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Research Article

Green ultrasonic-assisted extraction of mangiferin, antioxidants, and phenolic compounds from Mahkota Dewa (*Phaleria macrocarpa*)

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Abstract
This paper investigates ultrasonic-assisted extraction (UAE) of mangiferin from Mahkota Dewa (*Phaleria macrocarpa* (Scheff.) Boerl.) fruit and its antioxidant and phenolic compound characteristics, utilising water as a green solvent. The influence of sonication times (40–80 min), temperatures (35–70 °C), and ultrasonic power level (153–767 W) on mangiferin yield, antioxidant activity, and total phenolic content (TPC) was studied using the one-factor-at-a-time (OFAT) method. The mangiferin yield, antioxidant activity, and TPC characteristics of the extracts were analysed using high-performance liquid chromatography (HPLC), 1,1-diphenyl-2-picrylhydrazyl, and Folin–Ciocalteu assays, respectively. The highest mangiferin yield, 1.76 ± 0.05 mg/g, was obtained under conditions of 60 °C extraction temperature, Scale 4 (613 W) power, and 60 min extraction time with a TPC value of 388.61 ± 0.14 mg GAE/g Mahkota Dewa extract. The antioxidant activity of extracted Mahkota Dewa was moderate (IC_{50}) at 15.66 µg/mL. The result helps identify the best operating conditions for the UAE process and encourages the development of phenolic compounds and antioxidant extraction from Mahkota Dewa to obtain a high extraction yield using technique that is safer for the environment.

Keywords: Mahkota Dewa, ultrasonic waves, mangiferin, antioxidant activity, phenolic content

Introduction

Phaleria macrocarpa (Scheff.) Boerl., also commonly known as Mahkota Dewa by locals, is a valuable traditional herbal plant. The plant was discovered in the Indonesian archipelago's far eastern Papua Island, also known as the Irian Jaya archipelago [1]. Mahkota Dewa's leaves, seeds, fruits, and stems contain various bioactive substances, including mangiferin, mahkoside A, mahkoside B, phenolic compounds, terpenes, alkaloids, and benzophenone, all of which have medicinal properties. The bioactive substances possess anti-inflammatory, antimicrobial, and anticancer properties [2, 3]. Typically, conventional extraction techniques such as Soxhlet extraction and maceration extraction, which need an organic solvent (e.g., hexane, ethanol, and methanol), are used to extract the bioactive substances from the plant matrix. These processes require a considerable amount of time, ranging from a few hours to several days, when conducted at room temperature. Also, they necessitate a substantial amount of solvent [4, 5]. For instance, Jinoni et al. [3], reported the extraction of antibacterial and antioxidant activities from Mahkota Dewa seeds using Soxhlet extraction with hexane, ethyl acetate, and petroleum ether as solvents for 6 h.

The use of non-conventional extraction techniques such as ultrasonic-assisted extraction (UAE) [6], microwave-assisted extraction [7, 8], subcritical water extraction [9, 10], and supercritical fluid extraction [11] has gained popularity for extracting bioactive substances from the Mahkota Dewa plant matrix. Among these, UAE stands out as an eco-friendly and energy-efficient method, aligning with the green chemistry principle [12]. This is because UAE uses less energy than traditional methods like maceration and Soxhlet

extraction. UAE has been successfully used to extract alkaloids, flavonoids, phenolic acid, stilbene, glycosides, lignans, and coumarin due to its capacity to enhance the mass transfer of the substances from the solid material to the liquid phase [13]. Coelho et al. [14] demonstrated that UAE was more effective than conventional carotenoid extraction from cashew apple. The process was quicker, yielded a higher concentration of the extraction solution, and produced a greater quantity of carotenoids from all samples.

However, as shown by Habib and Ismail [6], the effectiveness of UAE can vary depending on the target compound. Their study revealed that the UAE was less efficient in extracting protein from Mahkota Dewa, likely due to cavitation-induced shear forces causing protein denaturation. In contrast, the UAE has proven highly effective in extracting small bioactive compounds such as phenolics and antioxidants, especially when suitable operating parameters and solvents, such as ethanol and choline chloride, were used [15]. Despite its widespread use in plant material extraction, the use of UAE with water as a solvent for extracting mangiferin, antioxidants, and phenolic compounds from Mahkota Dewa remains limited, underscoring the novelty and relevance of the current study.

This study aims to determine the best conditions for UAE, namely temperature, sonication power, and extraction time using water as solvent. The best extraction conditions are determined based on the mangiferin yield, antioxidant activity, and total phenolic content (TPC) characteristics analysed using high-performance liquid chromatography (HPLC) analysis, 1-diphenyl-2-picrylhydrazyl (DPPH) inhibitory concentration of (IC_{50}) assay, and Folin–Ciocalteu assay, respectively.

Materials and Methods

Materials and chemicals

Dried Mahkota Dewa fruits were acquired from Ethno Resources Sdn. Bhd, Selangor, Malaysia. Mangiferin analytical standard (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthan-9-one), methanol (99.9 wt %, HPLC grade), gallic acid, sodium carbonate, ascorbic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin–Ciocalteu reagents were supplied by Merck (Selangor, Malaysia). Deionised water was prepared using Milli-Q Ultrapure Water Purification System (Massachusetts, USA). The ultrapure purification system is equipped with a 0.22 μm filter. All the materials, chemicals, and deionised water were used without further purification.

Ultrasonic-assisted extraction process

The fruits were dried in the oven for 12 h at 40 °C. Next, the dried Mahkota Dewa were ground using a dry grinder and sieved to obtain powder with an average particle size of 500–520 μm. The sample was stored in a Ziploc bag at room temperature. Mahkota Dewa powder (6 g) was weighed and added to 100 mL deionised water in a beaker. The beaker was submerged in an ultrasonic bath (Crest Ultrasonic) for 60 min at 35 °C and ultrasonic power (Scale 3: 460 W). The solution was filtered into a Schott bottle and placed in the refrigerator at −4 °C until further usage. The procedures were repeated using different temperatures (40, 50, 60, 70 °C), ultrasonic power (Scale 1: 153 W, Scale 2: 307 W, Scale 4: 613 W, Scale 5: 767 W), and sonication times (40, 50, 70, 80 min) at an ultrasonic frequency of 50 kHz. Three duplicates of each experiment were performed.

Mangiferin analysis

The mangiferin yield quantification method using HPLC (Agilent 1200 series, Agilent Technologies, USA) was adapted from Kim et al. [4] and Hashim et al. [10] with minor modifications. The HPLC used is equipped with a solvent delivery pump, a column temperature control, and a diode array detector (DAD). Zorbax Eclipse plus, C18 column was used for chromatographic separation and mangiferin quantification. The DAD wavelength was set at 258 nm. The mobile phase consisted of HPLC-grade methanol and 0.1% phosphoric acid with a volume ratio of 31:69. The mobile phase flow rate and injection volume were set at 1.0 mL/min and 5 μL, respectively.

The extract sample was centrifuged for 10 min at 5000 rpm, sonicated for 5 min, and diluted with methanol. The diluted sample was then sonicated for 3 min to create a well-mixed solution and filtered using a 0.45 μm nylon syringe filter. The dilution is essential to ensure the sample and mobile phase have a similar strength and to avoid noise during analysis [16]. Mangiferin standard solution (500 ppm) was prepared by dissolving 5 mg mangiferin in 10 mL HPLC grade methanol. Then, it was diluted to obtain various concentrations from 5 to 40 ppm. The peak area at a retention time of 8.623 min, obtained from the HPLC analysis of the standard solutions, was used to generate a calibration curve. A linear relationship between peak area (y in mAU) and mangiferin concentration (x in ppm) was obtained with a regression coefficient (R^2) of 0.9937 and shown as **Equation 1**. The HPLC chromatogram and UV spectrum of mangiferin standard and Mahkota Dewa fruit extract are shown in **Figure 1**.

$$y = 16.458x - 18.778 \quad (1)$$

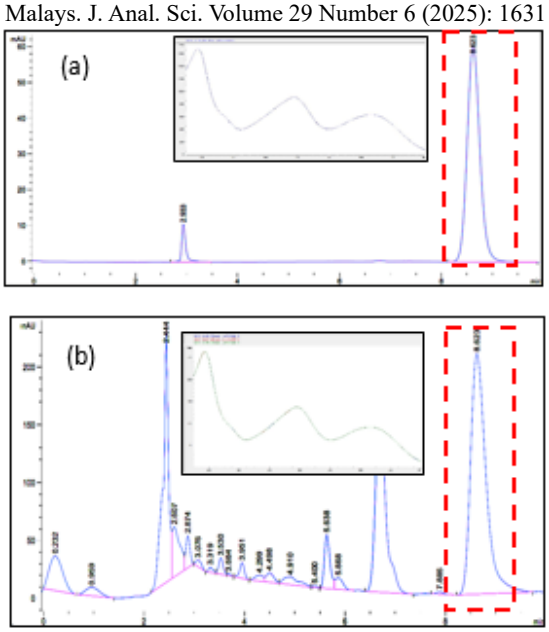


Figure 1. HPLC chromatogram of (a) mangiferin standard and (b) Mahkota Dewa fruit extracts

The actual mangiferin yield (mg/g) in the extract was calculated using **Equation 2**.

$$\text{Actual mangiferin yield (mg/g)} = \frac{(x)(V)(DF)}{m} \quad (2)$$

where *x, V*, *DF*, and *m* are the concentration of mangiferin obtained from Equation 1 (ppm), volume of the solvent used (L), dilution factor of the sample before analysis, and sample weight (g), respectively.

Total phenolic content (TPC) analysis

Total phenolic compound (TPC) was quantified by measuring the reaction of a phenolic compound with a calorimetric reagent, which can be measured throughout the visible portion of the spectrum [17]. The TPC analysis carried out by calorimetric assay methodology with slight modification [18]. Two reagents were prepared before analysis: 7.5% sodium carbonate (Na_2CO_3) reagent and Folin–Ciocalteu reagent. The reagent was prepared at a concentration of 0.5 mg/mL [18]. The same method was applied to gallic acid, which was used as the standard solution for TPC. It was prepared at a concentration of 1 mg/mL by diluting 10 mg gallic acid in 10 mL deionised water.

After preparing the reagents and solution, the standard and each sample were loaded in the first well for each row in the microplate. The standard gallic acid was then diluted into several concentrations ranging from 7.82 to 500 µg/mL by adding 75 µL deionised water to the second through seventh wells of the first three rows. The Folin–Ciocalteu reagent was added to the well, followed by100 µ of 7.5% Na_2CO_3. The mixture was allowed to react for 5 min before being stored in a dark place for 90 min at room temperature.

The absorbance value of the reaction mixture was measured at a wavelength of 765 nm using Tecan Infinite M200 Pro (Switzerland) microplate reader equipped with Magellan software. The values obtained were used to construct a standard curve of gallic acid versus the concentration of gallic acid. A linear equation with a regression coefficient R^2 value of 0.9922 (**Equation 3**) was used to estimate the TPC of the extracts.

$$y = 0.0009x - 0.0374 \quad (3)$$

where *y* is the absorbance value of the microplate reader at 765 nm and *x is* the gallic acid concentration in µg/mL.

Antioxidant analysis

A DPPH assay method was used to determine the antioxidant activity of the extracted sample towards the free radical DPPH. The antioxidant activity determination of OFAT screening of Mahkota Dewa fruit extract was carried out following [19, 20] with slight modification. Mahkota Dewa extracts (10 mg) were diluted in 10 mL of 90% methanol to obtain a concentration of 1 mg/mL (1000 µg/mL) per sample. These steps were repeated with ascorbic acid, which was used as the standard in this analysis. DPPH stock solution was prepared in 90% methanol. Each sample and standard was loaded into a microplate and diluted using 90% methanol into several concentrations (7.82 to 500 µg/mL). Next, 40 µL DPPH reagent was added into the wells and the plate was incubated in the dark at room temperature for 30 min. DPPH reagent was used

as the negative control , and ascorbic acid acted as a standard. The absorbance of the resulting solution was read at a wavelength of 517 nm by using Tecan Infinite M200 Pro (Switzerland) microplate reader with Magellan Software. The analysis was performed in triplicate, and the percentage of DPPH inhibition was calculated by using **Equation 4**:

$$\text{DPPH inhibition} = \frac{A_o - A_1}{A_o} \times 100\% \qquad (4)$$

where A_o is absorbance value of blank and A_1 is absorbance value of test sample and standard.

Statistical analysis of data

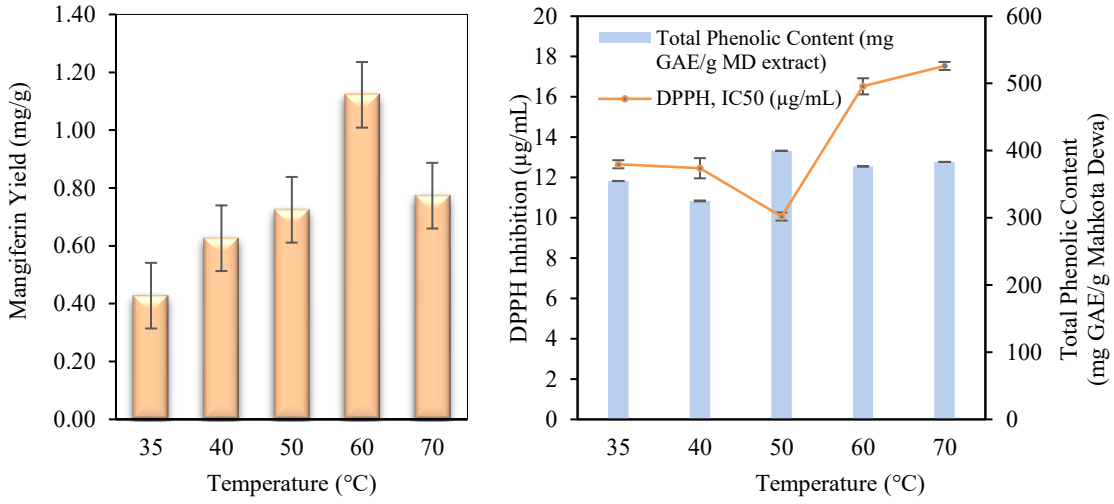
The experiments were conducted in triplicate and the results obtained were reported as average values. Data were analysed using Microsoft Excel [21]. One-way ANOVA was performed to determine the significance of the difference between the experimental results. A *p* value less than 0.05 indicates a significant difference in experimental data between means. The significance of all the independent variables was evaluated based on the *p* value, coefficients of determination (R^2), and mean squared error [21].

Results and Discussion

Effect of temperature

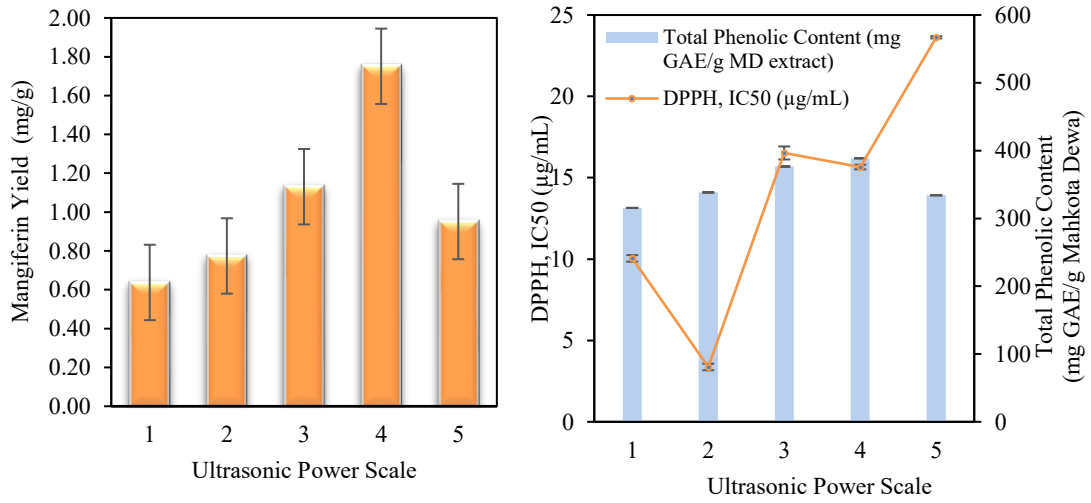
The extraction temperature significantly affects the yield of bioactive compounds from herbal plant and thus must be controlled. High temperature is recommended for UAE for extracting any plant material since low temperature may result in low diffusivity, consequently resulting in low extraction yield [22]. Spigno et al. [22] also mentioned that the temperature should be carefully optimised and controlled to avoid any degradation of compounds of interest. The mangiferin yield, TPC, and antioxidant activity of Mahkota Dewa fruit with different extraction temperatures are illustrated in **Figure 2**. As shown in **Figure 2(a)**, increasing the extraction temperature to 60 °C increased the mangiferin yield. However, further increases in temperature beyond 60 °C decreased the mangiferin yield. The highest mangiferin yield obtained was 1.12 ± 0.03 mg/g. The rise in temperature up to 60 °C increases the solubility and penetrability of the solvent, boosting the extraction speed and efficiency [22]. Furthermore, the increase in temperature decreases the viscosity and surface tension of the solvent [23]. The decrease in viscosity and surface tension enhanced the interaction between Mahkota Dewa powder and water, increasing the diffusivity of the bioactive compounds into water and thereby facilitating extraction. This can be seen in **Figure 4**, where the extracted Mahkota Dewa fruit has a higher mangiferin yield at 60 °C compared to other temperatures. This is probably due to the high diffusivity rate of the mangiferin into the solvent at 60 °C. The high temperature may also soften the surface of the Mahkota Dewa powder. Mangiferin is a type of xanthone that shows antioxidant behaviour [24, 25]. Although xanthone molecules are known to be heat-stable, a different behaviour was observed in this work. The observed changes may be attributed to the formation of xanthones from benzophenone derivatives, which are considered parents of xanthone C-glycoside [25]. Therefore, a relatively high temperature is recommended for the UAE for extracting any plant material, since low temperature may result in low diffusivity and low extraction yield [22]. Giacometti et al. [26] reported that the best yield of phenolics (Oleuropein 8.63%) for olive leaves was achieved using UAE with aqueous ethanol (60%) at 60 °C.

Figure 2(b) illustrates the DPPH inhibition concentration at 50% (IC_{50}) and TPC of the extract. The IC_{50} value decreased as the extraction temperature increased to 50 °C, before increasing as the temperature was further increased to 70 °C. The lowest IC_{50} of 10.06 µg/mL was obtained at 50 °C. However, TPC increased with increasing extraction temperature up to 50 °C, then decreased. The highest TPC of 399.64 ± 0.09 mg GAE/g was obtained at 50 °C. A lower IC_{50} value corresponds to higher scavenging or antioxidant activity [25, 27]. Based on the results, the Mahkota Dewa fruit extract with the highest IC_{50} was obtained at 50 °C extraction temperature with the lowest concentration at 10.06 µg/mL and the highest TPC value of 399.64 mg GAE/g. The TPC obtained in this work is slightly higher than the one reported by Alara et al. [8], who used microwave-assisted extraction (MAE) and water as the solvent. Mangiferin is a bioactive compound with an extensive range of pharmacological effects, including anti-inflammation, antioxidant, antidiabetic, antitumour, and anticancer activity [9]. Thus, it was hypothesized that a higher mangiferin yield would be associated with greater antioxidant activity and higher phenolic content. Unfortunately, the result of the DPPH inhibition concentration at 50% (IC_{50}) is not in line with the highest mangiferin yield obtained at 60 °C.



(a) (b)

Figure 2. (a) Mangiferin yield (mg/g), (b) DPPH inhibition (IC_{50}, µg/mL), and TPC (mg GAE/g Mahkota Dewa) at different temperatures. The ultrasonic power and extraction time were 460 W (Scale 3) and 60 min, respectively



(a) (b)

Figure 3. (a) Mangiferin yield (mg/g), (b) DPPH inhibition (IC_{50}, µg/mL), and TPC (mg GAE/g Mahkota Dewa) at different ultrasonic power scales. The extraction temperature was 60 °C and the extraction time was 60 min.

Effect of sonication power

The variation of mangiferin yield, TPC, and antioxidant activity with sonication power is illustrated in **Figure 3**. As seen in **Figure 3(a)**, the increase in sonication power from Scale 1 (153 W) to Scale 4 (613 W) increased mangiferin yield, with the highest mangiferin yield obtained at 1.76 ± 0.05 mg/g, before declining at Scale 5 (767 W). This corresponds to a power density increase from 1530 to 7670 W/L, based on the solvent volume of 100 mL which was used during the extraction. The initial increase in mangiferin yield is attributed to enhanced cavitation intensity, which promotes cell wall disruption and solute release. However, excessive power may lead to degradation of thermolabile compounds due to high shear forces and radical formations [27].

The IC_{50} value for antioxidant activity decreased with increasing power up to Scale 2 (307 W), reaching a minimum value of 3.37 µg/mL, indicating stronger radical scavenging capacity. Beyond this point, the IC_{50} value increased, suggesting possible degradation of antioxidant compounds at higher power level. Meanwhile, the TPC analysis of the sample shows that the increased sonication power up to Scale 4 (613 W) increased the phenol content, after which it

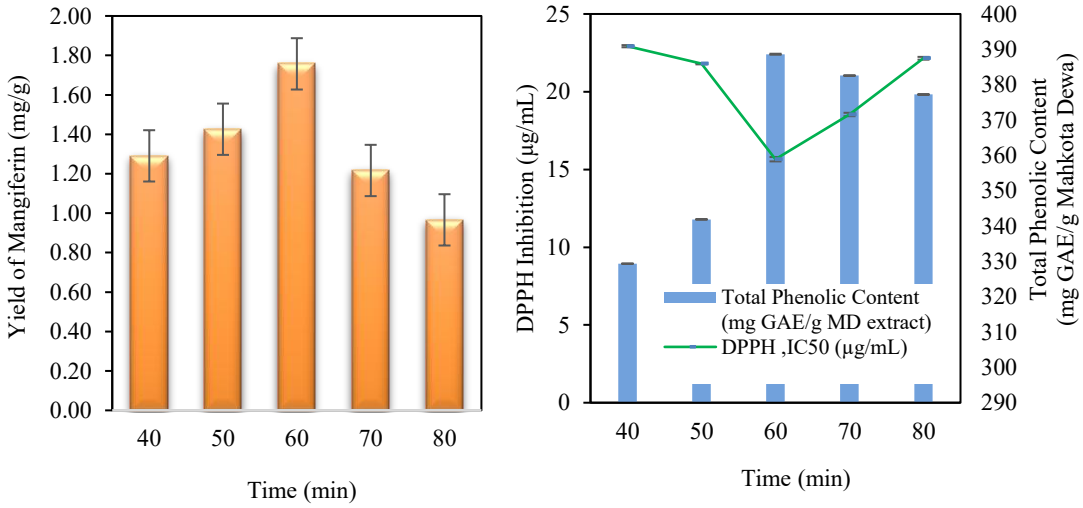
slightly decreased. The highest TPC recorded was 388.61 ± 0.07 mg GAE/g Mahkota Dewa extract. Both of these observations are illustrated in **Figure 3(b)**.

The efficacy of UAE is primarily driven by the acoustic cavitation; hence, sonication power may be considered the second most important component after temperature [28]. Acoustic cavitation occurs when ultrasound is introduced into a liquid medium, resulting from the interaction between the ultrasonic waves, the liquid, and dissolved gases. During this process, the frequent formation and collapse of cavitation bubbles cause a sudden increment in pressure and temperature as well as shock waves in the solvent. This process supplies additional energy to the extraction process. The collapse of cavitation bubbles may also create shear forces that disrupt the cell wall, further decreasing the particle size and increasing the surface area. Thus, it causes an increase in solubility and diffusivity of solutes, particularly mangiferin, antioxidant, and phenolic compounds obtained in this work. Zhang et al. [29] reported that ultrasonic waves have different effects on the extraction of bioactive compounds from plant material. This is because ultrasonic cavitation can produce free radicals, which then reduce the bioactive components. In this work, at higher ultrasonic power, up to Scale 4 (613 W), the ultrasonic waves improve the extraction; however, beyond that, it starts to decrease the mangiferin yield and TPC. This is probably due to the high shear effect that happens at ultrasonic power Scale 5 (767 W).

Effect of extraction time

Figure 4(a) shows that the increased extraction time of up to 60 min increased the mangiferin yield before it decreased. The highest mangiferin yield obtained was 1.76 ± 0.05 mg/g. As for the IC_{50}, the increase of extraction time up to 60 min decreased the IC_{50} value before it started to increase from 70 to 80 min. The lowest IC_{50} obtained was 15.66 µg/mL. Meanwhile, increasing extraction time up to 60 min increased TPC before it slightly decreased. The highest TPC was 388.61 ± 0.14 mg GAE/g of Mahkota Dewa extract. Both of these observations are illustrated in **Figure 4(b)**.

The lowest IC_{50} and the highest TPC were obtained at 60 min, which is similar with the extraction time for the highest mangiferin yield. The best extraction time obtained is consistent with the results obtained by Falleh et al. [30] and Medina-Tores et al. [31], who employed UAE to extract antioxidant and phenolic compounds from *Mesembryanthemum edule* L. and vegetables, respectively. Furthermore, the extraction time to obtain high yield is possibly less than 60 min, depending on other parameters such as intensity, temperature, and solid-to-solvent ratio [32, 33]. The reason is that in the ultrasonic extraction process, diffusion occurs rapidly through the membrane and the lyses and leads to the release of cell content within a short time [30].



(a) (b)

Figure 4. (a) Effect of different extraction times on mangiferin yield (mg/g) and (b) effect of different sonication powers on DPPH inhibition (IC_{50}, µg/mL) and TPC (mg GAE/g Mahkota Dewa) at 60 °C and sonication power at Scale 4 (613 W)

Table 1. One-way ANOVA result on the screening result obtained

Groups	Count	Sum	Average	Variance
Mangiferin yield (mg/g)	16	17.25	1.08	0.18
DPPH (IC_{50})	16	250.41	15.65	40.22
TPC (mg GAE/g MD extract)	16	5805.34	362.83	1002.4532

Source of Variation	SS	df	MS	F	*p* value	F crit
Between Groups	1341951.34	2	670975.67	1930.22	$2.43 \times ^{-44}$	3.20
Within Groups	15642.77	45	347.62			

In this work, the lowest IC_{50} is achieved with the highest mangiferin yield and TPC, rather than prolonged extraction. This is because keeping them longer during the extraction process would lead to degradation [32, 34]. Thus, the results suggest that an extraction time of 60 min, extraction temperature of 60 °C, ultrasonic power Scale 4 (613 W) are the best operating conditions, resulting in mangiferin yield of 1.76 ± 0.05 mg/g, lowest IC_{50} at 15.66 µg/mL, and maximum TPC at 388.61 ± 0.14 mg GAE/g of Mahkota Dewa extract. However, other work by Nawawi et al. [35] considered 30 min extraction time as the best duration to extract anthocyanins from dried blackcurrants, since prolonging the extraction time can lead to the degradation of anthocyanins.

Statistical analysis of data

One-way ANOVA was used to determine whether the results obtained from screening several parameters on mangiferin yield, antioxidant activity, and phenolic content are significant. A significant impact is indicated by a *p* value lower than 0.05. The *p* value obtained is 2.43×10^{-44} (**Table 1**), indicating that the result is significant.

Conclusion

This study successfully investigated ultrasonic-assisted extraction (UAE) of mangiferin, antioxidant, and total phenolic content from Mahkota Dewa under different process parameters (temperature, ultrasonic power, and extraction time) using one-factor-at-a-time (OFAT) method. The parameters investigated showed strong correlation with the mangiferin, antioxidant, and total phenolic content yields. Using water as the solvent, the best extraction conditions of 60 °C, power Scale 4 (613 W), and 60 min yielded 1.76 ± 0.05 mg/g mangiferin, 388.61 ± 0.14 mg GAE/g phenolic content, and 15.66 µg/mL DPPH inhibition at 50% (IC_{50}). Further investigation is needed to examine the sonication effect on the increment in solution temperature and the effect of solid–liquid ratio on extraction yield.

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