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Assessment on the antioxidant, photoprotective, and antimicrobial properties of *Siraitia grosvenorii*

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ABSTRACT: Exogenous antioxidants from plant-based sources, such as polyphenols, show potential in maintaining a balance of cellular oxidation in biological systems. Additionally, the rise of antimicrobial resistance and the harmful effects of UV radiation on skin necessitate the exploration of medicinal plants for drug development and photoprotective agents. This study specifically focused on *Siraitia grosvenorii* or Luo Han Guo, and aimed to assess its phenolic and flavonoid content, as well as its antioxidant, antimicrobial, and photoprotective properties. The study involved the preparation of *Siraitia grosvenorii* extracts from seeds and fruit skins, determination of total phenolic and flavonoid content using Folin-Ciocalteu reagent and $AlCl_3$, evaluation of antioxidant activity using the DPPH method, and analysis of antimicrobial activity against *Escherichia coli* using turbidity assays. Photoprotective activity was examined by calculating the Sun Protection Factor (SPF) using a UV spectrophotometer and a normalized function. The results showed that fruit skin of *Siraitia grosvenorii* had a higher total phenolic content than the seed, and no significant difference in flavonoid content between seed and fruit skin extract. Additionally, *Siraitia grosvenorii* fruit skin and seed had low SPF value and DPPH reduction. Both fruit skin and seed showed highest antimicrobial activity at the concentration of 100 ppm.

KEYWORDS: Antibacterial; flavonoid; DPPH; luo han guo; phenolic.

INTRODUCTION

In biological systems, reactive oxygen species (ROS) and reactive nitrogen species (RNS) can cause damage to DNA, leading to lipid and protein oxidation within cells. Normally, the body's antioxidant system can neutralize these radicals, by maintaining a balance between oxidation and antioxidation[1]. However, exposure to factors such as smoking, alcohol, radiation, or environmental toxins induces excessive production of ROS and RNS, disrupting the balance and resulting in various chronic and degenerative diseases. Exogenous antioxidants, primarily derived from plant-based foods and medicines, including fruits, vegetables, mushrooms, flowers, spices, and traditional medicinal plants, have the potential to serve as crucial natural antioxidant sources. The natural antioxidants from these plant materials are primarily polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids, and vitamins. In general, these natural antioxidants, particularly polyphenols and carotenoids, exhibit various biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer properties[2].

Besides ROS, diseases caused by pathogenic microorganisms pose a critical health issue, with antimicrobial resistance posing a serious threat to human well-being. Researchers are increasingly focusing on medicinal plants to develop improved drugs and antibiotic sources against microbial infections[3]. Also, Ultraviolet (UV) radiation, specifically UV-A and UV-B, can have various harmful effects, laying the groundwork for skin conditions such as erythema, sunburn, molecular-level damage (DNA or proteins), and even premature skin aging[4]. Therefore, protecting the skin from UV rays is crucial, involving the use of chemical compounds that absorb radiation. Currently, natural herbal compounds with antioxidant properties are applied in the field of phyto-cosmetics as sunscreens. These molecules could neutralize ROS and prevent skin damage from erythema and premature aging[5]. Photoprotective agents derived from plants can provide skin protection and offer health benefits.

Siraitia grosvenorii, originating from Southern China, is renowned for its fruit, Luo Han Guo, which is nearly 300 times sweeter than sucrose. It has been used as a natural sweetener in China for almost a millennium

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and has also been employed in traditional Chinese medicine for treating colds and sore throats[6]. Additionally, previous research reported that Luo Han Guo can effectively reduce inflammation caused by tracheal intubation during anesthesia[7]. Pharmacological studies on Luo Han Guo demonstrate its potential antidiabetic, anticarcinogenic, antibacterial, antioxidant, and antiallergic effects. Overall, *S. grosvenorii* holds promise as a significant source of pharmaceutical compounds and sweeteners for various food products[8].

The urgency of this research arises from the increasing prevalence of skin-related issues and the growing demand for natural products with multifunctional properties. *S. grosvenorii,* known for its traditional medicinal uses, has the potential to provide effective solutions for combating oxidative stress, skin inflammation, and microbial infections. Emphasizing the necessity for safe, natural alternatives in skincare and health products not only engages the audience but also underscore the relevance of our findings which focused on evaluating *Siraitia grosvenorii* for its total phenolic and flavonoid content, as well as its properties, including antioxidant, antimicrobial, and photoprotective activities.

▪ MATERIALS AND METHODS

Materials

Ethanol 96%, dimethyl sulfoxide (DMSO) (Merck, Darmstadt, German), Folin-Ciocalteu reagent (Merck, Darmstadt, German), sodium carbonate (Na_2CO_3) (Merck, Darmstadt, German), gallic acid (Merck, Darmstadt, German), aluminium chloride ($AlCl_3$) (Merck, Darmstadt, German), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, Missouri, USA), quercetin (Sigma, Missouri, USA).

Preparation of *Siraitia grosvenorii* extract

Seeds and fruit skins of *S. grosvenorii* were collected, powdered, and extracted with 70% ethanol in an ultrasonicator for 20 minutes. The extract was then filtered using filter paper and evaporated. After evaporation, the filtrate was dissolved in DMSO to achieve a final concentration of 1 g/mL as a stock solution. Stock solution was stored at room temperature for further analysis.

Determination of Total Phenolic Contents

Both skin and seed extract were made into a concentration of 500 ppm. A volume of 300 µL of both extracts were added with 300 µL 10% Folin-Ciocalteu reagent. After 5 min of incubation, 300 µL of 7.5% Na_2CO_3 and 120 µL of distilled water were added. The solution was incubated for 30 min under dark conditions. The absorbance of the solution was measured using a UV spectrophotometer at 765 nm. Gallic acid with concentration of 20, 40, 60, 80 ppm was used as a standard and distilled water was used as a blank.

Determination of Total Flavonoid Contents

Both skin and seed extract were made into a concentration of 500 ppm. Standard curve was made by quercetin with concentration 0 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm using distilled water. Extract and each concentration of standard curve were added 2% $AlCl_3$ with a volume ratio of 1:1. The sample was incubated for 10 minutes under dark conditions and the absorbance was measured using a UV spectrophotometer at 425 nm.

Antioxidant activity of *Siraitia grosvenorii*

Antioxidant activity was measured using the DPPH method. Both skin and seed extract were made into a concentration of 500 ppm, 400 ppm, 300 ppm, 200 ppm, and 100 ppm. The extracts were then centrifuged at 5000 xg for 15 minutes. Each extract concentration was added with 0.1 mM DPPH with ratio 1:1. Control was made with ratio 1:1 of DPPH and EtOH. Blank was made by mixture of extract and EtOH based on each concentration of extract. The sample was incubated for 30 minutes under dark conditions and measured the

absorbance using a UV spectrophotometer at 517 nm. Percentage of DPPH reduction was calculated by the following formula:

% DPPH reduction = (Absorbance Control − Absorbance Sample)/Absorbance Control x 100%

Photoprotective activity of *Siraitia grosvenorii*

The photoprotective activity was assessed based on the method from Mansur et al. [9]. Both skin and seed extract were made into a concentration of 5000 ppm using EtOH. The sample was centrifuged at 8000 xg for 15 minutes and the absorbance was measured using a UV spectrophotometer with a wavelength scan of 290-320 nm. The Sun Protection Factor (SPF) was calculated by the following formula:

$$SPF = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times sample\ absorbance$$

Note: CF = correction factor with value 10; EE = Erythemal Effect; I= Solar Intensity.

To calculate the SPF value, the photoprotective normalized function from Sayre et al. [10] was used, as seen in Table 1.

Table 1. Photoprotective normalized function

Wavelength (λ *nm*)	EE × I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Antimicrobial activity of *Siraitia grosvenorii*

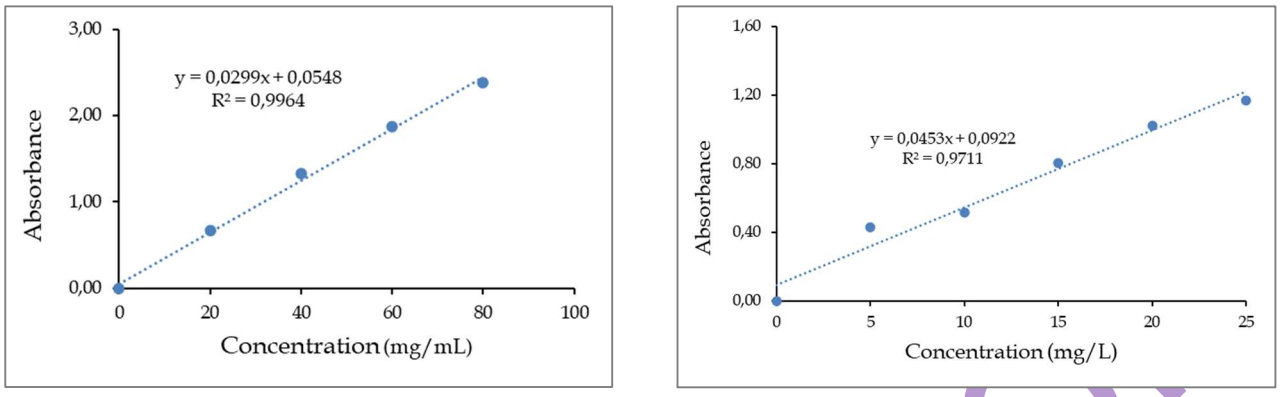
The antibacterial activity of *S. grosvenorii* against the growth of *Escherichia coli* was assessed with a turbidity assay. After 24 h of incubation in nutrient broth, the growth of *E. coli* in liquid culture was examined by checking the culture turbidity using a UV spectrophotometer at 600 nm. One percent of microorganism-containing broth was added into tubes containing 100, 200, 300, 400, and 500 ppm of seed and skin extract. The turbidity of each tube was measured using a UV spectrophotometer at 600 nm after 3 h, 6 h, 9 h, and 24 h of incubation. A tube with microorganism-containing medium without the extracts was used to serve as control. An uninoculated tube of growth medium was used as a blank.

▪ RESULTS & DISCUSSION

Total phenolic and flavonoid content

Total phenolic content of *S. grosvenorii* was estimated based on mg gallic acid equivalent per mg extract (mgGAE/mg extract), while total flavonoid was expressed as mg quercetin equivalents per g extract (mgQE/mg extract) (Figure 1). Based on the calibration curve, total phenolic and flavonoid concentrations in the sample were calculated. Table 2 shows the total phenolic difference between seed and fruit skin of *S. grosvenorii*. The seed and fruit skin contain 0.17 and 0.51 mgGAE/mg extract, respectively. Medicinal plants possess high amounts of polyphenols (phenolic acids and flavonoids). Phenolic compounds have reduction-oxidation (redox) properties that are considered as an important factor in contributing to antioxidant activity of plants [11]. Previous study showed that 95% ethanol extraction of whole *S. grosvenorii* fruit contains 2.38 mgGAE/mg extract [12]. It is expected for this study to show a lower phenolic content due to the use of

different *S. grosvenorii* fruit parts independently. Overall, this study suggested that the fruit skin of *S. grosvenorii* has a higher content of phenolic compounds than the seed.



(a) (b)

Figure 1. Calibration curve: (a) gallic acid and (b) quercetin.

Table 2. Total phenolic and total flavonoid of the seed and fruit skin of *Siraitia grosvenorii*

Sample	Total phenolic (mgGAE/g extract)	Total flavonoid (mgQE/mg extract)
Seed	0.17	0.18
Fruit skin	0.51	0.16

Flavonoids are ubiquitous polyphenolic compounds that have high medicinal value due to their activity in antioxidant, anti-inflammatory and anticancer [13]. Table 2 shows that the flavonoid content of seed and fruit skin of *S. grosvenorii* is 0.17 and 0.16 mgQE/mg extract, respectively. Interestingly, previous study of a whole *S. grosvenorii* fruit extracted with 95% ethanol only possessed a total flavonoid content of 13.452 µgQE/mg extract [12].

Antioxidant activity

The antioxidant capacity (expressed as a percentage of DPPH reduction) of seed and fruit skin extracts was investigated at different concentrations. As shown in Figure 2, higher extract concentrations resulted in increased antioxidant activity. Specifically, the DPPH reduction for the seed extract at a concentration of 500 ppm was 38.06%, while the fruit skin extract at 400 ppm exhibited a reduction of 39.79%. Based on the extrapolation of Figure 2, the IC50 values for the seed and fruit skin extracts were determined to be 637.88 ppm and 575.75 ppm, respectively. Sukweenadhi et al. screened the antioxidant activity of seven Indonesian herbal extracts using DPPH assay and found that meniran (*Phyllanthus niruri*) and kumis kucing (*Orthosiphon stamineus*) had IC50 of 102 ppm and 132 ppm, respectively, which are categorized as having moderate antioxidant activity. Antioxidant activity of a compound can be classified based on IC50 value: very strong (IC50 < 50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and weak (> 150 ppm) [14]. Thus, the antioxidant capacity of *S. grosvenorii* in this study was classified as weak.

Wuttisin and Boonsook reported that DPPH reduction can be negatively impacted by total phenolic content and the presence of non-phenolic compounds. Therefore, high total phenolic content might contribute to the low DPPH reduction observed in both seed and fruit skin of *S. grosvenorii* [15]. Interestingly, in this study, while fruit skin exhibited higher phenolic content compared to the seed, the DPPH reduction of fruit skin extract was greater than that of the seed extract. This discrepancy might be due to a higher content of non-phenolic compounds in the fruit skin compared to the seed. Additionally, the antioxidant activity of extracts can be influenced by the interaction of multiple compounds. If the specific compounds in the *Siraitia grosvenorii* extracts do not work synergistically, or if they are less effective than those found in other plants, this may result in a lower overall antioxidant capacity.Extraction method also influences the antioxidant activity of *S. grosvenorii*. Gong et al. found that four different extracts of *S. grosvenorii* were successfully prepared, and their antioxidant properties were assessed. The findings indicated that all four extracts

exhibited antioxidant and radical scavenging activities, with the effectiveness of their antioxidant properties ranked in the following order: ethyl acetate extract > water extract > methanol extract > ethanol extract [16].

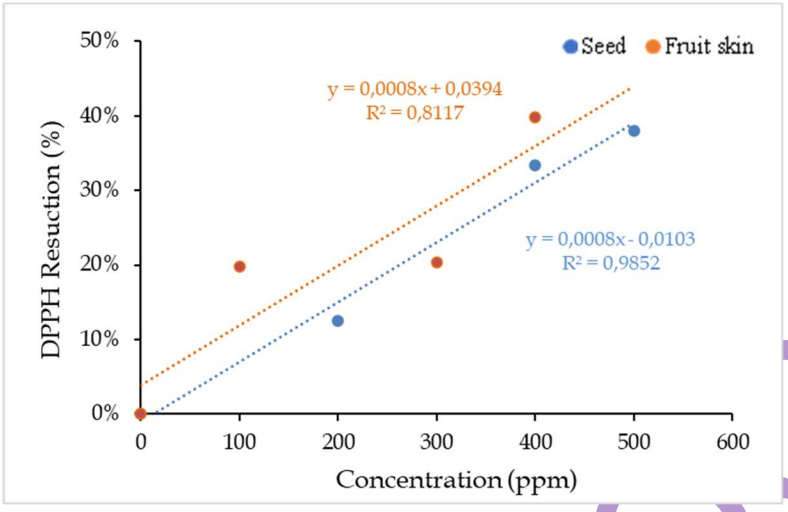


Figure 2. DPPH reduction by the seed and fruit skin extracts of *Siraitia grosvenorii*

Photoprotective Activities

UV radiation can have adverse effects on the skin, and one way to prevent these effects is by using sunscreen[17]. Table 3 shows that total SPF of the seed and fruit skin extract of *S. grosvenorii* differs. The SPF value of the seed and skins is 4.47 and 4.14, respectively. The Food and Drug Administration (FDA) recommends people to use a broad-spectrum sunscreen with an SPF of 15 or higher regularly and as directed [18]. However, the low SPF value of both extracts does not comply with the FDA's recommendation for sunscreen use. Therefore, *S. grosvenorii* is not suitable to be used as a sunscreen component to protect the skin from the sun's UV rays.

Table 3. Sun Protection Factor (SPF) of the seed and fruit skin of *Siraitia grosvenorii*

Sample	SPF
Seed	4.47
Fruit skin	4.14

Antimicrobial activity

The antimicrobial activity of fruit skin and seed extracts at various concentrations was investigated, with a particular focus on their impact on the growth of Escherichia coli. According to Figure 4, the seed extracts of *S. grosvenorii* exhibited better inhibitory activity towards *E. coli* compared to the fruit skin extract. All tested concentrations (100-500 ppm) of the seed extract could inhibit the growth of *E. coli* by 50%. On the other hand, the fruit skin extract generally exhibited no antimicrobial activity against *E. coli* after 24 h. It is interesting to note that the best inhibitory activity of both seed and fruit skin extracts was shown by low extract concentrations (100 ppm). In accordance with previous study, the results indicate that *S. grosvenorii*, particularly seed extracts, has a promising antimicrobial activity by inhibiting the growth of *E. coli* [19].

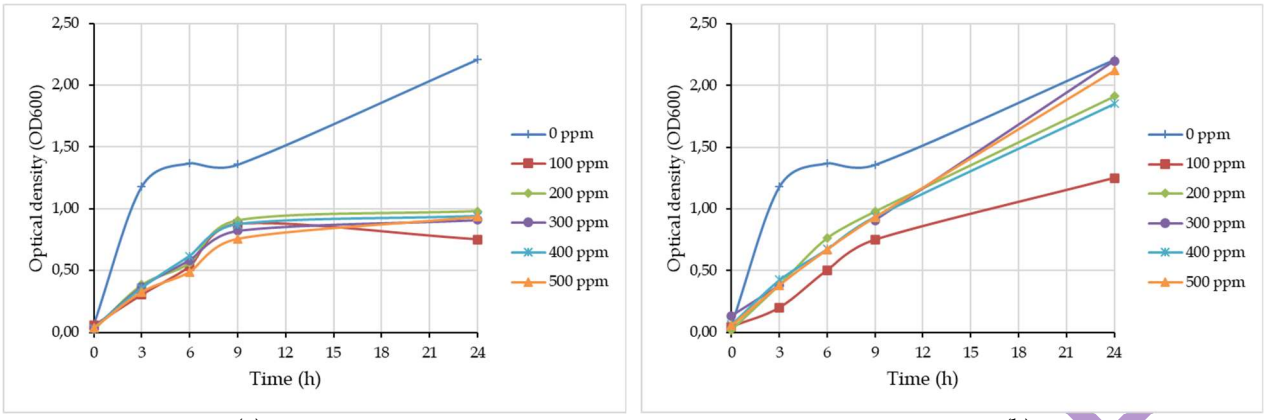


Figure 4. Antimicrobial activity of *Siraitia grosvenorii* against the growth of *Escherichia coli* BTCC: (a) seed extract and (b) fruit skin extract.

CONCLUSION

Our findings indicate that the fruit skin of *S. grosvenorii* possesses a higher phenolic content (0.51 mgGAE/ mg extract) compared to the seed (0.17 mgGAE/ mg extract). While there is a minimal difference in total flavonoid content between the seed and fruit skin extract, the seed has slightly higher flavonoid content (0.17 mgQE/mg extract) than the skin (0.16 mgQE/mg extract). Both seed and fruit skin extracts showed low SPF (4.47 and 4.14, respectively), thus they are not suitable to be used as sunscreen components. Low DPPH reduction was observed in both fruit skin (38.06% of DPPH reduction) and seed (39.79% of DPPH reduction) extracts. Additionally, both seed and fruit skin extracts exhibited the highest antimicrobial activity against *E. coli* at the concentration of 100 ppm.

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