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Total Phenolics and Antioxidant Properties of Red Hot Chili Peppers of Different Varieties in Malaysia as Potent Nutraceutical

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Introduction

Chili pepper is a plant that belongs to the genus of *Capsicum* originated from the family of *Solanaceae.* Despite the unstable taxonomy and the lack of a generally agreed upon nomenclature, only five species are domesticated. They are *Capsicum annuum* L., *Capsicum chinense* Jacq., *Capsicum frustescens* L., *Capsicum baccatum* L. and *Capsicum pubescens*[1]. In the current study, five different varieties of red hot chilli peppers are purchased. Among them, there are 4 different varieties that are derived from the species of *Capsicum annuum* L. (Kulai 151, Kulai 568 , Bara and Pelita), while Centil or bird eyes chilli is a variety that come from *Capsicum frustescens* L.. Kulai 151 and Kulai 568 are the varieties with larger size, with length of fruits ranging from 15-18cm. Bara, Pelita and Centil are small-sized chillies or known as Bird's eye chilli, with stronger pungent smell compared to Kulai varieties. Centil is commonly planted in urban areas in Malaysia. It is later cultured under program of 'Fertigasi' for better plantation quality. Its fruit length ranging from 2-3cm. Both of the variety of Bara and Peilta are small-sized chillies, ranging from 2-4cm in length. They are both originally breed in Indonesia. Lately, they are introduced and planted in Malaysia. According to the local farmers, Pelita is a new hybrid offspring from Bara variety.

All of these chilli peppers are widely marketed and consumed in Malaysia. They are important spices that are widely used in Malaysian cuisines. Except for culinary use, chilli peppers in country such as Iraq are also found to be a potential antioxidant agent due to its antioxidant content and significant antioxidant activities[2-5]. However, the antioxidant content and antioxidant activities in Malaysia chilli varieties have not been fully studied. Respective phenolic content and antioxidant activities in different parts (seed, pulp and whole fruit) of chilli pepper in Malaysia also have not been reported. This is the first report on determination the phenolic content and antioxidant activities in Malaysia local chillies through in-vitro studies.

Methods

Sample preparation and extraction

Five varieties of fully mature red hot chilli peppers (Centil, Kulai 151, Kulai 568, Bara, Pelita) were collected from Department of Agriculture, Serdang, Selangor, Malaysia. These chilli peppers are from two chilli species, *Capsicum annuum* (Kulai, Bara and Pelita) and *Capsicum frutescens* (Centil). They were separated into three parts, which were seeds, pulps and whole fruit. All the samples were freeze-dried and ground into fine powder using a Waring® blender and were kept in -20°C before extraction. The chilli pepper samples (4 g) were extracted with 70% ethanol. The extraction was repeated twice using 70% ethanol. Ethanol in pool extracts were removed using a rotary evaporator at 37°C. The concentrated extracts were kept in -20°C for further analysis. The extraction yield (%) for seed, pulp and whole fruit ranged from 2.16-6.82, 5.12-11.76 and 2.74-6.15, respectively.

Determination of total phenolics

TPC of chilli pepper extracts was determined according to the method described by Taegu *et al.*[6] Gallic acid was used as the standard and ethanol (70%) as the control. A gallic acid standard curve was constructed to determine TPC and expressed as gallic acid equivalent (mg GAE/kg) of extract.

TFC of chilli pepper extracts was determined according to the method described by Ozkok and Sorkun.[7] Quercetin was used as the standard and ethanol (70%) as the control. TFC of the extract (mg QE/kg) was calculated from the quercetin standard curve and expressed as quercetin equivalent (QE).

Antioxidant activity assays

FRAP value of chilli pepper extracts was determined according to the method described by Kenny *et al.*[8] Trolox was used as the standard and 70% ethanol was used as the control. FRAP value of the extracts was calculated from the Trolox standard curve. FRAP and expressed as mM Fe^{2+}/mg extract.

ABTS assay of chilli pepper extracts was performed according to the method described by Li et al.[9] Ferrous sulphate was used as the standard and 70% ethanol was used as the control. The ABTS value of extracts (at concentration of 0.03 mg/ml) was calculated based on the ferrous sulphite standard curve. The result was expressed as percentage of inhibition (%).

Statistical analysis

All data were presented as mean ± standard deviation of three replicates using Minitab version 17. Due to the homogenised data, one-way analysis of variance (ANOVA) was applied to compare mean differences among the different parts and different varieties of chilli pepper at $p<0.05$. Pearson correlation test was used to determine the correlations between total phenolics and antioxidant activities of the chilli pepper samples at $p<0.05$.

Results

Total phenolic of red hot chilli pepper samples

As shown in Table 1, the seed of all red hot chilli peppers had the highest TPC, followed the whole fruit. However, the TPC in both pulp and whole fruit was not significantly different. Comparing among the different varieties of red hot chilli peppers, the seed of both Kulai varieties had the significantly lowest TPC at $p<0.01$. The pulp of all chilli peppers had higher TPC than its whole fruit except the Kulai 151 variety. Nevertheless, TPC of the Kulai 151 whole fruit was not significantly higher than its pulp. Table 1 also showed TFC of the samples of red hot chilli peppers. The results shows that seed, pulp and whole fruit of chilli peppers of Pelita variety had the highest TFC, followed by Bara, Centil and Kulai varieties. Although TFC of all Kulai chilli pepper samples were low, however, the pulp of chilli pepper Kulai 568 had a high TFC which similar to the TFC in the pulp of chilli pepper Centil. For chilli pepper Kulai 151, no significant difference was found between the different parts of the chilli pepper.

Table 1 Total phenolic and total flavonoid content in different varieties and parts of chilli pepper

Sample		**TPC (mg GAE/kg)**	**TFC (mg QE/kg)**
Centil	seed	$57.65\pm9.12^{A,a}$	$230.00\pm39.71^{A,a}$
	pulp	$95.39\pm1.15^{A,b}$	$441.11\pm82.06^{A,b}$
	whole	$91.29\pm2.80^{A,B,b}$	$249.72\pm109.09^{A,a,b}$
Kulai 568	seed	$32.74\pm3.42^{B,a}$	$41.67\pm38.74^{B,a}$
	pulp	$95.76\pm0.45^{A,b}$	$227.64\pm6.14^{B,b}$
	whole	$93.81\pm1.13^{A,b}$	$160.97\pm20.57^{A,c}$
Kulai 151	seed	$19.05\pm8.46^{B,a}$	$146.81\pm9.14^{A,a}$
	pulp	$94.09\pm1.81^{A,b}$	$187.22\pm14.82^{B,a}$
	whole	$94.92\pm0.87^{A,b}$	$186.94\pm44.30^{A,a}$
Bara	seed	$55.72\pm5.02^{A,a}$	$387.92\pm39.87^{C,a}$
	pulp	$94.89\pm1.26^{A,b}$	$611.39\pm10.79^{C,b}$
	whole	$87.20\pm1.12^{B,C,c}$	$431.25\pm15.75^{B,a}$
Pelita	seed	$60.37\pm8.12^{A,a}$	$439.17\pm71.96^{C,a}$
	pulp	$93.15\pm2.67^{A,b}$	$835.56\pm9.17^{D,b}$
	whole	$84.33\pm3.91^{C,b}$	$793.06\pm8.08^{C,b}$

Different uppercase superscript letters ($^{A-D}$) and lowercase superscript letters ($^{a-d}$) represent significant differences between different varieties and between different parts of the chilli peppers respectively at $p<0.01$. GAE: gallic acid equivalent; QE: quercetin equivalent.

Antioxidant activities

Antioxidant activities of Malaysian red hot chilli peppers are shown in Table 2. The antioxidant activities were determined by FRAP and ABTS assays. The results showed that seed of all chilli peppers had significantly lowest FRAP values compared with pulp and whole fruit at p<0.01 except chilli pepper Centil. Also, no significant difference was found between FRAP values of pulp and whole fruit for all the chilli peppers. Moreover, FRAP value for the seed of Kulai 568 variety was significantly lower than the other samples at p<0.01.

On the other hand, there are significant differences in ABTS inhibition levels among the seed, pulp and whole fruit of Malaysian chilli pepper at p<0.01. Seed of the chilli peppers had the lowest ABTS inhibition level, however, the ABTS inhibition level of Bara seed was not significantly lower than the levels for pulp and whole fruit. Comparing among all the chilli pepper samples, the whole fruit of chilli pepper of Centil and Kulai varieties showed significantly highest ABTS inhibition levels compared with the seed and pulp (p<0.01). The pulp of both chilli pepper Bara and Pelita also had significantly higher ABTS inhibition levels compared to the pulp of the other chilli pepper varieties. In brief, the pulp and seed of chilli pepper Bara have the highest antioxidant activity determined by ABTS assay but not the whole fruit.

Table 2 FRAP value and ABTS value of different varieties and parts of chilli pepper

Sample		**FRAP value (mM Fe^{2+}/mg extract)**	**ABTS (Inhibition%)**
Centil	seed	$1.77±0.23^{A,a}$	$45.30±0.99^{A,a}$
	pulp	$2.08±0.01^{A,a}$	$59.93±0.79^{A,b}$
	whole	$2.06±0.01^{A,a}$	$87.36±1.18^{A,C,c}$
Kulai 568	seed	$1.44±0.12^{B,a}$	$51.72±2.23^{B,a}$
	pulp	$2.08±0.01^{A,b}$	$84.85±1.63^{B,b}$
	whole	$2.07±0.01^{B,b}$	$94.29±0.18^{B,c}$
Kulai 151	seed	$1.70±0.07^{A,B,a}$	$50.94±0.84^{B,a}$
	pulp	$2.07±0.01^{B,b}$	$62.43±1.04^{C,b}$
	whole	$2.07±0.01^{B,b}$	$90.29±0.52^{A,c}$
Bara	seed	$1.95±0.01^{A,a}$	$81.09±1.13^{C,a}$
	pulp	$2.08±0.01^{A,b}$	$94.16±0.37^{D,b}$
	whole	$2.07±0.01^{B,b}$	$87.22±1.77^{C,c}$
Pelita	seed	$1.87±0.01^{A,a}$	$53.29±0.87^{B,a}$
	pulp	$2.07±0.01^{C,b}$	$91.80±0.41^{E,b}$
	whole	$2.06±0.01^{C,b}$	$83.44±0.89^{D,c}$

Different uppercase superscript letters ($^{A-D}$) and lowercase superscript letters ($^{a-d}$) represent significant differences between different varieties and between different parts of the chilli peppers respectively at p<0.01. FRAP: ferric reducing antioxidant power; ABTS: 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid).

Correlation between total phenolic antioxidant activities

As shown in Table 3, there are significant correlations between total phenolics and antioxidant activities of the chilli pepper samples. The results showed that there was a very strong and positive correlation between TPC and FRAP value (R = 0.905; p<0.01). In addition, there was also a strong and positive correlation between ABTS value and TPC (R=0.699; p<0.01).

Table 3 Correlations between total phenolics and antioxidant activities of chilli pepper samples

Antioxidant capacity	**Total phenolic content (TPC)**		**Total flavonoid content (TFC)**	
	r-value	**p-value**	**r-value**	**p-value**
FRAP value	0.905**	0.000	0.465	0.081
ABTS	0.700**	0.004	0.397	0.143

** Correlation is significant at p<0.01.

Discussion

This study determined the antioxidant properties of seed, pulp and whole fruit of Malaysian red hot chilli of five different varieties. Chilli pepper of Kulai varieties differs physically from the other varieties of the studied chilli peppers. Chilli pepper Kulai is larger in size, whereas chilli pepper Bara, Centil and Pelita are small-sized chili or bird's eye chilli. In Malaysia, these small chilli samples are locally known as "cili padi" or Phrik k̄hī̂h̄nū in Thai words. Based on the information obtained from the local farmers, the Pelita variety is genetically modified from the Bara variety. Hence, the chilli pepper Pelita had TFC slightly higher than the

Bara variety. Bird's eye chilli is a typical chilli pepper having a higher phenolic acids and capsaicin content than the other chilli peppers which are larger in size such as red capsicum. These chilli peppers have stronger pungency compared with red capsicum or red bell pepper.

Conversely, the literature showed that Bird's eye chilli peppers (fresh samples) had the lowest total phenolics and antioxidant activities compared to sweet and bell peppers.[4]. The high antioxidant properties of these studied bird's eye chillies were probably due to the freeze dried samples used in this study, where removal of water from the chilli pepper significantly increased its antioxidant components.

Based on the results obtained, bird's eye chillies had a higher level of total phenolics than the big chilli. Although the seeds of these chilli peppers had total phenolics and antioxidant activities lower than the pulps, the TFC and antioxidant activities of the seed of chilli pepper Bara were comparable with its whole fruit. Therefore, chilli pepper seed was a potential source of nutraceutical as it contained a good amount of total phenolics. The fact was supported by a previous finding that the seed extract of red chilli was a potent pharmaceutical ingredient where it had antimicrobial activity and antioxidant activity.[5] Also, chilli pepper of Bara and Pelita varieties had high total phenolic content and antioxidant activities.

In the current study, the antioxidant activities in seed and pulp extracts were generally higher than whole fruit extract. This may be due to the different group of functional group in seed and pulp extract. In addition, the lower antioxidant activities of whole fruit may due to synergism effect between phenolic compound of seed and pulp.[10]The differences in the level of phenolic content among chilli peppers varieties were affected by environmental factors such as genotypic differences, climatic condition, cultural practices and harvesting methods. This will directly contributes to variation of antioxidant activities.[11]

Conclusion

This study shows that large-size chilli peppers such as Kulai 151 and Kulai 568 in their freeze-dried form had lower antioxidant properties than bird' s eye chilli peppers from both Capsicum species which including Centil, Bara and Pelita. The fruit of Bara variety had higher TPC than the other parts. Also, TPC of all chilli pepper samples was strongly correlated with the antioxidant activities. These lyophilised chilli pepper powders, especially the Bara and Centil varieties, were good sources of phenolic nutraceuticals and can be potentially used for various therapeutic purposes. Further in vitro and in vitro studies are needed for better understanding on the efficacy of Malaysian chilli peppers as nutraceutical for prevention of chronic diseases.

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