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Phenolics content and antioxidant properties of *Strobilanthes crispus* as affected by different extraction solvents

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ABSTRACT

Strobilanthes crispus or locally known as Pecah Kaca among Malaysian is a medicinal plant that belongs to the family Acanthaceae. *S. crispus* is an ethnomedicinal plant with high antioxidant content and is indicated in the treatment of diabetes, cancer, and hypertension. This study was conducted to study the phenolics content and antioxidant properties of *S. crispus* leaf as affected by different concentrations of extraction solvents. In this study, water and various concentrations (25, 50, 75 and 100%) of methanol and acetone in water were used as extraction solvent of *S. crispus* dried leaves. The antioxidant properties of *S. crispus* were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) (free radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. The highest polyphenols and phenolic acids content were recorded in 50% acetone extract with 10.80 and 33.86 mg GAE g^{-1} DW, respectively. Meanwhile, the highest total flavonoids content (4.98 mg QE g^{-1} DW) was obtained in 100% acetone extract. In the antioxidant analysis, the highest DPPH free radical scavenging activity was exhibited from 75% acetone extract with 24.88 mg TE g^{-1} DW and the highest FRAP value was obtained from 25% acetone extract with 47.21 mg TE g^{-1} DW. In conclusion, acetone was found to be the most suitable extraction solvent for phenolics content and antioxidant properties of *S. crispus* leaf in this study.

Keywords: *Strobilanthes crispus*, phenolic, flavonoid, antioxidant

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1 Introduction

For centuries, medicinal plants have been utilized as a source of alternative medicine to prevent and treat various diseases. Malaysia has been classified as one of the mega biodiversity countries in the world, one of the richest forests with more than 15,000 species (Ekor, 2014). The herbal industry in the world has expanded vigorously and generated approximately US$ 83.1 billion in annual income (Gunjan et al., 2015). In 2014, Malaysia's herbal industry has recorded US$ 2,077 million and US$ 441.7 million imports and exports values (Tnah et al., 2019). At present, the herbal industry has become one of the most important industries in Malaysia.

One of the popular medicinal plants in Malaysia is *Strobilanthes crispus* (Acanthaceae). In Malaysia, this plant is commonly known as Pecah Beling or Pecah Kaca. *S. crispus* is a woody spreading shrub with leaves in dark green, elliptical-shaped and arranged in opposite arrangement. *S. crispus* is a traditional medicine for Malaysian and Indonesian as an anti-diabetic, diuretic, antilithic and laxative for constipation treatment. Besides that, the leaves of *S. crispus* is normally boiled and taken as a tea among the local community and China (Ghasemzadeh et al., 2015). Based on the previous study, *S. crispus* extract possess several biological activities including anti-diabetic, antiangiogenic, wound healing activities, hypolipidemic effect and possess high antioxidant properties (Fadzelly et al., 2006; Muslim et al., 2010; Al-Henhena et al., 2011; Qader et al., 2011). An antioxidant is defined as the compounds responsible for delaying or

inhibits the formation of free radicals in the human body. The free radical is an unstable molecule that can cause significant damage to human cells and tissues. Hence, an antioxidant is needed to neutralize free radicals by donating the electron (Bratovcic, 2020). An antioxidant can be divided into natural and synthetic antioxidant. Natural antioxidant is widely present in plants. However, the amount is differed depending on various factors including genetic, climate, biotic and abiotic stresses and postharvest handling process. Meanwhile, synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used as food additive (Mohajer et al., 2019). However, the side effects and safety of these synthetic antioxidants are still not clear. Hence, natural antioxidant is more preferable as it is scientifically proven with very low or no side effects on human (Xu et al., 2017).

The extraction procedure is a critical step in maximizing the amount of phenolics extracted from the plant sample. One of the factors that affect the phenolic content and also antioxidant activity is an extraction solvent. Thus, this study is conducted to study the effect of extraction solvents on phenolic content and antioxidant activity of *S. crispus* leaves.

2 Materials and Methods

2.1 Plant materials

The leaves of *S. crispus* were collected from the local nursery located at Kuala Pilah, Negeri Sembilan, Malaysia. The aerial part of *S. crispus* leaves was used in this study. The leaves were cleaned under running tap water to remove the soils and debris. The leaves were dried in an oven for a week at a temperature of 50 °C. The dried leaves were ground using a commercial blender. The fine powder was kept in an airtight container and used for analysis.

2.2 Preparation of extract

The extraction procedure was carried out according to the method explained by Haida et al. (2020). Briefly, 0.25 g of *S. crispus* powder was placed in the vials. Then, 12.5 mL of different methanol and acetone concentrations (25, 50, 75 and 100%) were added. The vials were placed on an orbital shaker for an hour in the dark at room temperature. The samples were filtered using filter paper No. 1 and the extract was used for the analysis.

2.3 Total polyphenols content

The total polyphenols content was measured according to the method explained by Marinova et al. (2005). Briefly, a total of 100 µL of the extract was added to the vials. Then, 2.5 mL of Folin-Ciocalteu reagent which was diluted 10 times was added and the mixture was incubated for 5 minutes. After that, 2.5 mL of 7% sodium carbonate was added and the reaction mixture was incubated for 60 min at room temperature. The absorbance was measured at 725 nm using a UV-vis spectrophotometer. The standard curve of absorbance against different concentrations of gallic acid was constructed and the total polyphenols content was expressed as mg gallic acid equivalent per gram dry weight of the sample (mg GAE g^{-1} DW).

2.4 Total phenolic acids content

The total phenolic acids content was measured using a method as explained by Singleton and Rossi (1965). A total of 1 mL of extract was added with 9 mL of distilled water. Then, 1 mL of Folin-Ciocalteu reagent was added and the mixture was incubated for 5 minutes. After the incubation, 10 mL of 7% sodium carbonate was added and the final volume was adjusted to 25 mL by addition of 4 mL of distilled water. The reaction mixture was incubated for 90 minutes at room temperature and the absorbance was measured at 750 nm. The standard curve of absorbance against different concentrations of gallic acid and total phenolic acids content was expressed as mg gallic acid equivalent per gram dry weight of sample (mg GAE g^{-1} DW).

2.5 Total flavonoids content

The total flavonoids content was measured using a method as explained by Marinova et al. (2005). A total of 1 mL of sample extract was added with 4 mL of distilled water. After that, 0.3 mL of 5% sodium nitrite was added and the mixture was incubated for 5 min. Then, 0.3 mL of 10% aluminum chloride was added to the mixture. At the sixth minute, 2 mL of 1 M sodium hydroxide and 2.4 mL of distilled water were added. The reaction mixture was mixed thoroughly and absorbance was measured at 510 nm using a spectrophotometer. The standard curve of absorbance against different concentrations of quercetin was constructed and total flavonoids content was expressed as mg quercetin equivalent per gram dry weight of the sample (mg QE g^{-1} DW).

2.6 DPPH free radical scavenging activity

Quantification of DPPH free radical scavenging activity was conducted according to the method employed by Wong et al. (2006). Prior to experimentation, 0.1 mM DPPH was prepared in methanol and initial absorbance was measured at 515 nm. A total of 40 µL of the extract was added with 3 mL of 0.1 mM of methanolic DPPH solution. The mixture was incubated at room temperature for 30 min and a change

in absorbance was measured at 515 nm. The standard curve of absorbance against different concentrations of trolox was constructed and DPPH free radical scavenging activity was expressed as mg trolox equivalent per gram dry weight of the sample (mg TE g^{-1} DW).

2.7 Ferric reducing antioxidant power (FRAP)

The FRAP assay was conducted according to the method explained by Benzie and Strain (1996). Briefly, 200 µL of the extract was added in the test tube with 3 mL of FRAP reagent which was prepared with 300 mM of sodium acetate buffer (pH 3.6), 10 mM of 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM of FeCl.6H2O at the ratio 10:1:1. The reaction mixture was incubated in the water bath at temperature 37 °C for 30 min. After the incubation, the reaction mixture was cooled down to room temperature and absorbance was measured at 593 nm. The standard curve of absorbance against different concentrations of trolox was constructed and the FRAP value was expressed as mg trolox equivalent per gram dry weight of the sample (mg TE g^{-1} DW).

2.8 Statistical analysis

All the experiments were conducted in Completely Randomized Design (CRD) with three replications for each treatment. All data were analyzed using the Analysis of Variance (ANOVA) technique with the help of the Statistical Analysis System 9.4. The means comparison between treatments was conducted using Duncan's Multiple Range Test (DMRT) at P<0.05.

3 Results and Discussion

The study on total polyphenols and phenolic acids content were conducted using the Folin-Ciocalteu method and the results were tabulated in Table 1. The total polyphenols content recorded was ranged between 5.71 to 10.80 mg GAE g^{-1} DW. The highest total polyphenols content was significantly produced from 50% acetone (10.80 mg GAE g^{-1} DW). Meanwhile, the lowest total polyphenols content was recorded from 100% acetone (5.71 mg GAE g^{-1} DW). From the results obtained, the dried leaves of *S. crispus* extracted with different concentrations of methanol, the highest total polyphenols content was recorded from 75% methanol (9.75 mg GAE g^{-1} DW). As the concentration of methanol decreased to 50 and 25%, the total polyphenols content also was dropped from 8.00 to 7.21 mg GAE g^{-1} DW, respectively. In the different concentrations of acetone, the total polyphenols content obtained was the highest at concentration 50% acetone (10.80 mg GAE g^{-1} DW) and dropped when the acetone concentration was reduced to 25% with 9.46 mg GAE g^{-1} DW.

In total phenolic acids content analysis, the value recorded was in the range between 13.20 to 33.86 mg GAE g^{-1} DW (Table 1). The highest total phenolic acids content was significantly produced by treatment of 50% acetone with 33.86 mg GAE g^{-1} DW. Meanwhile, treatment of 75% methanol was recorded as the second-highest total phenolic acids with 28.20 mg GAE g^{-1} DW. The lowest total phenolic acids content was significantly recorded from the treatment of 100% acetone with 13.20 mg GAE g^{-1} DW. The total phenolic acids content recorded by the treatments showed the same trend as total polyphenols content.

The quantification of total flavonoids content was conducted using the Aluminium Chloride Colorimetric method. Based on the results in Table 2, the total flavonoids content recorded from *S. crispus* leaves extract was between 1.63 to 4.98 mg QE g^{-1} DW. The treatment of 100% methanol and 100% acetone showed that no significant differences between the treatments on total flavonoids content recorded from *S. crispus* leaves. The total flavonoids content recorded from 100% methanol and 100% acetone were 4.86 and 4.98 mg QE g^{-1} DW, respectively. Meanwhile, the lowest total flavonoids content also showed no significant difference between the treatment of 25% methanol and 25% acetone on total flavonoids content. The value recorded for 25% methanol and 25% acetone were 1.63 and 1.64 mg QE g^{-1} DW, respectively.

In the antioxidant analysis, the DPPH free radical scavenging activity and FRAP assays were conducted to measure the antioxidant potential of *S. crispus* leaves. Based on the results in Table 3, the highest DPPH free radical scavenging activity was exhibited from the treatment of 75% acetone with 24.88 mg TE g^{-1} DW. The lowest DPPH free radical scavenging activity was exhibited from the treatment of 25% methanol with 8.68 mg TE g^{-1} DW.

The antioxidant potential of *S. crispus* leaves was further quantified using the FRAP assay. The FRAP value recorded was ranged between 28.91 to 47.21 mg TE g^{-1} DW. The highest FRAP value was significantly exhibited from the treatment of 25% acetone with 47.21 mg TE g^{-1} DW. The treatment of 25% acetone was the only treatment that able to exhibited a FRAP value of more than 40 mg TE g^{-1} DW. In contrast, the treatment of 25% methanol was exhibited the lowest FRAP value with 28.91 mg TE g^{-1} DW.

In the present study, the leaves of *S. crispus* were extracted with different concentrations of methanol and acetone. Based on the results obtained, the leaves extracted with different concentrations of acetone were able to accumulate higher total polyphenols, phenolic acids and flavonoids content. In addition, higher antioxidant activities also were exhibited from the leaves extracted with acetone. According

Table 1. Total polyphenols and phenolic acids content of *S. crispus* as affected by different concentration of methanol and acetone

Treatment	Total polyphenols content (mg GAE g^{-1} DW)	Total phenolic acids content (mg GAE g^{-1} DW)
100% Methanol	6.38 ef	21.03 c
75% Methanol	9.05 bc	28.20 ab
50% Methanol	8.00 cd	27.20 bc
25% Methanol	7.21 de	25.78 bc
100% Acetone	5.71 f	13.20 d
75% Acetone	9.71 ab	31.95 ab
50% Acetone	10.80 a	33.86 a
25% Acetone	9.46 b	31.03 ab

GAE = gallic acid equivalent; DW = dry weight; Means followed by the same letter are not significantly different at P<0.05

Table 2. Total flavonoids content of *S. crispus* as affected by different concentration of methanol and acetone

Treatment	Total flavonoids content (mg QE g^{-1} DW)
100% Methanol	4.86 a
75% Methanol	3.71 c
50% Methanol	1.95 e
25% Methanol	1.63 f
100% Acetone	4.98 a
75% Acetone	4.64 b
50% Acetone	2.51 d
25% Acetone	1.64 f

QE = quercetin equivalent; DW = dry weight; Means followed by the same letter are not significantly different at P<0.05.

Table 3. DPPH free radical scavenging activity and FRAP value of *S. crispus* as affected by different concentration of methanol and acetone

Treatment	DPPH (mg TE g^{-1} DW)	FRAP (mg TE g^{-1} DW)
100% Methanol	10.01 cd	34.73 cd
75% Methanol	18.88 b	37.09 bc
50% Methanol	10.68 cd	32.64 de
25% Methanol	8.68 d	28.91 f
100% Acetone	12.68 cd	30.06 ef
75% Acetone	24.88 a	38.61 b
50% Acetone	18.35 b	36.22 bc
25% Acetone	13.15 c	47.21 a

DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant power; TE = trolox equivalent; DW = dry weight; Means followed by the same letter are not significantly different at P<0.05.

to a previous study by Ghasemzadeh et al. (2015), the aqueous extract of *S. crispus* leaves exhibited higher total phenolic content, total flavonoid content, DPPH free radical scavenging activity and FRAP value compared to ethanol extract. The same results also were previously found by Qader et al. (2011) which aqueous extract exhibited higher total phenolic content, DPPH free radical scavenging activity and FRAP value than ethanol extract of *S. crispus*. The previous studies showed that aqueous extract of *S. crispus* which has higher polarity than ethanol was more prominent by accumulated high phenolic and flavonoid content and exhibiting higher antioxidant activity. The findings were in contrast with this present study which acetone extract that has lower polarity than methanol was produced higher total phenolics, flavonoids and antioxidant activity than methanol extract. The differences obtained might be due to the different ages of plants or drying and extraction procedures. Thus, the secondary metabolites present in the sample are affected.

In a previous study by Bhebhe et al. (2015), the extraction solvents including water, 50% methanol, ethanol, 50% ethanol, acetone, 50% acetone and ethyl acetate were used to quantify the phenolic content and DPPH free radical scavenging activity of *Ilex paraguariensis* and *Camellia sinensis*. The study found that 50% of acetone was accumulated higher phenolic content and exhibited higher DPPH free radical scavenging activity compared to other solvents (Bhebhe et al., 2015). Besides that, the highest total phenolic content from blackcurrant also was reported from the sample extracted with aqueous-acetone solvent (Tabart et al., 2007). According to Galanakis et al. (2013), natural phenols present in plants possessed a solubility preference to solvents that have intermediate polarity such as alcohol and acetone compared to a more polar solvent such as water or less polar solvent such as ethyl acetate. In addition, polar protic solvents such as hydro-alcoholic mixtures or an aprotic solvent mixed with water are commonly been used in phenolic extraction from natural products as it was able to recover a higher amount of polyphenols from the plant (Lafka et al., 2007).

4 Conclusion

This study showed that different extraction solvents were largely influenced by the phenolics content and antioxidant activities of *S. crispus* leaves. The results found that acetone was the most suitable extraction solvent by recorded the highest total polyphenols, phenolic acids, flavonoids content and exhibited higher DPPH free radical scavenging activity and FRAP value. Hence, acetone is recommended for future research on *S. crispus* leaves. Besides that, other factors such as drying temperature and drying temperature need to be optimized as it will directly influence the phenolic content and antioxidant activity from the sample.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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