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Effect of Different Drying Methods on the Total Phenolic and Flavonoid Content and DPPH Free-Radical Scavenging Activity of *Pandanus amaryllifolius* Roxb. Planted in Mekong Delta

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aimed to investigate the total phenolic and flavonoid content and DPPH free-radical scavenging activity of *Vernonia amygdalina* planted in Mekong Delta. The optimized conditions for maceration of pandan leaves included drying method, ratio of pandan leaf powder-to-solvent, and extraction time.

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Methodology: The fresh pandan leaves were divided into two equal portions, subjected to different drying methods: shade and oven drying. The dried leaf powder was macerated in ethanol at room temperature. The maceration was conducted with 3 different ratios of pandan leaf powder-to-solvent (w/v) (1:10, 1:15 and 1:20), and the extraction time was 1, 2 and 3 days. The total flavonoid content was determined using aluminum chloride method whereas the total phenolic content was assessed using Folin-Ciocalteu assay. Meanwhile, the antioxidant activity of the plant extracts was quantitatively evaluated using DPPH test.
Results: The results indicated that the best conditions for maceration of pandan leaves were 1:10 shade-dried leaf powder-to-solvent ratio in 1-day extraction time. Accordingly, the total flavonoid and phenolic content was found to be the highest value of 130.02 ± 2.24 mg QE/g of dried extract and 100.67 ± 1.76 mg GAE/g of dried extract ($p < 0.05$), respectively. The lowest IC50 of DPPH free-radical scavenging activity of pandan leaf extract was found to be 0.90 ± 0.02 mg/mL ($p < 0.05$). In addition, the Pearson's correlation coefficient between IC50 of DPPH free-radical scavenge activity and total flavonoid content was $R^2 = 0.74$ compared to that of phenolic content with the value of $R^2 = 0.69$, indicating that the IC50 of DPPH free-radical scavenge capacity of pandan leaves was influenced chiefly by flavonoid compounds.
Conclusion: There was a significant difference in phenolic and flavonoid content and DPPH free-radical scavenging activity between shade-dried and oven-dried pandan leaf extracts.

Keywords: Pandanus amaryllifolius; flavonoid and phenolic content; DPPH free-radical scavenging.

1. INTRODUCTION

Pandanus amaryllifolius Roxb. (*P. amaryllifolius*), commonly known as pandan leaves, is a tropical plant of the family Pandanaceae [1]. It has high chlorophyll content that have been used as green-staining agent for food, drink, soap and cosmetics [2,3]. The leaves have sweet and delightful flavour which is widely used as a source of flavour enhancement for food such as desserts. The leaf extracts can also be applied for treatment of various diseases such as hair loss, hair whitening, dandruff, neurasthenia, anorexia, arthritis and different types of sore pains [4]. Besides these utilities, the leaves were also used as medicine for diuretic, cardio-tonic and anti-diabetic applications [5].

Fig. 1. *Pandanus amaryllifolius*

Pandan leaves contain a variety of phytol-components including steroids, carbohydrates, polyphenols, alkaloids, tannins, flavonoids and saponins [6]. Among the classes of bioactive compounds, flavonoids and phenolic compounds were intensively studied thanks to their potential benefits for human health [7]. They possess antioxidant properties capable of scavenging free superoxide radicals and reducing the risk of cancer based on its anti-aging properties [8]. Phenolics have the antioxidant activity due to their redox properties, hydrogen donators and singlet oxygen quenchers. Several studies have also found that flavonoids reduce blood lipids and glucose and enhance human immunity [9,10]. Furthermore, humans are protected by the enzyme system as a result of the effect of flavonoids [11].

It is noteworthy to know that the composition and quality of secondary metabolites extracted from the plants are affected by the selection of extraction method [12]. Excessive heat can damage the bioactivity of desired substances in the pandan leaves [13,14]. Therefore, maceration could be an appropriate method to extract bioactive substances in pandan leaves since the extraction is conducted at room temperature. Although it requires longer processing time and more solvents, maceration is easy to implement and does not require heating intervention. In this regard, this study primarily focused on the maceration of pandan leaves using ethanol as solvent. Factors involving the maceration included drying method, ratio of pandan leaf powder-to-solvent, and extraction time. The Pearson's correlation coefficients between DPPH free-radical scavenging activity and total flavonoid content, and total phenolic content were also investigated.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of plant materials

Pandan leaves were collected from a local garden in Mekong Delta. The plant was authenticated by The Institute of Tropical Biology Vietnam, and a voucher specimen of (No. AB Bio – 18 – 06 – 02) had been deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Biochemistry, School of Biotechnology, International University Vietnam National University – Ho Chi Minh City, Vietnam. The fresh pandan leaves were washed thoroughly with running water to remove any possible contaminants.

2.1.2 Chemicals

All chemicals were provided by Pharmaceutical Chemistry Laboratory and Applied Biochemistry Laboratory of Applied Biochemistry Department in International University HCMC. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagents were purchased from Sigma-Aldrich. All chemicals and reagents were stored in accordance with the most stringent regulations and freshly prepared with distilled water to the desired concentrations for experimental purposes.

2.2 Methods

2.2.1 Preparation of materials

The fresh pandan leaves were divided into two equal portions subjected to different drying methods: shade drying (SD) and oven drying (OD). Leaves from the first half were spread evenly on a tray and left dry at room temperature (26 ± 2^oC) in the shade. Meanwhile, leaves from the other half were placed in the oven at 40^oC. The completion of drying process was based on their constant weight. The dried leaves were then ground into fine powder using a blender, and later stored in a dessicator for later use.

2.2.2 Preparation of extracts

The dried leaf powder was macerated in ethanol at room temperature. The maceration was carried out with 3 different pandan leaf powder-to-solvent (P/S) (w/v) ratios (1:10, 1:15 and 1:20); and the extraction time was 1, 2 and 3 days. The extracts were filtered twice using Whatman filter paper. The filtrate was then evaporated under reduced pressure using a rotary evaporator until forming a brownish residue. The residue, known as total extracts, was weighted and kept in sealed vials at 4^oC until further usage.

2.2.3 Determination of total flavonoid content

The total flavonoid content (TFC) was determined using aluminum chloride method [15]. Quercetin was used as standard for the calibration curve. The assay was prepared by mixing 100 µL of plant extract (1.42 mg/mL), 860 µL of absolute ethanol followed by the addition of 20 µL of 10% $AlCl_3$ and 20 µL of 1 M CH_3COOK. The mixture was vortexed for 5 min and kept in the dark at room temperature for 30 min. Absorbance of sample was measured against the blank at 430 nm using a spectrophotometer and Biotek Synergy HT 96-well plate. Meanwhile, a calibration curve of known concentration of quercetin was made using the regression equation $y = bx + a$. TFC was expressed in terms of quercetin equivalent (QE) per gram of sample (mg QE/g of sample). All the experiments were triplicated, and the mean value of absorbance was recorded.

2.2.4 Determination of total phenolic content

The total phenolic content (TPC) was determined using Folin-Ciocalteu method [16]. Gallic acid was used as standard for the calibration curve. The assay was prepared by mixing 100 µL of plant extract (1.42 mg/mL), 500 µL of Folin-Ciocalteu reagent followed by the addition of 400 µL of 20% Na_2CO_3. The mixture was vortexed for 5 min and kept in the dark at room temperature for 30 min. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer and Biotek Synergy HT 96-well plate. Meanwhile, a calibration curve of known concentration of gallic acid was made using the regression equation $y = bx + a$. TPC was expressed in terms of gallic acid equivalent (GAE) per gram of sample (mg GAE/g of sample). All the experiments were repeated three times, and the mean value of absorbance was noted.

2.2.5 DPPH free-radical scavenging assay

The antioxidant activity of the plant extracts was quantitatively assessed using DPPH method [17]. Ascorbic acid was used as reference standard with seven different concentrations

ranging from 0.3 µg/mL to 30 µg/mL. The assay was prepared by mixing 250 µL of sample with 750 µL of 0.1 mM DPPH and then vortexed vigorously. The mixture was subsequently incubated for 30 min in the dark at room temperature. The absorbance of the mixture was spectrophotometrically measured against the blank at 517 nm using Biotek Synergy HT 96-well plate. The DPPH free-radical scavenging activity was expressed as IC50 which was the concentration of the sample required to inhibit 50% of DPPH free radicals.

2.3 Statistical Analysis

All the experiments were carried out in triplicate. The experimental data were analyzed with linear regression analysis to illustrate the correlation of the calibration curve by the Excel software. All the results were expressed as mean ± standard deviation (S.D.). The correlations between TFC, TPC and DPPH free-radical scavenging activity of samples were determined using Pearson's correlation test [18]. Analysis of variance (ANOVA) and Turkey's range test were used to identify the statistically significant differences at $p < 0.05$ between the samples through SigmaPlot software.

3. RESULTS

3.1 Total Flavonoid Content

Fig. 2 shows the value of TFC in various optimized conditions of eight selected samples. The results were derived from the calibration curve ($y = 1.1315x + 0.0141$, $R^2 = 0.9967$) (Fig. 3) of quercetin and expressed in milligram quercetin equivalents (QE) per gram of dried extract. Overall, samples of SD method had TFC higher than that of OD samples. In both drying methods, the highest value of TFC was found in samples of 1:10 P/S ratio in 1-day extraction time (130.02 ± 2.24 mg QE/g of dried extract of SD and 107.40 ± 0.95 mg QE/g of dried extract of OD) ($p < 0.05$). On the contrary, samples of 1:15 P/S ratio in 3-day extraction time displayed the lowest value of TFC in both drying methods with the value of 84.17 ± 4.42 mg QE/g of dried extract of SD and 20.89 ± 0.36 mg QE/g of dried extract of OD ($p < 0.05$).

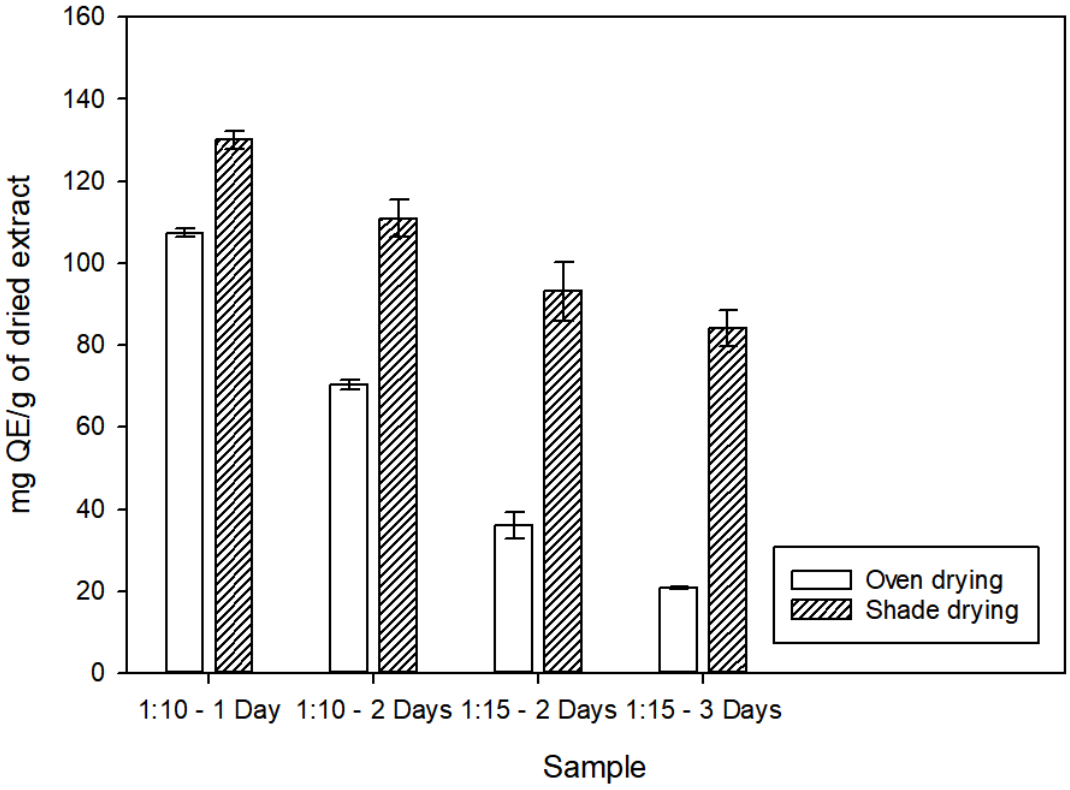

Fig. 2. Total flavonoid content in various optimized conditions of pandan leaves

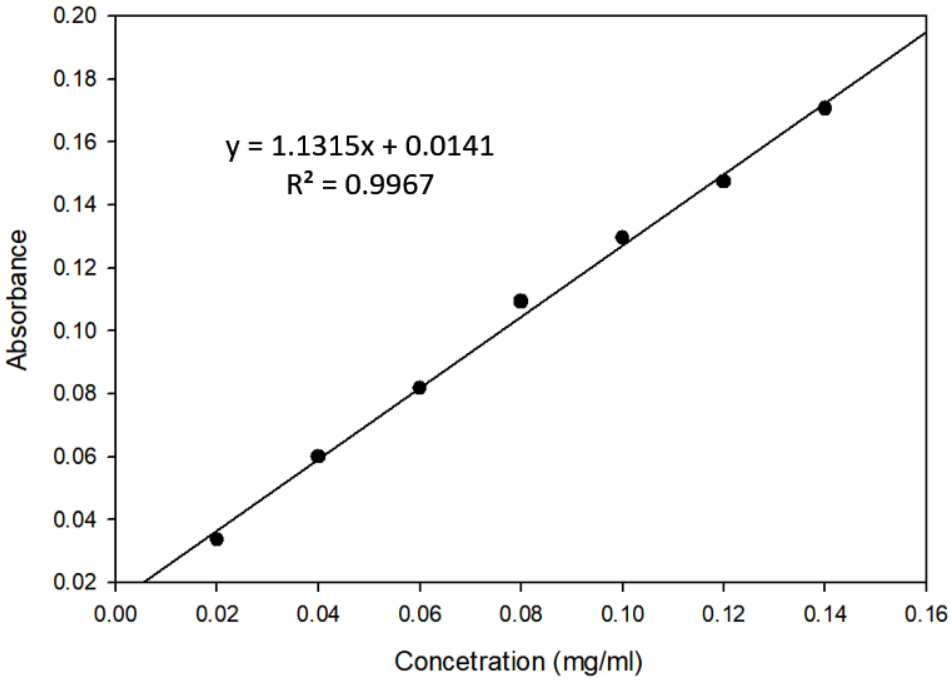

Fig. 3. Calibration curve of quercetin

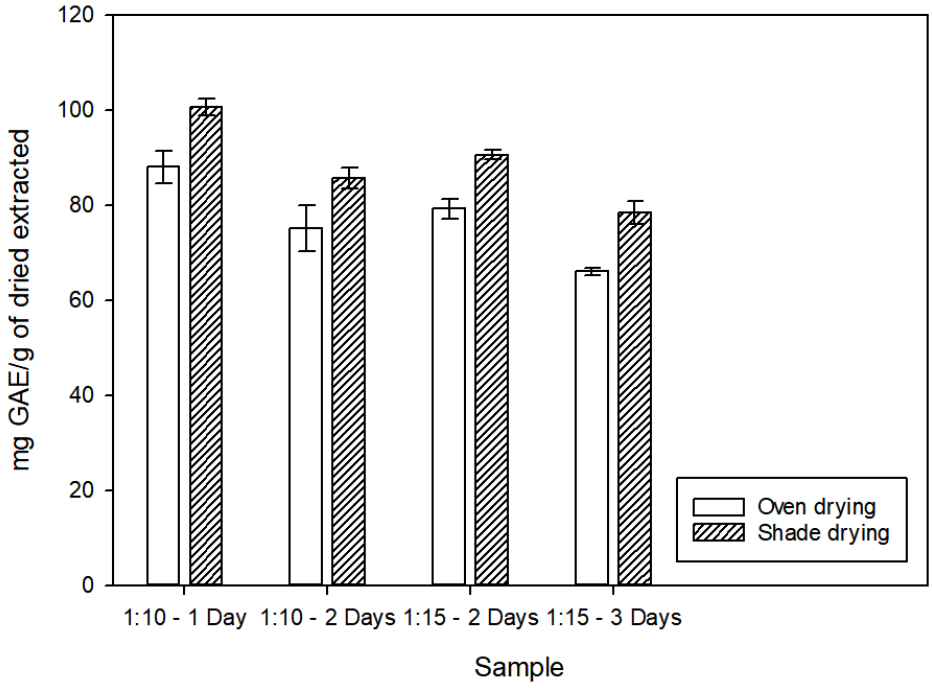

Fig. 4. Total phenolic content in various optimized conditions of pandan leaves

3.2 Total Phenolic Content

TPC in various optimized conditions of eight selected samples was illustrated in Fig. 4. The TPC values of these conditions were calculated using the gallic acid standard curve (y = 3.1006x – 0.0239, R^2 = 0.9973) (Fig. 5) and expressed in milligram gallic acid equivalents (GAE) per gram of dried extract. In general, samples of SD method had TPC higher than that of OD samples. Samples macerated with 1:10 P/S ratio in 1-day extraction time exhibited the highest

value of TPC in both drying methods (100.67 ± 1.76 mg GAE/g of dried extract of SD and 88.10 ± 3.35 mg GAE/g of dried extract of OD) (p < 0.05). Meanwhile, samples of 1:15 P/S ratio in 3-day extraction time demonstrated the lowest TPC with the value of 78.56 ± 2.39 mg GAE/g of dried extract of SD and 66.15 ± 0.80 mg GAE/g of dried extract of OD (p < 0.05).

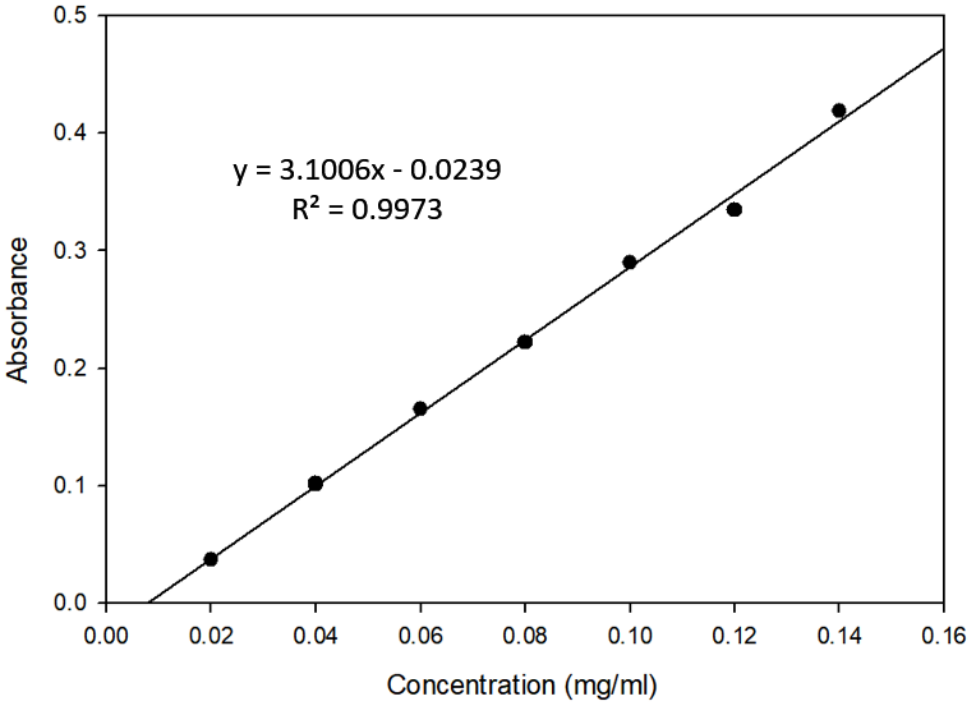

Fig. 5. Calibration curve of gallic acid

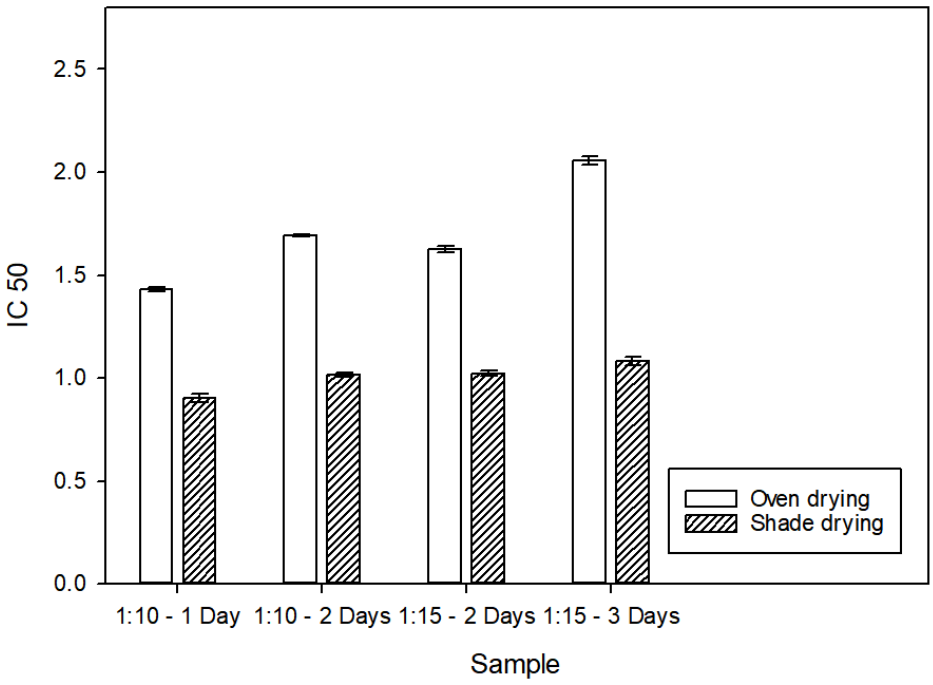

Fig. 6. DPPH free-radical scavenging activity of pandan leaves in various optimized conditions.

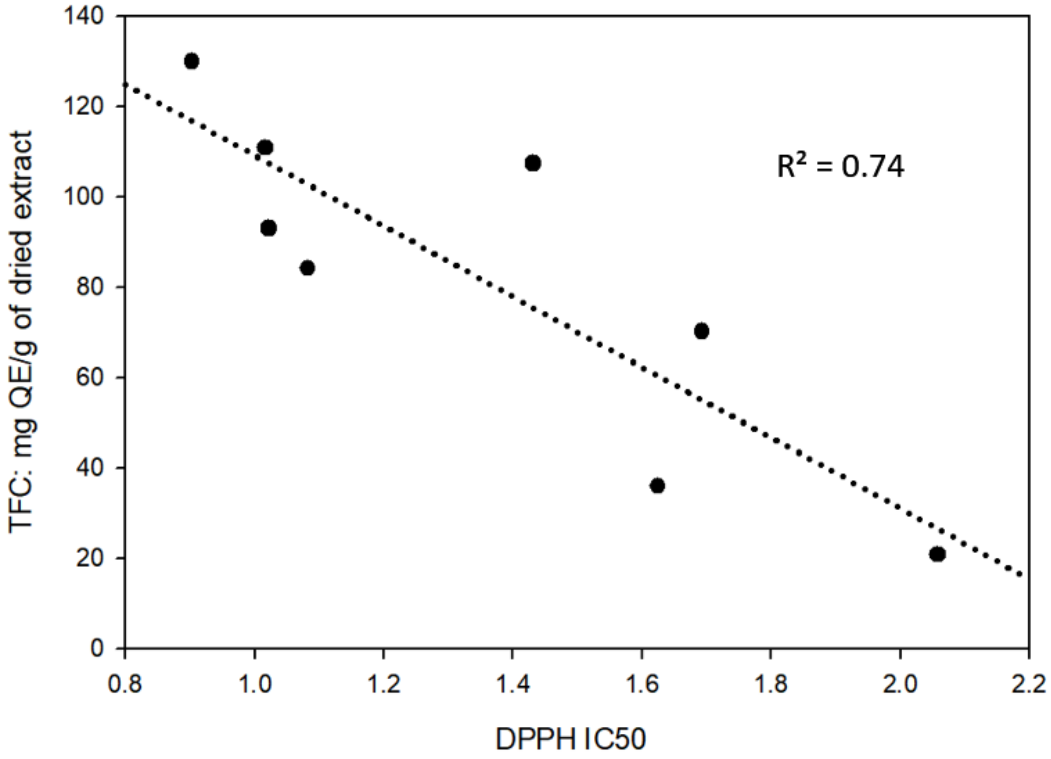

Fig. 7. Pearson's correlation coefficient between DPPH free-radical scavenging activity and total flavonoid content

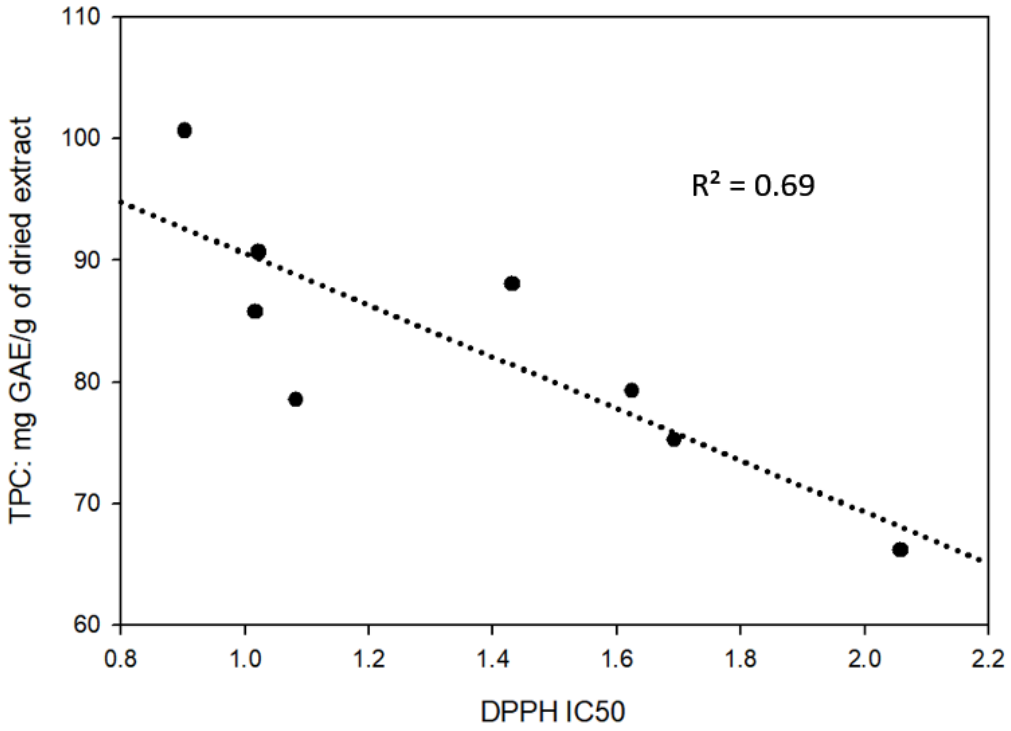

Fig. 8. Pearson's correlation coefficient between DPPH free-radical scavenging activity and total phenolic content

3.3 DPPH Free-Radical Scavenging Activity

Fig. 6 shows IC50 of DPPH free-radical scavenging activity of eight selected samples. Ascorbic acid scavenging ability to inhibit 50% of free radicals was calculated at $0.016 \pm 5\times10^{-4}$ mg/mL. Overall, samples of SD method exhibited lower IC50 than that of OD method. In addition, the less extraction time was, the lower IC50 was recorded. Accordingly, samples of 1:10 P/S ratio in 1-day extraction time achieved the lowest IC50 of 0.90 ± 0.02 mg/mL by SD method and 1.43 ± 0.01 mg/mL by OD method. On the contrary, the highest IC50 was found in samples of 1:15 P/S ratio in 3-day extraction time with the value of 1.08 ± 0.01 mg/mL by SD method and 2.06 ± 0.02 mg/mL by OD method.

3.4 Pearson's Correlation Coefficients Between DPPH Free-Radical Scavenging Activity and Total Flavonoid Content, and Total Phenolic Content

As shown in Fig. 7, the Pearson's correlation coefficient between DPPH free-radical scavenging activity of pandan leaf extracts and TFC was recorded $R^2 = 0.74$ compared to that of TPC with over value of $R^2 = 0.69$ (Fig. 8).

4. DISCUSSION

In this study, generally, the time for OD method was approximately 3 to 5 times faster than SD method. Conversely, the yield of total phenolic and flavonoid content from SD samples was significantly greater than that of OD when macerated at the same conditions including P/S ratio and extraction time. As can be seen from Fig. 2, the results showed that the 1:10 P/S ratio in 1-day extraction time was the optimum conditions for maceration of pandan leaves. Accordingly, the highest TFC was recorded 130 ± 2.24 mg QE/g of dried extract ($p < 0.05$). Meanwhile, the highest TPC was found to be 100.67 ± 1.76 mg GAE/g of dried extract ($p < 0.05$) (Fig. 4).

Phenolic and flavonoid compounds have been scientifically proven to possess ideal structural characteristics to scavenge free radicals. Their molecules are capable of donating hydrogen atoms or electrons to DPPH, deactivating free radicals [19]. The DPPH free-radical scavenging activity of pandan leaf extracts is presented in Fig. 6. The activity was assessed by determination of the IC50 values, which correspond to the concentration of pandan leaf extracts that can scavenge 50% of free radicals present in the reaction mixture. The low IC50 indicates the high capacity of scavenging free radicals. In this research, the IC50 of shade-dried pandan leaf extracts with 1:10 P/S ratio in 1-day extraction time was found to be the lowest value of 0.90 ± 0.02 mg/mL which was 56 times higher than IC50 value of ascorbic acid ($0.016 \pm 5\times10^{-4}$ mg/mL). Nevertheless, an investigation conducted by Jimtaisong et al. [2] had shown that the IC50 value of pandan leaves in Thailand was 0.81 ± 0.009 mg/mL, truly reflecting the nature of pandan leaves in DPPH free-radical scavenging activity.

Correlation between DPPH free-radical scavenging capacity of pandan leaf extracts and bioactive compounds was calculated by Pearson's correlation test. A correlation analysis showed that there was a significant ($p < 0.05$) relationship existed between flavonoids, phenolic compounds and antioxidant activity. As shown in the Figs. 7 and 8, the Pearson's correlation coefficient (R^2) of TFC and DPPH free-radical scavenging activity was higher than that of TPC and DPPH free-radical scavenging activity, apparently indicating the dominant contribution of flavonoids to the DPPH free-radical scavenging activity of pandan leaves compared to that of phenolic compounds. However, a report from Paixao et al. [20] pointed out that DPPH is known to react preferentially with low-molecular-weight phenolic compounds [20]. Moreover, the decline of antioxidant capacity of phenolic molecules can occur because the low-molecular-weight phenolic compounds are more susceptible to degradation if exposed to temperature, light and oxygen in a prolonged extraction time [21]. Furthermore, a study conducted by Tawaha et al. [22] indicates that the weak correlation between TPC and IC50 because the antioxidant activity does not incorporate necessarily from TPC [22]. Overall, the optimum conditions for ethanol maceration of shade-dried pandan leaf extracts were 1:10 P/S ratio in 1-day extraction time. The DPPH free-radical scavenging capacity was attributed primarily to the flavonoids present in pandan leaves.

5. CONCLUSION

It is conclusive that there was a significant difference in phenolic and flavonoid content and DPPH free-radical scavenging activity between

shade-dried and oven-dried pandan leaf extracts. However, drying pandan leaves in the shade took a significantly longer time to reach the constant weight. On the other hand, the phenolic and flavonoid content and DPPH free-scavenging capacity of pandan leaf extracts were also attributed to other factors including the ratio of pandan leaf powder-to-solvent and extraction time.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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