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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN *NYPA FRUTICANS* EXTRACTS

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Abstract: *Nypa fruticans* is a palm species commonly found inhabiting the brackish area or adjacent to the mangrove forest. The ability of this specie to inhabit in such extreme environment may have been assisted by a type of defence metabolites. In the present study, the content of total phenolic and antioxidant activity in matured leaves, young leaves and the husk of mature fruits were examined. Total phenolic was measured using Folin-Ciocalteu and antioxidant was based on free radical-scavenging activity. The results showed that the matured leaf contains the highest total phenolic (299.06 ± 0.02 mg/g dry wt. sample), followed by the young leaf (205.97 ± 0.02 mg/g dry wt. sample) and fruit-husk (30.77 ± 0.01 mg/g dry wt. sample) extracts. Both mature and young leaf contained high antioxidant activity, the IC_{50} values were 0.42 and 0.32 mg/ml, respectively. Antioxidant activities have a positive correlation with the total phenolic content (R^2 =0.687). Phenolics in *N. fruticans* leaves might play a role as defensive agent against the free-radical regenerated by stress.

Keywords: Quercetin, Folin-Ciocalteu, chlorogenic acid, protocatechuic acid, kaempferol.

Introduction

Nypa fruticans Wurmb (Palmae) or 'nipa palm' is the sole species in *Nypa*. This plant species inhabited the brackish area that often forms a wide border beyond the fringe of adjacent mangrove or swamp forest. Its ability to adapt and grow in this extreme environmental stress is amazing. It is known that plants produce an enormous number of secondary metabolites in specialised cell that are not directly essential for basic photosynthesis or respiratory metabolism during primary growth and development. But, these metabolites are vital for plant survival, interaction with the environment, reproductive strategy and defence mechanism (Cheynier *et al.,* 2013). During growth and development, plants undergo some forms of stress during the various stages of their life cycle. Activation of defence mechanism following imposition of environmental stress involves complex reiterative signal networks with extensive signal amplification and trade-offs (Suzuki *et al.,* 2004; Kono *et al.,* 1995). This includes developmental signal during lignification of new growth or the production of anthocyanin during fruit and flower development, and environmental signals for protection against abiotic and biotic stress (Suzuki *et al.,* 2004; Boscaiu *et al.,* 2009), which is a burden to the plants in the production of defence compounds (Cheynier *et al.,* 2013).

In the plant kingdom, phenolics are ubiquitously present and the most widely distributed secondary metabolites. Plant phenolics have also been implicated in many direct interactions with transport and signal transduction pathways. Its accumulation in plant tissues is a distinctive characteristic of plant stress (Boscaiu *et al.,* 2010), confers various physiological functions for plant survival (Kono *et al.,* 1995) and adaptation to environmental disturbances (Cheynier *et al.,* 2013). Defensive phenolic compounds appear to contribute to a general reduction of reactive oxygen species and therefore impact cellular processes sensitive to redox effects. Prasad and co-workers (2012; 2013) reported that the antioxidant activity of the edible part of immature and mature *Nypa* fruit*s* were related to the phenolics. Some of the identified phenolics in endosperm of *N. fruticans* are chlorogenic acid, protocatechuic acid and kaempferol (Prasad *et al.*, 2013). The objective of this paper is to investigate the total phenolic content and the antioxidant activity in *Nypa* plant parts; mature leaves and young leaves

and fruit-husk. This work is essential, since it would determine the potency of the extract at different plant parts and maturity levels. Furthermore, choosing of the most effective organic solvents for the extraction of phenolics is also crucial.

Methodology

Plant Materials and Chemicals

Leaves (young and mature) and mature fruits of *Nypa* palm were collected from mangrove area at Universiti Malaysia Terengganu (UMT), Kuala Terengganu, Malaysia.

2,2-diphenyl-2-picrylhydrazyl (DPPH) and quercetin were purchased from Sigma, (Germany). Folin-Ciocalteu reagent and sodium bicarbonate were obtained from Merck (Darmstadt, Germany). All reagents used were of analytical grade.

Extraction

The fresh plant materials were cut into small pieces, dried in an oven at 40 ^{0}C for three days and subsequently powdered using an electric blender. A 100-g of each powdered samples were extracted with 250 ml of hexane at ambient temperature (25 - 27 ^{0}C) for 1hour, followed with ethyl acetate and methanol, sequentially. The process was repeated twice for each solvent. Extracts from the same solvent were combined and concentrated using rotary evaporator at 40 ^{0}C. Ten milligram of recovered extract was dissolved in 1.0 ml of respective solvent prior used. The remaining crude extract was stored in -20 ^{0}C for further used.

Total Phenolic Content

The total phenolic content was quantified according to Maliauskas *et al.*, (2004) using Folin-Ciocalteu agent. Two hundred microliter extract was mixed with 1.0 ml Folin-Ciocalteu reagent and 0.8 ml of 7% (w/v) Na_2CO_3 solution. Subsequently, the mixture was incubated at room temperature (25 - 27 ^{0}C) for 1 hour. The absorbance was measured using spectrophotometer at 760 nm. The total phenolic acid content was expressed as gallic acid equivalents (GAE/g extract). The calibration curve ranged from 0.02 to 0.30 mg/ml of gallic acid.

Antioxidant Activity (DPPH Free Radical-scavenging Assay)

DPPH free radical-scavenging assay was measured according to modified method of Kanski *et al.*, (2002). The initial absorbance of DPPH was measured using spectrophotometer at 517 nm until absorbance remained constant. Briefly, 20 µl of dimethylsulfoxide (DMSO) was added into 96 wells plate except the well in the first lane which function as control. One hundred microliter of extract was added to the wells in the first and second lane. Two-fold serial dilution of extracts were pipetted into the wells in the second until seven lane. Twenty microliter of solution was discarded from well in lane seven. Subsequently, 200 µl of methanolic solution DPPH (6 x 10^{-5} M) was pipetted into all wells. The mixture was incubated at 30 ^{0}C for 30 minutes. Subsequently, the changes of absorbance was measured at 517 nm using ELISA reader under the dark conditions. Measurements were performed in triplicates. Quercetin (1 mg/ml) dissolved in DMSO was used as positive control. The percentage of inhibition was calculated as follows:

Percent of inhibition (%) = $[(A517_{control} - A517_{sample})/A517_{control}] \times 100$.

IC_{50} values denote the concentration of a sample required to reduce 50% of the absorbance at 517 nm.

Statistical Analysis

All experiments were conducted in triplicate. The data was statistical analysed using software DPS3.01user's guides. The data were presented as mean ± SD. Determination of significant differences of the means between various extracts of *Nypa* were performed by t-test.

Results and Discussion

Results showed that leaves of *N. fruticans* contains very high phenolic compound (Table 1). It was 25.6 % and 29.9% from the total dried weight of young and mature leaves, respectively. This was 8.3 and 9.7-fold higher than in fruit-husk. It was noticed that the highest total phenolic was in the ethyl acetate extracted. Phenolic compounds comprises from non-polar to very polar, thus choosing the most appropriate solvent is crucial in maximizing the extraction process. Ethyl acetate is the common solvent for extraction of polar phenolic compounds (Peschel *et al.,* 2006). The phenomenon suggested that, *Nypa* leaves might be dominated by the polar type of phenolic compounds. Meanwhile, ethanol was used in the extraction of phenolics from edible part of *Nypa* fruit (Prasad *et al.,* 2012; 2013) and oil palm mesocarp (Neo *et al.,* 2008) and areca seed (Zhang *et al.,* (2009). The content of total phenolic in *Nypa* fruit, oil palm mesocarp and areca seed are comparable to *Nypa* leaves.

The ethyl acetate fractions of mature and young leaves were exhibited the highest antioxidant activity compared to other crude extracts (Table 1). This was indicated by the DPPH scavenging activity, the lower IC_{50} values means the higher antioxidant activity. The IC_{50} values for ethyl acetate fractions of matured leaves (0.42 ± 0.07 mg/ml) and young leaves (0.32 ± 0.07 mg/ml) were not significantly different compared to quercetin (0.40 ± 0.07 mg/ml), the positive control. Ethyl acetate was also able to extract the compound that act as pro-oxidant (Prasad *et al.,* 2013; Yesilda *et al.,* 2000). Hence, the scavenging activity of the *Nypa* leaves might be contributed by the presence of both antioxidant and pro-oxidant compounds. On the other hand, the hexane fraction of the extracts contained the lowest phenolic content and antioxidant activity (Table 1). This finding is in agreement with Sultana *et al.,* (2009), where the antioxidant activity and phenolic content were influenced by extracting solvents. In addition, the current finding revealed that antioxidant activity was significantly correlated with the phenolic content (Figure 1). Jafri and co-workers (2014) reported a similar result on *Hedera nepalensis,* where antioxidant activity

Table 1: Total phenolic content and IC_{50} values based on DPPH scavenging activity of various extracts of *Nypa fruticans* using organic solvents

Sample or plant parts	Solvent used	Total phenolic (GAE mg/g extract)	DPPH scavenging IC_{50} (mg/ml)[¥]
Fruit-husk	Hexane	2.01 ± 0.01	3.58 ± 1.43[ab]
	Ethyl acetate	15.38 ± 0.01	1.54 ± 0.73[b]
	Methanol	13.39 ± 0.01	2.42 ± 0.27[ab]
Total		**30.78 ± 0.01**	
Matured-leaf	Hexane	71.13 ± 0.01	2.06 ± 0.37[b]
	Ethyl acetate	131.37 ± 0.02	0.42 ± 0.07[c]
	Methanol	96.56 ± 0.04	1.52 ± 0.06[b]
Total		**299.06 ± 0.02**	
Young-leaf	Hexane	29.87 ± 0.02	3.36 ± 0.03[a]
	Ethyl acetate	109.51 ± 0.02	0.32 ± 0.07[c]
	Methanol	66.59 ± 0.03	1.31 ± 0.20[b]
Total		**205.97 ± 0.02**	
quercetin[§]		-	0.40 ± 0.07[c]

Note: Each value was expressed as the mean ± SD (n=3).
Values with same alpha within the same column is not significantly different ($P < 0.05$)
[¥] Lower IC_{50} value indicated high antioxidant activity.
[§] Positive control for IC_{50}

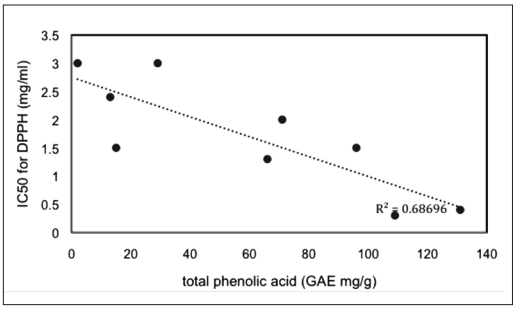

Figure 1: The negative correlation between IC_{50} values for DPPH radical scavenging activity against the total phenolic content of *Nypa frutican*

was significantly correlated with the amount of phenolic compounds present in the extract. Higher antioxidant activity was also reported in other palm trees that contains high phenolics (Prasad, 2012, 2013; Neo *et al.,* 2008: Zhang *et al.,* 2009). Nonetheless, there may not be always a linear correlation between these two activities. The antioxidant activities could be attributed to various mechanisms such as the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Cheynier *et al.,* 2013).

Conclusion

Total phenolic compounds present in *N. fruticans* varied between the plant parts. Leaves are the major site stored for phenolic compounds, which could be utilised as a source of substances in scavenging the radical ions and antioxidant. Thus, *Nypa* leaves are a potential source of antioxidant that could be commercialised. Ethyl acetate was the best solvent for extraction of phenolic compounds from leaves and fruit husk of *Nypa* palm.

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