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Research

Effect of Different Drying Methods on Retention of Colour, Total Phenolic Content, Flavonoid Content and Antioxidant Activity in *Pereskia bleo* Leaves

Nurain Nabilah Zulkipli[1], Iman Nur Sabrina Norasmadi[1], Suhaizan Lob[1], Wan Zaliha Wan Sembok[1,2], Nurud Iliani Suhaimi[1], Aidilla Mubarak[1,2]*

1. Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
2. Research Program of Postharvest Research and Innovation, Food Security Research Cluster, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

*Corresponding author: aidilla@umt.edu.my

ABSTRACT

Pereskia bleo, a widely cultivated plant known for its medicinal applications, contains abundant phytochemicals, including phenolics and flavonoids, predominantly in its leaves. The drying process, a standard practice for enhancing the shelf life, could affect the bioactive compounds within the leaves. Therefore, this study aims to evaluate the impact of various drying methods on the colour, total phenolic content, flavonoid content, and antioxidant activity of *P. bleo* leaves. The tested drying methods include shade, oven, microwave, and freeze-drying. The colour of fresh and dried leaves was assessed using a Minolta chromameter. The total phenolic content (TPC) and total flavonoid content (TFC) of the *P. bleo* leaves extracts were determined using the Folin-Ciocalteu's and aluminium chloride colourimetric assay, respectively. Antioxidant capacities were analysed with DPPH radical scavenging and ferric-reducing antioxidant power assay (FRAP). The results showed that microwave drying has significantly less impact than the other drying methods on the colour attributes of the leaves ($p<0.05$). Notably, microwave-dried *P. bleo* leaves demonstrated significantly higher TPC (77.31 ± 0.70 mg GAE/g dry extract) and TFC (35.79 ± 1.34 mg QE/g dry extract) compared to leaves dried using the other tested methods ($p<0.05$). Additionally, microwave-dried *P. bleo* leaves displayed the highest DPPH inhibition (91.62%) and exhibited the most potent IC_{50} value (76.90 ± 1.06 μg/mL) compared to oven and shade-dried leaves ($p<0.05$). *P. bleo* leaves dried with a microwave also recorded a significantly higher FRAP value (62.66 ± 0.10 μg TE/g dry extract) than oven-dried leaves ($p<0.05$). In conclusion, microwave drying emerged to be an efficient drying method in preserving the colour and antioxidant properties of the *P. bleo* leaves, suggesting its potential as a favourable drying technique for retaining bioactive compounds in medicinal plant materials.

Key words: Bioactive compounds, leaf drying methods, medicinal plant, microwave drying, phytochemicals

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INTRODUCTION

In recent decades, the continuous rise in the price of medicines has led consumers to turn to medicinal or herbal products. The utilization of medicinal herbs has increased worldwide, with communities relying on them for their beneficial phytochemical compounds. Most medicinal plants contain antioxidant, antibacterial, anti-inflammatory, antitumor and analgesic properties (Abdul Manaf *et al.*, 2014). Therefore, industry and consumers are looking for alternative treatments from these natural sources that can benefit health.

Pereskia bleo, locally known in Malay as 'Jarum Tujuh Bilah', in English as 'Rose Cactus', and in Chinese as 'Cak Sing Cam', meaning 'Seven Star Needle', is an underexplored medicinal plant that is widely found in the tropical area of Southeast Asia, particularly in Malaysia (Johari & Khong, 2019). *P. bleo* has thin and corrugated leaves, orangish-red flowers, and short spines. It is believed that the leaves of *P. bleo* have potential health benefits for humans, including treating diseases such as cancer, diabetes, and others

(Abdul Manaf *et al*., 2014). Hassanbaglou *et al*. (2012) noted that the extract of *P. bleo* leaves is high in antioxidants attributed to various phenolic and flavonoid constituents such as catechin, quercetin, epicatechin, myricetin, as well as ß-carotene and α-tocopherol. The leaves of *P. bleo* also consist of secondary metabolites such as alkaloids, fatty acids, glycosides, lactones, sterol, terpenoid and carotenoid compounds that also function as antioxidant agents (Zareisedehizadeh *et al*., 2014). Antioxidants are essential compounds that act as health-promoting factors by reducing the risks and hazards of diseases related to oxidative stress and have a health-promoting effect on human health (Adorjan & Buchbauer, 2010).

P. bleo leaves are usually preserved before use, and the common method is drying. Drying is an effective and crucial step in preserving and preparing the extraction process (Chua *et al*., 2019). Drying helps to control the moisture content of the freshly harvested leaves, minimize chemical degradation reactions, maintain leaf structure, control leaf density and porosity, and thus prolong the shelf life of the leaves (Abdul Manaf *et al*., 2014). There are many methods for dehydrating plants, specifically its leaves, such as oven drying, which is the simplest and faster than shade drying. Microwave drying offers the benefits of shorter drying time and lower water activity. On the other hand, freeze-drying has been reported as an effective method specifically used for the preservation of antioxidants and other biochemical compounds (Babu *et al*., 2018).

To our knowledge, the investigation of the effects of different drying methods on *P. bleo* leaves is scarce. The current drying methods used for traditional and laboratory practises of *P. bleo* leaves, which include oven drying and shade drying, are considered impractical because they require high temperatures and long drying time (Elshafi *et al*., 2020). Improper dehydration can significantly impact the leaves' physical appearance, colour, and stability of bioactive compounds, with the drying method and temperature playing crucial roles in determining the quality of the leaves, particularly their chemical and biological composition (Abdul Manaf *et al*., 2014; Lasano *et al*., 2018; Halim *et al*., 2019). The best drying method with minimal loss of physical quality and nutrient composition in *P. bleo* should be determined by comparing the effects of oven drying, shade drying, microwave drying and freeze drying on the colour, total phenolic content, flavonoid content and antioxidant activity of *P. bleo* leaves. This study aims to fill the knowledge gap on the best drying method to preserve the desirable constituents in *P. bleo* leaves.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents used were analytical grade. Methanol (CH_3OH), ethanol (C_2H_6O), sodium hypochlorite (NaClO), hydrochloric acid (HCl), acetic acid (CH_3COOH) and ferric chloride hexahydrate ($FeCl_3.6H_2O$) were obtained from R&M Chemicals, Malaysia. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium acetate trihydrate ($CH_3COONa*3H_2O$), aluminium chloride ($AlCl_3.6H_2O$), gallic acid ($C_7H_6O_5$), BHT ($C_{15}H_{24}O$) and quercetin ($C_{15}H_{10}O_7$) were purchased from Sigma Aldrich (USA). 2,4,6-tri(2-pyr-idyl)-s-triazine (TPTZ) was purchased from Thermo Scientific Chemicals (USA).

Plant collection and preparation of sample

The fresh and matured leaves of *P. bleo* were collected from Kampung Gong Pak Jin, Kuala Nerus, Terengganu, Malaysia. These matured leaves had reached the maximum growth stage before flowering (approximately 210 days), were dark green, and measured between 15 cm and 30 cm in length and 8 cm and 10 cm in width. Upon arrival at the Postharvest Technology Laboratory at Universiti Malaysia Terengganu, the leaves were first separated from the stalk. Then, the leaves were carefully washed with tap water and rinsed with distilled water to ensure the removal of any contaminants. The excess water was gently removed from the leaves using tissue paper and then subjected to drying immediately (Sharif *et al*., 2015). A voucher specimen of *P. bleo* leaves has been deposited in a herbarium for plant identification and reference.

Different drying methods for sample

Oven drying treatment of *P. bleo* leaf was applied following Mohd-Salleh *et al*. (2020), with slight modification by arranging the leaves on the trays and drying in the convection oven (Memmert, Model UN750plus, Republic of Germany) at 60°C for overnight. The shade drying method of *P. bleo* leaf was applied by arranging the leaves on the plastic at the table and drying them for two weeks at room

temperature (26 ± 2°C) in shade and ventilated condition (Rengganaten, 2013). For the microwave drying *P. bleo* leaf was dried by spreading them on a tray in a standard domestic microwave oven (Panasonic, model NN-GF560M, Malaysia) and the time was set for 8 min at 900 W, following the Lasano *et al*. (2018) method with some modification. Freeze drying of *P. bleo* leaf was applied according to the Chua *et al*. (2019) method. The leaves were kept in the ultra-low freezer (-80°C) for a week before being subjected to the freeze-drying process. After a week, the samples were placed in a freeze dryer (SP Scientific Virtis AdVantage 2.0 BenchTop Freeze Dryer, Model Advantage Plus ES-53) at 65 Pa and temperature -50°C for 4 days. All these drying methods were done until the weight of dried leaves achieved a constant dry weight sample with a moisture content of 11%. Once the drying process was complete, the leaves were manually crushed into pieces by hand, blended and ground by using a rock grinder (ROCKLABS, model IA-388, Auckland, New Zealand) to gain the smooth fine powder (250-500 µm particle size) and homogenous samples to allow for better contact between the sample surface and the solvent during the extraction procedure (Rahim *et al*., 2021).

Colour analysis of a sample

Colour changes in the fresh and dried *P. bleo* leaves were determined using the chromameter (Minolta CR-400b, Japan) by converting all colours within the human perceptual range into a standardised numerical code. This code uses colour designations of L^*, a^*, and b^*, which indicate lightness coordinate, greenness coordinate, and yellowness coordinate, respectively. The colour of *P. bleo* leaves was assessed before and after drying to observe the changes that occurred during the drying process. The Hunter-Scotfield equation (Equation 1) was used to calculate colour differences (ΔE) based on the L^*, a^*, and b^* parameters (Sarkhel *et al*., 2022).

Equation 1:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where, ΔL= difference of brightness; Δa = difference of redness and greenness; Δb= difference of yellowness and blueness.

Methanolic extraction of sample dried with different drying methods

The methanolic extraction of *P. bleo* dried leaves was performed according to Annegowda *et al*. (2012) and Abas *et al*. (2020). Ground *P. bleo* leaves were extracted with 80% methanol (ratio 1:10 w/v) in a Sonicator Ultrasonic Bath (Bransonic, Model 5510R-DTH, 42 kHz ± 6%, USA) for 30 min at room temperature. The extract was then filtered using No. 2 Whatman filter papers (Monotaro, Malaysia). The residue was re-extracted twice with 100 mL of 80% methanol (ratio 1:2 w/v), filtered and combined with the previous filtrate obtained. Then, the combined filtrate was concentrated under a rotary evaporator (Buchi, Rotavapor® R-300, Switzerland). The crude extracts obtained were stored in a dark bottle at 4°C for not more than a week before the evaluation of antioxidant activities (Hassanbaglou *et al*., 2012).

Analysis of total phenolic content (TPC) and total flavonoid content (TFC)

TPC in the samples was analysed using the Folin-Ciocalteu reagent method, as described by Aryal *et al*. (2019), with some modifications. All samples were assayed in triplicate. The crude extract of leaves and standard gallic acid were separately dissolved with 80% methanol and diluted to 1 mg/mL for the assay. Briefly, 200 µL of the diluted *P. bleo* leaves extract (1 mg/mL) was mixed with 1.0 mL of 0.10 M Folin-Ciocalteu reagent in a test tube. The mixture was allowed to stand at room temperature for 5 min. After 5 min, a volume of 1.5 mL of 7.5% sodium carbonate solution was added to the mixture. The mixture was vortexed for 30 sec and then incubated at room temperature for 45 min. The absorbance was measured using a spectrophotometer (Shimadzu, model UV-1800, Japan) at a wavelength of 765 nm after incubation. A calibration curve for the standard reference was established using gallic acid at concentrations ranging from 0.01 to 0.10 mg/mL. Results were expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

TFC was determined in the extracted samples following the method described by Aryal *et al*. (2019), with some modifications. All samples were assayed in triplicate. The crude extract of leaves and standard quercetin were dissolved with 95% methanol and diluted to 1 mg/mL for the assay. Briefly, 500 µL of diluted extract from the *P. bleo* leaves (1 mg/mL) was mixed with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 7.5% sodium acetate and 2.8 mL of distilled water. Following that,

the solution was vortexed for 30 sec and subsequently incubated in the dark at room temperature for 40 min. The absorbance was measured using a spectrophotometer (Shimadzu, model UV-1800, Japan) at a wavelength of 415 nm after incubation. The measurements were taken against a blank consisting of 95% methanol. A calibration curve was prepared using quercetin as the standard reference. The concentration range of quercetin used was 0.01 to 0.05 mg/mL. The result was expressed as mg/g quercetin equivalents in milligrams per gram (mg QE/g) of dry extract.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The DPPH radical scavenging assay was performed following Ali *et al*. (2011) and Aryal *et al*. (2019) with a few modifications. All samples were assayed in triplicate. The crude extract of leaves and standard BHT were separately dissolved in pure methanol and diluted to 1 mg/mL for the assay. Briefly, 2 mL of the diluted extract solution from the leaves of *P. bleo* at various concentrations (30-210 µg/mL) was mixed with 2 mL of DPPH solution (0.1 mM). The solution was vortexed for 30 sec and then left in the dark for 30 min. The decolourization of DPPH-donated protons was measured after 30 min using a spectrophotometer (Shimadzu, model UV-1800, Japan) at an absorbance of 517 nm. The measurements were taken against a blank consisting of an equal amount of DPPH and methanol. The antioxidant activity of each sample was assessed by calculating the percentage of DPPH· scavenging inhibition using Equation 2.

Equation 2:

$$\%\ \text{scavenging of DPPH}\cdot = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of each sample at different concentrations (30-210 µg/mL) was also evaluated to determine the inhibitory concentration 50 (IC_{50}) value. The IC_{50} value of the leaf extract and the standard (BHT) were calculated to determine the sample concentration needed to inhibit 50% of the radicals. The lower the IC_{50} values of samples, the higher their antioxidant activity.

Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was performed according to the methods outlined by Benzie and Strain (1996), Iqbal *et al*. (2015) and Lasano *et al*. (2018), with some modifications. All samples were assayed in triplicate. The working FRAP reagent was freshly prepared by mixing 50 mL of 300 mM acetate buffer with a pH of 3.6, 5 mL of 10 mM TPTZ solution, and 5 mL of 20 mM $FeCl_3$.6H2O solution (10:1:1 ratio). A 300 mM acetate buffer with a pH of 3.6 was prepared by dissolving 3.1 g of sodium acetate trihydrate in 500 mL of distilled water. A volume of 16 mL of acetic acid was added to the solution. Then, made up the combined solution to a total volume of 1 L using distilled water. The 10 mM TPTZ solution was prepared in 40 mM HCl, while 20 mM $FeCl_3.6H_2O$ was dissolved in distilled water. FRAP reagent was kept warm in a water bath at 37°C for 10 min. The crude extract of *P. bleo* leaves, and Trolox standard were dissolved with 80% methanol and diluted to 1 mg/mL for use in the assay. A calibration curve was prepared for the sample extract and Trolox, ranging from 0 to 100 µg/mL of concentrations. Briefly, 3 mL of the FRAP reagent was added into the cuvette, and a blank reading was taken at 593 nm using a spectrophotometer (Shimadzu, model UV-1800, Japan). Then, 100 µL of diluted extract from the *P. bleo* leaves (1 mg/mL) along with 300 µL distilled water and 3 mL of FRAP reagent were added into the test tubes. The mixture was incubated for 4 min, after which the second absorbance reading was taken at a wavelength of 593 nm. The FRAP values were determined by measuring the change in absorbance after 4 min from the initial blank reading. The results were expressed as µg Trolox equivalent (TE)/g extract.

Statistical analysis

All data were reported as mean ± standard deviation (SD) based on triplicate determinations. IBM SPSS Statistics software version 25 was used for the statistical analysis. When the normality test revealed that the data was normal ($p>0.05$), all data were analysed using one-way ANOVA, followed by Tukey's HSD multiple comparison test. Statistical significance is expressed as p-values less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Effect of different drying methods on the colour of *P. bleo* leaf

Table 1 compares the colour parameters of fresh and dried *P. bleo* leaves using different drying methods. Chromameter *L** represents the lightness coordinate, *a** represents the greenness coordinate, and *b** represents the yellowness coordinate to characterise colour changes that occur when food products undergo thermal processing. Values of fresh *P. bleo* leaves were 40.17 ± 1.08 (*L**), -16.19 ± 1.41 (*a**) and 22.03 ± 0.55 (*b**). The result shows that freeze drying gave the significantly highest *L** value (54.60 ± 1.20) and thus the lightest colour compared to fresh leaves (40.17 ± 1.08). However, microwave-dried *P. bleo* leaves showed no significant difference (39.23 ± 0.42) and a slight decrease in *L** value compared to the fresh leaves. This suggests that freeze drying changed the colour of *P. bleo* leaves from dark to lighter tones, while microwave drying did not provide much impact on the colour of the leaves. Meanwhile, the *P. bleo* leaves dried with shade and oven drying had a significantly lower *L** value (34.17 ± 0.70 and 31.24 ± 0.31, respectively) than the fresh leaves, indicating a darker colour after drying using the two methods.

Table 1. Table 1 Comparison of colour parameters of fresh and dried *P. bleo* leaves

Drying methods	*L** value	*a** value	*b** value	ΔE
Fresh	40.17 ± 1.08c	-16.19 ± 1.41a	22.03 ± 0.55c	-
Oven drying	31.24 ± 0.31a	-2.34 ± 0.22c	15.46 ± 0.47a	17.81 ± 1.20b
Shade drying	34.17 ± 0.70b	3.20 ± 0.70d	16.90 ± 0.61ab	20.95 ± 1.42c
Microwave drying	39.23 ± 0.42c	-14.88 ± 1.81a	20.76 ± 0.55c	2.14 ± 0.63a
Freeze drying	54.60 ± 1.20d	-8.61 ± 0.61b	18.16 ± 0.60b	16.80 ± 0.55b

Data is presented as means ± SD (*n*=3). Means followed by different superscript letters in the same column indicate significant differences at $p<0.05$.

As for the *a** value, the fresh leaves showed a green colour (-16.19 ± 1.41), while microwave-dried leaves had *a** value that was closest to the fresh leaves with no significant difference (-14.88 ± 1.81). Meanwhile, the oven and shade-dried leaves showed a higher *a** value (-2.34 ± 0.22 and 3.20 ± 0.70, respectively), which shows a more inclined value towards redness. This is because of oxidation, which results in non-enzymatic or enzymatic browning on the dried leaves, thus turning the samples less greenish (Youssef & Mokhtar, 2014). The *b** value, which indicated the yellowness colour of the sample, showed that microwave-dried *P. bleo* leaves had the highest yellow hue (20.76 ± 0.55) compared to other tested drying methods ($p<0.05$) and was found to be similar to the fresh leaves (22.03 ± 0.55). As a result, the microwave-dried leaves had a dark, green-bluish colour, but those freeze-dried ones had a higher luminosity and green-yellowish colour. The lower colour changes in microwave drying may result from a shorter drying time despite the high temperature, which hinders chlorophylls from turning olive-brown or colourless. Zambra *et al*. (2021) support that a shorter drying period can enhance nutrient retention while maintaining a higher quality of texture, colour, and flavour. The findings of this study align with the study conducted by Thamkaew *et al*. (2021), who discovered that increasing the microwave power from 360 W to 900 W resulted in a 64% reduction in parsley's drying time. Additionally, the parsley retained good colour retention, with only a slightly darker colour compared to fresh parsley. The result is also consistent with Snoussi *et al*. (2021), where microwave drying of myrtle leaves changed the leaf colour to a slightly light dark green compared to air and oven drying, which turned the leaf colour to a light brown with a green tinge.

The ΔE values relate to the total colour differences of dried *P. bleo* leaves using different drying methods also presented in Table 1. Microwave-dried *P. bleo* (2.14 ± 0.63) are significantly lower ($p<0.05$) in ΔE values compared to the other tested drying methods (16.80 ± 0.55, 17.81 ± 1.20 and 20.95 ± 1.42, freeze, oven and shade drying, respectively). Tezcan *et al*. (2021) reported that when the ΔE values were larger than 3, there was a noticeable variation in the colour of the tested food product. Based on the results reported in this study, significant ΔE values were observed in all drying treatments except microwave. This indicates that of all the tested drying methods, microwave-dried *P. bleo* leaf had a minimal colour change. In conclusion, microwave drying appeared similar to the fresh leaves by preserving their colour, as demonstrated by a lower ΔE value and no significant alterations in *L**, *a**, and *b** values. The breakdown of bioactive chemicals in leaf tissues may be associated with decreased leaf bioactivity (Wanyo *et al*., 2011). The breakdown of pigments such as chlorophyll during drying causes colour deterioration in dried herbs. Because of the increased exposure of the chlorophyll structure to heat, collapsing plant tissues may release chlorophyll molecules from the protein complex and convert chlorophylls to pheophytins (Thamkaew *et al*., 2021). As a result, this event could release substrates for enzymatic browning reactions in the surrounding areas.

Colour is widely acknowledged as an indicator of freshness and is also linked to nutritional value. Colour is a significant quality attribute in dried herbal products, influencing consumer perception and marketability. The colour changes can indicate the extent of degradation or preservation of bioactive compounds during drying. Change of colour in dried leaves is associated with hydrolysis, caramelization, and non-enzymatic and enzymatic reaction factors (Chua *et al*., 2019). These factors result from the elevated temperature and the duration of the drying process (Sarkhel, 2022). Green leaves contain high levels of chlorophylls. Chlorophylls are an unstable green pigment that is easily transformed or degraded during drying by changing to olive-brownish, greenish, or even colourless substances (Chua *et al*., 2019). Therefore, the colour of *P. bleo* leaves dried by different drying methods was analysed to observe the effects on the physical appearance of dried leaves, which helps understand the colour stability of the leaves for its potential use in the nutraceutical industry.

Effect of different drying methods on total phenolic content (TPC) and total flavonoid content (TFC)

The TPC assay is a method used to measure secondary metabolites, specifically phenolic compounds, in leaves. It involves an electron transfer-based assay that measures the reduction oxidant of specific compounds (Mubarak *et al*., 2019). Table 2 presents the effect of different drying methods on the TPC of *P. bleo* leaves. The TPC values in methanolic extracts of dried *P. bleo* leaves varied from 41.83 mg GAE/g to 77.31 mg GAE/g, showing an approximate two-fold variation. Microwave-dried *P. bleo* leaves showed a significantly higher TPC (77.31 ± 0.70 mg GAE/g) compared to the freeze-dried (70.06 ± 0.80 mg GAE/g), shade-dried (46.02 ± 0.60 mg GAE/g) and oven-dried leaves (41.83 ± 0.72 mg GAE/g) ($p<0.05$). Similarly, Halim *et al*. (2019) discovered that the microwave-dried stevia leaves showed significantly higher TPC than other drying methods (freeze, sun and oven drying). Snoussi *et al*. (2021) also found that microwave-dried myrtle leaves had a higher TPC than leaves dried using an oven or air-drying methods. Supported by Hihat *et al*. (2017), who revealed that microwave-dried coriander leaves at 900 W for 5 min showed a better TPC value (40 mg GAE/g) than oven-dried at 60°C for 4 hr (25 mg GAE/g). This suggests the potential of microwave drying for better retention of TPC in the leaves. The highest TPC in microwave-dried leaves compared to oven-drying may be attributed to the heat generated by microwaves. The heat from elevated temperatures leads to increased vapour pressure and temperature within the plant tissue, leading to the disruption of plant cell wall polymers. As a result, more phenolic compounds are extracted (Hihat *et al*., 2017). However, if the temperature exceeds the optimal level or the output power of 60°C and 900 W, it will decrease TPC (Hihat *et al*., 2017). Meanwhile, the lowest TPC value observed in oven-dried *P. bleo* leaves may be due to the breakdown and degradation of bioactive compounds. This could be caused by the activity of degradative enzymes, such as polyphenol oxidases, which are activated by elevated temperatures and extended drying times (Barimah *et al*., 2017).

Table 2. Effect of different drying methods on total phenolic content and total flavonoid content of *P. bleo* leaves extract

Drying methods	Total phenolic content (mg GAE/g dry extract weight basis)	Total flavonoid content (mg QE/g dry extract weight basis)
Oven drying	41.83 ± 0.72a	17.34 ± 0.52a
Shade drying	46.02 ± 0.60b	29.18 ± 0.70b
Microwave drying	77.31 ± 0.70c	35.79 ± 1.34c
Freeze drying	70.06 ± 0.80d	33.99 ± 0.36c

Data is presented as means ± SD (*n*=3). Means followed by different superscript letters in the same column indicate significant differences at $p<0.05$. All values are expressed as milligram gallic acid/quercetin equivalent per dried extract weight basis.

Concerning the comparison of the TPC values with a previous study, it was observed that the TPC of methanolic extract of oven-dried *P. bleo* leaves reported by Sim *et al*. (2010) had a lower value than the current study (27.88 mg GAE/g). Johari and Khong (2019) also demonstrated a similar result where the TPC of methanolic extract of shade-dried *P. bleo* leaves had a lower value than the current study, which is 40.82 ± 0.01 mg GAE/g extract. However, the study from Mustafa *et al*. (2010) recorded a higher TPC value of methanolic extract of oven-dried *P. bleo* leaves at 50°C for 24 hr (109 ± 0.84 mg GAE/ g extract) compared with the current study that was oven-dried for 60°C overnight. The varied results of TPC observed in the previous study can be related to different temperatures and duration of drying methods used to prepare the leaves, different extraction methods, as well as variation factors such as climate, season, and horticulture practices of collected *P. bleo* leaves (Chan *et al*., 2007; Calín-Sánchez *et al*., 2020).

Flavonoids are a type of secondary metabolites that exhibit antioxidant activity. This activity is determined by the presence and arrangement of free hydroxyl (OH) groups (Aryal *et al*., 2019). The impact of different drying methods on the TFC in *P. bleo* leaves are shown in Table 2. The TFC in methanol extracts of dried *P. bleo* leaves ranged from 17.34 GAE/g to 35.79 mg QE/g, showing an approximate two-fold variation. Microwave-dried *P. bleo* leaves had significantly highest content of TFC (35.79 ±1.34 mg QE/g) compared to oven drying and shade drying (17.34 ± 0.52 and 29.18 ± 0.70, respectively) ($p<0.05$). However, the TFC of microwave-dried leaves were comparable with the freeze-dried leaves (33.99 ± 0.36 mg QE/g).

Similarly, Snoussi *et al*. (2021) reported that microwave-dried myrtle leaves at 500 W for 5 min exhibited a significantly higher flavonoid content ($p<0.05$) than leaves that were air-dried at 16°C and oven-dried at 70°C. Hamrouni-Sellami *et al*. (2013) also observed similar results in microwave-dried sage leaves when using a microwave power of 850 W for 3 to 5 min compared to air-dried leaves at 22°C (shade and ambient temperature) and oven-dried leaves at 45°C and 65°C. Supported by Hihat *et al*. (2017) where the microwave-dried coriander leaves at 900 W power allowed the highest TFC value at 20 mg QE/g compared to oven-dried at 60°C which is 15 mg QE/g ($p<0.05$). The microwave-dried *P. bleo* leaves have higher preservation of total flavonoid compounds than the leaves dried using oven drying, parallel to the observation on TPC discussed earlier. According to Hihat *et al*. (2017), the loss of macromolecules, such as flavonoids, during heat treatment could be attributed to the harsh drying conditions. This statement indicated that TFC values may decrease when exposed to elevated temperatures combined with a long drying time.

Effect of different drying methods on DPPH radical scavenging activity

Antioxidants are well-known for their ability to inhibit lipid oxidation by scavenging free radicals. The scavenging of DPPH free radicals can effectively evaluate the antioxidant activity of specific compounds or extracts (Sim *et al*., 2010). Table 3 shows the effect of different drying methods on the DPPH radical scavenging activity of *P. bleo* leaf extract. The DPPH percentage of inhibition was measured at a concentration of 210 μg/mL, which was the highest concentration tested. The results show that the microwave-dried leaves exhibited the highest DPPH inhibition activity at 91.62%, followed by the freeze-dried leaves at 89.22%, the synthetic antioxidant (BHT) at 88.85%, the shade-dried leaves at 78.36%, and lastly the oven-dried leaves at 62.89%. The microwave-dried *P. bleo* leaves had a tendency for a higher antioxidant capacity measured by the ability to scavenge the free radical in this assay and was found to be comparable ($p>0.05$) with the BHT and freeze-drying. Besides, oven-dried leaves recorded a significantly lower antioxidant ability than BHT and other tested drying methods ($p<0.05$).

Table 3. Effect of different drying methods on DPPH percentage inhibition and inhibitory concentration 50 (IC_{50}) value of *P. bleo* leaves extract

Drying methods	DPPH percentage of inhibition (%)	DPPH IC_{50} value (μg/mL)
Oven drying	62.89 ± 0.80a	160.6 ± 0.44b
Shade drying	78.36 ± 0.63b	131.6 ± 1.05b
Microwave drying	91.62 ± 0.42c	76.90 ± 1.06a
Freeze drying	89.22 ± 0.27c	93.59 ± 0.75a
BHT	88.85 ± 0.16c	58.79 ± 1.36a

Data is presented as means ± SD (n=3). Means followed by different superscript letters in the same column indicate significant differences at $p<0.05$. $IC_{50.}$ Inhibitory concentration at 50%. BHT act as a reference standard.

Data in Table 3 also demonstrated the DPPH IC_{50} value from the different drying methods on *P. bleo* leaf extract. A higher IC_{50} value represents a lower antioxidant activity, whereas a lower IC_{50} value represents higher antioxidant potency. IC_{50} from this study ranged from 58.79 μg/mL to 160.6 μg/mL, representing approximately three-fold variation. Microwave-dried leaves recorded the lowest IC_{50} value, which is 76.90 ± 1.06 μg/mL, thus showing the highest antioxidant potency in comparison to freeze-dried, shade-dried and oven-dried *P. bleo* leaves (93.59 ± 0.75 μg/mL, 131.6 ± 1.05 μg/mL and 160.6 ± 0.44 μg/mL, respectively), but was lower than synthetic antioxidant, BHT (58.79 ± 1.36 μg/mL). However, no significant difference was found between microwave, freeze-drying, and BHT. Therefore, microwave and freeze drying positively affect the antioxidant activity of *P. bleo* leaves, which shows potential to be used as an effective drying method.

Similarly, Halim *et al*. (2019) found that microwave and freeze-dried stevia leaves showed a higher potency of antioxidant activity than oven and sun-dried leaves. This finding aligns with the results of Snoussi *et al*. (2021), which showed that microwave-dried myrtle leaves had the highest IC_{50} value than

air and oven drying methods. Supported by Hamrouni-Sellami *et al*. (2013) findings, microwave-dried sage leaves at 800 W for 5 min showed the lowest IC_{50} value than shade drying at 22°C and oven drying at 65°C. The lowest radical scavenging ability of *P. bleo* leaves dried with oven drying, as seen in this current study, suggests that the high temperature used in the drying process might inflict losses on the antioxidant compounds in the leaves. Phenolic content has been reported to be highly attributed to the scavenging capacity of the radicals (Hassanbaglou *et al*., 2012). Elshaafi *et al*. (2020) have found that drying leaves at 60°C and above may cause a lowering of the phenolic content and antioxidant activity due to thermal degradation. Chua *et al*. (2019) have also stated that the antioxidant activity of *S. crispus* leaves could be preserved at the optimum temperature of 50°C. The previous result was associated with the current result, where the higher IC_{50} value was noted for oven and shade-dried *P. bleo* leaves.

Concerning the comparison of the DPPH analysis with a previous study, it was observed that the lowest IC_{50} value could be achieved in the methanolic *P. bleo* leaf extract dried using the air-drying method, which is at 33.83 µg/mL and can be as high as 277.5 µg/mL by using oven drying method (Hassanbaglou *et al*., 2012; Johari & Khong, 2019). The varied results of antioxidant activity observed in the previous study can be linked to the different temperatures and duration of drying used to prepare the leaves, different extraction methods, as well as variation factors such as climate, season, and horticulture practices of collected *P. bleo* leaves (Chan *et al*., 2007; Calín-Sánchez *et al*., 2020). Furthermore, there is a positive relationship between TPC, TFC and antioxidant activities. A high TPC and TFC lead to a high antioxidant capacity, as there is a linear correlation between these three parameters. This is supported by many previous studies, which reported an excellent correlation between the antioxidant capacity with the phenolic and flavonoid compounds of the *P. bleo* leaves extract (Sim *et al*., 2010; Mustafa *et al*., 2010; Hassanbaglou *et al*., 2012; Johari & Khong, 2019). Thus, the microwave-dried product showed higher retention of antioxidant activity than the leaves dried using oven, shade and freeze drying, parallel to the observation on TPC and TFC discussed earlier.

Effect of different drying methods on ferric reducing antioxidant power (FRAP)

The FRAP assay is a method used to measure the ability of samples to convert ferric ions into the ferrous form of TPTZ (2,4,6-tripyridylstriazine) (Hassanbaglou *et al*., 2012). Table 4 shows the FRAP of *P. bleo* leaf extract dried with the different drying methods. The analysis shows that the microwave-dried *P. bleo* leaves had the highest FRAP value (62.66 ± 0.10 µg TE/g), followed by freeze-dried leaves (62.60 ± 0.01 µg TE/g), shade-dried leaves (62.57 ± 0.01 µg TE/g) and oven-dried leaves (62.27 ± 0.02 µg TE/g). The values of FRAP for all dried leaves are quite similar but oven-dried recorded a significantly lower value than microwave and freeze-dried leaves ($p<0.05$). The data obtained showed that all the leaf extracts from different drying methods had similar abilities in reducing Fe3+/ferric cyanide complex to the ferrous form.

Table 4. Effect of different drying methods on ferric reducing antioxidant power assay (FRAP) of *P. bleo* leaves extract

Drying methods	Ferric reducing antioxidant power (µg TE/g dry extract weight basis)
Oven drying	62.27 ± 0.02a
Shade drying	62.57 ± 0.01ab
Microwave drying	62.66 ± 0.10b
Freeze drying	62.60 ± 0.01b

Note: Data is presented as means ± SD (*n*=3). Means followed by different superscript letters in the same column indicate significant differences at *p*<0.05. All values are expressed microgram trolox equivalent per dried extract weight basis (µg TE/g dry extract weight basis).

Similarly, Halim *et al*. (2019) reported oven drying had the lowest FRAP value, which is at 6.69 ± 0.23 µM $FeSO_4$ equivalent, meanwhile, freeze-dried and microwave-dried stevia leaves showed a good reducing capability Fe^{3+} into Fe^{2+}. The current findings are consistent with a previous study conducted by Saifullah *et al*. (2019), where the FRAP result of sun drying (2 days) and shade drying (2 weeks) of lemon myrtle dried leaves was significantly lower than microwave drying at 960 W for 7 min and freeze drying at -50°C for 48 hr. The finding also matches the observation on TPC, TFC and DPPH described earlier. It can be reported that microwave and freeze-drying methods have been found to effectively preserve the TPC, TFC, and antioxidant capacity of plant materials. These methods are superior to other drying methods as they prevent the degradation of nutrients within the plant material. The highest FRAP value in microwave-dried leaves compared to oven-drying may be attributed to the heat generated by microwaves. This heat increases vapour pressure and temperature inside plant tissue, disrupting plant cell wall polymers. Thus, higher antioxidants can be extracted in a short time (Hihat *et al*., 2017). Taking into consideration FRAP content in freeze-dried *P. bleo* leaves that was

recorded with no significant difference from microwave-dried might be due to the cell disruption caused by ice crystal formation during the freezing process, the release of certain enzymes that prevent the degradation of phenolic compounds and antioxidant activity (Halim *et al*., 2019). Thus, the antioxidant activity of *P. bleo* can be preserved. Youssef and Mokhtar (2014), showed that freeze-dried at -20°C for 24 hr and microwave-dried at 900 W for 25 min could exhibit low adverse effects on the antioxidant activity of the *Portulaca oleracea* leaves compared to hot drying treatments at 60°C.

Concerning the comparison of the FRAP values of *P. bleo* leaves with a previous study, it was observed that the FRAP of oven-dried *P. bleo* leaves reported by Hassanbaglou *et al*. (2012) had a lower value than the current study (45.2 ± 1.2 μg TE/g extract). However, a study by Sim *et al*. (2010) showed a higher value of FRAP for oven-dried *P. bleo* leaves than the current study (645 μg TE/g extract). The variations in antioxidant activity observed in the plant extracts from the previous study can be related to several factors, such as the temperatures and conditions used for drying the leaves, the extraction methods employed, the composition of the extracts, and the culture practices during the collection of *P. bleo* leaves (Chan *et al*., 2007; Hassanbaglou *et al*., 2012; Calín-Sánchez *et al*., 2020).

CONCLUSION

In conclusion, microwave drying at 900 W for 8 min resulted in *P. bleo* leaves having the closest colour to the fresh leaves. The highest TPC, TFC, DPPH and FRAP values were also observed in *P. bleo* leaves dried with microwave drying, followed by freeze, shade and oven drying. Despite having a statistically comparable value of TFC, DPPH and FRAP, microwave drying can be an excellent alternative to freeze drying since freeze drying involves a costly operation. This study, therefore, highlights the potential of microwave drying in optimizing the retainment of phytoconstituents of *P. bleo* leaves, which increases its effectiveness for utilization in the nutraceutical industries and indirectly helps in the expansion of *P. bleo* cultivation.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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