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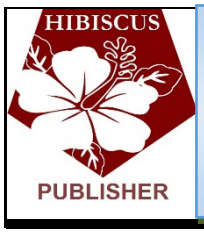

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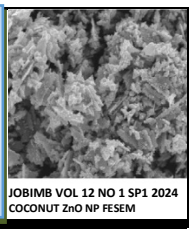

Extraction of Total Phenol and Antioxidant Activity of Butterfly Pea Flower (*Clitoria ternatea* L.) Extracts by Ultrasound-Assisted and Maceration Extraction

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ABSTRACT

Butterfly pea flower, also known as Asian pigeon wings, has been widely used as an ingredient in food and medicine due to the high content of various types of antioxidants that are beneficial to health. One of the technologies used to extract the bioactive compound from the plant is the ultrasound-assisted extraction (UAE) method. This study aimed to investigate the effect of different extraction times from 10 min to 30 min and types of solvent: water, aqueous methanol and aqueous ethanol on the phenolic compound and antioxidant activity present in the butterfly pea flower. The total phenolic content (TPC) was determined using Folin-Ciocalteu assay while the antioxidant was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Based on this study, the optimal condition to increase the extract yield from butterfly pea flower was found to be at a 20-min sonication, while the highest TPC and antioxidant activity were achieved at a 30-min sonication using aqueous methanol. Compared with maceration, UAE demonstrated better results in percentage yield, TPC and antioxidant activity when using aqueous methanol. This approach indicated that UAE is an efficient technique for obtaining bioactive compounds from butterfly pea flowers.

INTRODUCTION

Butterfly pea flower or scientifically known as *Clitoria ternatea* L. belongs to Fabaceae family. It is one of the sources of natural colourant used nowadays as it contains anthocyanins and other flavonoids, which are particularly useful as antioxidants [1]. Butterfly pea flowers are rich in phenolic compounds such as kaempferol, quercetin and ternatin that are sensitive to high temperature. In order to gain all the benefits from the butterfly pea flower, extraction has been done on the flower by using different types of methods. Conventional methods for obtaining plant extracts, including Soxhlet extraction, maceration, and percolation have several disadvantages. These conventional methods often require long extraction times, consume large amounts of plant material and solvents and have a considerable negative environmental impact. One of the non-conventional extraction techniques used is ultrasound-assisted extraction (UAE). The processes used in the UAE are considered to be cleaner, more ecologically friendly, and more energy-efficient [3]. UAE is also claimed to be able to retain the functionality of the bioactive compound as it can be conducted at low temperatures and shorter extraction time with higher yield [4]. Since the phenolics are sensitive to harsh conditions, optimum parameters are required to obtain high extract yield at high concentrations. The objective of this study is to investigate the effect of types of solvent and extraction time on extraction yield, total phenolic content and antioxidant activity. The second objective is to evaluate the efficiency of extraction between maceration and ultrasound extraction.

MATERIALS AND METHOD

Sample collection

A 5 kg of healthy and fresh butterfly pea flowers were collected from a local plantation in the area Perumahan Awam Seri Perlis 2, Kuala Lumpur. After collection, the flowers were cleaned and dried in a smoke oven at 55°C for 6 hours until a consistent weight was achieved. Samples were ground into powder and transferred into polyethylene plastic before vacuum-sealed and kept in a chiller at 4°C for further analysis.

Ultrasound and Maceration Extraction of Butterfly Pea Powders

Butterfly pea powders were subjected to ultrasound-extracted extraction. A 1.0 g powder mixed with 200 mL solvent at different sonication time (10, 20 and 30 min) and repeated for each water, 50% ethanol and 50% methanol. For maceration, 1.0 g powder was homogenized with the 200 mL solvent in a magnetic stirrer for 30 min and repeated for different types of solvent (water, 50% ethanol and 50% methanol) [5]. Then, the mixture was filtered, and the supernatant was evaporated at 50°C using a rotary evaporator. The weight of the extract was recorded.

Total Phenolic Content and Antioxidant Activity of Extracts

The percentage of yield was calculated as ratios of extract residue weights to sample weights. The total phenolic content (TPC) was determined using Folin-Ciocalteu assay while the antioxidant was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay [6]. TPC was conducted by mixing 0.2 mL of 10-fold dilution extract with 1 mL of Folin–Ciocalteu reagent for 8 min. Next, 2 mL of 7.5% sodium and distilled water was added before the mixture was kept at room temperature in the dark for 2 h. For antioxidant analysis, 1 mL of 10-fold dilution extract was mixed with 3 mL of DPPH solution (0.1 mol/L in 95% methanol). The absorbance of TPC and antioxidant activity were measured using UV–vis spectrophotometer at 765 nm and 517 nm respectively. The analysis was conducted in triplicate by using Minitab to conduct a 2-way analysis of variance (ANOVA) and Tukey's test with a significant level of 95% (P<0.0.5).

RESULT AND DISCUSSION

Effect of Different Types of Solvent and Extraction Time on Percentage Yield

Fig. 1 shows the percentage yield of butterfly pea flower extract by using different types of solvent and extraction time. Based on the figure, ultrasound extraction by using aqueous methanol with 20 min of sonication was the most efficient in extracting the butterfly pea flower compared to water and aqueous ethanol. Methanol is the ideal solvent to use for extraction because methanol has higher polar organic characteristics and the extracts are more soluble in polar solvents [7]. Ultrasound is thought to be able to increase extraction yield because of high-intensity and frequency ultrasound waves that induce cavitation bubbles to form and burst [8]. A previous study reported that methanol had the highest extraction yield (33.2%) out of all the solvents tested, followed by distilled water (27.0%) and ethanol (12.2%) in extracting the content of phytochemical constituents, antioxidants and in vitro anti-inflammatory constituents from the branches of *Severinia buxifolia* [9].

The extraction yield percentages were higher for extraction with water compared to ethanol. Butterfly pea flower may contain more hydrophilic compounds which dissolve better in water than in ethanol. The percentage of yield decreased significantly from 20 min to 30 min for both water and methanolic extract. A longer sonication period initially results in a higher yield; however, as time evolves, the yield decreases because prolonged ultrasonic exposure damages the solute structurally and lowers the extraction yield [10]. The percentage of yield was not significantly different for all solvents at 10 min due to insufficient extraction time to effectively extract all the compounds.

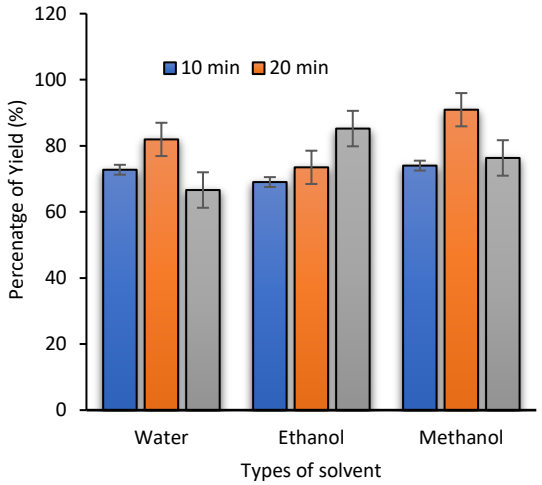

Fig. 1. Percentage yield of butterfly pea flower extract by using different types of solvent and extraction time. Values not sharing similar letters are significantly different (P<0.05).

Effect of Different Types of Solvents and Extraction Time on Total Phenolic Content (TPC)

Fig. 2 shows the total phenolic content of butterfly pea flower extract by using different types of solvent and extraction time. Based on the result, extraction by using aqueous methanol at 30 min sonication resulted in significantly higher TPC compared to the other solvent. When phenolic compounds are extracted in methanol, complexes including large molecular weight molecules may develop resulting in higher TPC values in higher methanol concentration [11]. The TPC yield rises linearly as the extraction time increases because longer extraction times improve the extraction of polyphenolic compounds and provide sufficient time for the solute to be exposed to the release medium [12].

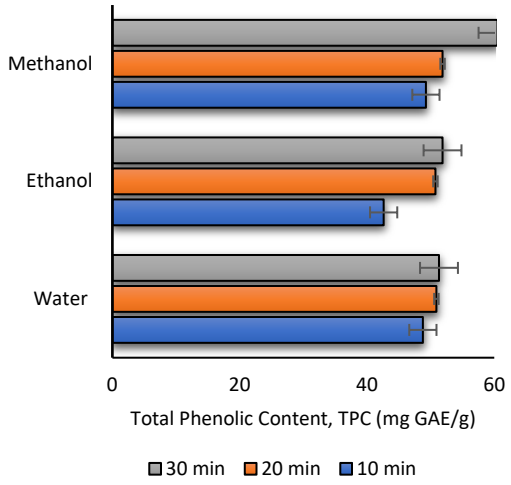

Fig. 2. Total phenolic content of butterfly pea flower by using different types of solvent and extraction time. Values not sharing similar letters are significantly different (P<0.05).

Effect of Different Types of Solvents on Antioxidant Activity

The result of the antioxidant activity of butterfly pea extract by using different types of solvent and extraction time is shown in **Fig. 3**. Based on the figure, the best antioxidant activity was in the treatment in which butterfly pea flower was extracted by using aqueous methanol as a solvent at 30 min extraction time. This is because the mixture of water and methanol increases the dielectric constant which will increase the solvent's polarity [13]. However, there was no significant increase between the different sonication times for methanol extraction. It is suggested that a 10-min extraction is adequate to get a scavenging level similar to that of 30 min. Extraction with ethanol resulted in a decrease in the percentage of radical scavenging with time, likely due to prolonged extraction leading to increased oxidation of extracted compounds in the extract, while extraction with water showed an increase in radical scavenging activity over time, likely due to a different mechanism or stability of compounds in water extraction.

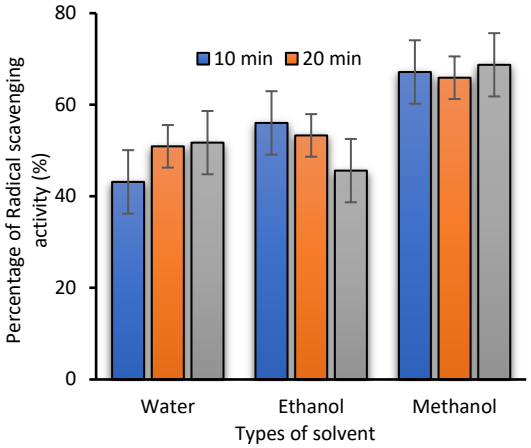

Fig. 3. Antioxidant activity of butterfly pea extract by using different types of solvent and extraction time. Values not sharing similar letters are significantly different (P<0.05). * The antioxidant activity was measured by using 5 mg/mL extract.

Effect of Maceration Extraction on Percentage Yield, TPC, and Antioxidant Activity

Fig. 4 and **Fig. 5** shows the percentage yield and TPC of butterfly pea extract by using maceration and UAE respectively. The comparison was made between the highest data of ultrasound with the maceration to study the differences between the best result between the two extraction methods. The results demonstrate ultrasound treatments produced a significantly higher percentage of yield and TPC compared to maceration (P<0.05) for all types of solvent butterfly extract. Based on **Fig. 5,** the TPC value increased by 41.38% when the extraction was changed from maceration to 30 min ultrasound extraction for aqueous ethanol. This is due to the leaching process that occurred by using different extraction methods.

In addition, the generation of acoustic waves causes solvent and sample molecules to migrate, facilitating the leaching of both organic and inorganic substances [14].

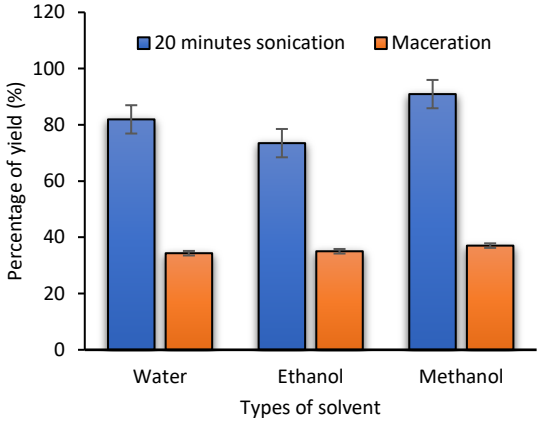

Fig. 4. Percentage yield of butterfly pea extract by using maceration and UAE. Values not sharing similar letters are significantly different (P<0.05).

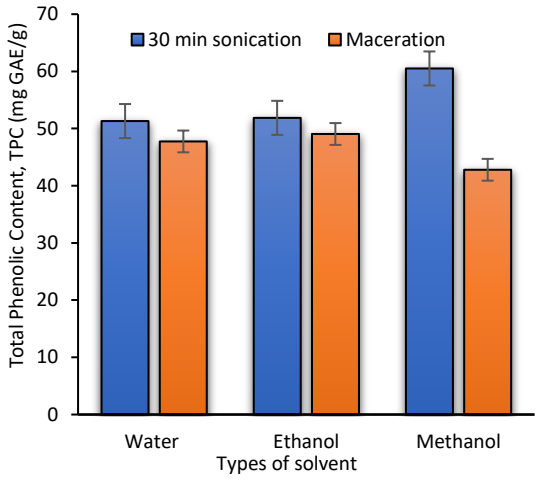

Fig. 5. Total phenolic content of butterfly pea extract by using maceration and UAE. Values not sharing similar letters are significantly different (P<0.05).

Fig. 6 shows the antioxidant activity of butterfly pea extract by using maceration and UAE. Based on the **Fig.**, 30 min sonication had the highest antioxidant activity with no significant difference compared with maceration for aqueous methanol extract (P>0.05). The antioxidant activity of maceration is higher than in UAE might be due to the degradation that occurred during the sonication process. UAE is an alternative to maceration, but strong ultrasonic waves might produce free radical production, which can unfavourably modify the retrieved components [15].

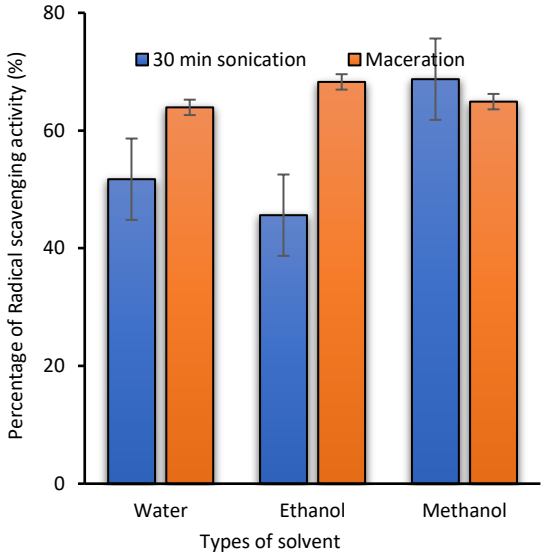

Fig. 6. Antioxidant activity of butterfly pea extract by using maceration and UAE. Values not sharing similar letters are significantly different (P<0.05). *The antioxidant activity was measured by using 5 mg/mL extract.

CONCLUSION

Extraction solvent and time factors significantly affected ultrasound-assisted extraction on the total phenolic content and antioxidant activity present in butterfly pea flower. In addition, the best condition to increase the extract yield from butterfly pea flower was at 20-min sonication while TPC and antioxidant activity were at 30-min sonication by using aqueous methanol. This study also proved that ultrasound by using aqueous methanol showed better results compared to maceration in percentage of yield, TPC and antioxidant activity. In the future, research should focus on improving extraction by considering measures to limit the heat created during the UAE, since ultrasonic has the power to generate significant heat, which dramatically affects the bioavailability, extraction efficiency, and extraction yield.

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