Total Phenolic, Flavonoid Contents and Antioxidant Activity of *Siraitia grosvenorii* Fruits Extracts

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Abstract

The fruit of *Siraitia grosvenorii* (Luo hanguo) is commonly used as a beverage material and traditional medicine. *S. grosvenorii* has been used in China as a folk remedy for the treatment of common cold, pharyngitis, and pulmonary congestion. Previous studies showed that *S. grosvenorii* extracts possess properties of anti-asthmatic, anti-cancer, anti-diabetic, anti-obesity, anti-oxidative, anti-inflammation and immunoregulating effects, but the possibility of *S. grosvenorii* for using as cosmetic ingredient has not been investigated. The purpose of this study was to find a suitable solvent to produce *S. grosvenorii* extract with contained antioxidant property. *S. grosvenorii* fruit was extracted by macerating in solvents including distilled water, propylene glycol, 95% ethanol, ethyl acetate, and *n*-hexane. The extracts were evaluated for their total phenolic content, total flavonoid content, antioxidant activities and physical color properties. The results showed that 95% ethanol extract exhibited the highest total phenolic content (2.387 ± 0.063 mg GAE/mg solid crude) compared to other solvents. However, distilled water extract showed the greatest amount of total flavonoid content (25.229 ± 0.904 µg QE/mg solid crude) and the highest value of antioxidant activities determined by DPPH assay (47.396 ± 1.946 µg TEAC/mg solid crude) and ABTS assay (53.997 ± 0.155 µg AEAC/mg solid crude). These results indicated that the antioxidant activity of *S. grosvenorii* extracts might be attributed to the presence of flavonoid compounds. It can be concluded that distilled water extract of *S. grosvenorii* might be suitable to apply as an antioxidant ingredient for further applications.

Keywords: ABTS, DPPH, Flavonoids, Phenolic compound, *Siraitia grosvenorii*

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1. Introduction

Siraitia grosvenorii (Luo hanguo or monk fruit) is a member of the Cucurbitaceae family (Xia *et al*., 2018). The fruit of *S. grosvenorii*, has been used for centuries as beverage material and traditional medicine (Qing *et al*., 2017). *S. grosvenorii* has been used in China as a folk remedy for the treatment of common cold, pharyngitis, and pulmonary congestion (Li and Zhang, 2000). The studies have shown that *S. grosvenorii* extracts possess properties of anti-asthmatic, anti-cancer, anti-diabetic, anti-obesity, anti-oxidative, anti-inflammation and immunoregulating effects (Lin *et al*., 2007; Zhang *et al*., 2006; Prakash and Chaturvedula, 2014; Wang *et al*., 2014; Zhang *et al*., 2011). *S. grosvenorii* is a good source of triterpenoids, vitamins, proteins, polysaccharides, and volatile oil (Zhang and Li, 2011). The presence of various phenolic compounds, phenolic acid, anthraquinones, alkaloids, sterols, aliphatic acids and flavonoids such as kaempferol, quercetin and vanillic acid in the fruit, leaves, stems, and flowers of *S. grosvenorii* has been reported (Ji, 2016; Li *et al*., 2014). The ripe fruit of *S. grosvenorii* contains mogrosides, for example, mogroside III, mogroside IV, siamennoside I, mogroside V and 11-oxomogroside V (Li *et al*., 2006; Zhang *et al*., 2012). Mogrosides are purified and used as a non-caloric, non-sugar sweetener in the United States and Japan, since they are estimated to be approximately 300 times more than sucrose (Qing *et al*., 2017). *S. grosvenorii* was also used as a substitute sugar for obese and diabetic patients (Zhang *et al*., 2006). Currently, *S. grosvenorii* products have been approved as dietary supplements in Australia, Japan, New Zealand and the United States (Li *et al*., 2014).

The reactive oxygen species (ROS) and free radicals can cause considerable damage to human health and cause many diseases like cancer, coronary artery disease, arteriosclerosis, inflammatory disorders, and aging diseases (Wang *et al*., 2007). ROS can occur in human skin that induce with ultraviolet radiation, leading to skin aging such as age-spots, loss of skin tone, photo-aging, sagging skin and wrinkle (Yasui and Sakurai, 2003; Lephart, 2016). Moreover, ROS can damage skin cell, leading to mutation that contribute to skin cancer (Watson *et al*., 2016; Robertson and Fitzgerald, 2017). Antioxidants could decrease or forbid the oxidation of macro molecules in cell by inhibiting the oxidative chain reactions (Young and Woodside, 2001). The natural antioxidants are found in foods, fruits, vegetables, cereals, spices, and herbs which have shown strong free radical scavenging abilities (Xu *et al*., 2017). The *in vitro* study with different solvents extraction (water, ethanol, ethyl acetate, and chloroform) from the stem of *S. grosvenorii* exhibited excellent antioxidant activity superior to the control butylatedhydroxytoluene (BHT) (Li *et al*., 2014). However, there is no report about the antioxidant activity in *S. grosvenorii* fruit using different extracting solvents.

Since *S.grosvenorii* has long been used as traditional herbal medicine and dietary supplement, but the possibility of *S. grosvenorii* for using as cosmetic ingredient has not been investigated. Therefore, the aims of this study were to find the suitable solvents for *S. grosvenorii* extraction, determination of total phenolic and flavonoids contents as well as evaluated the properties such as antioxidant activity and color. The results of this study might be supported for further use of *S. grosvenorii* in cosmetic.

2. Materials and Methods

2.1 Chemicals

Folin-Ciocalteu reagent, gallic acid, quercetin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8,-tetramethyl chroman-2-carboxylic acid (Trolox), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. All other chemicals and solvents used are analytical grade.

2.2 Plant material

Dried *S. grosvenorii* fruits were purchased from Siam Makro Public Company Limited (Chiang Rai, Thailand).

2.3 Preparation of *S. grosvenorii* extracts

Dried *S. grosvenorii* fruits were ground in a blender (Panasonic MX-J210GN), then the powder was macerated with different solvents (distilled water, *n*-hexane, ethyl acetate, 95% ethanol and propylene glycol) by sample to solvent ratio of 1.5 (w/v). All samples except water extracted one were vigorous shaken (120 rpm) for 24 h at room temperature (25 °C). The extracts were then filtrated by using suction filtration with Whatman® paper filter no 1. For water extraction, *S. grosvenorii* powder was boiled in 80°C distilled water (1.5 w/v) for 1 h. The filtrate was collected using suction filtration through filter paper (Liu *et al*., 2013). All extracts were covered with aluminum foil and stored at 4 °C.

2.4 Determination of total phenolic content (TPC)

The total phenolic content was determined by modified method from Kanlayavattanakul *et al*. (2012). The extracts (20 µL) were mixed with Folin-Ciocalteu reagent (100 µL) in a 96-well plate, followed by the addition of sodium carbonate (7.5% w/v, 80 µL). The volume of the mixture was adjusted to 200 µL with distilled water. The solution was then incubated at ambient temperature for 1 h. The absorbance was measured at 765 nm by using microplate reader (SPECTROstar Nano, BMG LABTECH). Gallic acid (0.001 to 0.01 mg/mL) was used as a standard. The total phenolic content in each extract was compared with the standard curve and expressed as mg of gallic acid equivalents per 1 mg of solid crude extract (mg GAE/mg solid crude). The procedure was repeated in triplicate.

2.5 Determination of total flavonoid content (TFC)

Total flavonoids content was determined according to the colorimetric assay modified from Kamtekar *et al*. (2014). Quercetin was used as a standard at concentrations of 0.008 to 0.08 mg/mL. For determination, 0.5 mL of extract was mixed with 3.0 mL of distilled water, 0.3 mL of 5% (w/v) sodium nitrite was properly mixed and allowed to stand for 5 min at room temperature. Then, 0.6 mL of 10% (w/v) aluminium chloride was added followed by 2 mL of 1 M sodium hydroxide and incubated for 6 min. The absorbance was read at 510 nm using UV/Vis spectrophotometer (Libra S22, Biochrom). A standard curve was obtained by plotting absorbance against the quercetin concentration. Results were expressed as µg of quercetin equivalents per 1 mg of solid crude extract (µg QE/mg solid crude). The procedure was repeated in triplicate.

2.6 Determination of DPPH radical scavenging activity

Antioxidant activity was assessed by using the DPPH assay as method modified from Kanlayavattanakul *et al*. (2012). Trolox was used as a standard and prepared in 95% ethanol at concentrations of 1.25 to 12.50 µg/mL. For determination, 50 µL of extract was well mixed with 150 µL of 0.3 mM $DPPH^{\bullet}$. The mixture was then incubated 30 min in the dark. A microplate reader was used to monitor the reduction of $DPPH^{\bullet}$ at 517 nm. The content of antioxidants in the extract was determined as a percentage decrease of color intensity (% inhibition) and calculated according to the formula:

$$\% \text{ inhibition} = [A_{control} - A_{sample}]/A_{control} \times 100$$

where A_{sample} is the absorbance of $DPPH^{\bullet}$ solution mixed with extract, and $A_{control}$ is the absorbance of $DPPH^{\bullet}$ solution without extract.

The total antioxidant capacity of extract was expressed as a Trolox equivalent antioxidant capacity (TEAC) per mg of solid crude extract (µg TEAC/mg solid crude).

2.7 Determination of $ABTS^{\bullet+}$ scavenging activity

Radical scavenging activity against ABTS assay was conducted by the method of Re *et al*. (1999) with a slight modification. 7 mM ABTS was dissolved in distilled water, and mixed with 2.45 mM potassium persulfate. The mixture was incubated in the dark at room temperature for 16 h to obtain ABTS radical cation ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted with phosphate buffer (50 mM, pH 7.40) to an absorbance of 0.700 ± 0.020 at 734 nm. For determination, 180 µL of $ABTS^{\bullet+}$ solution was well mixed with 20 µL of sample. The mixture was then incubated for 30 min. Ascorbic acid was used as the standard antioxidant that were prepared in distilled water at concentrations of 0.05 to 0.4 µg/mL. A microplate reader was used to monitor the reduction of $ABTS^{\bullet+}$ at 734 nm. The content of antioxidants in the extract was determined as a percentage decrease of color intensity

(% inhibition) and calculated according to the formula:

$$\% \text{ inhibition} = [A_{control} - A_{sample}]/A_{control} \times 100$$

where A_{sample} is the absorbance of $ABTS^{\bullet+}$ solution mixed with extract, and $A_{control}$ is the absorbance of $ABTS^{\bullet+}$solution without extract.

The total antioxidant capacity of extract was expressed as an ascorbic acid equivalent antioxidant capacity (AEAC) per 1 mg of solid crude extract (μg AEAC/mg solid crude).

2.8 Physical color determination

The color of the crude extract was determined using chromameter (Konica Minolta/CR-400). Color difference was defined as L*, a*, and b* color system. L*, a*, and b* color scale were recorded, L* shows the lightness of the color from black to white (L* = 0-100), a* shows the position between red (+) and green (-), and b* shows the position between yellow (+) and blue (-). The numerical total color difference ΔE was used to indicate the overall color differences from the water extract and calculated according to the formula:

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

where ΔE between 2.00 to 3.50 represents medium difference, and obvious to an untrained eye. ΔE between 1.00 to 2.00 represents very small difference, and only obvious to a trained eye (Ji, 2016).

2.9 Statistical analysis

One-way analysis of variance (ANOVA), Post Hoc multiple comparisons by Duncan's multiple-range test was used to evaluate the difference between groups. The level of significance was at $p < 0.05$. A linear correlation analysis was performed in order to determine relationship between TPC, TFC and antioxidant activities.

3. Results and Discussion

3.1 Total phenolic content (TPC)

The total phenolic content was estimated and expressed as mg GAE/mg solid crude. Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee et al., 2005). The total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Their free radical scavenging ability is facilitated by their hydroxyl groups. From Table 1, the results showed that the total phenolic content ranged from 0.152 ± 0.024 to 2.387 ± 0.063 mg GAE/mg solid crude. The different solvents have different polarities. 95% Ethanol extract showed the highest phenolic content (2.387 ± 0.063 mg GAE/mg solid crude) compared to other extracts. The total phenolic content for each solvent was different because of the different solvent polarities. The solvent polarity is very important in increasing the solubility of phenolic compounds. *n*-Hexane is nonpolar solvents and produce

lower phenolic content than ethyl acetate, distilled water, propylene glycol, and 95% ethanol. Due to *n*-hexane and ethyl acetate extracts provided the significant lower amount of TPC as compared to other solvents, then they were not determined in further experiments. Water is the most polar solvent but it not suitable solvent for phenolic extraction in this study. Other studies revealed that ethanol and water mixture were more effective in extracting phenolic compounds than water (Tomsone *et al*., 2012). Ethanol and water mixtures are commonly used for the extraction of phenols from plant materials due to the wide range of phenols that the ethanol and water mixtures can dissolve (Alothman *et al*., 2009). Li et al. (2014) found that vanillic acid is an important phenolic compound in *S. grosvenorii*.

Table 1. Total phenolic content, total flavonoids content and antioxidant activity of *S. grosvenorii* fruit extracts.

Extract	Total phenolic content (mg GAE/mg solid crude)	Total flavonoids content (µg QE/mg solid crude)	DPPH assay (µg TEAC/mg solid crude)	ABTS assay (µg AEAC/mg solid crude)
Distilled water	1.112 ± 0.031^{c}	25.229 ± 0.904^{b}	47.396 ± 1.946^{c}	53.997 ± 0.155^{c}
Propylene glycol	1.289 ± 0.073^{d}	11.825 ± 1.097^{a}	25.604 ± 0.825^{b}	47.464 ± 1.719^{b}
95% ethanol	2.387 ± 0.063^{e}	13.452 ± 0.452^{a}	5.430 ± 0.001^{a}	1.097 ± 0.028^{a}
Ethyl acetate	0.382 ± 0.063^{a}	-	-	-
n-hexane	0.152 ± 0.024^{b}	-	-	-

Note: - = no test. Each value was expressed as the mean ± S.D. (n=3). Different letters in the same column indicate significant differences ($p < 0.05$).

3.2 Total flavonoid content (TFC)

The total flavonoids content in the extract was determined by spectrophotometric method with aluminum chloride. The most abundant flavonoid which has a good antioxidant property is quercetin. The flavonoids content was expressed in terms of µg QE/mg solid crude (Table 1). The flavonoids concentration in *S. grosvenorii* extracts ranged from 11.825 ± 1.097 to 25.229 ± 0.904 µg QE/mg solid crude. The highest content of flavonoid was from distilled water extract (25.229 ± 0.904 µg QE/mg solid crude). The results presented that the solvent polarity is very important in increasing the solubility of flavonoid compounds. Previous study reported that the main flavonoids compound isolated from *S. grosvenorii* are kaempferol and quercetin derivatives (Li *et al*., 2014). However, further research is needed to identify the water soluble flavonoids components in *S. grosvenorii*.

3.3 Determination of DPPH radical scavenging activity

The free radical scavenging activities of the extracts were determined using DPPH assay and the results were displayed in Table 1. DPPH assay, radical scavenging method offers the first approach for evaluating the antioxidant potential of plant extract. This assay measures the ability of the plant extract to donate an electron or H^+ ion. The $DPPH^•$ is a stable free radical and shows maximum absorbance at 517 nm. $DPPH^•$ accepts an electron or a hydrogen from antioxidant molecules to become a stable molecule resulting in a decrease in absorbance (Ahmed *et al*., 2013). In this study, the results were expressed with TEAC range from 5.430 ± 0.001 to 47.396 ± 1.946 µg TEAC/mg solid crude. The highest antioxidant activity is from distilled water extract (47.396 ± 1.946 µg TEAC/mg solid crude) which has the highest polarity as compared with propylene glycol and ethanol. Then, it can be concluded that $DPPH^•$ scavenging activity increase by an increased polarity of solvent (Marinova and Yanishlieva, 1997; Tomsone *et al*., 2012).

3.4 Determination of $ABTS^{•+}$ scavenging activity

ABTS assay depends on the antioxidant compound ability to scavenge $ABTS^{•+}$. This assay can measure antioxidant capacity of hydrophilic compounds. In the same way, $ABTS^{•+}$ inhibition mechanism was similar to DPPH assay. The highest antioxidant activity is from distilled water extract (53.997 ± 0.155 µg AEAC/mg solid solid crude) followed by propylene glycol extract (47.464 ± 1.719 µg AEAC/mg solid crude) and 95% ethanol extract (1.097 ± 0.028 µg AEAC/mg solid crude). This result was in consistent with that of DPPH assay. The antioxidant activities of extracts are dependent on the solvent polarity, due to the different antioxidant potentials of compounds with different polarity (Marinova and Yanishlieva, 1997; Tomsone *et al*., 2012). The results revealed that distilled water extract exhibited the highest antioxidant activities, then it is the most interesting for further used as antioxidant ingredients in cosmetic.

3.5 Physical color determination

The color measurements of the extracts were shown in Table 2. There were significant differences ($p < 0.05$) between the L*, a* and b* values of the extracts obtained from different solvents. L* value shows the lightness of the color from black to white (L* = 0-100). The data in Table 2 showed propylene glycol extract presences the lowest L* value which the most darkened color when compared with other solvents. In contrast, the highest L* value is 95% ethanol extract which shows the lightness color. The a* value shows the position between red (+) and green (-). It was found that all of the extracts have negative value with green shade. The last value is b* that shows the position between yellow (+) and blue (-). The b* value of all solvent extracts were positive value that presence of yellow shade. The ΔE values indicated that 95% ethanol (ΔE = 2.724 ± 0.703), propylene glycol (ΔE = 2.064 ± 0.483), and ethyl

acetate (ΔE = 2.022 ± 0.528) extracts showed medium difference with obvious to an untrained eye when compared with water extract. While *n*-hexane extract (ΔE = 1.687 ± 0.528) exhibited very small difference only obvious to a trained eye (Ji, 2016).

Table 2. Physical color of *S. grosvenorii* fruit extract

Extract	Color parameter			ΔE
	L* value	a* value	b* value	
Distilled water	24.027 ± 0.801^{b}	-0.053 ± 0.006^{d}	0.283 ± 0.006^{c}	-
Propylene glycol	21.970 ± 0.625^{a}	-0.063 ± 0.015^{d}	0.117 ± 0.015^{a}	2.064 ± 0.483
95% Ethanol	26.717 ± 0.422^{d}	-0.470 ± 0.046^{b}	0.230 ± 0.010^{b}	2.724 ± 0.703
Ethyl acetate	25.693 ± 0.227^{cd}	-0.230 ± 0.010^{c}	0.417 ± 0.015^{e}	2.022 ± 0.528
n-Hexane	25.110 ± 0.716^{bc}	-0.630 ± 0.020^{a}	1.400 ± 0.026^{d}	1.687 ± 0.893

Note: Each value was expressed as the mean ± S.D. (n=3). Different letters in the same column indicate significant differences ($p < 0.05$).

Additionally, the color of the extracts was dark brown. The physical colors of the extracts were obviously different by naked eyes depending on chemicals which were extracted by each solvent. The organic solvents used to extract active molecules from a plant also extract the molecules responsible for the plant's color (Plainfossé, *et al*., 2018). Propylene glycol and distilled water extract are slightly different that propylene glycol extract is darker than distilled water extract. 95% ethanol extract has golden yellow color. Ethyl acetate and *n*-hexane extracts were slightly similar pale yellow and clear translucent. However, natural extract with high intensity color cannot be directly applied to the cosmetic because of their color may display undesirable color which are generally unacceptable in skin care products (Byun, *et al*., 2004). Therefore, it is recommended to further processing, including a discoloration procedure or find the suitable concentration for adding *S. grosvenorii* in cosmetic.

3.6 Correlation between total phenolic, flavonoids content and antioxidant activity

The correlation between the phenolic and flavonoid content, and their antioxidant activities were done and displayed in correlation coefficients (R^2) values from linear regression analysis. The results showed that TPC has negative correlation with $DPPH^{\bullet}$ antioxidant activity (R^2 = 0.8369) and $ABTS^{\bullet+}$ antioxidant activity (R^2 = 0.9997). These results suggest that the antioxidant activity might be attributed to the presence of non-phenolic compounds and crude extract might be contained other synergistic or antagonistic compounds (Tomsone *et al*., 2012). On the other hand, TFC has positive correlation with $DPPH^{\bullet}$ antioxidant activity (R^2 = 0.6698) but no correlation with $ABTS^{\bullet+}$ antioxidant activity (R^2 = 0.2523). These results suggested that

the antioxidant activity of *S. grosvenorii* extracts might be attributed to the presence of flavonoid compounds. Furthermore, flavonoids are more react with $DPPH^{\bullet}$ better than $ABTS^{\bullet+}$. Thus, the total flavonoids content can be used to predict the antioxidant activity of *S. grosvenorii*. However, further investigation is needed to perform to evaluate the presence of non-phenolic type antioxidants.

4. Conclusion

S. grosvenorii fruit showed good result in total phenolic content, total flavonoids content, and antioxidant activity. The solid crude extract by distilled water found to be the most interesting for further used as antioxidant ingredients in cosmetic due to the present of the highest antioxidant activity. Further studies need to perform for improvement of this study such as determination of suitable condition for water extraction. In addition, other techniques or other instruments are recommended for determining of active compounds in *S. grosvenorii* fruit extract.

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