ASEAN Journal of Chemical Engineering 2024, Vol. 24, No. 1, 24 – 35

Dehydration Impact on Antioxidant Potential and Phenolic Content of Lemon Myrtle *(Backhousia citrodora)* Leaves

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Submitted 15 August 2023 *Revised* 4 April 2024 *Accepted* 6 April 2024

Abstract. The most popular method for food preservation is dehydration. To enhance the overall quality and prolong the shelf life of herbal products, it is imperative to choose optimal dehydration conditions carefully. The study aimed to determine the best dehydration method with maximum retention of biochemical content in *Backhousia citriodora* leaves. The dehydration of *Backhousia citriodora*, also known as lemon myrtle leaves (LML), was conducted via three distinct techniques: conventional dehydration (CD) at temperatures of 40, 50, and 60°C (referred to as CD40, CD50, and CD60, respectively); vacuum dehydration (VD) at the same temperature as conventional dehydration with a pressure of 50 mbar; and heat pump dehydration (HPD) at a constant temperature of 45°C. The antioxidant capacities, specifically the radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP), along with the total phenolic content (TPC), were evaluated. HPD samples came up second to VD samples regarding TPC retention, DPPH activity, and FRAP test, whereas CD samples had the lowest biochemical content across all dehydration conditions. The TPC and antioxidant activity in the CD sample exhibited a substantial reduction as the dehydration temperature increased. After dehydration, the CD60 sample had the largest TPC, DPPH, and FRAP values reduction. Therefore, VD was the best method for lemon myrtle leaves dehydration with maximum retention of antioxidant ac comparable to the HPD method. Maximizing the retention of biochemical content is one of utmost importance in post-harvest processing as it serves as an indicator of greater retention. Therefore, selecting appropriate dehydration techniques and conditions is critical in achieving this objective.

Keywords: Antioxidant, *Backhousia citriodora*, Heat Pump Dehydration, Total Phenolic Content, Vacuum Dehydration

DOI: 10.22146/ajche.9433

INTRODUCTION

Backhousia citriodora, also known as lemon myrtle (Fig. 1) is commonly employed in its dried form as a flavoring ingredient and as herbal tea in Australia. Its commercial distribution has been approved in the European Union under the new food category (Sultanbawa, 2016). Because of their strong antioxidant activity, *Backhousia citridora* have many potentials in the food, pharmaceutical, and cosmetic industries. According to (Sandrang *et al*., 2014), lemon myrtle has two possible products: [1] essential oil derived from the leaves and [2] dehydrated leaves produced by the dehydration process of fresh *Backhousia citriodora*. From these semi-finished goods, products of cosmeceuticals, nutraceuticals, and medicines may be developed. Commonly, the dehydrated LML are processed for exportation to foreign countries as LML owns the properties of antibacterial (Cock, 2013; Chao *et al*., 2008; Dupont *et al*., 2006), antimicrobial (Zrustova *et al*., 2006; Pattnaik *et al*., 2016) and antioxidant (Xia *et al*., 2013).

Fig. 1: *Backhousia citriodora* (lemon myrtle) plantation plot in MARDI Research Station

According to Buchaillot et al. (2009), reducing the moisture level of a substance guarantees that microbial development is suppressed and that the degradation of biochemical processes is limited. This research used a heat pump, vacuum and conventional dehydration techniques to dehydrate *Backhousia citriodora*. The most popular drier in the food industry is where there is a considerable and unavoidable loss of quality and bioactive substances in the conventional dehydrator. As the dehydration temperature rose during conventional dehydration on several goods, including Galega kale (Oliveira *et al*., 2015) and *Angelica sinensis* (Wu *et al*., 2013) significant nutritional losses and product degradation were measured. The use of heat pump dryers has lately received a lot of interest and attention in the food processing sector due to the increased energy efficiency. Heat-pump aided dehumidified air dehydration of *Moringa oleifera* leaves preserved and maintained at acceptable levels the phytochemicals, antioxidant capacity, and color (Potisate *et al., 2015)*. Vacuum dehydration is another technique that is often used on materials that are sensitive to heat. The lack of a dehydration medium is thought to prevent the goods' biological content from oxidizing. Rosemary and thyme, collard leaves, nettle leaves, and lemon balm all had greater TPC and FRAP levels after vacuum dehydration (Alibas, 2007, 2009; Argyropoulos and Müller, 2014; Hossain *et al*., 2010).

The herb was dried until its moisture content reached 10% or below, which is ideal for the dried product (Alharbi et al., 2021). The dehydration of *Backhousia citriodora* is commonly achieved with the application of heated air (at a temperature of around 40°C) within the dehydration chamber; nevertheless, this method is associated with a prolonged duration. The extended exposure of dried *Backhousia citriodora* to elevated

temperatures has been observed to deteriorate its quality, mostly due to an increased likelihood of biochemical degradation. Application of a suitable dehydration method and condition for *Backhousia citriodora* leaves' is crucial since the functionality and market acceptability depends on the bioactive material's preservation. Hence, the objective of this study was to investigate the impact of different dehydration conditions and methods namely, conventional dehydration, vacuum dehydration, and heat pump dehydration on the total phenolic content and antioxidant potential of (*Backhousia citriodora)* lemon myrtle leaves. This study also determined the correlation between TPC and Backhousia citriodora's antioxidant properties in dehydrated leaves.

MATERIALS AND METHODS

Sample

Fresh *Backhousia citriodora* leaves of the Linpinwood B. variety were harvested at the MARDI Research Station in Serdang, Selangor, Malaysia, and promptly prepared for the subsequent dehydration experiment. The leaves and stalks were separated before the dehydration process. Subsequently, the fresh leaves were cleaned and examined to detect any signs of insect bites without any size reduction process before dehydration. The sample schedule was also constant between 8 and 9 a.m. for each sampling to guarantee that the leaves had the same initial moisture content, the.

Dehydration of Lemon Myrtle Leaves

A total of 500 g of *Backhousia citriodora* samples were subjected to three different dehydration processes: conventional dehydration (CD), vacuum dehydration (VD), and heat pump dehydration (HPD). The specimens were stored on a tray made from perforated wire mesh. The dehydration experiments were conducted in triplicate. The samples were dried for three measurements until the weight remained consistent. The dried goods were weighed at their bone-dry weight and kept in airtight plastic containers at room temperature, in the dark.

Conventional dehydration was carried out at CD40, CD50, and CD60 temperatures. In a conventional vacuum (Memmert, Germany; Model V200), vacuum dehydration was carried out at 40, 50, and 60°C with a pressure setting of 50 mbar. I-Lab Sdn. Manufactured the heat pump (HPD) Bhd. in Selangor, Malaysia.

The moisture content of dried samples was determined through the conventional oven-drying method. Briefly, samples were dried in an oven at 105°C until they reached a constant weight (AOAC, 2005).

Antioxidant Analysis

Extraction for Biochemical Activities

The extraction of dehydrated *Backhousia citriodora* was conducted using the method described by Abd Razak et al. (2015)with slight modifications. One gram of dried *Backhousia citriodora* was mixed with 10 mL of deionized water in a water bath set at a temperature of 55°C for 10 min. The water used in this experiment was obtained from the Milli-Q water purification system (Pall C, Illinois, United States). Before centrifuging the solution for 10 minutes at 10,000 rpm (Model: Centrifuge 5810 R, Eppendorf, Germany), the solution was refrigerated. The substance was extracted, and the supernatant was separated by filtration using Whatman No. 1 filter paper before further analysis.

Determination of Total Phenolic Content

With slight adjustments, the Folin-Ciocalteu technique, as reported by Abd Razak *et al*. (2015), was used to estimate the total phenolic content. In summary, 4 mL of sodium carbonate (Sigma-Aldrich; Missouri, United States of America) with a concentration of 7.5% (w/v) was mixed with 5 mL of the Folin-Ciocalteau reagent (Sigma-Aldrich; Missouri, United States of America) that had been diluted. The resulting mixture was allowed to react at ambient temperature for 2 h. The absorbance of the mixture was determined using a spectrophotometer (Model: Cary50 UV-Vis, Varian, United States of America) set at a wavelength of 765 nm. Regarding gallic acid equivalent (GAE), the findings were given as mg/g of sample.

Determination of 2,2-diphenyl-1-1picicrylhydrazyl Radical Scavenging Activity

A technique published by Thaipong et al. (2006) was modified to test the extract's capacity to scavenge free radicals. The 2-2-diphenyl-1-picrylhydrazyl (DPPH) stock was prepared by combining 24 mg of DPPH with 100 mL of methanol in an amber container. The spectrophotometer utilized for this experiment was the Cary50 UV-Vis model (Varian, United States of America). The absorbance of the solution at a wavelength of 515 nm was measured to be 1.1 ± 0.02 units. To prepare the working solution, 10 mL of the stock solution was combined with 45 mL of methanol. A total volume of 150 mL of extract was mixed with 2,850 mL of a working solution of DPPH obtained from Sigma-Aldrich (analytical grade, Missouri, United States of America). The mixing process was conducted in a dark environment for 30 min. The absorbance at a wavelength of 515 nm was measured using a spectrophotometer. The following equation was employed to determine the scavenging activity:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] x\ 100\%$$

where:

A_{blank} = Absorbance of blank sample (Absorbance without extract)

A_{sample} = Absorbance of sample

Determination of ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant potential (FRAP) values were examined as another antioxidant activity in *Backhousia citriodora* powder. The FRAP test used a modified version of the Benzie and Strain (1996) technique. The FRAP stock solution was prepared by mixing 300 mM acetate buffer consisting of 3.1 g of sodium acetate trihydrate ($C_2H_3NaO_2{\cdot}3H_2O$) (Sigma-Aldrich; Missouri, United States of America) and 16 mL of acetic acid (Sigma-Aldrich; Missouri, United States of America) at pH 3.6. Additionally, the solution included 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid (HCl) (Merck & Co; New Jersey, United States of America), as well as 20 mM iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) (Sigma-Aldrich; Missouri, United States of America). Acetate buffer, TPTZ solution, and $FeCl_3.6H_2O$ solution were each mixed with 25 mL of acetate buffer to provide the working solution for this experiment. The working solution combination was warmed to 37°C before the FRAP analysis. Then, 2,850 µl of FRAP solution was combined in the dark with 150 µl of extract. The mixture's absorbance was calculated using a spectrophotometer set at 593 nm after 30 minutes. Plots and measurements were made of the FRAP solution's absorbance

throughout 30 minutes at various ascorbic acid concentrations. FRAP's ascorbic acid equivalent (AAE) value was given as mg/g of material.

Statistical Analysis

Every set of data is presented as mean standard deviation. The data were analyzed using one- and two-way analysis of variance (ANOVA) with the aid of SAS software (Version 9.4, S.A.S. Institute Inc. Cary, North Carolina, United States of America). Tukey's honest significance difference (HSD) test was employed at a significance level of p <0.05 to evaluate differences in means.

RESULTS AND DISCUSSION

Impact of Dehydration Methods on Total Dehydration Time and Final Moisture Content

Table 1 displays the impact of dehydration techniques on the dehydration time and moisture content of dried *Backhousia citriodora*. The presented data provide unambiguous evidence that the dehydration process's duration decreases as the dehydration temperature increases, regardless of whether conventional or vacuum dehydration methods are utilised. The dehydration time of CD60 is the shortest, followed by HPD, CD50, VD60, VD50, CD40, and VD40. Dehydration times were shortened due to quicker dehydration rates in both conventional and vacuum dehydration at higher temperatures.

All CD conditions led to a shorter dehydration time, but all VD conditions led to a longer one. When the temperature was raised to 60°C, the dehydration time for both CD and VD decreased accordingly. This might be explained by air convection near the *Backhousia citriodora*, which created a bigger temperature difference and accelerated moisture removal in the CD, especially at higher operating temperatures.

Table 1. Impact of dehydration methods and conditions on dehydration time and final moisture content

Sample	Dehydration time (h)	Final moisture content (% w.b)
Fresh	-	63.13 ± 4.44[a]
CD40	52	10.36 ± 1.38[b]
CD50	32	9.76 ± 1.15[b]
CD60	13	9.24 ± 1.67[b]
VD40	76	7.39 ± 0.83[b]
VD50	52	8.42 ± 1.15[b]
VD60	34	5.62 ± 0.77[b]
HPD	30	10.36 ± 0.89[b]

Mean values ± standard deviation (n=3 replications) within the same column with the same letter are not significantly different (p>0.05)

Impact of Dehydration Methods on Total Phenolic Content

Figure 2 displays the total phenolic content (TPC) of *Backhousia citriodora* under several dehydration settings, including fresh, heat pump-dried, and various conventional- and vacuum-dehydration methods. To assess the influence of various dehydration methods and environmental factors on the retention of total phenolic compounds (TPC) in dried *Backhousia citriodora*, the losses incurred were calculated and evaluated. TPC measurements were conducted on both fresh and dried leaves. Based on the data presented in Figure 2, it can be observed that the total phenolic compound (TPC) concentration varied between 43.71 and 63.97 mg GAE/g samples across different dehydration procedures and conditions. In comparison, the TPC concentration of fresh *Backhousia citriodora* was 74.18 mg GAE/g sample. The VD60 sample had the highest

TPC value, whereas the CD60 sample had the lowest TPC value. The lower TPC value after *Backhousia citriodora* was exposed to CD points to a greater loss during CD.

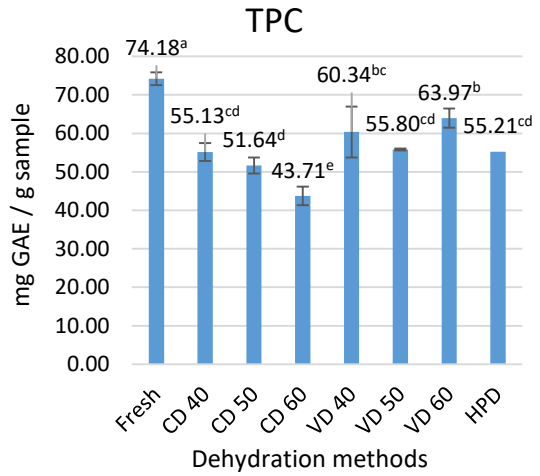


Fig. 2: Impact of dehydration methods and conditions on total phenolic content. Vertical bars indicate standard deviation and values marked by the same letter are not significantly different ($p>0.05$).

Increasing the dehydration temperature in the instance of CD led to a higher decline in TPC value. This explains why the sample's phenolic concentration decreased after being subjected to high temperatures. The pattern of the TPC reduction of *Backhousia citriodora* in VD was different from that of CD. This could be connected to the temperature and dehydration time. In the VD50 treatment, the TPC was further reduced due to an extended dehydration period of 52 h and an elevated dehydration temperature of 50°C. In contrast, dehydration time decreased as the temperature increased, which led to a lower TPC drop in VD60. Figure 2 depicts how dehydration conditions affect the loss of TPC. The loss of phenolic compounds could be affected by dehydration time and temperature. Cells get disturbed, and oxidative and hydrolytic enzymes that may oxidize phenolic compounds are produced when they are subjected to high temperatures during dehydration. Moreover, the enzymatic activities of polyphenol oxidases and peroxidases, which play a crucial role in the oxidation of polyphenols in plant materials, are deactivated when exposed to elevated dehydration temperatures (Lim & Murtijaya, 2007). The TPC may have decreased after dehydration due to the interaction of polyphenols with other molecules that altered their molecular structure, making them difficult to extract (Mrad *et al.*, 2012). The influence of the dehydration temperature in the conventional on the overall phenolic content of *Angelica sinensis* leaves is significant (Wu *et al.*, 2013). *Ben Haj Said et al.* (2015) determined that increasing the dehydration temperature led to a notable decrease in the TPC of *Allium roseum* leaves, from 60% to 69%. Similar to how the lengthy dehydration period would have caused the TPC to decrease. Long-term dehydration air exposure might lead to phenolic content degradation, leading to a greater reduction in TPC in dried leaves.

Impact of Dehydration Methods on DPPH Free Radical Scavenging Capacity

The percent suppression of the DPPH radical measures of the extracts' antioxidant activity (Alothman *et al.*, 2009). According to Lavanya et al. (2010), this method is based on the production of non-radical DPPH-H during the reaction, which causes a reduction in the amount of DPPH solution. The decline is evidenced by the alteration of the DPPH's color, changing from purple to yellow due to decolorization. The quantification of the reducing ability is commonly expressed as the percentage of scavenging, which denotes the free radical DPPH inhibition.

Figure 3 displays the percentage of radical scavenging activity, as measured by the DPPH assay, for fresh and dehydrated *Backhousia citriodora* samples subjected to HPD and different CD and VD conditions. *Backhousia citriodora* extracts have a 77 to 90% scavenging activity in conventional, vacuum and heat pump dehydration. Scavenging was most prevalent in VD40, and least prevalent in CD60. More antioxidants in the extract neutralized more free radicals, suggesting a greater percentage of scavenging.

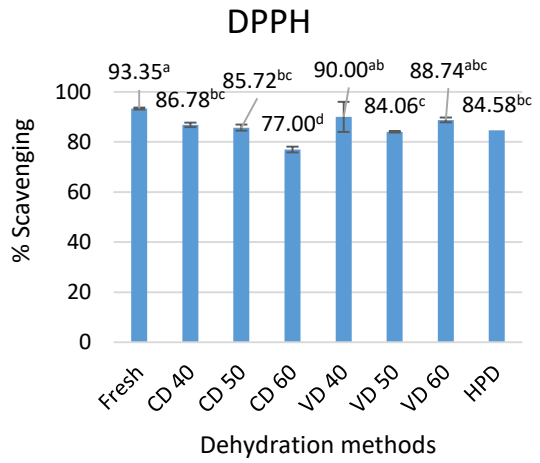


Fig. 3: Impact of dehydration methods and conditions on radical scavenging analysis (DPPH). Vertical bars indicate standard deviation and values marked by the same letter are not significantly different ($p>0.05$).

The DPPH levels varied considerably across dehydration techniques and environmental factors. The DPPH scavenging activity (%) decreased as the dehydration temperature increased. The drop-in antioxidant activity brought on by CD demonstrated the loss of endogenous antioxidants, which are often fragile. This result is in line with a report by Latiff et al. (2020) that reported a reduction of antioxidant value (% DPPH) in oven-dried *Cosmos caudatus* by 12% as the drying temperature increased from 40 to 60°C. Additionally, structural changes brought on by dehydration leaves may cause plant cell components to adhere without water, making it difficult to extract antioxidants from dried samples (Garau *et al*., 2007). It appears that increased dehydration temperatures are associated with decreased antioxidant activity. This phenomenon may be attributed to the fact that employing a lower temperature during the dehydration process results in decelerating the enzymatic breakdown of the antioxidant component (Abdul Kahar, 2021). The dehydration duration significantly impacted how heat treatment affected bioactive chemical scavenging and degradation (Mediani *et al*., 2014). Antioxidant activity may be decreased by degradation due to prolonged dehydration.

Impact of Dehydration Methods on Ferric Reducing Antioxidant Power (FRAP) Assay

DPPH and FRAP are used to determine the extract's antioxidant activity; however, the methods are not equivalent. The evaluation of the antioxidant's radical scavenging activity was conducted by the DPPH test, whereas its potential to function as a reducing agents was assessed using the FRAP assay (Orphanides *et al*., 2013). The presence of the ferrous tripyridyltriazine complex, which exhibited absorbance at a wavelength of 595 nm, was observed upon the combination of the FRAP solution with the sample extract, resulting in a transformation of the solution into a dark blue color (Benzie & Strain, 1996; Rabeta & Lin, 2015). The observed reaction depends on the molar concentration of the antioxidants. The ascorbic acid equivalent (AAE) of each gramme of material is used to calculate the FRAP value.

Figure 4 displays the FRAP values of fresh and dehydrated *Backhousia citriodora* samples subjected to HPD treatment under varying CD and VD settings. Figure 3 demonstrates that the FRAP values for fresh *Backhousia citriodora* were 88.04 mg AAE/g sample, while the values for dehydrated leaves varied from 56.19 to 81.24 mg AAE/g sample. More ferrous tripyridyltriazine with a higher FRAP value was produced when *Backhousia citriodora* extract was reacted with the FRAP solution. The production of additional ferrous tripyridyltriazine through the reaction increased the antioxidant activity (FRAP) of the extract. The FRAP values of the CD, VD, and HPD samples exhibited a decrease after the dehydration process in the following sequence: VD60 > VD40 > VD50 > CD40 > HPD > CD50 > CD60. The FRAP value for the VD60 sample was the highest, whereas the FRAP value for the CD60 sample was the lowest.

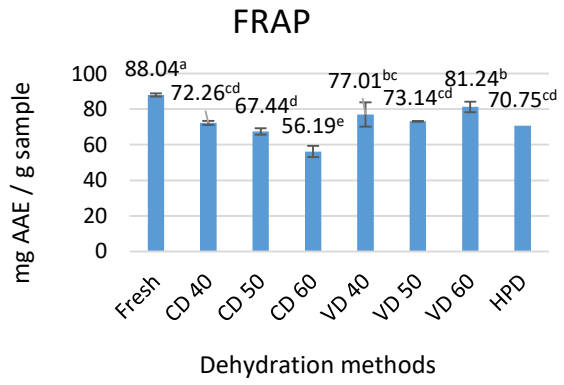


Fig. 4: Impact of dehydration methods and conditions on the FRAP value. Vertical bars indicate standard deviation and values marked by the same letter are not significantly different (p>0.05).

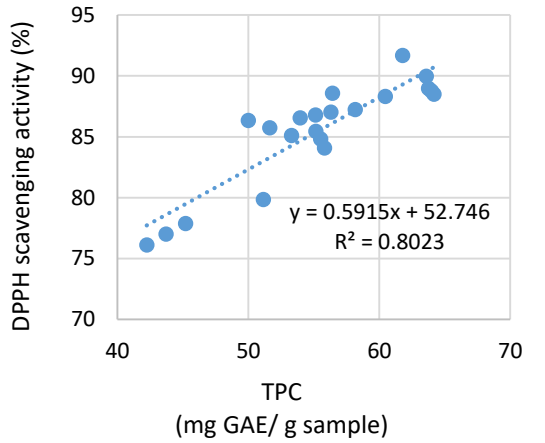
Based on the FRAP values presented in Figure 4, it can be observed that various dehydration procedures and environmental conditions have a substantial impact on the antioxidant capacity of *Backhousia citriodora*, as indicated by a *p*-value of 0.05. The FRAP value for VD, which ranged from 73.14 to 81.24 mg AAE/g sample, was greater than that for the other two dehydration procedures. One possible explanation for this phenomenon is that vacuum dehydration facilitates the preservation of more antioxidant chemicals. Additionally, the removal of oxygen during this process may contribute to the delay of potential oxidation. Oxygen in the dehydration air during CD and HPD may contribute to the observed discrepancy, as oxygen can initiate enzymatic oxidation, particularly through redox enzymes, leading to the conversion of antioxidant molecules (Nguyen *et al*., 2009). Like TPC and DPPH, an increase in dehydration temperature leads to a greater reduction in the FRAP value in CD. The decrease in the FRAP value of the extract can be attributed to the degradation of antioxidant components induced by elevated temperatures (Abdul Kahar, 2021).

Furthermore, the degradation of heat-unstable compounds and volatile molecules that escape into the surrounding environment are destroyed through exposure to elevated temperatures (Yen & Vu, 2017). According to Alibas, 2009, vacuum dehydration is the preferred technique for dehydration collard leaves compared to hot air dehydration. This is also consistent with the findings of Yen & Vu, 2017 who discovered that vacuum and convective dehydration of *Limnophila aromatica* resulted in less antioxidant chemicals.

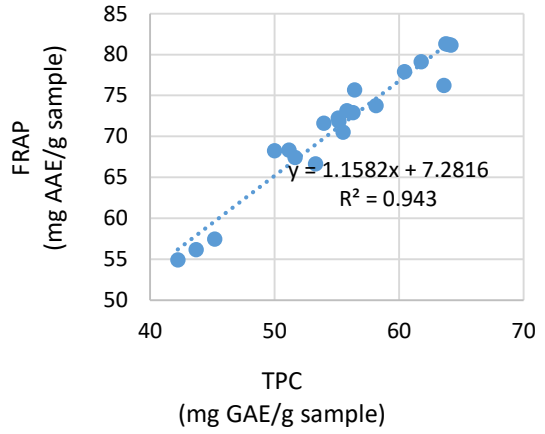
Correlation of Total Phenolic Content and Antioxidant Activities

The antioxidant capacity of *Backhousia citriodora* may potentially be associated with the quantity of total phenolics that are present. These chemicals exhibit the ability to

scavenge free radicals generated by oxidation processes. A correlation was established between the antioxidant capacity and TPC to ascertain the impact of phytochemical substances on the antioxidant capacity of *Backhousia citriodora*. Figures 5(a) and (b), indicate how TPC affects FRAP and DPPH scavenging activity.



(a)



(b)

Fig. 5: Correlation of total phenolic content (TPC) on (a) DPPH scavenging activity and (b) FRAP in lemon myrtle leaves

The coefficient of determination (R^2) values for FRAP and DPPH scavenging activity were 0.8023 and 0.943, respectively. The R^2 is lower than the DPPH score of 0.9. This proved that dried *Backhousia citriodora* treated with CD, VD, and HPD exhibited a slight association between their FRAP value and TPC. The FRAP value of 0.943 demonstrates that it is a reliable predictor of the TPC in different dried *Backhousia citriodora* samples, compared to the DPPH value. The more antioxidant activity the higher the TPC. Yap *et al*., 2020 and Youssef and Mokhtar, 2014 determined that the R^2 values for the correlation between the TPC with ABTS and DPPH in papaya leaves and purslane leaves were 0.935 and 0.9536, respectively. These values, ranging from 0.9043 to 0.9885, are almost identical to our findings.

According to these results, phenolic retention is essential for preserving *Backhousia citriodora*'s antioxidant activity. The strong correlation coefficient value indicates that phenolic compounds are a major factor in the antioxidant activities of *Backhousia citriodora*. Phenolic compounds may be further isolated and identified since they play a significant role in *Backhousia citriodora* 's antioxidant ability to understand more about their biological characteristics and health benefits.

CONCLUSION

The preservation of biochemical contents varied depending on the techniques and conditions used. VD60 resulted in better dehydration method for lemon myrtle leaves in terms of high TPC and antioxidant activity retention after dehydration, followed by HPD and CD. The dehydration temperature and dehydration period influenced the variation in biochemical retention of dried *Backhousia citriodora*. The reduction in the biochemical content in *Backhousia citriodora* was seen as a result of higher dehydration temperatures and prolonged exposure. This can be attributed to the increased loss of polyphenols and antioxidants due to

oxidation and enzymatic reactions. The antioxidant effects of *Backhousia citriodora* are primarily ascribed to phenolic compounds, as evidenced by the high correlation coefficient.

ACKNOWLEDGEMENT

The authors thank the University of Nottingham Malaysia (UNM) and the Malaysian Agricultural Research and Development Institute (MARDI) for financial support.

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