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Effects of Different Drying Methods and Solvents on Biological Activities of *Curcuma aeruginosa* Leaves Extract

(Kesan Kaedah Pengeringan dan Pelarut Berbeza pada Aktiviti Biologi Ekstrak Daun *Curcuma aeruginosa*)

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ABSTRACT

Curcuma aeruginosa *Roxb. is one of the plants from the* Zingiberaceae *family which the rhizome has been used for medicinal purposes. However, the biological properties of the leaves have not been fully explored. Therefore, this study was conducted to evaluate the effects of different drying methods and solvents on total phenolic content, antioxidant and anti-hyperglycemic activities of* C. aeruginosa *leaf extract. Samples were dried by oven drying (OD) and freeze drying (FD), and then extracted using different ratios of ethanol:water (100:0, 50:50 and 0:100). The amount of phenolic content (TPC) was determined using a spectrophotometer. Antioxidant activity was tested using the Free Radical Scavenging (DPPH) test and the Ferric Reducing Antioxidant Power assay (FRAP), while the anti-hyperglycemic test was evaluated by determining the percentage of α-glucosidase inhibition. The results showed that FD at 100:0 and 50:50 concentrations had the highest phenolic content (30.88 and 33.06 mg GAE/g extract, respectively) and highest antioxidant activity value (38.24 and 42.46 mg TEAC/g extract, respectively). For DPPH, FD at 50:50 showed the highest inhibition of 71.48% compared to other extracts. Whereas FD at 100:0, 50:50 and 0:100 showed the highest α-glucosidase inhibition of 25.65, 30.78 and 27.65%, respectively. However, compared to Quercetin, the extract showed mild anti-hyperglycemic activity. The results indicated that FD is the best method of drying while 50:50 showed as the best solvents. Positive correlation between TPC with antioxidant and anti-hyperglycemic activities showed that* C. aeruginosa *leaf has potential as a source of natural antioxidant with the presence of phenolic compounds.*

Keywords: Anti-hyperglycemic; antioxidant; freeze drying; oven drying; phenolic

ABSTRAK

Curcuma aeruginosa *Roxb. merupakan tumbuhan daripada famili* Zingiberaceae *yang mana rizomnya telah digunakan secara tradisi untuk tujuan perubatan. Namun begitu, aktiviti biologi daripada bahagian daun masih belum diterokai secara meluas. Oleh itu, penyelidikan ini dijalankan untuk mengkaji kesan kaedah pengeringan dan penggunaan nisbah pelarut yang berbeza terhadap jumlah kandungan fenol, aktiviti antioksidan dan anti-hiperglisemik ekstrak daun* C. aeruginosa*. Pengeringan ketuhar (PK) dan pengeringan sejuk beku (PB) telah digunakan untuk mengeringkan daun dan kemudiannya diekstrak menggunakan etanol:air dengan nisbah berbeza (100:0, 50:50 dan 0:100). Spektrofotometer telah digunakan untuk menentukan jumlah kandungan fenol (TPC) pada ekstrak. Aktiviti antioksidan pula telah diuji dengan menggunakan ujian Pemerangkapan Radikal Bebas (DPPH) dan ujian Penurunan Ferik (FRAP), manakala ujian anti-hiperglisemik pula dinilai dengan menentukan peratusan perencatan α-glukosidase. Hasil menunjukkan bahawa PB pada kepekatan 100:0 dan 50:50 mempunyai kandungan fenol tertinggi (masing-masing 30.88 dan 33.06 mg GAE/g ekstrak) dan nilai aktiviti antioksidan paling tinggi (masing-masing 38.24 dan 42.46 mg TEAC/g ekstrak). Bagi DPPH, PB 50:50 menunjukkan peratusan perencatan tertinggi sebanyak 71.48% apabila dibandingkan dengan ekstrak lain. PB pada 100:0, 50:50 dan 0:100 menunjukkan perencatan α-glukosidase tertinggi masing-masing sebanyak 25.65, 30.78 dan 27.65%. Namun begitu, apabila dibandingkan dengan Kuersetin, ekstrak menunjukkan aktiviti anti-hiperglisemik yang rendah. Keputusan ini menunjukkan bahawa PB adalah kaedah pengeringan terbaik manakala pelarut 50:50 merupakan pelarut paling sesuai untuk pengekstrakan daun* C. aeruginosa*. Korelasi positif antara TPC dengan aktiviti antioksidan dan anti-hiperglisemik menunjukkan bahawa* C. aeruginosa *mempunyai potensi sebagai sumber antioksidan semula jadi dengan kehadiran sebatian fenol.*

Kata kunci: Anti-hiperglisemik; antioksidan; fenol; pengeringan beku; pengeringan ketuhar

Introduction

Curcuma aeruginosa or also known as 'pink and blue ginger' in Malaysia, is among the herbs that is used traditionally to treat various diseases such as diarrhea, bloating, parasitic infections, and dengue fever (Choudhury et al. 2013; Moektiwardoyo et al. 2014). Studies on *C. aeruginosa* are gaining attention as extracts from this plant can also act as antioxidant, antibacterial, anti-inflammatory, and antipyretic agents (Safitri et al. 2017; Simoh et al. 2018). Jarikasem et al. (2005) and Thaina et al. (2009) reported that there are sequential bioactive compounds in the rhizome extracts such as zedoalakton A, zedoalakton B, zedoarondiol, zedoarol, curcumenol, isocurcytol, furanodien, isofuranodien, curzerenone, 1.8-sineol, and β-pinen. According to Mathela and Joshi (2012), curzerenone acts as one of the antioxidant and anti-bacterial agents. This is supported by George and Britto (2015) who also stated that this plant is indeed a high source of antioxidants such as flavonoids, curcumin, alkaloids and polyphenols. While most research was done on the rhizome of the plants, the biological activities of the leaves is yet to be explored.

Antioxidants are compounds that prevent oxidative processes due to the production of free radicals that can damage cells. The potential for antioxidants as therapeutic agents for oxidative stress-related diseases has gained much attention. Furthermore, plants have high potential to be commercialised as they are readily-available and inexpensive (Simoh & Zainal 2015). Bioactive substances that act as antioxidant agents are essential to prevent free radical damage, as these reactions can occur in either the human body or the food system (Kumar & Pandey 2013). According to Asif (2015), free radical reactions or reactive oxygen species (ROS) can cause biomolecular damage, which is the cause of cell death in the human body.

Studies on the relationship of free radical reactions to human health have long been observed because free radicals are thought to be contributing to diseases such as asthma, cancer, cardiovascular, and even diabetes (Phaniendra et al. 2015; Pizzino et al. 2017). This is supported by various findings which showed that polyphenol compounds are able to protect cells from oxidative damage through various mechanisms, therefore, lowering the risk of disease resulting from such oxidative stress (Hussain et al. 2016). In addition, oxidative stress responsible in pathogenesis of diabetes by the disruption of enzymatic systems, causing reduction in vitamin C levels, lipid peroxidation as well as impaired glutathione metabolism (Asmat et al. 2016). Previous studies suggested that plants with high phenolic content have strong antioxidant properties (Anuduang et al. 2020; Ismail et al. 2018; Ling et al. 2020). This occurs when these phenolic compounds bind to free radical ions and thus, inhibit the formation of ROS (Panche et al. 2016). Therefore, natural substances such as plants that have the potential to reduce oxidative stress or have antioxidant properties are said to be able to treat diseases caused by ROS including diabetes and cancer.

Previous studies have shown that the amount of polyphenols and antioxidant activity in a plant depends on biological factors (genotype, organ, and growth rate) and the environment (temperature, water, and light levels) (Medini et al. 2014). However, the amount or antioxidant content that can be extracted from a plant also depends on the type and concentration of the solvent used during the extraction process. According to Dent et al. (2013), polyphenol compounds have a wide range of polarities ranging from low to high polarity. Therefore, to obtain the optimal amount of extracts, various types of solvents can be used based on the solubility of the polyphenols such as water, ethanol, methanol, acetone or any mixture of water and alcohol. Ethanol has been known as a good solvent for polyphenol extraction and it is safe for human consumption. Ethanol is reliable and can consistently produce potent extractions with minimal fuss.

In addition, the pre-extraction drying process also affects the amount of bioactive and phytochemical content of plants. This is because bioactive substances are usually degraded due to high temperatures, high oxygen content, and exposure to radiation (Nguyen et al. 2015). It is important to know the appropriate solvent concentration and drying method during the phenolic determination process in order to enhance or optimise the phenolic content of a substance. Therefore, the objective of this study was to determine the effect of drying method and solvents on phenolic content, antioxidant and antihyperglycemic activities of the *C. aeruginosa* leaf extract.

Materials and Methods

Materials

All chemicals used in this study are of analytical grade. The chemicals used were ethanol, aluminium chloride ($AlCl_3$) (Hamburg Chemicals, Germany), 2,2-diphosphate-2-prylhydrazyl (DPPH), Folin Ciocalteu reagent (Vetec, Brazil), sodium carbonate, $FeCl_3.6H_2O$, 2,4,6-tripiridyl-1,3,5-triazine (TPTZ), sodium acetate trihydrate ($CH_3COONa.3H_2O$), p-nitrophosphate-α-D-

glucopiranoside, Quercetin (Merck, Germany) and α-glucosidase (Megazyme, Australia).

PROXIMATE COMPOSITION

Fresh sample materials were used for quantitative determination of proximate composition such as moisture content, total ash, protein, fat, fiber, and carbohydrate. These physicochemical studies were done according to standard procedure of the Association of Official Analytical Chemists (AOAC 2000); meanwhile the carbohydrate content was estimated by subtracting the moisture, ash, protein fat and fiber values from 100. All analyses were carried out in triplicate and results obtained were reported in percentages.

SAMPLE PREPARATION

C. aeruginosa leaves were obtained from the Seri Suboh Herbal Park located in Kuala Pilah, Negeri Sembilan, Malaysia and identified by botanists from the Herbarium, Universiti Kebangsaan Malaysia (Voucher specimen ID: 026/2020). The fresh leaf samples were cleaned and dried using a clean dry tissue and kept at 4 °C (TSB-Inverter, Toshiba, Malaysia). The drying methods used in this study were oven drying (Memmert UN55, Germany) and freeze drying (Alpha LD Plus, CHR 101521, Germany). For oven drying, the sample is placed in an oven at 40 °C until constant weight of the sample was achieved. For freeze-drying, the samples were kept in the refrigerator at -20 °C for 48 h and then dried using a freeze dryer before the constant weight of the sample was obtained. The dried samples were ground into powder (Waring Blender 7011S, America) and sieved using a 300 µm siever (mesh size: No. 50) before being stored at -20 °C for analysis.

SAMPLE EXTRACTION

The dry powder of *C. aeruginosa* leaves was extracted using the method performed by Rahman et al. (2017) with slight modifications. Samples were extracted using ethanol:water as the solvent at 100:0, 50:50 and 0:100 ratios (v/v). The dried sample (10 g) was extracted with 150 mL of solvent and left for 24 h in an incubator shaker (Infors HT Ecoton 4103, Switzerland) and kept at 25 °C. The extract was then filtered using a Whatman No. 1 filter paper and the process was repeated three times for 24 h at a time. Solvent evaporation was performed using a rotary evaporator (Laboratoria 4000 efficient, Heidolph, Germany) at 40 °C. The product from the evaporation process was then frozen so that the solvent and water content are completely removed. The extract was weighed and the percentage of recovery calculated using the following formula and stored at -20 °C before further analysis:

$$\text{Recovery (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of fresh leaves (g)}} \times 100$$

TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content of *C. eruginosa* extract was determined based on the method of Hashim et al. (2019) with some modifications. Gallic acid was used as a standard (0 - 250 µg/mL). Extracts of 100 µL of 0.2 N Folin Ciocalteu reagent, 20 µL of *C. eruginosa* extract and 80 µL of 7.5% sodium carbonate were added in 96-well microplates and allowed to react at room temperature for 2 h. Absorbance readings were obtained using the EpochTM Microplate Spectrophotometer, Biotech Instrument (USA) at 765 nm where complex blue was formed. The standard curve is plotted with gallic acid and phenolic content is expressed as gallic acid equivalents in mg per g extract (GAE/g extract). The calibration equation for GA was $y = 0.0062x + 0.1039$ ($R^2 = 0.999$) where y refers to absorbance and x represents GA concentrations (mg/mL).

FREE RADICAL SCAVENGING TEST (DPPH)

Determination of antioxidant activity was performed using the method of Sin et al. (2018) with some modifications. Ascorbic acid and butylated hydroxyanisole (BHA) are used as positive controls. A total of 40 µL extract of different concentrations were mixed with 280 µL of DPPH solution provided in methanol (25 µg/mL). All mixtures were prepared in microplates and allowed to react for 30 min at room temperature in a dark. Absorption was measured using EpochTM Microplate Spectrophotometer, Biotech Instrument (USA) at 517 nm.

FERRIC REDUCING ANTIOXIDANT POWER ASSAY TEST (FRAP)

Determination of the ferric reducing antioxidant power assay test (FRAP) was performed using method by Mirghani et al. (2018) with some modifications. Ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution, 10 mM 2,4,6-tripiridyl-s-triazine (TPTZ) solution in 40 mM HCl

and acetate buffer solution (CH_3COONa) were prepared separately. A total of 5.4058 g of $FeCl_3.6H_2O$ powder was mixed with distilled water until completely dissolved and transferred into a 100 mL volumetric flask to produce 20 mM $FeCl_3.6H_2O$. The 10 mM TPTZ solution was prepared by mixing 0.3121 mg of TPTZ powder with 40 mM HCl and then diluted to 100 mL using the same HCl. The solution of acetic acid buffer with a concentration of 0.3 M, pH 3.6 was prepared by mixing 0.31 g of sodium acetate trihydrate ($CH_3COONa.3H_20$) and 1.6 mL of glacial acetic acid and then diluted to 100 mL with distilled water. Acetate buffer solution, 10 mM TPTZ solution and 20 mM $FeCl_3.6H_2O$ solution at a volume ratio of 10:1:1 were mixed to produce fresh FRAP solution. Next, 1950 µL of fresh FRAP solution was mixed with 50 µL of sample extract for analysis. After 30 min, the absorption readings were taken at 593 nm wavelength using an EpochTM Microplate Spectrophotometer, Biotech Instrument (USA). The standard curve was plotted with Trolox (0 - 250 µg/mL) and the value of the ferric reducing power is expressed as trolox equivalents in mg per g extract (mg TEAC/g extract). The calibration equation for Trolox was $y = 0.0034x + 0.1413$ ($R^2 = 0.9816$) where y is absorbance and x is mg/mL of Trolox.

α- GLUCOSIDASE INHIBITORY ACTIVITY

α-glucosidase inhibition was performed according to the method of Zayapor et al. (2020) with some modifications. The substrate solution (10 mM p-nitrophosphate-α-D-glucopiranoside) was prepared in 50 mM phosphate buffer (pH 6.5). Then, 10 µL of sample, 130 µL of 30 mM phosphate buffer solution and 10 µL of α-glucosidase (3 U/mL) were pre-incubated in 96 plates at 25 °C for 5 min. Then, 50 µL of the substrate was added and the reaction mixture was incubated for 15 min at 25 °C. 50 µL of 2 M glycine (pH 10) was added and the reaction stopped. Blank samples were prepared using the same method as the experimental sample, but the enzyme and substrate solutions used in the experimental sample were replaced with (50 µL) 30 mM buffer phosphate solution while glycine was replaced with 50 µL distilled water. Absorption readings were taken using the EpochTM Microplate Spectrophotometer, Biotech Instrument (USA) at 405 nm. Percentage inhibition (%) was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\Delta A_n - \Delta A_s}{\Delta A_s} \times 100$$

where ΔA_n is the difference between a negative control (with an enzyme) and blank (without an enzyme); and ΔA_s is the difference in absorption between sample (with enzyme) with blank (without enzyme).

STATISTICAL ANALYSIS

All analytical methods to determine phenolic content, antioxidant and anti-hyperglycemic activity were performed in three replications (n = 3). All data were analysed using Analysis of Variance (ANOVA) and Tukey's test to determine significant differences between samples. Pearson correlation tests were used to determine the correlation between phenolic content and antioxidant and anti-hyperglycemic activities between each extract. All statistical tests were performed using Minitab version 17.0 software at the significance level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION *C. aeruginosa*

Currently, findings on nutrient composition of *C. aeruginosa* is limited to the flower and rhizome. Therefore, the composition of important nutrients found in *C. aeruginosa* leaves was determined and is shown in Table 1. The results showed that the fresh leaves of *C. aeruginosa* had the highest moisture (85.38%) compared to other nutrient compositions. The fresh leaves also contain 6.94% carbohydrate, the second highest after moisture content. High carbohydrate content indicates the plant's ability to produce high carbon substrates during primary metabolism, thus producing other phytochemical substances during the metabolism of secondary products (Irwan & Shahreda 2014). Meanwhile, the fresh leaves of *C. aeruginosa* were found to have 2.69% protein. The leaf parts of a plant contain many essential amino acids for the production of enzymes that function during defense mechanisms from stress and danger (Edelman & Colt 2016). In addition, this analysis also showed that *C. aeruginosa* leaves have a fat content of 1.55%, which is the major building block of nutrition. Results showed that leaves of *C. aeruginosa* are a good source of mineral elements as they contain a high percentage of ash (0.89%). The fiber content (2.55%) in this plant may be influenced by the amount of cellulose present in the tissue of the plant organ. Cellulose is responsible for preserving plant-derived proteins, which play an important role in supporting leaf growth under stress (He et al. 2015).

TABLE 1. Proximate composition (%) for the *C. aeruginosa* leaf

Composition	(%)
Moisture	85.38 ± 0.47
Ash	0.89 ± 0.08
Fat	1.55 ± 0.29
Carbohydrate	6.94 ± 0.48
Protein	2.69 ± 0.20
Fiber	2.55 ± 0.32

EFFECT OF DIFFERENT SOLVENT RATIOS AND DRYING METHODS ON THE RECOVERY PERCENTAGE OF *C. aeruginosa* LEAF EXTRACT

Proper drying is required to obtain optimal extraction yield, while at the same time, minimizing changes to maintain the functional properties of the extract. Percentage recovery for FD was significantly higher ($p < 0.05$) than OD at all solvent concentrations (Table 2). This finding is consistent with those reported by Siti Zulaikha et al. (2017) on the percentage of extracts of gooseberry tree (*Phyllanthus acidus*) from freeze-drying using ethanol which was higher than oven drying, with 45.64% (FD) and 28.52% for OD. Freeze-drying has a higher extraction efficiency than thermal extraction due to the breakdown of cell structure by crystalline ice formed in the plant matrix (An et al. 2016). This causes degradation of the cell wall structure, causing the intracellular product to be released into the solution (Zhang et al. 2018). In addition, if heat extraction is used, organic compounds such as protein, fat, and carbohydrates are released into carbon dioxide gas and water during the combustion process (Irwan & Shahreda 2014). Thus, this condition affects the amount of compound left in the sample, reducing the amount of extraction obtained from oven drying.

TABLE 2. Percentage recovery of *C. aeruginosa* leaf extract samples with different drying method and solvent ratios

Drying method	Recovery (%)		
	100:0	50:50	0:100
FD (Freeze Drying)	13.54 ± 0.95^{a}	13.89 ± 0.15^{a}	10.83 ± 0.76^{b}
OD (Oven Drying)	3.68 ± 0.81^{d}	8.92 ± 0.17^{c}	8.51 ± 0.42^{c}

Ratios above indicated the ratio of ethanol to water. Values represent the mean ± standard deviation (n = 3). $^{a-d}$ Different letters show significant differences ($p < 0.05$) analyzed with Tukey's test

The percentage of the extracts recovery can also be influenced by the nature of the solvent used. The selection of appropriate types of solvents is required as each compound present in the cell structure has different chemical properties and polarity (Stramarkou et al. 2017). Based on the results, there was a significant difference ($p < 0.05$) between the percentage of extract recovery for 100:0 and 50:50 with 0:100 for FD samples. For OD samples, 50:50 and 0:100 showed higher ($p < 0.05$) recovery percentage compared to 100:0 extracts. The extraction ability of the solvent depends on the solubility of the compound in the solvent itself, the kinetics of the mass transfer of the product and the interaction strength of the soluble substance or matrix although all these factors are affected by the presence of heat (Dhanani et al. 2017).

TOTAL PHENOLIC CONTENT (TPC)

Polyphenols are the largest group of phytochemicals that act as antioxidant agents in plants. The total phenolic content found in *C. aeruginosa* leaves using different drying methods and solvent ratios is shown in Table 3. Results indicated that FD 50:50 had a significantly higher phenolic content of 33.06 ± 0.36 mg GAE/g extract ($p < 0.05$) compared to other extracts except for FD 100:0 extract (30.88 ± 3.09 mg GAE/g extract). The lowest TPC value ($p < 0.05$) was seen in the OD 100:0 extract. The findings of this study are in line with the total value of phenolic content of turmeric (*Curcuma longa*), which was the highest in extracts using aqueous alcohol mixture (678.76 mg GAE/g extract in 60:40), compared to the extraction using only water (496.76 mg GAE/g extract) (Nisar et al. 2015). Overall, from this experiment, the FD technique produced extracts with the highest phenolic content compared to the OD technique especially in the 100:0 and 50:50 extracts. This indicates that the mixture of water and alcohol could provide the maximum extraction of phenolic compounds.

TABLE 3. Total phenolic content of *C. aeruginosa* leaf extract with different drying methods and solvent ratios

Drying method	Total phenolic content (mg GAE/g extract)		
	100:0	50:50	0:100
FD (Freeze Drying)	30.88 ± 3.09^{ab}	33.06 ± 0.36^{a}	23.65 ± 3.31^{c}
OD (Oven Drying)	13.31 ± 0.46^{d}	26.70 ± 0.30^{bc}	26.13 ± 1.80^{bc}

The sample concentration used in the calculation of total phenolic content was 1000 µg/mL. Values represent the mean ± standard deviation (n = 3). $^{a-d}$ Different letters show significant differences ($p < 0.05$) analyzed with Tukey's test

A study by An et al. (2016) found that the amount of phenolic content in ginger (*Zingiber officinale* Roscoe) rhizome extract was the highest using the freeze-drying method compared to oven drying (13.83 mg GAE/g FD extract versus 9.69 mg GAE/g OD extract). The difference in phenolic content obtained might be due to heat and dehydration factors in the oven drying process, which could potentially destroy the structure of the phenolic compounds. Increases of temperature during the extraction process causes the bioactive compounds present in the extract to decompose (Hossain et al. 2013).

FREE RADICAL SCAVENGING TEST (DPPH)

The results from the DPPH analysis were expressed as the percentage of free radical inhibitory activity. Table 4 shows the results of the capture of DPPH free radicals by *C. aeruginosa* leaf extract by different drying methods and solvent ratios.

TABLE 4. Inhibition percentage of free radicals of *C. aeruginosa* leaf extract by different drying methods and solvent ratios

Drying method / Standard	DPPH (% inhibition)		
	100:0	50:50	0:100
FD	57.35 ± 4.29^{b}	71.48 ± 8.57^{a}	55.18 ± 5.67^{b}
OD	33.02 ± 2.18^{c}	24.89 ± 6.62^{cd}	17.65 ± 4.30^{d}
AA	78.23 ± 1.06^{a}	80.19 ± 0.94^{a}	77.44 ± 0.70^{a}
BHA	64.36 ± 2.24^{ab}	63.07 ± 1.79^{ab}	64.22 ± 0.83^{ab}

FD = freeze drying; OD = oven drying; AA = ascorbic acid, BHA = Butylated hydroxyanisole. The sample concentration used in calculating the percentage of free radicals was 2000 µg/mL while 40 µg/mL was used for positive controls (AA and BHA). Values represent the mean ± standard deviation (n = 3). $^{a-d}$ Different letters show significant differences ($p < 0.05$) analyzed by Tukey's

The results showed that the free radical scavenging activities of extracts were higher ($p < 0.05$) in FD compared to OD for all solvent ratios tested. FD 50:50 showed the highest percentage of activity among *C. aeruginosa* leaf extracts ($p < 0.05$) of 71.48 ± 8.57%. However, there was no significant difference ($p > 0.05$) between the percentage of free radical scavenging activities of FD 100:0 and 0:100 extracts, which was 57.35 ± 4.29% and 55.18 ± 5.67%, respectively. This indicates that the FD sample extracted using 50:50 ethanol:water have a higher ability to trap free radicals compared to 100:0 and 0:100 extracts. These results clearly indicate that the type of solvent and the drying methods used during the freezing process affects the rate of DPPH-free radical scavenging activity. A study by Assefa and Keum (2016) showed that yuzu (grapefruit) extracted using mix of alcohol and water exhibited the highest percentage of free radical scavenging activity compared to those extracted with pure alcohol or water. Results obtained from this study also showed that pure solvents are found to be less effective in extracting antioxidative compounds compared to a mixture of solvents.

The results of this experiment also showed that all OD extracts (100:0, 50:50 and 0:100) were not able to scavenge 50% of DPPH free radical activity. In addition, heat degradation causes the structure of the bioactive component of the compound to be destroyed, reducing the amount of phenolic in the extract, and thus, reducing the rate of free radical scavenging activity (Mokrani & Madani 2016). This may explain the low percentage of the capture activity in extracts from OD.

FERRIC REDUCING ANTIOXIDANT POWER ASSAY TEST (FRAP)

The ability of a sample extract to act as an antioxidant can also be traced through its capability to stabilize the high-energy electrons. The results of the ferric reduction power test for the solvent concentrations and different types of drying methods on *C. aeruginosa* leaf extraction are shown in Table 5. Overall, the total ferric reducing power of different drying methods and solvent concentrations in *C. aeruginosa* leaf samples showed an increase in the order of 0% OD <100% OD <50% OD <0% FD <100% FD <50% FD.

A similar trend with the TPC results can be seen in Table 5 whereby the FD samples showed higher ($p < 0.05$) antioxidant activity compared to OD samples for all extracts (100:0, 50: and 0:100). FD 50:50 and 100:0 extracts recorded the highest ($p < 0.05$) ferric reduction power of 42.46 ± 1.02 and 38.24 ± 0.77 mg TEAC/g extract compared to OD 100:0 and 0:100 extracts having the lowest ferric reduction power with 8.60 ± 2.5 and 4.84 ± 2.07 mg TEAC/g extract. For FD, 50:50 showed higher FRAP value compared to 100:0 ($p > 0.05$) and 0:100 ($p < 0.05$). The same trend was obtained from OD, where 50:50 extract showed significantly higher antioxidant capacity compared to both100:0 and 0:100 extracts.

Higher ferric reduction power indicates higher antioxidant capacity to counteract the oxidation process. *C. aeruginosa* leaf extraction using freeze drying and a 50:50 solvent concentration has the highest potential compared to other extracts to act against the oxidation process. A report by Ngo et al. (2017) found that *S. chinensis* (berry magnolia) root extract obtained with water and alcohol mixtures also had the highest reducing power compared to pure water and alcohol, with higher order values such as water <methanol <ethanol <50% methanol <50% ethanol.

According to Sun et al. (2015), the type and concentration of solvent used strongly influence the number of phenolic compounds that can be extracted, thus, directly affecting the antioxidant activity in the sample. There are several other factors that influence the ability of antioxidant activity in an extract, including the preparation process such as cutting, slicing, and grinding (Sathishkumar et al. 2009), temperature, storage time, light and moisture (Zou et al. 2016), and extraction methods such as types of maceration, types and concentrations of solvent as well as the types of drying method used (Azwanida 2015). Therefore, it is important to know the type of drying and the appropriate concentration of solvent to be used during the extraction process, so that optimal results on the amount and activity of antioxidants in the extract can be obtained.

TABLE 5. Ferric reduction power of *C. aeruginosa* leaf extract with different drying methods and solvent ratios

Drying method	Ferric reducing power (mg TEAC/ g extract)		
	100:0	50:50	0:100
FD (Freeze Drying)	38.24 ± 0.77[a]	42.46 ± 1.02[a]	29.39 ± 2.08[b]
OD (Oven Drying)	8.60 ± 2.53[d]	14.76 ± 1.76[c]	4.84 ± 1.59[d]

The sample concentration used in the calculation of FRAP was 1000 µg/mL. Values represent the mean ± standard deviation (n = 3). [a-d] Different letters show significant differences ($p < 0.05$) analyzed with Tukey's test

α-GLUCOSIDASE INHIBITION TEST

The potential of *C. aeruginosa* leaves as one of the ingredients to inhibit α-glucosidase activity and indirectly prevent diabetes is tested using the α-glucosidase inhibition test. The percentage of α-glucosidase inhibition for the leaf extract of *C. aeruginosa* is shown in Table 6. The results showed that the leaf extract of *C. aeruginosa* dried using the FD method possessed a higher percentage of α-glucosidase inhibition activity, 30.78 ± 2.88% (FD 50:50), followed by 0:100 and 100:0 of 27.65 ± 2.46% and 25.65 ± 3.63%, respectively, compared to the OD method. The lowest inhibition percentage was detected in the 0:100 OD extract (10.32 ± 1.38%). Significant differences ($p < 0.05$) can only be seen between drying types, where freeze drying (FD) showed a higher percentage of α-glucosidase inhibition activity compared to oven drying (OD).

However, a study conducted by Sajak et al. (2016) found that extract of *Ipomoea aquatica* (spinach) using 100% ethanol had a higher α-glucosidase inhibition than ethanol and water mixture. These discrepancies may be due to the structural properties of the metabolite compounds present in the extract, whether they have high polarity to organic or aqueous solutions. Furthermore, among other factors affecting the rate of α-glucosidase inhibition activity includes the type of solvent used, the nature of the plant itself, the temperature during extraction process and the amount of original metabolites that capable of inhibiting the process of glucose decomposition (Murugesu et al. 2019).

TABLE 6. Percentage of α-glucosidase inhibition of *C. aeruginosa* leaf extract by different drying methods and solvent ratios

Drying method / Standard	α-glucosidase inhibition (%)		
	100:0	50:0	0:100
FD (Freeze Drying)	25.65 ± 3.63^{b}	30.78 ± 2.88^{b}	27.65 ± 2.46^{b}
OD (Oven Drying)	16.17 ± 1.8^{cd}	18.01 ± 0.54^{c}	10.32 ± 1.38^{d}
Quercetin	66.00 ± 2.03^{a}	63.51 ± 1.81^{a}	64.89 ± 0.86^{a}

The sample concentration used in calculating the percentage of α-glucosidase inhibition was 10000 µg/mL whereas the positive control (Quercetin) used was 125 µg/mL. Values represent the mean ± standard deviation (n = 3).$^{a-d}$ Different letters show significant differences ($p < 0.05$) analyzed by Tukey's test

In this study, the positive control used was Quercetin. The percentage of α-glucosidase inhibition for Quercetin was 66%, which was significantly higher ($p < 0.05$) compared to all *C. aeruginosa* leaf extracts. Quercetin is a bioflavonoid commonly used to regulate hyperglycemia activity and to aid in the process of glucose homeostasis in the blood (Riyaphan et al. 2017). However, the results showed that the *C. aeruginosa* leaves extract exhibited lower inhibitory activity compared to Quercetin. This was expected because Quercetin is an active pure compound while *C. aeruginosa* extract is a crude extract consisting of active and inactive compounds.

CORRELATION BETWEEN PHENOLIC COMPOUNDS WITH ANTIOXIDANTS AND ANTI-HYPERGLYCEMIC ACTIVITIES

The Pearson correlation test showed positive correlation between TPC with FRAP, DPPH and α-glucosidase for 100:0 extracts ($r = 0.978$, $p < 0.001$; 0.980, $p < 0.001$ and 0.856, $p < 0.05$) and 50:50 ($r = 0.995$, $p < 0.001$; 0.958, $p < 0.01$ and 0.960, $p < 0.01$) extracts. Higher phenolic content indicates higher antioxidant activity values as well as its ability to inhibit the α-glucosidase enzyme. This indicates that phenolic content is a good indicator of antioxidant and anti-hyperglycemic activities. Phenolic compound such as flavonoids (i.e. kaempferol, Quercetin, morin and rutin) are well known as antioxidant and have been previously reported to possess α-glucosidase inhibitory activity (Proença et al. 2017). Nonetheless, there are a few studies that only found a correlation between TPC and antioxidant properties but not with antidiabetic properties (Quan et al. 2019; Yao et al. 2012). The differences between those studies and this study is probably due to the phenolic compound in those plants were not the major compounds that contributed to the antidiabetic property (Quan et al. 2019).

CONCLUSION

The results showed that the overall best drying method was FD followed by OD. FD at 100:0 and 50:50 concentrations produced the highest phenolic content and antioxidant activity when measured using FRAP assay. For DPPH-free radical scavenging activity, FD 50:50 showed the highest percentage inhibition when compared to other extracts. In addition, FD 100:0, 50:50 and 0:100 showed the highest α-glucosidase inhibition of 25.65, 30.78, and 27.65%, respectively. This study also found that there is a very high correlation between phenolic content with antioxidant and anti-hyperglycemic activities of 100:0 and 50:50 *C. aeruginosa* leaf extracts. To conclude, results indicated that FD is the best method of drying while a solvent of 50:50 ethanol and water is considered as best solvent covering all bioassays measured in this study.

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