	13.03 ± 1.16
70% w/w ethanol	10°C	30.72 ± 2.88	16.09 ± 0.64	53.10 ± 1.26	15.56 ± 0.50
	25°C	29.75 ± 1.57	17.86 ± 1.43	52.74 ± 0.56	15.91 ± 0.68
	40°C	37.58 ± 2.13	19.29 ± 0.88	52.11 ± 2.21	14.31 ± 1.56
50% w/w ethanol	10°C	33.28 ± 3.28	19.62 ± 0.69	52.27 ± 1.55	16.75 ± 0.97
	25°C	34.59 ± 2.40	22.51 ± 1.15	55.48 ± 0.33	17.05 ± 0.50
	40°C	41.01 ± 4.17	27.74 ± 0.48	56.13 ± 0.63	17.41 ± 1.61
Trolox (20 ug/mL)	-	47.10 ± 0.46	-	-	-
Trolox (40 ug/mL)	-	-	22.79 ± 2.00	-	-
Kojic acid (20 ug/mL)	-	-	-	50.47 ± 2.10	-



Conclusion and Suggestions

Solvent extraction is a widespread technique to obtain a crude extract from various plants. The optimum extraction conditions for individual plants, such as temperature, extraction time, and polarity of the solvent, were investigated. These extraction factors significantly influenced the extracts' yield and biological activities, especially the plants containing polyphenol compounds. Some polyphenol compounds were less stable and could degrade throughout the extraction process. Overheating may be affected by the loss of bioactivities, possibly relating to the decomposition of polyphenols, resulting in a decrease in total phenolic content and loss of antioxidant activity [18]. From previous reports, the effect of temperature on the extraction of *G. atroviridis* by solvent has not been reported. Only the drying temperature of slice *G. atroviridis* fruit was reported that using a high temperature greater than 50 °C could reduce vitamin C and acid contents [20]. This study found that only slight differences in the total phenolic content were displayed by increasing extraction temperatures. This may be because of the other compounds presenting in *G. atroviridis* fruit. Organic acids and sugars were the primary chemical composition [13]. There was citric acid, tartaric acid, malic acid, ascorbic acid, and so on [13]. Because of their high polarity, their solubilities in the high polar solvent could increase when raising the temperatures resulting in higher percentage yields but could not affect the total phenolic content.

The significant increase in extract yield, antioxidant activities, tyrosinase inhibition, and the total phenolic content of the crude was revealed

by increasing the solvent polarity of the extraction, reducing the ethanol ratio, and replacing it with water. The reason could be from polyphenol compounds and many organic acids present in *G. atroviridis* fruit. In conclusion, the suitable extraction condition of *G. atroviridis* fruits was using 50% ethanol as a solvent and 40 °C as an extraction temperature. This condition provided the highest yield, antioxidant capacity, tyrosinase inhibition, and total phenolic content. From the above data, antioxidant and tyrosinase inhibition activities of *G. atroviridis* fruit extract that are beneficial as active ingredients for cosmetic products.

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