Antioxidant activities of *Pandanus amaryllifolius* leaves extracted under four designed extraction conditions.

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Abstract

Pandanus amaryllifolius is a tropical plant in Pandanaceae family, which customarily used for food coloring and flavoring. Leaves of *P. amaryllifolius* provide a rich source of essentials oil, carotenoid, vitamin E and other bioactive compounds such as antioxidants. High quantity of total phenolic compounds (TPC) and antioxidants (as being detected by 2,2-diphenyl-1-picry hydrazyl radical (DPPH) and linoleic acid peroxidation methods) were previously reported. Nevertheless, limited information on antioxidant activities of *P. amaryllifolius* leaves regarding extraction conditions is available. Thus, the purpose of this study was to investigate the effect of extraction conditions of *P. amaryllifolius* leaves regarding its antioxidant activity. Freeze-dried powder of *P. amaryllifolius* leaves were extracted under designed extraction conditions including extraction times (15–240 minutes), concentrations of aqueous ethanol (0–100% v/v), extraction temperatures (30–90°C) and solid-to-liquid ratios (1:20–1:60 v/w). The antioxidant activities were measured using ferric reducing antioxidant power (FRAP) method. As results, it was found that the antioxidant activities of *P.amaryllifolius* leaves extracted under investigated experiments were ranged from 9.54–23.16 mg trolox equivalent (TE) per 1 g dry weight. The optimized extraction conditions of *P. amaryllifolius* leaves were 15 minutes of extraction time, 80% (v/v) aqueous ethanol, and 1:60 (v/w) of solid-to-liquid ratio, while extraction temperature was an insignificant factor for extraction conditions. The information received from this research would support further investigation on optimized of condition for extraction by using response surface methodology and isolation of bioactive compounds from leaves of *P. amaryllifolius*.

Keywords: antioxidant activity, ferric reducing antioxidant power (FRAP), bioactive compounds, *Pandanus amaryllifolius*

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1. Introduction

Free radicals are naturally produced in biological systems as results of oxidative stress induced environments. These compounds play a significant role in pathogenesis of several human diseases and are the causes of various degenerative disorders such as carcinogenesis, cardiovascular genesis, gene mutation and especially ageing (Singh and Singh, 2008). Antioxidants are the compounds acting against the free radicals by forming interactions with oxidants and terminating free-radical chain reactions. Antioxidants are important tools for preventing oxidative stress related diseases and preserving good health. These antioxidants could be produced from biological systems or occurred naturally in many foods, especially plant based food such as fruits, vegetables, spices and herbs (Carlsen *et al.*, 2010).

Pandanus amaryllifolius, a member of Pandanaceae Family, is an herb that is widely distributed in tropical area including South Asia, Africa and Australia (Nor *et al.*, 2008). Leaves of *P. amaryllifolius* are used for food favoring, aromatherapy and medical applications (Cheeptham and Towers, 2002). Abundant quantity of essentials oil, carotenoid, vitamin E and other bioactive compounds are available as being previously reported (Lee *et al*., 2004). Among these, antioxidants were detected using 2,2- diphenyl-1-picry hydrazyl radical (DPPH) scavenging assay and linoleic acid peroxidation method (Yan and Asmah, 2010; Jimtaisong and Krisdaphong, 2013). The first is based on the measurement of the scavenging capacity of antioxidants towards oxidant compounds (or in this case, DPPH radicals). The interaction of DPPH radicals and antioxidants could be measured by color change from purple to yellow (Kedare and Singh, 2011). This method is quick but provides low sensitivity reaction. The latter is based on the measurement of the lipid peroxidation rate of linoleic acid in the presence of a constant radical source. The kinetics of oxygen consumption accompanying linoleic acid oxidation was followed by oxygen electrode in a thermostated oxygraphic cell (Rossetto *et al.*, 2002). Again, even though this method is quick and sensitive, it is not specific since linoleic acid could react with other components present in biological samples.

Nevertheless, limited information on extraction conditions of *P. amaryllifolius* leaves regarding its corresponded antioxidant activities is available. Thus, the purpose of this study was to investigate the antioxidant activities using ferric reducing antioxidant power (FRAP) method on *P. amaryllifolius* leaves that extracted under different extraction conditions. The FRAP method is a pre-method for analyzed antioxidant activity in this plant with several benefits including being simple, time and economically efficiency and widely used for preliminary measurement of the ability of compounds that act as free radical scavengers. The information received from this study would support further investigation on optimized extraction

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conditions by using response surface methodology and isolation of bioactive compounds from *P. amaryllifolius* leaves.

2. Materials and Methods

2.1 Materials

Chemicals including 2,4,6–tripyridyl–*s*–triazine (TPTZ), Trolox and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were received from Sigma Aldrich (St. Louis, MO, USA). Solvent including acetate, ethanol and sodium acetate trihydrate were purchased from RCI Labscan (Bangkok, Thailand). Glacial acetic acid was supplied from Merck (Damstadt, Germany).

2.2 Sample preparation

Fresh leaves of *P. amaryllifolius* were collected in June, 2013 from Nakorn Pathom province, Thailand. The samples were washed with deionized water and cut into small pieces. Next, the clean samples were freeze-dried for approx. 40–50 hours and then ground into fine powder by a cyclotex sample mill (series 1903 with 200–240 V and 50/60 Hz from FOSS, Höganäs, Sweden). The moisture content of the freeze-dried samples was analyzed by AOAC (2000) method and was found to retain approx. 7.73±0.09% of moisture. All samples were kept in vacuum bag and stored at –20°C.

The sample (particle size < 0.18 mm) was extracted by varying extraction times (15, 30, 60, 120 and 240 minutes), concentrations of aqueous ethanol (0, 20, 40, 60, 80 and 100% v/v), extraction temperatures (30, 50, 70 and 90°C) and solid-to-liquid ratios (1:20, 1:30, 1:40, 1:50 and 1:60 v/w). The mixture after extraction was centrifuged at 2,810 g for 15 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper. The filtrate was then kept at -20°C for further analysis.

2.3 Determination of antioxidant activity

The FRAP assay was determined according to the method of Benzie and Strain (Benzie and Strain, 1996) with some modifications. The FRAP reagent containing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM) in HCl (40 mM) and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a ratio of 10:1:1 was warmed at 37°C before use. The samples (20 µL) were mixed with FRAP reagent (150 µL) in the 96–well flat–bottom microplate and incubated at room temperature for 8 minutes. The reaction was monitored using the microplate reader (BioTek Instruments, Inc., Winooski, VT) at a wavelength of 595 nm. Trolox solution (7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 µM) was used as a control. The FRAP values for the samples

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were determined using a standard curve of trolox solution and expressed as Trolox equivalent (TE) per 1 g dry weight of sample.

2.4 Statistical analysis

All data were expressed as mean of triplicate experiments ± standard deviation (SD). The data was analyzed by One-way analysis of variance (One-way ANOVA) and Tukey's-b *post hoc* tests. They were performed to determine significant differences between values (p<0.05). All statistical analyses were carried out using SPSS software for window (version 16.0, SPSS Inc., IL, USA).

3. Results and Discussion

The results of antioxidant activities as being measured by FRAP assay showed that *P.amaryllifolius* leaves extracted under investigated extraction conditions ranged from 9.54–23.16 mg Trolox equivalent (TE) per 1 g dry weight. The optimized extraction conditions of *P. amaryllifolius* leaves were 15 minutes of extraction time, 80% (v/v) aqueous ethanol and 1:60 (v/w) of solid-to-liquid ratio, while antioxidant activities under investigated time and temperatures showed no differentiation (Table 1).

It was found that the highest antioxidant activity was observed at the particular percentage of ethanol concentration (80% v/v), which might be due to the solubility of *P. amaryllifolius* bioactive compounds. It was previously reported that the bioactive compounds in *P. amaryllifolius* leaves were flavonoids, alkaloids, saponin, and tannin, which likely dissolved in solvent with higher polarity than water but lower than absolutely ethanol.

Interestingly, the optimized antioxidant activity was observed with high liquid-to-solid ratio (1 g of *P. amaryllifolius* powder per 60 mL solvent), suggesting that the antioxidants required large volume of extraction solvent to dissolve. This matter could be explained in term of the increase in driving force for mass transfer or increased solvent-sample interface area.

Besides, antioxidant activities of *P. amaryllifolius* leaves extracted under various extraction times and extraction temperatures were similar, suggesting that time and temperature were insignificant factors for extraction of antioxidants in this study. Thus, the time and temperature were fixed at 15 minutes and 30 °C, respectively, the conditions that used to extract sample.

Under these optimized extraction conditions, it was show that *P. amaryllifolius* leaves exhibited 23.16±0.23 mg trolox equivalent (TE) per 1 g dry weight as being measured by FRAP assay. The FRAP method is wildly employed for measuring the quantity of antioxidants based on reduction of ferrion analogue, the Fe^{3+} complex of tripyridyltriazine [$Fe(TPTZ)^{3+}$], into

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its blue reduced counterpart, $Fe(TPTZ)^{2+}$ (Benzie & Strain 1996). This method possesses several benefits including being quick, simple, economically efficient and widely used for preliminary measurement of the ability of compounds that act as free radical scavengers. This method is also employed for evaluating antioxidant activity in foods. It can also be used to quantify antioxidants in complex biological systems or for solid or liquid samples in several different solvent systems. These preliminary FRAP values will be further employed for designing extraction conditions of bioactive compounds from *P. amaryllifolius* leaves using response surface methodology.

Table 1 The FRAB values of *P. amaryllifolius* leaves extracted with various extraction conditions

Extraction condition of *P. amaryllifolius* leaves				FRAP value
Time (minutes)	Ethanol (% v/v)	Temperature (°C)	Solid-to-liquid ratio (% v/w)	(μmol TE per 1 g dry weight)[#]
15	40	50	1:40	10.95 ± 0.44 [a]
30				11.52 ± 0.56 [a]
60				12.17 ± 0.94 [a]
120				11.34 ± 0.39 [a]
240				12.49 ± 0.51 [a]
15	0	50	1:40	9.63 ± 0.91 [e]
	20			10.58 ± 0.47 [d]
	40			13.42 ± 0.19 [c]
	60			17.23 ± 0.45 [b]
	80			19.36 ± 0.27 [a]
	100			9.54 ± 0.17 [e]
15	80	30	1:40	19.65 ± 1.17 [a]
		50		21.37 ± 0.68 [a]
		70		20.30 ± 0.49 [a]
		90		20.98 ± 0.66 [a]
15	80	30	1:20	16.02 ± 0.19 [d]
			1:30	18.20 ± 0.48 [c]
			1:40	20.20 ± 0.05 [b]
			1:50	20.74 ± 0.70 [b]
			1:60	23.16 ± 0.23 [a]

Note: [#]Each value was represent as mean ± SD (n = 3). Mean within a column in each tested condition was shown with difference superscript letters, which was significantly different ($p<0.05$).

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4. Conclusion

The optimized extraction conditions of *P. amaryllifolius* leaves were 15 minutes of extraction time, 80% (v/v) aqueous ethanol and 1:60 (v/w) of solid-to-liquid ratio. The information received from this research would support further investigation on the optimization of extraction conditions using response surface methodology (RSM) and the development on efficient isolation of bioactive compounds from *P. amaryllifolius* leaves.

Acknowledgements: This research was performed at the Institute of Nutrition, Mahidol University.

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