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The effect of various drying strategies on the greenness, chlorophyll, bioactive compounds, antioxidant activity, and anti-tyrosinase of dried *Acanthus ilicifolius* L. leaves

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Abstract

The most essential process of *Acanthus ilicifolius* L. tea production is drying. The current convective drying method (*e.g.*, hot-air drying) of producing dried *A. ilicifolius* is inadequate due to its high energy consumption and long drying time, thus leading to undesirable final product quality. The objective of the present work was therefore to evaluate how the different standard drying methods and conditions namely hot-air drying (HAD), microwave drying (MWD), microwave-vacuum drying (MWVD), and freeze drying (FD) affect the greenness, chlorophyll content, antioxidant content, antioxidant activity, and anti-tyrosinase activity of *A. ilicifolius* leaves. MWD increased the drying rate of *A. ilicifolius* leaves by 6.7 times as compared to HAD. The logarithmic model best described moisture evolution during MWD and MWVD. The coefficient of diffusivity for MWD and MWVD was 10 times higher than that of HAD. The greenness of samples dried using HAD, MWD, and MWVD was 58.6 - 78.9, 51.7 - 73.9, and 56.4 - 68.8% lower than that of FD, respectively. As compared to HAD at 80°C, the greenness of the sample dried using MWD and MWVD at 700 W was 16.63 and 5.18% higher, respectively. MWD at 700 W was the best condition for minimising decomposition of the flavonoids (26.0%) and antioxidant activity namely DPPH assay (27.9%), FRAP scavenging (37.1%), reducing power (29.8%), Fe^{2+} chelating ability (22.9%), and Fe^{2+}-1,10-Phenanthroline reducing (33.6%) of these medicinal leaves as compared to FD. Chlorophyll content, total phenolics, ABTS assay, and tyrosinase enzyme inhibition of leaves dried at MWD 700 W were not significantly different from those of the FD sample.

Introduction

Acanthus ilicifolius L. (sea holly), known in Thai as *ngueak-plaa-moo*, is a medium shrub, found in the mangrove areas of Southeast Asia. The leaves have long been used to treat skin allergies, chronic hepatitis, and helminthic infestation (Sardar *et al.*, 2018). The plant is a rich source of phytochemical compounds such as benzoxazinoid glucosides, saponins, phenols, and catechols (Govindasamy and Arulpriya, 2013). These important antioxidant properties make it an important resource for improving human health. When extracting these naturally nutritional and functional compounds from medicinal plants, such as *A. ilicifolius*, drying is often the preferred method due to its ability to reduce high moisture contents before further processing (*e.g.*, tea production). However, due to a variety of driving forces and drying mechanisms, different drying methods can affect the nutritional and functional compounds.

Drying is a process that results in the reduction of microbial growth and prevention of microbial spoilage (Rahimmalek and Goli, 2013). This common preservation method also minimises packaging requirements and reduces bulk weight for easier transportation. Several conventional drying methods have been widely used to reduce moisture in plants namely sun drying and hot-air drying due to their cost-effectiveness. However, convectional air drying often results in a significant colour change of the final product due to elevated temperature and longer

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drying duration (Hamrouni-Sellami *et al*., 2013). This thus causes a reduction in the retention of phytochemical compounds in the dried products (Onwude *et al.*, 2016). Furthermore, in comparison to hot air convective drying, both microwave and microwave-vacuum drying can reduce the drying times while maintaining the product quality (Hamrouni-Sellami *et al*., 2013; Bualuang *et al*., 2019).

Although there have been several studies on the antimicrobial activity and antioxidant properties of *A. ilicifolius* leaves (Khajure and Rathod, 2010), based on our knowledge, there have been no studies on the use of microwave and microwave-vacuum drying methods to produce dried *A. ilicifolius* leaves with better nutritional qualities. Besides, studies on quantifying the greenness, chlorophyll content, bioactive content, antioxidant activity, and anti-tyrosinase enzyme of dried *A. ilicifolius* do not yet exist. Therefore, the objective of the present work was to evaluate the effects of different drying methods (hot-air drying, microwave drying, microwave vacuum drying, and freeze-drying) on the qualities of dried *A. ilicifolius* leaves as functions of operating conditions (drying temperature and microwave intensity). These nutritional and functional qualities include the greenness value, chlorophyll content, bioactive contents (carotenoid, phenolic, and flavonoid), antioxidant activities (DPPH, ABTS, FRAP, reducing power, metal chelating, and 1,10-phenanthroline), and tyrosinase enzyme inhibition.

Materials and methods

Sample preparation

Mature, fresh, and dark green leaves of *A. ilicifolius* (Figure 1) were harvested from Phehla, Klongtom district, Krabi province, Thailand on the 10th of November 2017. The leaves were cleaned and cut into 1 - 1.5 cm lengths. The method prescribed by the Association of Official Agricultural Chemists method (AOAC, 2007) was referred to determine the moisture content of the samples prior to drying.

Drying methods

The leaves (100 g) with an initial moisture content of 369.74 ± 0.09% d.b. were exposed to four different drying methods, namely microwave drying (MWD), microwave-vacuum drying (MWVD), hot air drying (HAD), and freeze-drying (FD). The sample mass was weighed every two minutes until a final moisture content of 10.07 ± 0.01% d.b. was achieved (Rumaisa *et al.*, 2018). Experiments at each interval were done in triplicate. The details of the drying methods were as follows:

i. MWVD was performed using a microwave oven (Samsung MS23F301E) connected to a vacuum pump (New IM-TECH IM125D) with a pumping rate of 0.108 m^3/min (Bualuang *et al.*, 2019). Then, 100 g of *A. ilicifolius* leaves were placed into the vacuum container, and into the oven cavity. The microwave oven was turned on as the pressure inside the chamber reached 100 mbar. Intermittent microwave heating at 300, 450, 600, and 700 W was used. Due to overheating, the microwave power was turned off, and the samples were weighed every two minutes during the drying process until the desired final moisture content was achieved. An infrared thermometer (Amprobe, Model RS1327) was used to determine the drying temperatures during the drying process.

ii. MWD was performed similarly as MWVD (Figure 2) but without connecting it to the vacuum pump. Intermittent microwave heating at 300, 450, 600, and 700 W was used.

iii. HAD was performed using a hot-air oven (Memmert, UF30 model, 32 L capacity, Germany). Then, 100 g of *A. ilicifolius* leaves were placed into a 750 mL glass container, and dried at 80, 100, 120, and 140°C. The range of drying temperatures chosen was based on Rumaisa *et al.* (2018). The sample weight was measured every two minutes during the drying process until the desired final moisture content was achieved.

iv. FD (as a control group for comparing the product quality to other methods) was performed using a freeze dryer (Labconco, benchtop model, 2.5 L capacity, China) at a vacuum pressure of 65 Pa. Then, 100 g of *A. ilicifolius* leaves was frozen at -24°C in a freezer (Sanden Intercool, SNH-0205 model, Japan) for 24 h, dried under vacuum, and sublimated at 30°C for 48 h.

Figure 1. *Acanthus ilicifolius* leaves.

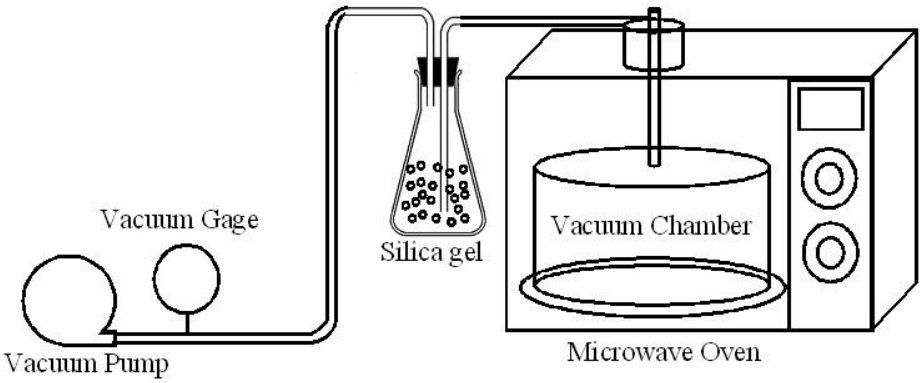

Figure 2. Experimental setup of microwave vacuum drying.

Drying kinetics and diffusivity coefficient

The moisture content changes during drying was expressed in terms of moisture ratio (MR), and derived using Eq. 1 (Bualuang *et al.*, 2019):

$$MR = (M_t - M_e) / (M_0 - M_e) \quad \text{(Eq. 1)}$$

where. M_t = moisture content at any drying time, M_0 = moisture content of the sample before drying (% d.b.), M_e = equilibrium moisture content which was ignored due to its insignificance. Therefore, Eq. 1 was simplified as (Aksoy *et al.*, 2019):

$$MR = M_t / M_0 \quad \text{(Eq. 2)}$$

Fick's second law of diffusion was used to determine the effective diffusivity coefficient (D_{eff}) found within *A. ilicifolius* leaves. Some assumptions were used, which neglected shrinkage and resistance to surface moisture transfer (Crank, 1975). Samples with long lengths and narrow widths were considered

to have infinite slab geometry, as in *A. ilicifolius* leaves. The effective diffusivity was expressed using Eq. 3:

$$MR = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \left[\frac{1}{(2n+1)^2} \exp\left(-(2n+1)^2 \frac{\pi^2 D_{eff} t}{4L^2} \right) \right] \quad \text{(Eq. 3)}$$

where, D_{eff} = effective diffusivity (m^2/s), t = drying time (min), n = 1, 2, 3,….., and L = half the thickness of sample leaves (m). D_{eff} was evaluated using the slope method.

The moisture content changes were predicted using three empirical equations widely used for agriculture materials (Aghbashlo *et al.,* 2008) due to their ability to describe drying behaviour, and are shown in Eqs. 4 – 6:

Newton's equation;

$$MR = \exp(-kt) \quad \text{(Eq. 4)}$$

Page's equation;

$$MR = \exp(-kt^n) \quad \text{(Eq. 5)}$$

Logarithmic equation;

$$MR = a \exp(-kt) + c \quad \text{(Eq. 6)}$$

where, a, c, k, and n = drying constants solved by using non-linear regression analysis, and t = drying time (min).

Dimensionless root mean square error (RMSE), correlation coefficient (R^2), and chi-square (χ^2) were used to evaluate the suitability of the empirical equation (Bualuang *et al.,* 2019). These parameters were expressed using Eqs. 7 – 9:

$$R^2 = \sum_{i=1}^{N} \frac{\left(MR_{pre,i} - MR_{exp,i}\right)^2}{\left(MR_{exp,i} - \overline{MR_{pre}}\right)^2} \quad \text{(Eq. 7)}$$

$$RMSE = \left[\frac{1}{N} \sum_{i=1}^{N} \left(MR_{pre,i} - MR_{exp,i}\right)^2 \right]^{1/2} \quad \text{(Eq. 8)}$$

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(MR_{exp,i} - MR_{pre,i}\right)^2}{N - n} \quad \text{(Eq. 9)}$$

where, $MR_{pre,i}$ = moisture ratio predicted using the mathematical model, $MR_{exp,i}$ = moisture ratio obtained from the experiment, MR_{pre} = average moisture ratio predicted using the mathematical model, n = number of constant values in each equation, and N = total data number.

Greenness analysis

The greenness of *A. ilicifolius* leaves was investigated following Wang *et al.* (2004). Dried leaves were ground into fine particles using a grinder (WF-10B, China), and sieved using a 100-wire mesh screen. Five points of the sample on the Petri dish were determined using a chromameter CR-410 (Konica Minolta, Osaka, Japan), and greenness was reported as *a** values.

Phytochemical evaluation

Sample extraction

Sample extraction followed the methods of Bualuang *et al.* (2019) and Özcan and Matthäus (2017) with some modifications. Briefly, the dried sample leaves were ground with a grinder into fine particles. The particles (3.0000 g) were extracted twice with 100 mL of 80% (w/w) methanol for 6 h, stirred continuously with a shaker, and then filtered with a paper filter. The remaining solids were re-extracted using the same procedure. Both filtrates were pooled, and the solvent was removed using a rotary evaporator at 40°C (Rotavapor R-215 Buchi, Switzerland). Finally, the concentrated extract was dissolved with 25 mL of 80% methanol before being stored in a brown bottle at -25°C until further analyses.

Total phenolic determination

The total phenolic compound (TPC) was analysed following Srisook *et al.* (2010). Briefly, 20 µL of diluted extract solution was pipetted and transferred to a test tube, where 60 µL of distilled water and 20 µL of 50% (v/v) Folin-Ciocalteu reagent was added. After 5 min, 100 µL of 8% (w/v) Na_2CO_3 was added to the mixture. Next, the test tube containing the reaction was incubated at room temperature for 30 min. Then, the reaction's absorbance was measured at 749 nm using a microplate spectrophotometer (BMG LABTECH, Germany). Gallic acid was used as standard solution. All experiments were done in quintuplicate. The TPC was expressed as mg gallic equivalent per g dry weight (mg GAE/g d.w.).

Total flavonoid determination

The total flavonoid content (TFC) was analysed following Srisook *et al.* (2010). The diluted extract (20 µL) was mixed with 80 µL of distilled water, and 6.6 µL of 5% (w/v) $NaNO_2$. After 5 min of the reaction, 6.6 µL of 10% (w/v) $AlCl_3$ was added. The absorbance of the mixture was analysed after 10 min at 510 nm using UV spectroscopy. The TFC was expressed as mg catechin equivalent per g dry weight (mg CAE/g d.w.). All experiments were done in quintuplicate.

Total chlorophyll content

The absorbance of the methanolic extracted solution of dried *A. ilicifolius* leaves was measured at 653 and 666 nm using a 96 well microplate reader (Pessarakli, 1997). All experiments were done in quintuplicate. The total chlorophyll content was expressed as mg per g dry weight (mg/g d.w.), calculated using Eqs. 10 – 12:

$$\text{Chlorophyll (Chl.) a} = (15.65\ A_{666} - 7.34\ A_{653}) \quad \text{(Eq. 10)}$$

$$\text{Chlorophyll (Chl.) b} = (27.05\ A_{653} - 11.21\ A_{666}) \quad \text{(Eq. 11)}$$

$$\text{Total chlorophyll} = \text{Chl. a} + \text{Chl. B} \quad \text{(Eq. 12)}$$

where, A_{653} and A_{666} = absorbance of the extracts at 653 and 666 nm, respectively.

Antioxidant activity determination

DPPH radical scavenging determination

DPPH scavenging was examined following Srisook *et al.* (2010). Briefly, DPPH was dissolved with methanol, forming a purple solution. The purple intensity was decreased by an electron-accepting DPPH• from antioxidant compounds in the extract. Next, a diluted extract of 5 µL was mixed thoroughly with 195 µL of 6×10^{-5} M DPPH• solution for 30 min. Then, the mixture's absorbance was measured at 515 nm using UV spectroscopy. DPPH scavenging activity was expressed as mg Trolox equivalent per g dried weight (mg TE/g d.w.). All experiments were done in quintuplicate.

ABTS radical scavenging determination

The $ABTS^{+}\cdot$ solution was prepared by mixing 5 mL of 7.5 mM ABTS with 0.088 mL of 0.0378 g/mL potassium persulphate before being incubated in the dark for 12 - 16 h (Srisook *et al.*, 2010). Next, $ABTS^{+}\cdot$ solution (195 mL) was added to a diluted extract of 5 µL, and mixed thoroughly. After 7 min of incubation in the dark, the absorbance of the mixture was measured at 745 nm. The ABTS radical's scavenging capacity was expressed as mg Trolox equivalent per g dried matter (mg TE/g d.w.). All experiments were done in quintuplicate.

Ferric reducing antioxidant power

According to Srisook *et al.* (2010), a diluted extract of 0.50 mL was added to 1.50 mL of FRAP reagent in test tubes, which then underwent vortex mixing. The absorbance of the extract was measured at 593 nm after 10 min of incubation in a 37°C water bath. FRAP values were expressed as mg ferrous equivalent per g of dried sample (mg FE/g d.w.). All experiments were done in quintuplicate.

Reducing power determination

An extracted sample (0.50 mL) was mixed with 1.25 mL of 0.2 mM phosphate buffer (pH 6.6) and 1.25 mL of $K_3Fe(CN)_6$ (1%, w/v), and then incubated at 50°C for 30 min, followed by immediate cooling in an ice bath for 1 min. Next, 1.25 mL of CCl_3COOH (10%, w/v) was added and mixed thoroughly. After 5 min, 1.25 mL of supernatant was pipetted and mixed with 1.25 mL of distilled water and 0.25 mL of $FeCl_3$ (0.1%, w/v). Following 10 min incubation, the absorbance of the reaction was measured at 700 nm (Srisook *et al.*, 2010). Reducing power capacity was expressed as mg gallic acid equivalence per g dried weight (mg TE/g d.w.). All experiments were done in quintuplicate.

Fe^{2+} chelating ability determination

Antioxidant compounds can act as chelating agents. Chelation of Fe^{2+} with the extract was investigated following Srisook *et al.* (2010). The diluted extract (0.05 mL), 0.05 mL of phosphate buffer (pH 6.0), and 0.40 mL of distilled water were mixed. Later, 2 mM $FeCl_2$ (0.05 mL) and distilled water (3.00 mL) were added to the mixture. Then, 0.1 mL of ferrozine (5 mM) was added to terminate the reaction, and the mixture was shaken thoroughly. Following incubation at room temperature for 10 min, the absorbance was determined at 562 nm. The chelating activity was expressed as mg EDTA equivalence per g of dried weight (mg EDTA/g d.w.). All experiments were done in quintuplicate.

Fe^{2+} chelation with 1,10-phenanthroline

Antioxidant capacity can be investigated by chelation of Fe^{2+} with 1,10-phenanthroline as described by Minotti and Aust (1987). Diluted extracts (0.3 mL) were added to 0.50 mL of 0.2% (w/v) $FeCl_3$, followed by 0.25 mL of 0.5% (v/v) 1,10-phenanthroline and 3.95 mL of methanol, and mixed thoroughly. The absorbance was read at 510 nm after 20 min of incubation in the dark at room temperature. The antioxidant activity was expressed as mg $FeSO_4$ equivalent per g of dried weight (mg FE/g d.w.). All experiments were done in quintuplicate.

Tyrosinase inhibition activity

To investigate the anti-tyrosinase activity of dried *A. ilicifolius* leaves, a modified dopachrome method was used. L-DOPA was employed as a substrate, and ascorbic acid as a standard inhibitor compound; the measurement was taken with a microplate spectrophotometer as described by Neagu *et al.* (2018). Briefly, extracted solution, sodium phosphate buffer pH 6.8 (0.2 M, 80 µL) and tyrosinase enzyme (100 unit/mL, 40 µL) solution in buffer were mixed. After 10 min of reaction time in the dark at room temperature, 80 µL of 12 mM L-DOPA was added and mixed. The absorbance was read at 479 nm after being incubated at 37°C for 15 min. Tyrosinase inhibition activity was expressed as mg ascorbic acid equivalent per g of dried weight (mg AAE/g d.w.). All experiments were done in quintuplicate.

Morphology of dried samples

The morphology of the leaves after drying by various methods was assessed using a scanning electron microscope (SEM; JSM-5600, JEOL). Briefly, the leaves were cut into squares, placed on aluminium stubs, and coated with a layer of vaporised gold ions. The SEM image was observed at 20 kV.

Statistical analysis

All experiments were conducted in quintuplicate, and variance of the average values was evaluated using SPSS with one-way ANOVA tests ($p < 0.05$).

Results and discussion

Drying rate and moisture diffusivity coefficient

Acanthus ilicifolius leaves with an initial moisture content of 369.74 ± 0.09% d.b. was reduced to 10.07 ± 0.01% d.b. using HAD, MWD, and MWVD methods. The drying kinetics and moisture diffusivity coefficient of *A. ilicifolius* leaves is depicted in Table 1 and Figure 3.

The changes in moisture ratios, sample temperatures, and drying proportion with drying time during the drying processes of *A. ilicifolius* leaves are illustrated in Figures 3 to 6. As expected, longer times were required at lower temperatures and microwave power. The times needed to eliminate moisture within the samples to the desired level were 24 - 10 min and 24 - 9 min for 300 - 700 W of MWD and MWVD,

Table 1. Effective diffusivity (D_{eff}) of *Acanthus ilicifolius* leaves dried using hot air (HAD), microwave (MWD), and microwave-vacuum (MWVD) drying methods.

Drying strategy	Sample temperature (°C)		Final moisture content (%d.b.)	Drying time (min)	D_{eff} (m^2/s)	R^2
	Maximum	Average				
HAD 80°C	81.0	79.9	10.07	400.0	1.822×10^{-11}	0.9354
HAD 100°C	101.0	99.8	10.08	230.3	3.044×10^{-11}	0.9314
HAD 120°C	122.0	119.6	10.05	139.0	4.726×10^{-11}	0.9274
HAD 140°C	142.0	139.6	10.07	137.3	5.655×10^{-11}	0.9393
MWD 300 W	75.0	64.9	10.08	24.0	2.270×10^{-10}	0.8734
MWD 450 W	90.0	78.5	10.08	14.4	4.272×10^{-10}	0.8965
MWD 600 W	95.0	83.7	10.08	11.1	6.088×10^{-10}	0.9073
MWD 700 W	100.0	87.6	10.08	10.5	7.208×10^{-10}	0.9185
MWVD 300 W	66.0	60.1	10.08	24.0	2.564×10^{-10}	0.9002
MWVD 450 W	72.0	63.5	10.08	15.3	4.307×10^{-10}	0.9057
MWVD 600 W	72.0	63.4	10.08	12.0	5.936×10^{-10}	0.9135
MWVD 700 W	83.0	63.0	10.08	9.3	8.259×10^{-10}	0.9185

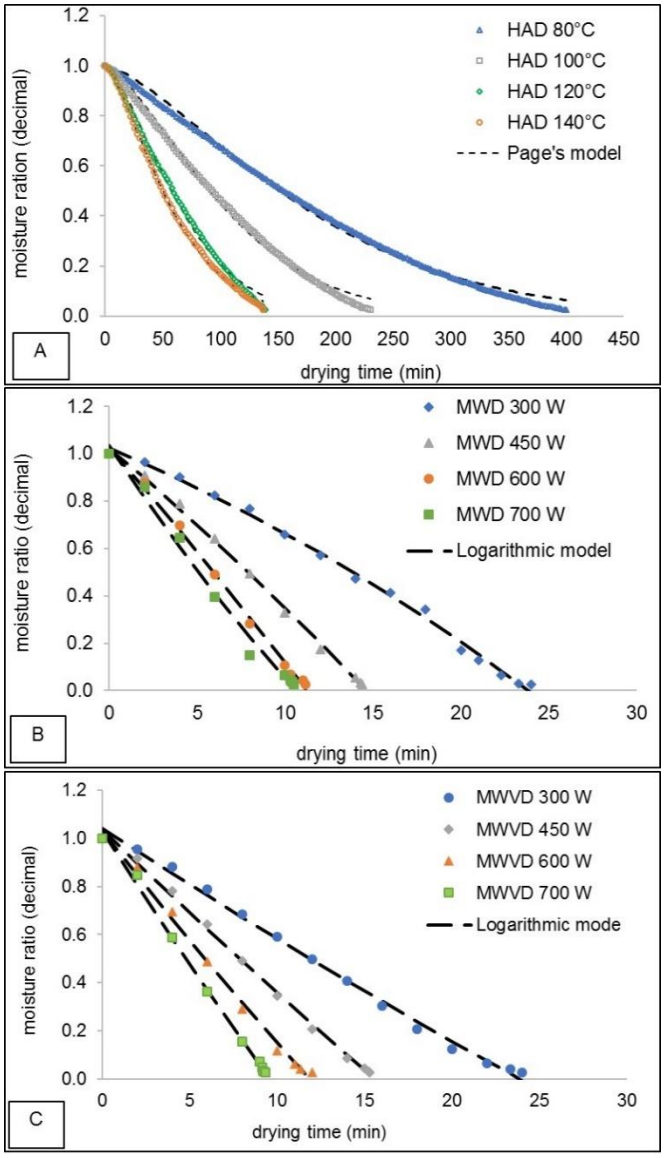

Figure 3. Changes in moisture ratios at different drying methods for **(A)** hot air at 80 - 140°C, **(B)** microwave drying at 300 - 700 W, and **(C)** microwave vacuum at 300 - 700 W.

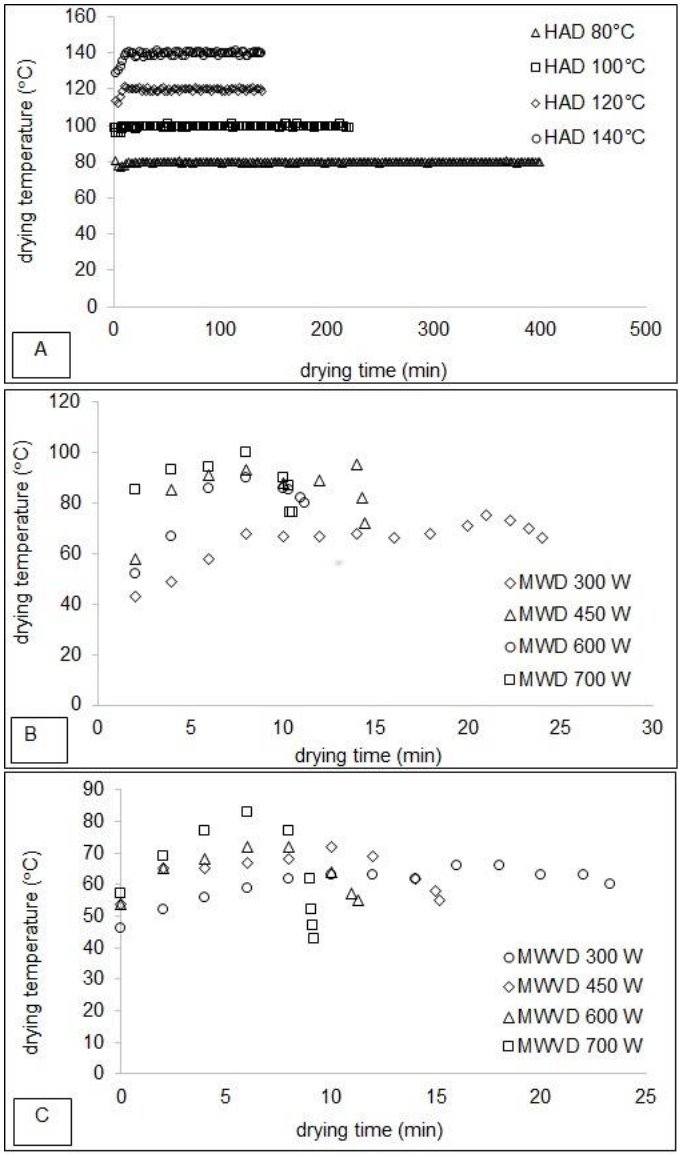

Figure 4. Changes in temperatures at different drying methods for **(A)** hot air at 80 - 140°C, **(B)** microwave drying at 300 - 700 W, and **(C)** microwave vacuum at 300 - 700 W.

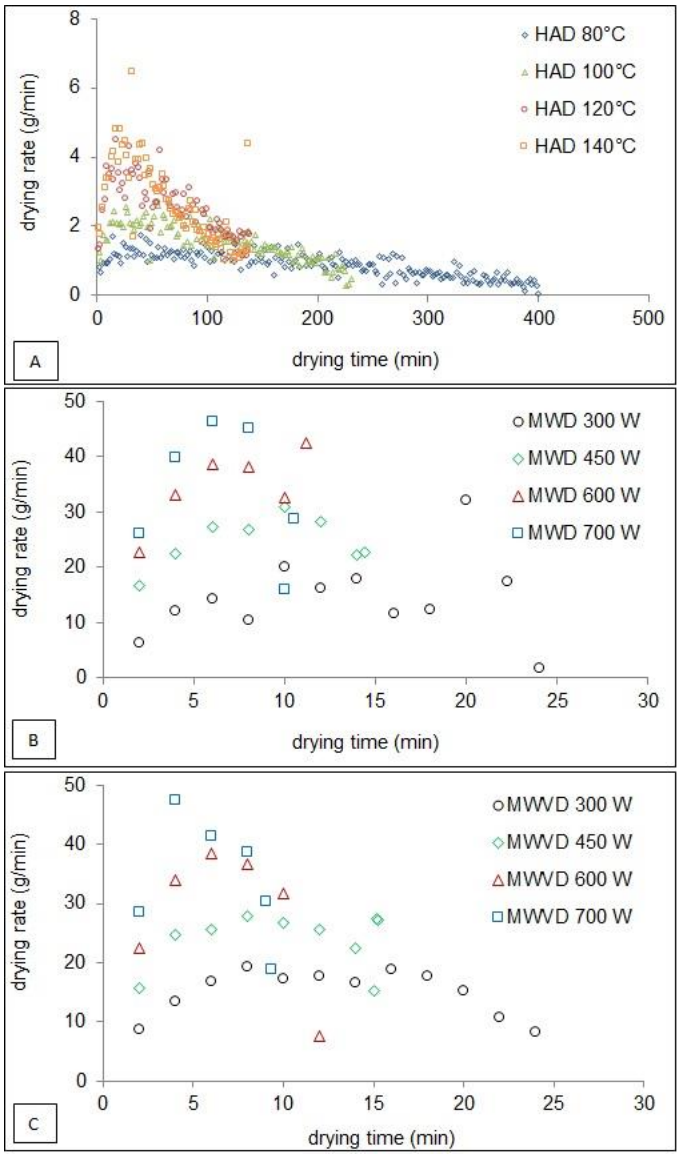

Figure 5. Changes in drying rates at different drying methods for **(A)** hot air at 80 - 140°C, **(B)** microwave drying at 300 - 700 W, and **(C)** microwave vacuum at 300 - 700 W.

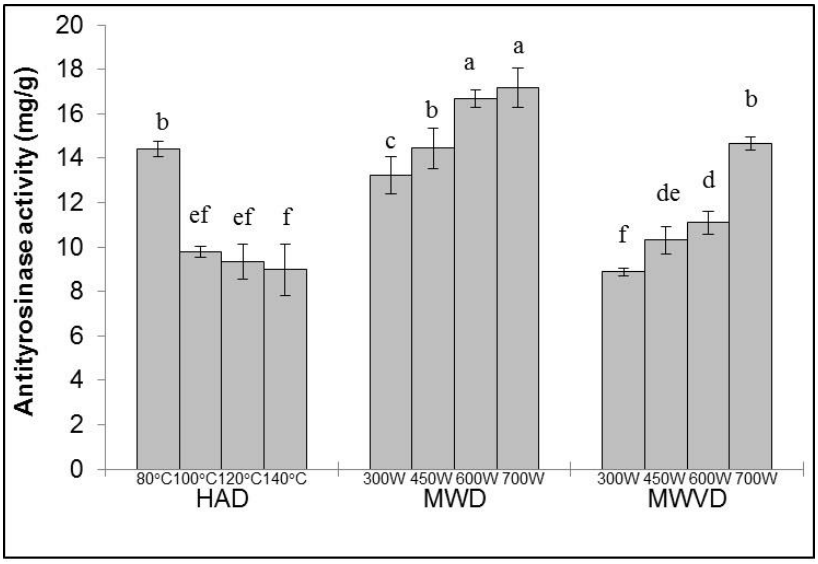

Figure 6. Effect of different drying methods on the anti-tyrosinase activities of dried *Acanthus ilicifolius* leaves. Values within each drying method with different lowercases are significantly different ($p < 0.05$).

respectively. Increasing the microwave intensity from 300 to 700 W decreased the drying time by 58%. For HAD, the times required were 400 - 137 min at 80 - 140°C.

Regarding the drying behaviour (Figure 3) at low microwave powers (300 and 450 W) and drying temperatures (80 and 100°C), drying curves showed three stages in the drying process, including warming up time, constant rate, and falling rate. During the warming up period, the surface moisture was rapidly removed as a result of a great difference between air temperature and sample surface temperature. The constant rate period (short duration) was due to the moisture inside the sample being controlled by the diffusion mechanism, and transferred via the porous structure of the sample surface (Gazor and Minaei, 2005). When drying at higher temperatures (120 and 140°C) and microwave powers (600 and 700 W), there was no constant rate period; here, drying proceeded directly to the falling rate stage. These results were in accordance with those reported by Onwude *et al.* (2016). The drying rate of MWVD was slightly higher than that of MWD due to a lower pressure, thus resulting in a lower boiling point.

The drying kinetics of *A. ilicifolius* leaves using various drying methods were described based on the selected drying models Newton, Page, and Logarithmic. The result of the kinetic model showed that the moisture removal behaviour for HAD could be best predicted using Page's model, which had the highest correlation coefficient (R^2), and the lowest root mean square error (RMSE) and chi-square (χ^2), while the logarithmic model was the best fit for both MWD and MWVD. Turkiewicz *et al.* (2019) reported that Page's model is the best fitting model for several agricultural crops during HAD. The logarithmic model has been popular in describing the moisture transfer model of bitter gourd flakes and pumpkin (Chauhan *et al.*, 2018).

The effective moisture diffusivities were calculated under some assumptions such as the infinite slab of sample shape, a lack of shrinkage, and resistance to surface moisture transfer, as shown in Table 1. An increase in drying temperature and microwave power resulted in increasing D_{eff} values due to increased volumetric thermal energy. These results corresponded to those reported by Onwude *et al.* (2016), which stated that the D_{eff} of many food stuffs were within the range of 10^{-11} - 10^{-9} m^2/s. The low boiling point of water (45.6°C at 100 mbar) resulted in greater D_{eff} for MWVD than that of MWD and HAD. Additionally, the lower pressures associated with MWVD prevented overheating during microwave drying (Orikasa *et al.*, 2018).

Effect of drying methods on greenness and total chlorophyll

The greenness of the samples is an important quality parameter. The reduction of greenness during the drying process is not only caused by the decomposition of colorants, chlorophyll, and carotenoids, but also by enzyme action and chemical reactions such as the browning reaction (Zielinska and Michalska, 2016). The changes in the greenness (*a**) parameter of *A. ilicifolius* during various drying methods is illustrated in Table 2.

The increased *a** value indicated a reduction in greenness. The greenness of FD samples was higher than that of those dried using other methods, but the method requires longer drying times and higher energy consumption; thus, it is not often applied at the industrial level (Turkiewicz *et al.*, 2019; Xu *et al.*, 2020). The greenness of samples dried under HAD was significantly decreased by 49.17% as air-drying temperature increased from 80 to 140°C, which could be attributed to the Maillard non-enzymatic reaction (Motevali *et al.*, 2011), and pigment degradation during the drying process (Xu *et al.*, 2020). Accordingly, similar results were reported by Turkiewicz *et al.* (2019). They found that the greenness of Japanese quince fruit decreased when the drying temperature of HAD increased from 50 to 70°C. The result was in accordance with a previous study (Therdthai and Zhou, 2009) that investigated the effect of MWVD and HAD on the greenness of mint leaves. They reported that increased drying temperature resulted in the colour changing from green to red, thus resulting in a dark green-brown colour. Moreover, they found that after 15 min of MWVD, the colour of mint leaves began to show a green-yellow colour due to the decomposition of chlorophyll. They attributed this to the loss of magnesium from the chlorophyll structure at high temperatures, causing the conversion of chlorophyll to pheophytins.

After both MWD and MWVD, the greenness changed in different ways. The greenness of the sample increased with an increase in microwave power because of its resultant shorter drying time. The influence of microwave power on colour change

was in accordance with Chauhan and Srivastava (2009).

FD provided the best product quality in terms of colour, followed by MWD and MWVD at 700 W. Considering the effect of drying methods on greenness deterioration, the results showed decreasing greenness values during the drying process when using HAD at 80°C, MWD at 700 W, and MWVD at 700 W with 58.6, 51.7, and 56.4% reduction as compared to FD sample, respectively. The result is in agreement with those obtained by Xu *et al.* (2020) and Therdthai and Zhou (2009). Maskan (2001) stated that the colour compounds could degrade at high drying temperatures; also, the authors found that microwave drying can both accelerate drying rates and reduce the decay of colour constituents.

The chlorophyll compound, a green colour composition, was investigated in all drying methods. The degradation of chlorophyll was influenced by the drying period, temperature, and thermal strategies (Xu *et al.*, 2020). The results of various drying methods on chlorophyll content are illustrated in Table 2. It was apparent that chlorophyll content degradation can be prevented by drying under a lower temperature (80°C) using HAD, and high microwave intensity (700 W) using MWD. The effect of temperature during HAD and microwave power during MWD and MWVD on the chlorophyll content showed a trend similar to that of greenness in all drying methods. Increasing either the air temperature or microwave power during drying caused a significant increase in greenness and chlorophyll content when not exceeding 600 W using MWVD. During thermal processing, chlorophyll can be degraded to pheophytin via the first-order reaction (Rahimmalek and Goli, 2013). The chlorophyll content of the samples after MWVD at 700 W was lower than at 600 W. This may result from a high vapour pressure difference between the leaves and the sample surface, which damaged the sample, thus leading to the degradation of chlorophyll content. The total chlorophyll content of MWVD was found to be higher than that of MWD at the same microwave level, except for MWVD at 700 W. This may be attributed to the activity of chlorophyllase and lipidase, which was higher for MWVD samples dried at 700 W due to the shorter drying time, higher dehydration rate, and lower oxidation rate for MWVD under appropriate conditions (Xu *et al.*, 2020).

Effect of drying methods on TPC and TFC

The effects of various drying methods on important phytochemical properties of *A. ilicifolius* are listed in Table 3 in which the TPC and TFC varied. With no heating effect (FD), *A. ilicifolius* leaves had a TPC of methanolic extract of 218.84 ±

Table 2. Greenness and total chlorophyll of *Acanthus ilicifolius* leaves dried using hot air (HAD), microwave (MWD), and microwave-vacuum (MWVD) drying methods.

Drying strategy	Greenness (*a**)	Total chlorophyll (mg/g)
FD	-13.06 ± 0.06^{a}	3.90 ± 0.06^{b}
HAD 80°C	-5.41 ± 0.07^{d}	4.55 ± 0.14^{a}
HAD 100°C	-3.20 ± 0.09^{j}	4.01 ± 0.13^{b}
HAD 120°C	-2.89 ± 0.07^{k}	3.31 ± 0.23^{de}
HAD 140°C	-2.75 ± 0.03^{l}	3.50 ± 0.08^{cd}
MWD 300 W	-3.47 ± 0.09^{i}	3.13 ± 0.02^{e}
MWD 450 W	-4.82 ± 0.07^{g}	3.33 ± 0.02^{d}
MWD 600 W	-4.96 ± 0.04^{f}	3.58 ± 0.21^{c}
MWD 700 W	-6.31 ± 0.09^{b}	3.81 ± 0.08^{b}
MWVD 300 W	-4.08 ± 0.09^{h}	3.54 ± 0.07^{c}
MWVD 450 W	-4.94 ± 0.04^{f}	3.54 ± 0.07^{c}
MWVD 600 W	-5.09 ± 0.01^{e}	3.93 ± 0.05^{b}
MWVD 700 W	-5.69 ± 0.04^{c}	3.57 ± 0.01^{c}

Values within each column with different lowercase superscripts are significantly different ($p < 0.05$).

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) retention of *Acanthus ilicifolius* leaves dried using hot air (HAD), microwave (MWD), and microwave-vacuum (MWVD) drying methods.

Drying strategy	TPC (mg GAE/g d.w.)	TFC (mg CAE/g d.w.)
FD	218.84 ± 2.09^{a}	248.54 ± 4.52^{a}
HAD 80°C	129.69 ± 4.96^{e}	114.44 ± 2.40^{e}
HAD 100°C	51.43 ± 0.87^{i}	54.96 ± 1.83^{g}
HAD 120°C	43.56 ± 0.40^{j}	48.25 ± 6.60^{gh}
HAD 140°C	44.25 ± 0.42^{j}	45.64 ± 2.11^{h}
MWD 300 W	24.68 ± 0.20^{k}	129.68 ± 11.45^{d}
MWD 450 W	142.02 ± 4.28^{d}	145.98 ± 1.40^{c}
MWD 600 W	212.05 ± 2.36^{b}	183.08 ± 7.82^{b}
MWD 700 W	221.79 ± 1.72^{a}	183.93 ± 4.72^{b}
MWVD 300 W	56.97 ± 3.53^{h}	66.96 ± 1.22^{f}
MWVD 450 W	62.98 ± 2.07^{g}	68.36 ± 2.49^{f}
MWVD 600 W	68.61 ± 0.79^{f}	71.59 ± 0.46^{f}
MWVD 700 W	166.25 ± 6.46^{c}	144.42 ± 3.62^{c}

Values within each column with different lowercase superscripts are significantly different ($p < 0.05$).

2.09 mg GAE/g d.w., which was similar to results reported by Thirunavukkarasu *et al.* (2011). However, many other researchers have reported different results for both TPC and TFC and their respective antioxidant activities regarding *A. ilicifolius* which could result from diverse climates, regions, treatment methods, genetics, and plant varieties (Biswas *et al.*, 2019).

The TPC decreased after HAD from 80 - 140°C, and the decrease was 40.74, 76.50, 80.10, and 79.78%, respectively. For TFC, the decrease was 53.96, 77.89, 80.59, and 81.64%, respectively. This decreasing trend of TPC is similar to the previous work reported by Srivastava and Kaur (2020). They studied the effect of microwave and oven treatments on Moringa oil, and found that higher temperatures and longer heating times increased the rate of polyphenol oxidation. In the case of MWD, increasing the microwave power from 300 to 700 W of MWD led to lower degradation rates by 88.9 and 29.5% for TPC and TFC, respectively. A similar trend was observed in the report by Inchuen *et al.* (2010), which looked at the effect of microwave treatment ranging from 180 - 540 W on Thai red curry powder, where they found an increase of 13.0% of the TPC. Hamrouni-Sellami *et al.* (2013) also found that increasing microwave power (600 to 800 W) caused the TPC of *Salvia officinalis* L. to increase, which they attributed to the disturbance of the plant tissue by microwave intensity penetration, thus causing an increase in the release of phenolic compounds. For MWVD, increasing the microwave intensity to 600 W caused the antioxidant level to increase, but the bioactive compounds decreased at 700 W.

It was found that MWD and MWVD had significantly higher TPC and TFC as compared to HAD at higher temperatures. Similar results were found by Özcan *et al.* (2020) who studied the effects of microwave and oven-heating on antioxidant activity, total phenols, and phenolic compounds in kiwi and pepino fruits. They reported that microwave drying led to increased amounts of both antioxidant activity and TPC.

Effect of drying methods on antioxidant activity

The antioxidant activity cannot be evaluated using only one method because of the complexity of the composition of plants, and possible reactions between them and their structure (Valadez-Carmona *et al.*, 2016). Therefore, in the present work, the antioxidant activity found in extracted leaves was determined by DPPH, ABTS, FRAP, reducing power, Fe^{2+} chelating ability, and the Fe^{2+}-1,10-phenanthroline method as shown in Table 4. The highest and lowest antioxidant activity values were obtained for samples dried using MWD at 700 W and HAD at 140°C, respectively. Moreover, antioxidant ability was reduced in HAD at higher temperatures, and in MWD and MWVD at decreased microwave

Table 4. Antioxidant activities of *Acanthus ilicifolius* leaves dried using hot air (HAD), microwave (MWD), and microwave-vacuum (MWVD) drying methods.

Drying strategy	DPPH (mg TE/g d.w.)	ABTS (mg TE/g d.w.)	FRAP (mg FE/g d.w.)	Reducing power (mg GAE/g d.w.)	Metal chelating (mg EDTA/g d.w.)	1, 10-Phenanthroline (mg FE/g d.w.)
FD	861.66 ± 3.20^{a}	986.99 ± 44.06^{a}	195.53 ± 1.61^{a}	6.31 ± 0.01^{a}	36.43 ± 0.31^{a}	308.20 ± 16.15^{a}
HAD 80°C	503.82 ± 42.36^{c}	954.99 ± 26.97^{a}	128.62 ± 12.85^{b}	4.10 ± 0.01^{c}	22.66 ± 0.47^{d}	192.49 ± 13.54^{bc}
HAD 100°C	457.09 ± 35.67^{d}	543.66 ± 29.28^{ef}	55.73 ± 0.39^{e}	1.24 ± 0.00^{i}	14.35 ± 0.95^{h}	83.14 ± 6.96^{f}
HAD 120°C	25.25 ± 5.27^{i}	485.59 ± 20.33^{fg}	58.50 ± 0.05^{e}	1.05 ± 0.00^{j}	11.60 ± 0.30^{j}	52.25 ± 4.09^{g}
HAD 140°C	17.20 ± 4.66^{i}	456.89 ± 15.34^{g}	47.37 ± 0.13^{f}	1.05 ± 0.00^{j}	12.51 ± 0.50^{ij}	76.22 ± 8.79^{f}
MWD 300 W	410.84 ± 24.61^{e}	796.27 ± 59.44^{bc}	93.25 ± 0.26^{d}	2.98 ± 0.03^{f}	19.66 ± 0.30^{f}	148.47 ± 8.41^{d}
MWD 450 W	450.89 ± 21.62^{d}	836.05 ± 41.36^{b}	106.48 ± 0.52^{c}	3.70 ± 0.03^{d}	21.12 ± 0.37^{e}	173.79 ± 4.19^{c}
MWD 600 W	597.60 ± 9.32^{b}	930.69 ± 23.06^{a}	123.09 ± 3.29^{b}	4.10 ± 0.01^{c}	21.26 ± 0.14^{e}	204.02 ± 7.41^{b}
MWD 700 W	621.22 ± 32.41^{b}	935.95 ± 34.48^{a}	122.91 ± 0.49^{b}	4.43 ± 0.04^{b}	28.08 ± 0.21^{b}	204.67 ± 6.46^{b}
MWVD 300 W	95.68 ± 6.11^{h}	539.81 ± 23.35^{ef}	53.35 ± 0.35^{ef}	1.59 ± 0.03^{h}	12.98 ± 0.12^{i}	81.55 ± 12.49^{f}
MWVD 450 W	137.34 ± 23.13^{g}	568.16 ± 37.26^{de}	59.57 ± 0.15^{e}	1.86 ± 0.02^{g}	16.61 ± 0.34^{g}	108.63 ± 13.96^{e}
MWVD 600 W	200.21 ± 28.83^{f}	619.17 ± 54.78^{d}	59.13 ± 1.99^{e}	1.61 ± 0.01^{h}	17.52 ± 0.30^{g}	110.12 ± 17.70^{e}
MWVD 700 W	465.49 ± 19.09^{cd}	770.64 ± 28.06^{c}	100.71 ± 0.56^{c}	3.27 ± 0.03^{e}	24.31 ± 1.34^{c}	177.61 ± 7.33^{c}

Values within each column with different lowercase superscripts are significantly different ($p < 0.05$).

intensity, which is in accordance with Turkiewicz *et al.* (2019).

Effect of drying methods on anti-tyrosinase activity

Tyrosinase is a multifunctional copper-containing enzyme found in plants, fungi, and animals (Huang *et al.*, 2006). Tyrosinase enzyme acts as a producer of melanin pigment by activating the oxidation reaction of L-tyrosine to dopaquinone. These dopaquinones are highly reactive and polymerise into melanin pigments which are brown in colour. The tyrosinase enzyme was inhibited by antioxidant compounds exist in the extracted dried leaves. The activity of anti-tyrosinase was expressed as mg ascorbic acid equivalent per g of dried weight, as illustrated in Figure 6.

The anti-tyrosinase activity of extract dried under FD was 17.05 ± 0.21 mg AAE/g d.w., which was not significantly different from that of samples dried using MWD at 600 (16.69 ± 0.40 mg AAE/g d.w.) and 700 W (17.16 ± 0.87 mg AAE/g d.w.). Results showed that the anti-tyrosinase activity of dried samples significantly decreased when hot-air drying temperature was increased, and microwave power was decreased. These results were attributed to lower degradation of chlorophyll, phenolics, and flavonoids due to higher drying temperatures of HAD, and shorter drying times and lower average temperatures of MWD and MWVD (not over 90°C for all MWD and MWVD treatments, as seen in Table 1). Lech *et al.* (2018) reported that the safest temperature for microwave vacuum drying process of pumpkin slices to preserve antioxidant compounds is below 90°C. In the present work, the extracted leaves dried using MWD at 700 W possessed more antioxidant compounds, and exhibited greater anti-tyrosinase activity than that of HAD at 80°C due to shorter drying time. MWVD at 700 W showed that a lower anti-tyrosinase activity could cause the deformation of the sample structure due to high vapour pressure within the sample. However, there is no available information about the effects of vacuum pressure within the sample on bioactive compounds and tyrosinase inhibitory activity of plant materials. The inhibition mechanisms of bioactive components were described by Therdphapiyanak *et al.* (2013). They stated that one possible way to inhibit the tyrosinase enzyme is by using bioactive substances, acting as inhibitors, bound to the active sites of copper; this would also prevent the oxidation reaction of the electrochemical process. Different bioactive substances have different inhibition mechanisms. Flavonoids can bind the copper atoms in the active sites of the enzyme. The electrochemical process of L-DOPA was inhibited by quercetin (Therdphapiyanak *et al.*, 2013). Moreover, Kaewnarin *et al.* (2016) assumed that hydroxyl groups of phenolic compounds could form a hydrogen bond to the active sites of the enzyme, thus causing the inhibition of the tyrosinase enzyme, and that antioxidant activity may be one of the mechanisms essential for tyrosinase inhibition.

Effect of drying methods on morphology

Microstructure changes of *A. ilicifolius* leaves dried using various treatments were investigated using a scanning electron microscope (SEM) with a magnifying power of 200×, as shown in Figure 7. The SEM showed a fine structural view of the salt glands and the stomata on the leaves' bottom surface. Wong and Ong (1984) described that salt glands, in which no holes are present on both the dorsal and ventral sides of the leaf, are rich on the dorsal surface. Each stoma has a length and width of 20 and 10 µm, respectively, and the salt glands have diameters of approximately 40 - 50 µm. Different drying methods have significant effects on the microstructure of the leaves.

Leaves dried using FD (Figures 7a - 7b) and MWD (Figures 7c - 7f) showed a smooth surface and less shrinkage of cellular tissue. Salt glands were more abundant on the dorsal side for FD than for MWD. This could result from the glands being destroyed by the high temperature and the microwave intensity penetration of MWD. HAD dried leaves (Figure 7c - 7d) exhibited more shrinkage of fibre structure when compared with FD, MWD, and MWVD. This may be due to the longer drying time when compared with the other treatments. MWVD dried products (Figure 7g - 7h) revealed less shrinkage than those of HAD. The salt glands of leaves dried by MWVD were obviously deformed, and there was an aperture of the glands and penetration to the other side.

Conclusion

The present work demonstrated the best drying methods for *A. ilicifolius* based on various quality characteristics such as phenolics, flavonoids, antioxidant activity, chlorophyll, and final colour of

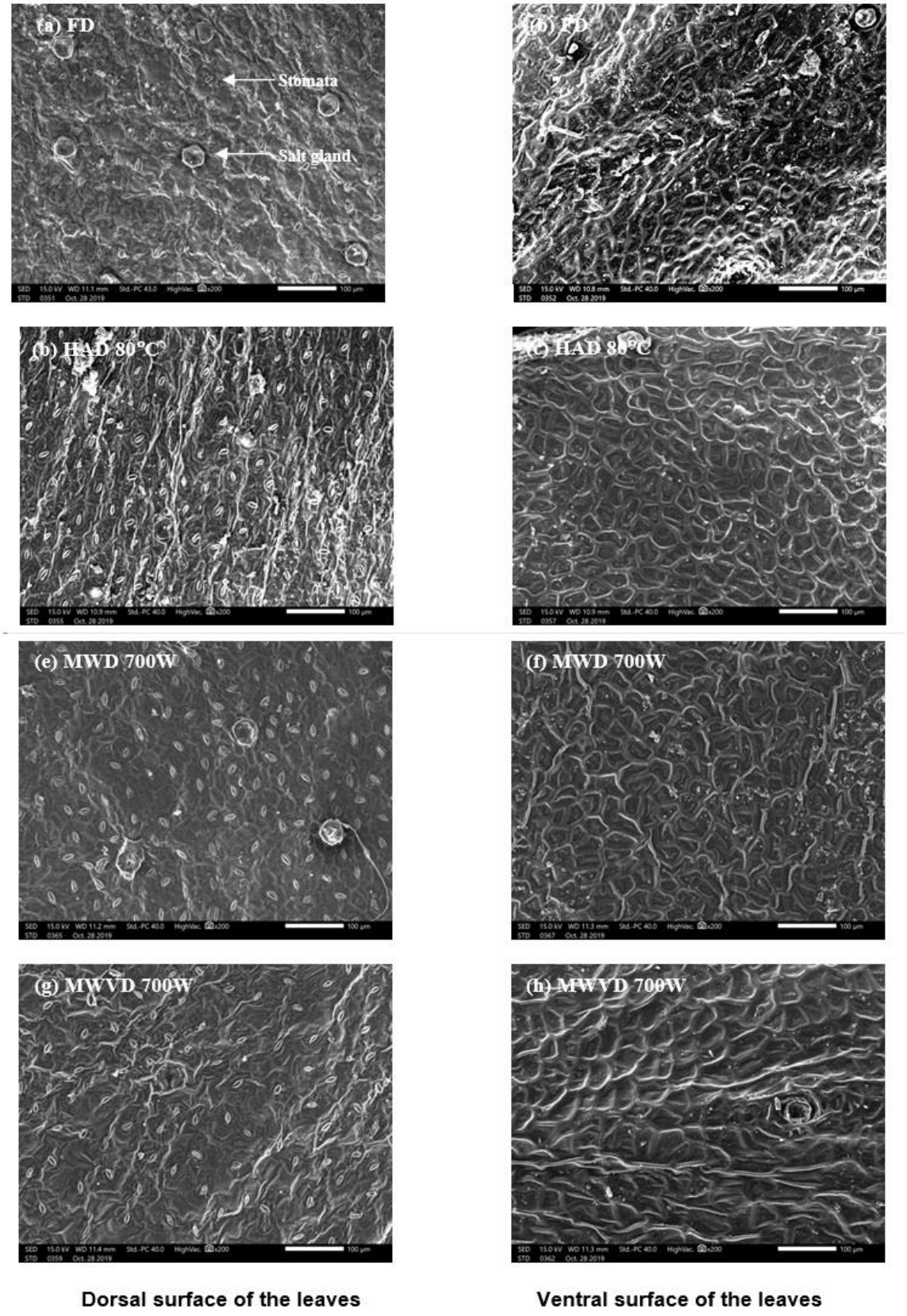

Figure 7. Scanning electron micrographs of *Acanthus ilicifolius* leaves dried using four different drying methods. FD = freeze-drying, HAD 80°C = hot-air drying at 80°C; MWD 700 W = microwave drying at 700 W; MWVD 700 W = microwave vacuum drying at 700 W.

the dried product. High air temperatures and longer drying time increased colour degradation of *A. ilicifolius*. Microwave drying (MWD) best preserved the greenness (51.7%), flavonoids (26.0%), and antioxidant activity, including DPPH assay (27.9%), FRAP scavenging (37.1%), reducing power (29.8%), Fe^{2+}-Metal chelating ability (22.9%), and Fe^{2+}-1,10-phenanthroline reducing (33.6%) of these medicinal leaves at 700 W as compared to freeze-drying (FD). There was no significant difference between the chlorophyll contents, total phenolics, ABTS assay, and tyrosinase enzyme inhibition of the dried sample under MWD 700 W and those of the FD sample. This vital information on the advantages of MWD over other standard commercial drying methods could pave the way to adopting this technology for the industrial drying of medicinal plants.

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