

Phytochemical Contents of *Capsicum Frutescens* (Chili Padi), *Capsicum Annum* (Chili Pepper) and *Capsicum Annum* (Bell Peper) Aqueous Extracts

Rohanizah Abdul Rahim⁺ and Ishak Mat

Unit Kanser MAKNA-USM, Animal Research Centre, Advanced Medical & Dental Institute, Universiti
Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract. *Capsicum* is a genus of flowering plant from family Solanaceae and is widely cultivated in South- East Asian and Latin-American countries. The fruits of *Capsicums* were extracted using aqueous and tested for their total phenolic contents, total flavonoid contents and antioxidant activity. The antioxidant activities were analyzed using DPPH scavenging assay. Total phenolic contents and total flavonoid contents range were from 0.739 to 1.291 umol of gallic acid equivalent to 1g of fresh sample and 0.306 to 0.551 umol of quarcetin equivalent to 1g of fresh samples respectively. Total phenolic contents were correlated with the antioxidant activities.

Keywords: *Capsicum*, Antioxidant activity, Biological activity

1. Introduction

There have been an increasing number of chronic degenerative disease such as cancer, diabetes and cardiovascular disease. Many study showed that there is a relationship between consumption of fruits and vegetables and the development of these disorders [1]. Epidemiological studies showed that antioxidant compounds in the fruits and vegetables can terminate a free radical chain reaction [2]. Therefore it can prevent diseases such as cardiovascular disease, cancer and diabetes [2].

This antioxidant compound was the plants secondary metabolites such as phenolic acid, alkaloid, flavonoid, capsaicinoid and carotenoid [3]. *Capsicum* fruit was one of the plant products that have a rich source of antioxidants compounds [4] and commonly eaten as spice herbs in Malaysia. There are five species of *Capsicum* native to the New World which is *Capsicum pubescens*, *Capsicum baccatum*, *Capsicum annum*, *Capsicum frutescens* and *Capsicum chinense*. Capsaicinoid is phenolic pungent compounds that have been found mostly in *Capsicum* fruits and contribute to 90% of pungency of pepper fruits. The objective of this study is to investigate the phytochemical contents of *Capsicum* between *Capsicum frutescens* (Chili padi) and *Capsicum annum* (Chili pepper and Bell pepper).

2. 2. Material and Methods

2.1. Samples Preparation

The whole *Capsicum* fruits were washed using tap water and cut into small slices. Then the samples were put in the conventional oven at 40°C. The dried samples were then, blended and extracted with purify water in a shaker water bath at 45°C for 7 hours. Then the supernatants were collected and evaporated to dry using freeze dryer and kept at -20°C for further analysis.

2.2. Determination of Total Phenolic Content

Corresponding author. Tel.: + 60126095047; fax: + 6045622086.
E-mail address: rohanizah@amdi.usm.edu.my.

Total phenolic content of samples were determined according to Folin-Ciocalteu method described by Fernandez et al., (2011) [5] with some modifications. 0.3 ml of samples (100 mg/ml) was mixed with 0.5 ml Folin Ciocalteu reagents. The mixture were homogenized and incubated for 5 minutes at room temperature before the addition of dysodium carbonate solution (1ml, 7.5% w/v). The absorbance of the mixture were measured at 725 nm using UV/VIS spectrophotometer (Varian,USA) after incubated for 30 minutes at room temperature. 0.3 ml gallic acid solutions in the concentration of 0-0.2 mg/ml were used to prepare the calibration curve. This estimation of phenolic content in the extracts was carried out in triplicate. Results are express as gallic acid equivalents (GAE), in μmol per gram of sample.

2.3. Determination of Total Flavonoid Content

The total flavonoid content of samples were determined by method modification of Basma et al., 2011[2]. 75 μl 10% alumunium chloride (AlCl_3) was mixed with 250 μl of extracts (100 mg/ml). Then the solutions were incubated for 15 minutes before the absorbance were measured at 430 nm using spectrophotometer. The total flavonoid content were determined using quarcetin standard calibration curve at concentration 0-1 mg/ml. Total flavonoid content was expressed as quarcetin equivalents (QE) in $\mu\text{mol/g}$ samples.

2.4. DPPH Free Radical Scavenging Assay (DPPH Assay)

Samples were tested for their DPPH assay with modification method of Chang et al., 2002[6]. 2 ml extracts were added with 2 ml DPPH and incubated at room temperature for 15 minutes before the absorbance was read at 517 nm using UV/VIS spectrophotometer. The capability of samples to scavenge the DPPH radical was calculated by using the following equation:

$$\text{Scavenging effect (\%)} = (1 - \text{Absorbance of sample} \times 100) / \text{Absorbance of control}$$

2.5. Statistics and Data Analysis

Experiments were analyzed in triplicate. Statistical significance was determined by one way ANOVA and Scheffe Multiple Range Test. Significance differences were considered at $P < 0.05$.

3. Results and Discussions

3.1. Antioxidant Activity

In this study, the antioxidant properties of samples were detected using DPPH radical scavenging assay. Radical scavenging is the main mechanism by which antioxidants act in food. This method was based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant that shows a strong absorption band at 517 nm and appears as deep violet colour [2]. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [7]. Table 1 and Figure 1 (A) showed that *Capsicum* fruits had strong antioxidant activity at concentration of 4 mg/ml. Bell pepper scavenged the highest radicals at 92% followed by Chili pepper 91%, Chili padi, 90%, while standard (Butylated hydroxyanisole) BHA scavenged 97% radicals. The antioxidant activity of extracts increased with the increasing number of total phenolic compounds in the extracts with good correlation value at $r^2 = 0.935$. This finding is in agreement with the findings of Basma et al., 2011[2]. Therefore, our results indicate that the hydroxyl groups existing in the phenolic compounds may provide a major component as a radical scavenger to *Capsicum* samples.

Table 1: Total phenolic content, total flavonoid content and antioxidant activity of *Capsicum* extracts

| Samples | Total Phenolic Content (μmol GAE/g)* | Total Flavonoid Content (μmol Q/g)* | Antioxidant capacity (μmol BHA/g)* |
|--------------|--|---|--|
| Bell pepper | 1.291 \pm 0.969 | 0.451 \pm 0.001 | 1.893 \pm 0.036 |
| Chili pepper | 0.759 \pm 0.003 | 0.306 \pm 0.001 | 1.229 \pm 0.020 |
| Chili padi | 0.739 \pm 0.004 | 0.551 \pm 0.003 | 0.795 \pm 0.049 |

*Values are expressed as mean \pm standard deviation of triplicate measurements.

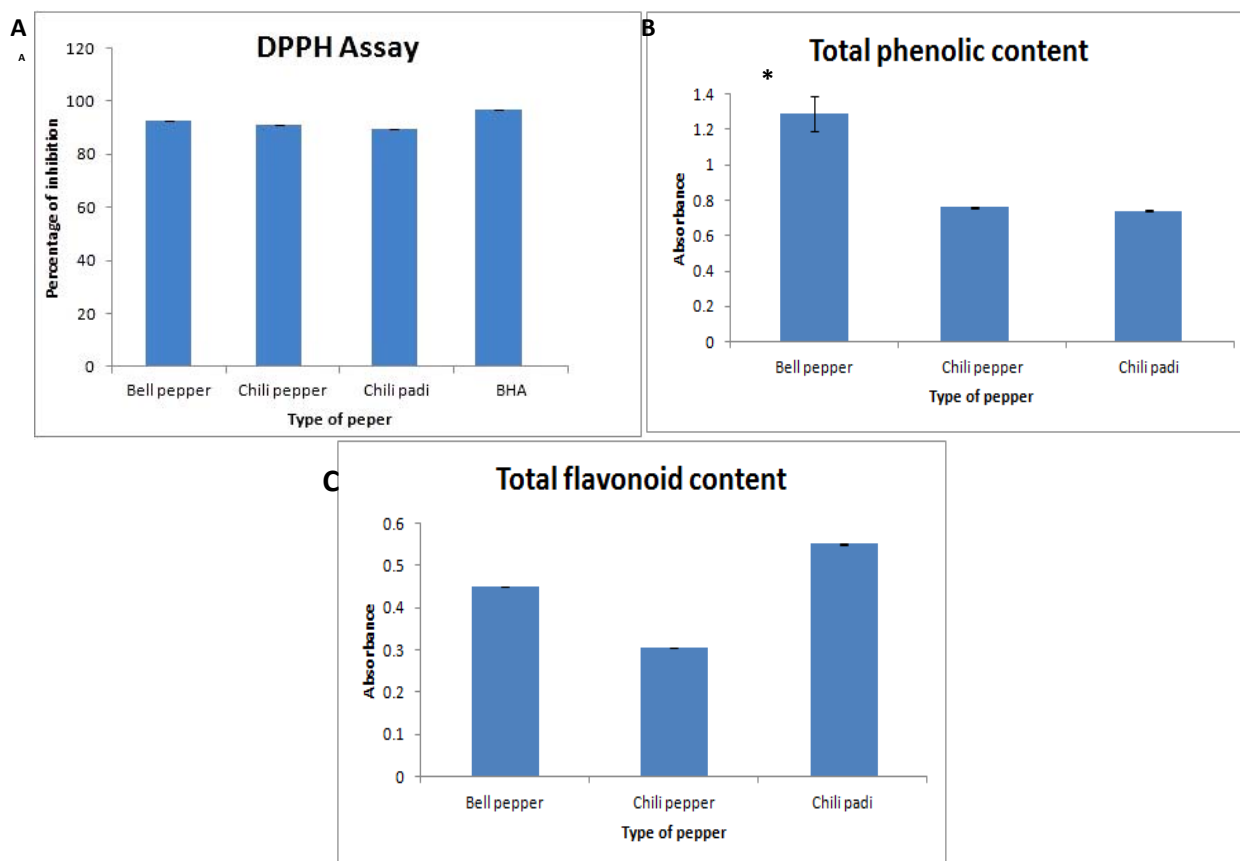


Fig. 1: Chart (A) antioxidant activity of sample and BHA standard (B) total phenolic content, (C) total flavonoid content [bar chart represents of standard error] and * Significant ($p < 0.05$) when compared to chili pepper and chili padi.

3.2. Total Phenolic Content and Total Flavonoid Content

The radical scavenging properties of samples could be due to the present of phenolics compounds [1]. Study by Miller and Rice-Evans (1997) [8] showed that 80% of the total antioxidant activity was contributed by phenolic compounds. Therefore, in this study the total phenolic content was determined based on the phenolic substances which were oxidized by Folin-Ciocalteu reagent. The reagent was partly reduced from the production of complex molybden-tungsten blue that was measured spectrophotometrically [9]. Folin-Ciocalteu method allows the estimation of the total phenolic content that included the flavonoids, anthocyanins and nonflavonoid phenolic compounds. The total flavonoid content was determined by the Alumunium Chloride colorimetric method. The alumunium chloride will form a stable acid complexes with flavones and flavonols group that measured at 430nm [5].

The total phenolic content is reported as gallic acid equivalents by references to standard curve ($y = 2.34925x + 0.07559$, $r^2 = 0.99321$) while total flavonoid content is reported as quarcetin equivalents by reference to standard curve ($y=1.1062x+0.0124$, $r^2 = 0.9957$). As shown in Table 1 and Figure 1(B) and (C) respectively, Bell pepper contain the highest amount of total phenolic content followed by Chili pepper and Chili padi while Chili padi contain the highest amount of flavonoid followed by Bell pepper and Chili pepper. Results showed that there were significant differences ($p < 0.05$) for total flavonoid content for all samples. While for total phenolic content there were significant differences ($p < 0.05$) between Bell pepper to Chili pepper and Chili padi and no significant differences ($p > 0.05$) between Chili pepper and Chili padi. There was no correlation between total phenolic content and total flavonoid content with $r^2 = 0.0734$. Therefore it can be concluded that the flavonoid compound did not contribute as a major compound to the high total of phenolic content in this *Capsicum* samples.

In conclusion, the variability of the composition of phytochemical compounds in this fruits indicate the important in choosing suitable foods that have rich source of antioxidant capacity as a prevention to the

development of chronic disease such as diabetes and cancer. Further work is required to isolate and identify the compounds that may have contributed to the high antioxidant activities in *Capsicum* samples.

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5. References

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