Effects of solvent system and drying on the total phenolic content and antioxidant activities of leaf extract of *Pereskia bleo*

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ABSTRACT *Pereskia bleo* (*P. bleo*), a plant traditionally consumed in parts of China, Malaysia, and Panama, is believed to have a variety of medicinal properties, including anti-cancer, anti-tumour, anti-rheumatic, anti-ulcer, and anti-inflammatory effects. The total phenolic content (TPC) and antioxidant activities of the leaves of *P. bleo* were determined using three extraction solvents of different polarities: water, methanol and dichloromethane. The effects of drying in a drying oven at 40˚C on the TPC and antioxidant activities were also determined. TPC was measured using the Folin-Ciocalteu reagent, and antioxidant activities were determined using ferric reducing antioxidant power (FRAP) assay and beta-carotene bleaching (BCB) assay. While the aqueous extract exhibited the highest TPC ($p<0.05$) (34.20 mg GAE/g extract for fresh leaves and 71.24 mg GAE/g extract for dried leaves), the methanolic extract showed the strongest antioxidant activities ($p<0.05$) for both the FRAP assay (332.17mmol Fe (II)/g extract for fresh leaves and 270.59 mmol Fe (II)/g extract for dried leaves) and the BCB assay (69.86% for fresh leaves and 60.50% for dried leaves). Drying resulted in a lower TPC ($p<0.05$) for the methanol and dichloromethane extractions (-5.39% and -15.67%, respectively) but an increased TPC ($p<0.05$) for the water extraction (+52.00%). The FRAP and BCB activities decreased ($p<0.05$) after drying, with reductions ranging from -12.82% to -34.53% and -13.31% to -65.52%, respectively.

KEYWORDS: *Pereskia bleo*; Total phenolic content; Antioxidant activity; Ferric reducing antioxidant power; Beta-carotene bleaching
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INTRODUCTION

Non-communicable diseases, including cardiovascular conditions (such as heart attacks and strokes), cancers, chronic respiratory diseases (like chronic obstructive pulmonary disease and asthma), and diabetes, are the leading causes of death worldwide. The production of free radicals in the body causes oxidative stress, which may contribute to the development of non-communicable diseases (Lai & Lim, 2011). Free radicals are reactive molecules or molecular fragments containing one or more unpaired electrons (Wong *et al*., 2014). Antioxidants, both natural and synthetic, are compounds that reduce or prevent oxidative damage caused by free radicals through neutralization, involving the donation of electrons to the free radicals. Antioxidants encompass a variety of compounds, including phenolic acids, flavonoids, catechin derivatives, ascorbic acid, tocopherols, tocotrienols, carotenoids, and other phytochemicals (Krishnaiah *et al*., 2007; Shahidi *et al*., 1992).

Pereskia bleo (*P. bleo*), commonly known as `Jarum Tujuh Bilah' in Malaysian language and `Cak Sing Cam' in Cantonese, is a member of the botanical family Cactaceae. In parts of Malaysia and China, *P. bleo* is traditionally consumed either raw as a vegetable or as a brewed concoction made from fresh leaves. The plant is believed to possess various medicinal properties, including anti-cancer, anti-tumour, anti-rheumatic, anti-ulcer, and anti-inflammatory effects (Khor *et al*., 2013). Besides being used as a traditional remedy for headaches, ulcers, haemorrhoids, gastric pain, and atopic dermatitis, the leaves of *P. bleo* are consumed to alleviate discomfort and refresh the body (Sim *et al*., 2010). The current study investigated the effects of solvent system and drying on the total phenolic content and antioxidant activities of the leaf extract of *P. bleo*.

METHODOLOGY

Sample Collection, Preparation and Extraction

Sample collection

Fresh, mature *Pereskia bleo* was sourced from a local farmer in Kota Kinabalu, Sabah. Upon harvest, the sample was delivered immediately to the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah in Kota Kinabalu, Sabah. The sample was processed within 24 hours.

Sample preparation

The leaves (10-15 cm in length) were separated from the stems before being cleaned using distilled water. For the dry sample, the leaves were dried in a drying oven (Thermoline Scientific, TD-78T-2-SD) at 40 °C until a constant weight was obtained. The samples (fresh and dried) were subject to grinding, and kept in an air-tight amber bottle before being stored at -20 °C.

Extraction

The ground samples were extracted with three solvents: water, methanol and dichloromethane. Twenty g of the ground fresh sample was added with 400 mL of solvent giving a sample-to-solvent ratio of 1:20. For the dried sample, five g of the ground sample was added with 100 mL of solvent. The sample was placed on an incubator shaker for 2 hr at 40 °C. The extracts from the methanol and dichloromethane extractions were recovered through filtration using Whatman No. 1 filter paper. The aqueous extract was obtained through centrifugation at 8000 rpm for 15 min (Viacava *et al*., 2015) with subsequent concentration and drying using a rotary evaporator (Heidolph, Germany) at 40 °C.

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extracts obtained from the three solvents was determined using the Folin-Ciocalteu method described by Singleton & Rossi (1965) and Sim *et al*. (2010) with slight modifications. The calibration curve was constructed using a series of gallic acid solutions of different concentrations (0.01-0.10 mg/mL). The assay was based on the absorbance at 765 nm using a UV-Vis spectrophotometer (Perkin Elmer Lambda 25, USA). The TPC, expressed as mg gallic acid equivalents/g extract (mg GAE/g extract), was determined based on the following equation:

$$C = c \; x \; \frac{V}{m}$$

where C = total phenolic content in mg GAE/g plant extract; c = concentration of gallic acid in mg/mL; V = volume of extract in mL; m = weight of plant extract in g.

Analyses of Antioxidant Activities

Ferric reducing antioxidant power (FRAP)

The assay was based on the method described by Benzie and Strain (1996) with slight modifications. The calibration curve was prepared using a series of freshly prepared Fe (II) solutions of different concentrations (0.10-0.45 mg/mL). The FRAP antioxidant activity was assayed based on the absorbance at 593 nm using a UV-Vis spectrophotometer (Perkin Elmer Lambda 25, USA).

Beta-carotene bleaching (BCB)

The beta-carotene bleaching assay was carried out according to the method described by Hassanbaglou *et al*. (2012) and Sim *et al*. (2010) with slight modifications. The antioxidant activity was determined based on the absorbance at 470 nm using a UV-Vis spectrophotometer (Perkin Elmer Lambda 25, USA). The rate of degradation of the beta-carotene was calculated using the following equations:

$$R = [\ln (A_0/A_t)] / t$$

where R = the rate of degradation (bleaching) of the beta-carotene; ln = natural logarithm; A_0 = absorbance at time t=0; A_t = absorbance at time t=120 min; t = 120 min).

The antioxidant activity (expressed as the inhibition in %) was calculated in terms of percentage inhibition relative to the control using the equation below:

$$\text{Antioxidant activity} = \left[\frac{(R_C - R_S)}{Rc}\right] \times 100$$

where *Rc* = the rate of beta-carotene bleaching of the control sample; *Rs* = the rate of beta-carotene bleaching of the extract.

Statistical Analysis

All analyses were carried out in triplicates and all results are reported as mean ± standard deviation. Statistical analysis of data was carried out using SPSS (Statistical Package for Social Sciences version 21.0., IBM SPSS Statistics, USA). The data on the effect of solvent system were analyzed using one-way ANOVA and the Tukey test. The data on the effect of drying were analyzed using T-test. A 95% confidence level ($p<0.05$) was used for statistical significance.

RESULTS AND DISCUSSION

The total phenolic contents (TPC) of the extracts obtained using three different solvents (water, methanol and dichloromethane) from the fresh and dried leaves are shown in Table 1. Among the three solvents, water is the most polar solvent with a polarity index of 10.2, followed by methanol (5.1) and dichloromethane (3.1). Water is a very polar solvent. Methanol is also a polar solvent but is less polar than water. Dichloromethane is slightly polar and is the least polar among the three solvents. The three solvents were selected to cover a wide range of polarities - low, medium, and high, ensuring the extraction of phenolic compounds across this broad polarity spectrum. The amounts of TPC extracted by the three solvents followed the order: water > methanol > dichloromethane, for both fresh and dried leaves. For the fresh leaves, the highest TPC was 34.20 mg GAE/g extract (water extraction), followed by 30.00 mg GAE/mg extract (methanol extraction) and 14.09 mg GAE/g extract (dichloromethane extraction). The results suggest that more than 80% of the phenolic compounds extracted from the fresh leaves, based on weight, were of high polarity. After drying, the TPC values were 71.24, 25.30 and 13.33 mg GAE/g extract for the water, methanol and dichloromethane extractions, respectively. The TPC decreased after drying for the extractions using methanol and dichloromethane. Phenolic compounds degrade upon exposure to heat due to their heat sensitivity and susceptibility to oxidation (Lim & Murtijaya, 2007). The loss of TPC after drying may be due to the degradation of phenolic compounds by enzymatic reactions involving polyphenol oxidase during drying (Cavalcanti *et al*., 2006). The reduction rate in TPC after drying was lower for the extraction using dichloromethane (-5.39%) compared to that of the extraction using methanol (-15.67%). The results suggest that the phenolic compounds extracted with dichloromethane had a higher thermal stability than those extracted with methanol. For the extraction using water, the TPC showed an increase (52%) after drying. The increase may be due to the formation of new antioxidant compounds during drying through the Maillard reaction, or as a result of lipid oxidation products (Pérez-Jiménez *et al*., 2008; Nicoli *et al*., 1999). The TPC of 25.30 mg GAE/g extract from the methanolic extract of the dried sample in the present study is slightly lower than those reported by Johari & Kong (2019) (40.82 mg GAE/g extract) and Zulkipli *et al*. (2024) (41.83 – 77.31 mg GAE/g extract). The TPC of *Preskia bleo*, like that of other plants, is influenced by factors such as geographical location, seasonality, climate, drying temperature and method, as well as the extraction solvent and conditions (Chan *et al*., 2007; Calín-Sánchez *et al*., 2020).

Table 1. Total phenolic content (TPC) of the fresh and dried leaves of *Pereskia bleo*.

Extraction solvent	Total phenolic content (mg GAE/g extract)	
	Fresh	Dried
Water	$34.20 \pm 0.70^{A,b}$	$71.24 \pm 1.69^{A,a}$ (+52.00%)
Methanol	$30.00 \pm 0.35^{B,a}$	$25.30 \pm 0.24^{B,b}$ (-15.67%)
Dichloromethane	$14.09 \pm 0.26^{C,a}$	$13.33 \pm 2.69^{C,b}$ (-5.39%)

Values shown are means ± standard deviations (n=3). Different superscript uppercase letters (A-C) in the same column denote significant differences ($p<0.05$). Different superscript lowercase letters (a-b) in the same row denote significant differences ($p<0.05$).

The antioxidant activities based on FRAP are shown in Table 2. The highest activity for the fresh leaves was observed for the extraction using methanol (332.17 mmol Fe (II)/g extract), followed by the extraction using dichloromethane (249.26 mmol Fe (II)/g extract) and the extraction using water (207.68 mmol Fe (II)/g extract). Despite having the highest TPC values, the extract obtained using water showed the lowest FRAP activities. The results suggest that not all of the very polar phenolic compounds present in the aqueous extract contributed to the FRAP activities. TPC is a measure of the quantity of phenolic compounds but does not account for their types or structures. Different phenolic compounds have varying antioxidant potentials. For example, flavonoids and phenolic acids may have different efficacy depending on their chemical structures, such as the number and position of hydroxyl groups. The relatively less polar phenolic compounds present in the extracts obtained using methanol and dichloromethane contributed more significantly to the FRAP activities than those more polar phenolic compounds from the aqueous extract.

Table 2. Ferric reducing antioxidant power (FRAP) of the fresh and dried leaves of *Pereskia bleo*.

Extraction solvent	FRAP (mmol Fe (II)/g extract)	
	Fresh	Dried
Water	$207.68 \pm 2.0^{C,a}$	$135.96 \pm 1.90^{C,b}$ (-34.53%)
Methanol	$332.17 \pm 1.72^{A,a}$	$270.59 \pm 1.59^{A,b}$ (-18.54%)
Dichloromethane	$249.26 \pm 1.29^{B,a}$	$217.30 \pm 2.82^{B,b}$ (-12.82%)

Values shown are means ± standard deviations (n=3). Different superscript uppercase letters (A-C) in the same column denote significant differences ($p<0.05$). Different superscript lowercase letters (a-b) in the same row denote significant differences ($p<0.05$).

The FRAP activities for all three extracts decreased after drying. The extract from the water extraction (-34.53%) recorded the highest reduction, followed by the methanol extraction (-18.54%) and the dichloromethane extraction (-12.82%). The results may suggest that the phenolic compounds contributing to the FRAP activities from the dichloromethane extraction had the highest thermal stability, followed by those obtained through the methanol extraction and those from the water extraction.

The antioxidant activities based on BCB are shown in Table 3. Similar to the trend in FRAP, the highest BCB activity for the fresh leaves was observed for the extract obtained through methanol

extraction (69.85%), followed by the extract from dichloromethane extraction (45.48%) and the extract from water extraction (23.97%). Again, the results may suggest that, despite having the highest TPC, not all of the phenolic compounds present in the aqueous extract contributed to the BCB activities. A deterioration in the BCB activities after drying was observed for all three extracts. For the methanol and dichloromethane extractions, the decrease in the BCB activities after drying aligns with the reduction in the TPC due to the degradation of phenolic compounds during drying. For the water extraction, it was demonstrated again that not all of the phenolic compounds present in the extract contributed significantly to the BCB activities. The reduction rates in BCB activities after drying for the three extracts followed the order: dichloromethane (-65.52%) > water (-49.94%) > methanol (-13.31%). The results suggest that the phenolic compounds contributing to the BCB activities in the methanolic extract had the highest thermal stability, followed by those in the aqueous extract and those obtained through the dichloromethane extraction. The three extracts showed the same trend in the antioxidant activities based on FRAP and BCB: methanol extraction > dichloromethane extraction > water extraction. The results showed that the relatively less polar phenolic compounds in the extracts from the methanol and dichloromethane extractions were effective antioxidants and contributed significantly to antioxidant activities based on FRAP and BCB.

Table 3. Beta-carotene bleaching (BCB) of the fresh and dried leaves of *Pereskia bleo*.

Extraction solvent	**BCB (Inhibition, %)**	
	Fresh	**Dried**
Water	$23.97 \pm 0.58^{C,a}$	$12 \pm 2.00^{C,b}$ (-49.94%)
Methanol	$69.86 \pm 01.20^{A,a}$	$60.50 \pm 9.47^{A,b}$ (-13.31%)
Dichloromethane	$45.48 \pm 0.036^{B,a}$	$15.68 \pm 2.60^{B,b}$ (-65.52%)

Values shown are means ± standard deviations (n=3). Different superscript uppercase letters (A-C) in the same column denote significant differences ($p<0.05$). Different superscript lowercase letters (a-b) in the same row denote significant differences ($p<0.05$).

CONCLUSION

In comparison to the extractions using methanol and dichloromethane, the water extraction resulted in the highest total phenolic content (TPC) ($p<0.05$) from the leaves of *Pereskia bleo*. Drying led to a decrease in TPC ($p<0.05$) for the methanol and dichloromethane extractions. The TPC increased ($p<0.05$) after drying for the water extraction. The antioxidant activities of the leaves (fresh and dried) based on FRAP and BCB were in the order ($p<0.05$): methanol extraction > dichloromethane extraction > water extraction, indicating that the phenolic compounds obtained through methanol and dichloromethane extractions were more efficient as antioxidants than those obtained through the aqueous extraction. Drying resulted in lower antioxidant activities ($p<0.05$) for both FRAP and BCB.

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