

Effect of Different Drying Methods on Colour, Total Phenolic Content, Flavonoid Content, and Antioxidant Activity Retention of *Strobilanthes crispus* Leaves

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ABSTRACT

Strobilanthes crispus, a medicinal herb, is recognised for its abundant phytochemicals, notably in its leaves, contributing to its high antioxidant activity. However, the crucial step of drying, aimed at extending shelf life, can impact the stability of these bioactive compounds. This study evaluates the impact of different drying methods, which include oven, microwave, freeze drying, and air drying, on the colour, phenolic and flavonoid content, and antioxidant activities of *S. crispus* leaves. The colour analysis of the fresh and dried leaves was assessed using the chromameter. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using Folin-Ciocalteu's and aluminium chloride colourimetric assays, respectively. Antioxidant capacities were analysed via ferric-reducing antioxidant power (FRAP) and a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results showed that microwave-dried *S. crispus* leaves exhibited minimal

alterations in colour attributes L^* , a^* , and b^* , closely resembling the fresh leaves ($p > 0.05$). Microwave drying significantly preserved TPC (145.42 ± 1.61 mg GAE/g), TFC (117.27 ± 5.10 mg QE/g), FRAP activity (258.92 ± 0.15 μ g TE/g extract), and displayed the most potent DPPH scavenging half-maximal inhibitory concentration (7.58 ± 0.48 μ g/ml) compared to other methods ($p < 0.05$). Notably, the DPPH scavenging potency surpassed that of the synthetic

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antioxidant butylated hydroxytoluene. In conclusion, microwave drying appeared to be an efficient method for preserving the colour and antioxidant properties of *S. crispus* leaves. It highlights its potential as a favourable drying technique for conserving bioactive compounds in medicinal plant materials, offering promising applications in the nutraceutical and pharmaceutical fields.

Keywords: Bioactive compounds, leaves drying methods, medicinal plant, microwave drying, phytochemicals, *Strobilanthes crispus*

INTRODUCTION

Strobilanthes crispus, locally known as *pecah kaca*, is one of the traditional folklore herbal medicines found locally in Malaysia and Indonesia. It is used as an infusion or concoction and is intended for treatments as antidiabetic, anticancer, antilytic, diuretic, and laxative agents (Nurraihana & Norfarizan-Hanoon, 2013). The leaf extract of *S. crispus* has been reported for its pharmacological properties, such as reducing the glucose level in the blood (antihyperglycemic), improving the lipid profile (antilipidemic), and lowering the threat of cardiovascular diseases (Mohd Fadzelly et al., 2006). This plant has further displayed notable anticancer activity when it was reported to inhibit cancer cell proliferation (Bakar et al., 2006) and antibacterial properties as a bactericidal agent (Muskhazli et al., 2009). Bakar et al. (2006) and Ismail et al. (2000) mentioned that those therapeutic activities were

attributed to its mineral contents, vitamins, alkaloids, polyphenol content, as well as phytochemicals such as catechin that were reported to be in abundance in this plant. The mixture of the bioactive constituents present in *S. crispus* results in a synergistic positive effect for chronic conditions, for example, hyperglycaemia, hypertension, and cancer (Nurraihana & Norfarizan-Hanoon, 2013). *Strobilanthes crispus* leaf extract has been reported to contain high antioxidant activity attributed to various phenolic constituents, for example, catechin, caffeic acid, kaempferol, and luteolin, which contribute to its radical scavenging activity and ability to eradicate oxidative stress reactions (Al-Henhena et al., 2015; Liza et al., 2010). Antioxidant is a significant compound that acts as a health-protecting factor by lowering the risks and dangers of oxidative stress-related diseases as well as giving a health-enhancing effect on human health (Adorjan & Buchbauer, 2010), which corresponds with local lifestyle practices of using *S. crispus* as herbal tea and concoctions for increasing overall well-being (Chua et al., 2019).

Drying has become an indispensable step in processing herbal medicinal plants (Poós & Varju, 2017) because the process reduces the risk of microbial spoilage and enhances the shelf life of herbs for beneficial purposes while providing advantages such as reducing transportation and storage costs simultaneously (Barimah et al., 2017). However, drying may result in alterations in the aroma and physical characteristics of the samples with a loss of essential

phytochemicals, particularly antioxidant properties, which can affect the utmost plant quality (Orphanides et al., 2013). Various studies have reported that drying methods can have an impact on the antioxidant activity of plant products (Kuljarachanan et al., 2009; Park et al., 2006). Hajimehdipoor et al. (2012) suggested that each plant requires a specialised drying method due to differences in plant constituents in different plant species, which may result in varying levels of bioactive compounds as a result of methodological differences. After that, the ideal drying method is often debated as it becomes the earlier crucial step in preserving raw materials. It could have adverse effects by degrading the bioactive compounds and polyphenols (Chua et al., 2019). It is especially critical for heat-sensitive compounds, as their degradation may occur at specific high temperatures unique to each sample. If the drying process is unsuitable, these compounds are at risk of being lost. It is of concern when there is a considerable loss of compounds during processing, resulting in a waste of raw materials. Hence, many studies have discussed drying methods to minimise the degradation due to heat treatment so that the compounds can be optimised when extracted for various beneficial purposes. Additionally, plant species, cost, final colour, and nutritional value of dried material, as well as the time cost, should be taken into consideration when choosing the best drying method (Roshanak et al., 2016).

A previous report has compared microwave and oven drying for *S. crispus*

leaves (Lasano et al., 2018) but did not address the possible difference between microwave drying and freeze drying, a method highly reported for its efficiency in preserving bioactive compounds and antioxidant properties in dried leaves (Babu et al., 2018; Thamkaew et al., 2021). Moreover, there is insufficient data on air drying for *S. crispus* compared to other methods, despite air drying being the common approach for drying herbal leaves (Babu et al., 2018).

Therefore, this study compares microwave, freeze, air, and oven drying for *S. crispus* leaves. Since *S. crispus* leaves can be a potentially useful source of many bioactive compounds, it is crucial to determine the best drying method to preserve the phytochemical and bioactive compounds in *S. crispus*. This study, therefore, may contribute to the body of knowledge in determining the efficient drying method for retaining the desirable compounds specifically for *S. crispus* leaves and become a factor in optimising the potential utilisation of this natural source in various fields to reap its benefits.

MATERIALS AND METHODS

Chemical Reagents

Methanol, hydrochloric acid (HCl), acetic acid (C₂H₄O₂), and iron (III) chloride hexahydrate (FeCl₃.6H₂O) were purchased from R&M Chemicals (Malaysia). Meanwhile, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, sodium carbonate (Na₂CO₃), Trolox, sodium acetate

trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$), aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), gallic acid, butylated hydroxytoluene (BHT), and quercetin were obtained from Sigma Aldrich Company (USA). As for 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), it was obtained from Alfa Aesar Company (USA). All chemicals used were of analytical grade.

Collection and Preparation of Plant

Material

The *S. crispus* leaves were collected from Kampung Serdang Baru, Kuala Terengganu, Malaysia. During the collection, the leaves were separated from the stalks (Ismail et al., 2000) and transported to the laboratory. The leaves were cleaned briefly with wet tissue paper and immediately subjected to different drying methods (Lasano et al., 2018). A specimen of the plant was also deposited in a herbarium for plant identification and reference.

Drying Methods

Strobilanthes crispus fresh leaves were subjected to four different drying methods, which include oven drying (OD), microwave drying (MD), freeze-drying (FD), and air drying (AD). In oven drying (OD), the leaves were arranged and spread on the trays or sheets of paper and, after that, dried in the convection oven drying chambers (Memmert, Model UN750 Plus, Germany) at $60 \pm 2^\circ\text{C}$ overnight (Ismail et al., 2000). In microwave drying (MD), the method was slightly modified from Lasano et al. (2018), where the leaves were spread

on the provided metal rack in a standard domestic microwave (Panasonic Model, NN-GF560M, Malaysia) and dried at 900 W for 5 min. In freeze drying (FD), the fresh leaves were pre-frozen at -80°C prior to freeze drying. The leaves were then freeze-dried in a freeze dryer (SP Scientific Virtis AdVantage 2.0 BenchTop Freeze Dryer/Lyophilizer Model Advantage Plus ES-53, USA) at a pressure of 65 Pa and a temperature of $-50 \pm 2^\circ\text{C}$ for 4 days (Chua et al., 2019). Air drying of *S. crispus* fresh leaves was performed at $27 \pm 2^\circ\text{C}$ for a fortnight by arranging the leaves in thin layers on plastic sheets on an even surface such as a table and leaving them to dry in a room (Koay et al., 2013). After drying, the dried leaves were crushed instantly into pieces by an electrical dry blender (Panasonic, Model MX-SM1031, Malaysia), and lastly, ground by a rock grinder (ROCKLABS, Model 1A, New Zealand) to gain the smooth, fine powder leaves ($250 \mu\text{m}$ particle size) for further extraction (Ahmed et al., 2011; Zayed et al., 2014).

Colour Assessment of Fresh and Dried *S. crispus* Leaves

Chromameter (CR-400, Konica Minolta Sensing, Japan) analysed fresh and all dried leaves. Photographs were obtained to compare leaves that had dried by different drying methods (Chua et al., 2019). Colour differences (ΔE) of colour values were calculated using Equation 1 (Tezcan et al., 2021).

$$\Delta E = \sqrt{\Delta L^*{}^2 + \Delta a^*{}^2 + \Delta b^*{}^2} \quad [1]$$

where, $\Delta L^* = L^*_{\text{dried sample (2)}} - L^*_{\text{fresh/standard (1)}}$,
 $\Delta a^* = a^*_{\text{dried sample (2)}} - a^*_{\text{fresh/standard (1)}}$, $\Delta b^* =$
 $b^*_{\text{dried sample (2)}} - b^*_{\text{fresh/standard (1)}}$.

Extraction of Dried *S. crispus* Leaves

Strobilanthes crispus powdered leaves were extracted with 80% methanol (1:10) in a sonicator (Bransonic, Model 5510R-DTH, 42 kHz \pm 6%, USA) for 30 min at room temperature (27 \pm 2°C) (Abas et al., 2020). The extract was then filtered with No. 2 Whatman filter paper (MonotaRO, Malaysia) with a vacuum pump (Rocker, Model Rocker 300, Taiwan) for faster filtration. The residue from the filtration was re-extracted twice with 80% methanol, which was then filtered and combined with the previous filtrate obtained. The filtrate was then concentrated under reduced pressure in a rotational evaporator (Buchi, Rotavapor® R-300, Büchi, Switzerland), and the obtained crude extracts were stored at 4°C in a chiller (Hassanbaglou et al., 2012).

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The analysis of TPC was carried out following Aryal et al. (2019), with some modifications. An amount of 200 μ l *Strobilanthes crispus* leaves crude extract (1 mg/ml reconstituted in 80% methanol) was mixed with 1.0 ml of 0.10 M Folin-Ciocalteu's reagent in the test tube and allowed to stand for 5 min at room temperature. A volume of 1.5 ml

of 7.5% sodium carbonate (Na_2CO_3) was added, agitated using a vortex for 30 s, and incubated at room temperature (27 \pm 2°C) for 45 min. After that, the absorbance readings at 765 nm were measured using a spectrophotometer (Shimadzu, UV-1800, Japan) against a blank (80% methanol). The assay was carried out in triplicate. A standard reference calibration curve was established using gallic acid with a concentration ranging from 0.01 to 0.05 mg/ml. The TPC values were expressed as mg/g of gallic acid equivalents in milligramme per gramme (mg GAE/g) of dry extract.

TFC was assessed using an aluminium chloride colourimetric assay following Aryal et al. (2019) and Formagio et al. (2014), with a few adjustments. An aliquot of 500 μ l of sample (1 mg/ml) *S. crispus* leaf extract was mixed with 1.5 ml of 95% methanol, 100 μ l of 10% aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), 100 μ l of 7.5% sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$), and 2.8 ml of distilled water. The solution in the test tubes was mixed evenly using a vortex for 30 s and kept in the dark for 40 min. After the incubation, the absorbances were taken using a spectrophotometer (Shimadzu, UV-1800, Japan) at 415 nm, and assays were carried out in triplicate. A standard quercetin calibration curve was made using the same steps as the sample extract in the 0.01–0.07 mg/ml concentration range. The TFC values were expressed as mg/g of quercetin equivalents in milligramme per gramme (mg QE/g) of dry extract.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activity of *S. crispus* leaf extract dried with different drying methods was measured using the DPPH radical scavenging assay following Aryal et al. (2019) with slight modifications. Two (2.0) ml of *S. crispus* extract solution (10-210 µg/ml in methanol) was mixed into 2.0 ml of DPPH (0.1 mM) solution, vortexed for 15 s, and kept in the dark for 30 min. Decolourisation of DPPH-donated protons was determined by measuring the absorbance at 517 nm by a spectrophotometer (Shimadzu, UV-1800, Japan) against an equal amount of DPPH and pure methanol as a blank. The assay was carried out in triplicate. The percentage of DPPH scavenging activity was calculated using Equation 2, and the half maximal inhibitory concentration (IC₅₀) values for samples were determined. A sample with a smaller IC₅₀ value was considered to exhibit stronger antioxidant activity. The scavenging activity was compared with the synthetic antioxidant BHT.

$$\text{DPPH scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

[2]

where, A₀ = absorbance of the control and A₁ = absorbance of the test extracts.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted following Benzie and Strain (1996), Iqbal et al. (2015), and Lasano et al. (2018), with

slight adjustments. The FRAP assay was measured based on the rapid reduction of ferric-tripyridyltriazine (Fe (III)-TPTZ) by antioxidants present in the *S. crispus* leaf extract, forming ferrous-tripyridyltriazine (Fe (II)-TPTZ), a blue-coloured product (Payne et al., 2013). All samples were assayed in triplicate. The reagents used for stock solutions contain 300 mM acetate buffer in pH 3.6 (3.1 g sodium acetate trihydrate, C₂H₃NaO₂·3H₂O in 500 ml distilled water, and 16 ml acetic acid C₂H₄O₂, marked up to 1 L), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution in 40 mM HCl (hydrochloric acid) and 20 mM FeCl₃·6H₂O (Iron (III) chloride hexahydrate) solution. The FRAP reagent was prepared beforehand by mixing 50 ml of 300 mM acetate buffer, 5 ml of TPTZ solution, and 5 ml of 20 mM FeCl₃·6H₂O solution (ratio 10:1:1). The FRAP reagent was warmed at 37°C in a water bath for 10 min. After that, 3 ml of FRAP reagent was added to the cuvette, and a blank reading was taken at 593 nm using a spectrophotometer (Shimadzu, UV-1800, Japan). Then, 100 µl of *S. crispus* leaf extract (0–125 µg/ml) mixed with 300 µl of distilled water were added together into the 3 ml of FRAP reagent in the test tubes. The mixture was then kept in the dark for 4 min, and the second absorbance reading was conducted at 593 nm. The change in the absorbance value determined the FRAP values for all samples after 4 min from the initial blank reading. The concentration of FRAP content in the extract was determined as µg Trolox equivalent (TE)/g extract basis.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics package (version 26). The data were analysed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) multiple comparison test for the parametric test. In contrast, as for the non-parametric test, the data were analysed using the Kruskal-Wallis H test and further determined with multiple pairwise comparisons. The values were recorded as means with a standard deviation from three replicates. The significant difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Colour of Fresh and Dried *S. crispus* Leaves

Fresh and dried *S. crispus* leaves were assessed for their colour using physical evaluation and instrumental measurement using the chromameter. Figure 1 shows the difference in colour of the dried leaf powder obtained by different drying techniques. Leaves dried by microwave drying (MD) and freeze-drying (FD) methods showed the brightest colours, while leaf powders dried by air-drying (AD) and oven-drying (OD) methods tended to show darker colours.

Table 1 shows the data obtained from the chromameter evaluation of the dried leaf powder colour. The results from the chromameter measurement support the visual images shown in Figure 1. This instrument's colour assessment was also compared on fresh leaves. Chromameter gives the value of L^* , which indicates

lightness with lighter (+) and darker (-) values. Based on the results, freeze-dried leaves show a significantly higher L^* value (45.22 ± 2.37), which illustrates a lighter colour than the fresh leaves (35.52 ± 1.18) ($p < 0.05$). Leaves dried with FD were also found to be lighter than the leaves with other tested drying methods, which are MD (31.75 ± 1.50), AD (29.03 ± 1.60), and OD (28.33 ± 1.47) ($p < 0.05$). In contrast, the L^* value of microwave-dried leaves is statistically insignificant ($p > 0.05$) compared to fresh leaves. In fact, the MD method showed no distinct difference in the L^* value from the fresh leaves. On the other hand, the leaves dried with AD and OD show a significantly lower L^* value ($p < 0.05$), indicating a darker colour than the fresh leaves. From these observations, FD, AD, and OD were found to have an impact on the lightness of the leaves, while MD was able to retain the fresh leaves' colour even after the drying process.

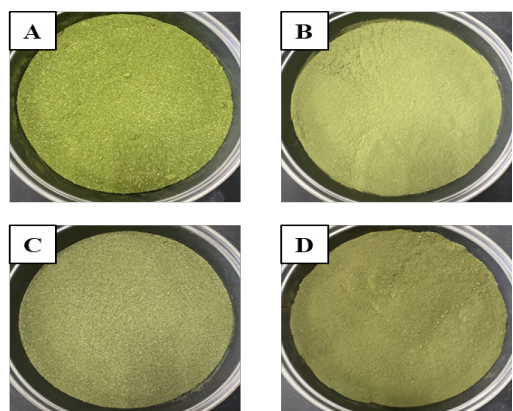


Figure 1. Ground dried *Strobilanthes crispus* leaves after drying by different drying methods: (A) microwave drying, (B) freeze drying, (C) air drying, (D) oven drying, respectively

Table 1

Colour parameters L^* , a^* , and b^* of *Strobilanthes crispus* affected by drying methods

Drying methods	Colour parameters			
	L^*	a^*	b^*	ΔE
Fresh leaves	35.52 ± 1.18 ^b	-12.95 ± 1.04 ^a	15.95 ± 1.54 ^b	-
MD	31.75 ± 1.50 ^{ab}	-10.08 ± 1.00 ^a	14.93 ± 0.84 ^b	5.24 ± 1.01 ^a
FD	45.22 ± 2.37 ^c	-10.63 ± 0.31 ^a	17.67 ± 1.54 ^b	10.28 ± 1.38 ^b
AD	29.03 ± 1.60 ^a	-3.36 ± 1.40 ^b	6.72 ± 1.35 ^a	14.87 ± 0.56 ^c
OD	28.33 ± 1.47 ^a	-3.10 ± 1.53 ^b	8.99 ± 0.88 ^a	14.07 ± 0.91 ^c

Note. Data is represented as means ± SD (n = 3); Values followed with different superscript letters in the same column indicate significant differences at $p < 0.05$; MD = Microwave drying; FD = Freeze drying; AD = Air drying; OD = Oven drying; L^* = Lightness; a^* = Red/green coordinate; b^* = Yellow/blue coordinate; ΔE = Colour difference

The value a^* depicts the colour of red (+) and green (-) present in the samples, with the outcome of fresh leaves (-12.95 ± 1.04) showing the greenest colour, followed by leaves dried with FD (-10.63 ± 0.31), MD (-10.08 ± 1.00), AD (-3.36 ± 1.40), and lastly OD (-3.10 ± 1.53). Freeze-dried and microwave-dried leaves recorded statistically insignificant different values ($p > 0.05$) of a^* compared to fresh leaves. This finding manifests MD as a drying method that can retain the green colour of fresh leaves, apart from FD. Meanwhile, the a^* value for air-dried and oven-dried leaves increased considerably ($p < 0.05$) compared to fresh, microwave-dried and freeze-dried leaves, implying that the natural green of leaves dried with AD and OD underwent some losses. Meanwhile, the b^* value, which indicates yellow (+) shade in the sample, shows the highest tint of yellow in freeze-dried leaves (17.67 ± 1.54), followed by fresh leaves (15.95 ± 1.54), leaves dried with MD (14.93 ± 0.84), OD (8.99 ± 0.88), and lastly AD (6.72 ± 1.35).

Freeze-dried and microwave-dried leaves have no significant difference in b^* values ($p > 0.05$) from the fresh leaves. Conversely, the b^* value for oven-dried as well as air-dried leaves reduced significantly ($p < 0.05$) in comparison to fresh leaves, suggesting that the initial yellowish tint in the leaves has been lost and there was a tendency to a darker colour on the dried leaves.

The results observed from this study are in accordance with studies by Chua et al. (2019) and Roshanak et al. (2016), where the L^* and b^* values of freeze-dried leaves were reported to show the highest value when compared to other treatments, including the fresh leaves themselves. For the a^* value, previously mentioned reports recorded the a^* value of freeze-dried leaves as being the greenest compared to the fresh leaves. However, the observation from the present results shows that freeze-dried leaves (-10.63 ± 0.31) could not be compared to the potent green colour of fresh leaves when fresh leaves recorded the highest green a^* value (-12.95 ± 1.04).

Therefore, freeze-dried leaves appeared to be lighter and yellower. Meanwhile, oven-dried leaves produced the darkest leaves among all the tested drying treatments, with a substantial loss of green pigments. It is probably caused by prolonged exposure to high drying temperatures (60°C), which may have accelerated chlorophyll degradation (Chua et al., 2019). Low a^* (-) values in OD (-3.10 ± 1.53) and AD (-3.36 ± 1.40) indicated that there is a significant loss of green colour, which is associated with chlorophyll degradation as well as low retention of chlorophyll *a* (Rubinskienė et al., 2015).

Chlorophylls are unstable green pigments that are easily transformed or degraded, producing derivatives of olive-brownish, greenish, or even colourless substances (Chua et al., 2019). *Strobilanthes crispus* leaves are green herbal medicinal plants, where chlorophyll degradation is the most common occurrence during drying that may change the colour of leaves (Thamkaew et al., 2021). Drying with prolonged heat temperature led to the release of chlorophyll, a molecule from the protein complex, which could promote the transformation of chlorophylls into pheophytins (olive-brown) due to greater exposure of the chlorophylls' structure to heat (Thamkaew et al., 2021). Meanwhile, similarly to FD, MD is a drying method that retained the colour of *S. crispus* leaves without major alterations compared to fresh leaves based on the recorded L^* , a^* , and b^* values.

The ΔE value, which refers to the total colour differences of dried *S. crispus* leaves

in comparison to fresh leaves, was calculated and reported in Table 1. The ΔE value is vital to determining the differences, especially for comparing different tested drying methods. Previous research attested that greater ΔE values of more than 3 delineate distinct colour differences (Pathare et al., 2013; Tezcan et al., 2021). The ΔE values measured in the current study show that all tested drying methods resulted in a distinct colour difference compared to the fresh leaves (MD: 5.24 ± 1.01 , FD: 10.28 ± 1.38 , AD: 14.87 ± 0.56 , OD: 14.07 ± 0.91). However, despite the noticeable total colour difference, *S. crispus* dried with MD recorded the lowest ΔE value, statistically different ($p < 0.05$) from the rest of the drying methods. It is indicated that microwave-dried leaves showed a similar appearance as fresh leaves, as determined by the smallest value of ΔE , with insignificant differences in the data of L^* , a^* , and b^* regarding the fresh leaves. These data suggest that MD can be a better and more efficient drying method to reduce colour changes from the fresh leaves. Ultimately, the modifications within colour attributes are greatly influenced by the leaves' properties and the drying methods used to carry out the process, as supported by Rubinskienė et al. (2015).

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in Dried *S. crispus* Leaves

The TPC and TFC values of all dried *S. crispus* leaf extracts, expressed in mg gallic acid equivalents (GAE) and quercetin equivalents (QE) per gramme of dry extract,

are shown in Table 2. The result shows that the *S. crispus* leaves dried with MD contained the highest TPC, followed by FD, AD, and lastly OD (145.42 ± 1.61 , 137.02 ± 6.90 , 133.92 ± 5.29 , and 19.05 ± 1.39 mg GAE/g, respectively). *Strobilanthes crispus* leaves dried by OD have a significant TPC reduction ($p < 0.05$), down to a double-digit value compared to MD, FD, and AD. Although the TPC value in microwave-dried leaves was found to have an insignificant difference ($p > 0.05$) with leaves dried with FD and AD, there was a tendency for better retention of TPC using this MD method. This occurrence coincided with Lasano et al. (2018), who recorded a better TPC value in microwave-dried leaves.

According to Chua et al. (2019), the enzymes associated with degradation in *S. crispus* may reach optimal productivity at 50°C . For example, polyphenol oxidase expedites the disintegration of phenolic compounds if activated by high-temperature drying methods, as notably occurred in OD, where the bioactive compounds are possibly degraded (Barimah et al., 2017). As a result, phenolics were most likely

disintegrated when *S. crispus* leaves were subjected to temperatures beyond 50°C for many hours in the OD. Moreover, extreme thermal processing may affect the cell microstructure, thus accelerating compound migration and further losing polyphenols (Barimah et al., 2017). Apart from that, the chemical structure of polyphenols might undergo some changes, causing the compounds to adhere to other constituents, such as proteins, resulting in difficulties in the extraction of the polyphenol compounds, thus consequently leading to low values of TPC (Barimah et al., 2017). In accordance with the TPC level in oven-dried *S. crispus* leaves, this implies that OD may not be an efficient method, or perhaps it needs some adjustments where the temperature should be lower or the drying duration should be shortened.

In terms of comparison of the TPC values with the previous reports, the MD and OD showed a great difference, where higher values of TPC were observed compared to the previous report (Lasano et al., 2018). The current study uses methanol as the extraction solvent, while Lasano et

Table 2
Total phenolic content and total flavonoid content of dried Strobilanthes crispus leaves

Drying methods	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
MD	145.42 ± 1.61^b	117.27 ± 5.10^c
FD	137.02 ± 6.90^b	64.10 ± 1.75^b
AD	133.92 ± 5.29^b	49.95 ± 4.58^a
OD	19.05 ± 1.39^a	48.92 ± 1.91^a

Note. GAE = Gallic acid equivalents; QE = Quercetin equivalent; Data is represented as means \pm SD ($n = 3$); Values followed with different superscript letters in the same column indicate significant differences at $p < 0.05$; MD = Microwave drying; FD = Freeze drying; AD = Air drying; OD = Oven drying

al. (2018) used boiling water. The drying techniques employed were also slightly different from the previous study (Lasano et al., 2018), which used OD for 10 min at a higher temperature (95–100°C) and MD for 2 min, a shorter drying time than the current study. Therefore, the differences in solvent extraction, temperature, and drying duration may explain the varied phenolic content in the *S. crispus* leaves. Furthermore, the ultrasonic extraction method used in this study may also account for the higher total phenolic contents. The same report was further noted by Nantitanon et al. (2010), where ultrasonication extraction produced a significantly high TPC as well as antioxidant activity in guava leaf extract. Through ultrasonication, a high frequency produced by the ultrasonic bath disturbs the plant cell wall structure, thus leading to increased contact between the solvent used during extraction and the extracted plant material (Nantitanon et al., 2010). Therefore, the dissolution of active compounds desired from the plant cell was enhanced, resulting in higher phytochemicals.

In terms of the TFC, *S. crispus* leaves dried with MD contained a significantly higher ($p < 0.05$) value (117.27 ± 5.10 mg QE/g) compared to the other drying methods. The TFC value was followed by leaves dried with FD (64.10 ± 1.75), AD (49.95 ± 4.58), and OD (48.92 ± 1.91). This observation has a similar trend to that of TPC discussed previously and is supported by Hayat (2020), where the TFC of microwave-dried peppermint leaves was significantly higher than that of oven-dried leaves. Apart from

that, it is observed that FD is the second best at containing TFC in *S. crispus* leaves besides MD, which is significantly ($p < 0.05$) better than both AD and OD based on the TFC amounts obtained. It can be explained by the fact that FD allows the ice crystal formation, which lies inside the plant matrix, to rupture the microstructure of the plant, thereby increasing the extractability of the phytochemicals into solvent extraction without subjecting them to any heat treatment that can further degrade the phytochemicals (Bernard et al., 2014).

Lasano et al. (2018) recorded insignificant TFC between MD and OD leaves and unfermented and fermented *S. crispus* leaves, which contradicts the findings in this study. This present study recorded a significant difference in TFC value between the dried leaves of MD and OD, with higher TFC value as well as TPC results than the reported data by Lasano et al. (2018). The different temperatures, drying durations, and extraction techniques used in this study may account for the different results obtained from the previous study. The methanol solvent used in this study as an extraction solvent has been reported to be a better solvent for extracting polyphenol constituents in comparison to chloroform, ethyl acetate, and water, and it is also satisfactory for extracting flavonoids from the microstructure of cells (Yao et al., 2004). Since *S. crispus* leaves have been reported to contain abundant amounts of catechin and epicatechin (Liza et al., 2010), the methanol solvent used would contribute to the higher TFC determined from this

study, which may lead to higher antioxidant activity. Other factors contributing to the disparate values may also be the different climates and horticulture practices of *S. crispus* leaves harvested from the sampling location because catechin content in the flavonoids group varies depending on those factors (Chan et al., 2007). The mentioned factors are further supported by Ghasemzadeh et al. (2015), who reported higher flavonoid content, including catechin, in *S. crispus* leaves harvested from Kelantan in north-east Malaysia compared to other states, which are Penang and Selangor. According to Ismail et al. (2000), catechin was abundant in *S. crispus* leaf extract, and other flavonoids were present, contributing to its high antioxidant properties.

Antioxidant Activity in Dried *S. crispus* Leaves Measured by DPPH Radical Scavenging Assay and FRAP Assay

Table 3 illustrates the antioxidant activity of dried *S. crispus* leaves assessed using the DPPH radical scavenging and FRAP assays. The ability of dried *S. crispus* leaf extracts to quench DPPH free radicals was expressed as an inhibitory concentration, IC_{50} , which is the half-maximal inhibitory concentration to measure the potency of extracts in inhibiting free radicals. It denotes that the smaller IC_{50} value reflected the high potency of the antioxidants assessed in the extract. *S. crispus* leaves dried with MD revealed the highest potency of DPPH scavenging activity with an IC_{50} value of $7.58 \pm 0.48 \mu\text{g/ml}$ compared to the other drying methods ($p < 0.05$). *Strobilanthes crispus* leaves dried

with MD also show more potency than the synthetic antioxidant BHT ($p < 0.05$), followed by FD and AD, which were also more potent than the BHT (IC_{50} values of 26.59 ± 2.78 , 42.28 ± 0.45 , and $55.98 \pm 2.75 \mu\text{g/ml}$, respectively). Meanwhile, *S. crispus* leaves dried with OD showed the highest IC_{50} value of $438.73 \pm 0.76 \mu\text{g/ml}$, indicating the weakest antioxidant potency.

The findings in this study strongly suggest that there is a good scavenging activity in *S. crispus* leaves dried using MD compared to other tested drying methods, which may be attributed to the higher preservation of phenolic and flavonoid content as observed in the analysis of TPC and TFC described earlier. Phenolic and flavonoid constituents can inhibit free radicals because their antioxidant activity is primarily due to their redox attributes, which exert an important duty of adsorbing and neutralising the free radicals, as well as quenching singlet and triplet oxygen or even decomposing peroxides (Hajimehdipoor et al., 2012; Zheng & Wang, 2001). Also, microwave-dried *S. crispus* leaves exhibited higher scavenging activity than oven-dried leaves, agreeing with Lasano et al. (2018). Furthermore, according to Lasano et al. (2018), the moisture evaporated faster during MD as a result of an elevation inside the inner temperature with a bigger vapour pressure produced, which aided the release of phytochemicals out of the samples during extraction. Based on the same trend, $MD > FD > AD > OD$, between TPC, TFC, and antioxidant activity determined by the DPPH radical scavenging assay, it can be

Table 3

DPPH free radical scavenging IC_{50} and ferric reducing antioxidant power of dried *Strobilanthes crispus* leaves

Drying methods	DPPH• scavenging IC_{50} ($\mu\text{g/ml}$)	FRAP value ($\mu\text{g TE/g extract}$)
Standard - BHT	55.98 ± 2.75^d	-
MD	7.58 ± 0.48^a	258.92 ± 0.15^c
FD	26.59 ± 2.78^b	256.40 ± 0.04^{bc}
AD	42.28 ± 0.45^c	256.13 ± 0.04^{ab}
OD	438.73 ± 0.76^e	84.72 ± 0.04^a

Note. Data is represented as means \pm SD ($n = 3$); Values followed with different superscript letters in the same column indicate significant differences at $p < 0.05$; IC_{50} = Half maximal inhibitory concentration; TE = Trolox equivalent; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant power; BHT = Butylated hydroxytoluene; MD = Microwave drying; FD = Freeze drying; AD = Air drying; OD = Oven drying

suggested that there is a direct interaction, which has been established by previous reports (Barimah et al., 2017; Hayat, 2020; Zheng & Wang, 2001).

Regarding the FRAP values of dried *S. crispus* leaves, microwave-dried leaves show the highest value of antioxidant activity ($258.92 \pm 0.15 \mu\text{g TE/g extract}$), followed by leaves dried with FD (256.40 ± 0.04), AD (256.13 ± 0.04), and OD (84.72 ± 0.04), which aligns with the observation on DPPH scavenging activity discussed earlier. It is also notable that microwave-dried leaves are significantly higher ($p < 0.05$) than oven-dried leaves. These observations coincide with a previous report that determined that green tea leaves dried via microwave demonstrated higher FRAP values than other commercial green tea samples tested (Chan et al., 2007). Lasano et al. (2018) have further acknowledged that microwave-dried, fermented, and unfermented *S. crispus* leaves have higher FRAP values than oven-dried leaves. Despite the higher value of FRAP in MD-dried leaves,

the difference with freeze-dried leaves was statistically insignificant ($p > 0.05$). MD allows microwave energy to accelerate the liberation of most phenolic constituents, which reside in the plant matrix in conjugated-bound form (Hayat, 2020). Furthermore, MD also provides intense heating that elevates the inner vapour gradient as well as the temperature within the microstructure tissues and further disrupts the cell wall, causing more extraction of bioactive compounds and contributing to high antioxidant activity (Hayat, 2020). Meanwhile, FD involves low temperatures, thus avoiding the possible extreme loss of bioactive compounds, especially heat-sensitive ones (Barimah et al., 2017).

Meanwhile, the lowest FRAP value in oven-dried leaves can result from higher temperatures and thermal treatment durations for this particular drying treatment. As previously discussed, lower values of TPC and TFC were recorded in the oven-dried leaves, resulting in low antioxidant

activity. This occurrence is aligned with the suggestion by Hayat (2020), where antioxidant activity coincides with TPC as well as TFC values, which explains that antioxidant activity was at least partly due to the total phenols and flavonoids of the samples. It is supported by Franke et al. (2004), who reported that flavonoid catechin and epicatechin, apart from vitamin C present, are potent antioxidants and synergistically contribute to antioxidant activity. Apart from that, the preference selection of *S. crispus* leaves for mature old dark green leaves used in the current study might contribute to better antioxidant properties than the younger leaves at the apex, as suggested by Bakar et al. (2006). This circumstance is caused by the buildup of chemical constituents, particularly phenolics, in the physiology of old mature leaves (Bakar et al., 2006). Therefore, mature old leaves of *S. crispus* might be considered for further practices or industrial applications.

CONCLUSION

Observing the impacts of the different drying methods on *S. crispus* leaves, it can be concluded that MD produced the closest appearance to fresh leaves of *S. crispus* without major alterations to the physical colour. Apart from that, leaves dried with MD showed the highest total phenolic content, flavonoid content, and antioxidant activity compared to the values in leaves dried with FD, AD, and OD. Despite a few reports by previous studies that claimed FD as a better method for drying a few plant

species (Barimah et al., 2017; Orphanides et al., 2013; Roshanak et al., 2016), current research has proven otherwise, which is in accordance with the view of Hajimehdipoor et al. (2012), who opined that each plant requires a specialised drying method due to differences in plant constituents in different plant species. This suggestion concluded that one drying method may not be suitable for all plant species because methodological differences may result in varying phenolic content and antioxidant levels (Hajimehdipoor et al., 2012). This study illustrates the excellent antioxidant activity of *S. crispus*, particularly when dried with MD. MD can, therefore, be a better alternative for drying *S. crispus* leaves since FD is a method that requires high maintenance and high cost. A lab-scale MD was utilised in this study due to the accessibility and affordability at this stage of the research. There is indeed a potential limitation in capacity. However, the findings demonstrated that even with this lab-scale equipment, MD outperformed other methods, including FD, in retaining the antioxidant properties of the leaves. Given the cost-effectiveness of lab-scale MD compared to FD, there is a need for future research to explore industrial-scale microwave drying for large quantities of samples. Despite the limitations, this study provides a valuable foundation for further investigation into the potential benefits of microwave drying on a larger scale. This study highlights the potential of MD in optimising the retention of phytoconstituents in *S. crispus* leaves, which

thus increases its effectiveness for utilisation in herbal or nutraceutical applications and could contribute to a larger scale of *S. crispus* cultivation.

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