A Comparative Study of the Phytochemicals and Antioxidant Activity of Pruned Harumanis Mango Leaves Using Microwave-Assisted Extraction

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Abstract Harumanis mango is one of the best mangoes in the world, and it is only produced in Perlis, Malaysia. Pruning of the Harumanis plant is an important and routine step to maximize both the quantity and quality of the fruits. During pruning season, a lot of leaves and branches are typically discarded. The leaves can however be upcycled into a source of phytochemicals and antioxidants, and this may be used to partially offset the cost of producing the Harumanis. Therefore, in line with zero waste practice, this study examines the phytochemical content and antioxidant activity of young and matured pruned Harumanis leaves. Microwave-assisted extraction (MAE) with water as the solvent was used to extract the phytochemical and antioxidant compounds. The total phenolic compounds (TPC) and total flavonoid compounds (TFC) were then quantified, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to determine antioxidant activity. It was found that ground leaves sieved to a

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particle size of 200 mesh, a sample-to-solvent ratio of 2:50 (g/mL), and a sample size of 2 g yielded the best results for extracting antioxidants and phytochemical compounds from young and matured leaves. The matured leaves were found to contain higher TPC and TFC values (11.05 ± 1.10 mg GAE/g and 46.98 ± 0.03 mg QE/g, respectively) compared to young leaves (10.30 ± 0.03 mg GAE/g and 39.34 ± 0.05 mg QE/g, respectively). However, DPPH activity was found to be higher in young leaves compared to matured leaves (75.78 ± 0.48% and 70.52 ± 0.83%, respectively). It is concluded that both young and matured Harumanis leaves are a good source of phytochemicals and antioxidants.

Keywords Harumanis mangoes · Phytochemical compounds · Antioxidant activity · Mango leaves

1 Introduction

Mango, or *Mangifera indica*, is a fruit that contains a high concentration of phytochemical compounds [1]. The phytochemical compounds of mango are mainly phenolic acids, and they can be obtained from various parts of the plant such as fruit, kernel (stone), leaves, and bark. Numerous other phytochemical compounds, including mangiferin, gallic acids, gallotannins, quercetin, isoquercetin, ellagic acid, flavonoids, ascorbic acid, carotenoids, tocopherols, among others are also found in the leaves of the plant [2]. Phytochemicals, sometimes referred to as secondary metabolites, are naturally occurring bioactive molecules. They serve to protect plant cells from pollution, stress, drought, exposure to UV light, and attacks by pathogens [3].

Mango leaves contain phytochemicals that are known to have antioxidant, antidiabetic, antibacterial, immunomodulatory, antipyretic, anti-inflammatory, and analgesic properties [4–6]. The antioxidant activity in mango leaves is due to the presence of phenols and flavonoids. There are several varieties of mangoes grown in Malaysia, but the Harumanis mango is one of the most well-known mango varieties grown in the country. The Harumanis mango is only grown in Perlis, a small state located in northern Malaysia, and it is in high demand in the commercial market [7].

Phytochemical and antioxidant compounds can be extracted using a variety of extraction techniques. Traditional extraction methods generate large amounts of solvent waste, causing many environmental and health problems. By extracting antioxidants using green extraction methods such as microwave-assisted extraction (MAE), less solvent is needed, and less waste is produced during extraction as a result [8].

In this study, MAE is chosen as the extraction method to use due to its advantages, including low solvent consumption, minimal operation time, high recovery yield, high selectivity, and low sample manipulation [9]. This study evaluates the phytochemicals content and antioxidant activity of matured and young Harumanis mango leaves.

2 Materials and Methods

2.1 Chemicals and Materials

2,2-diphenyl-1-1picrylhydrazy (DPPH) and ethanol were purchased from Fisher Scientific Limited. Folin–Ciocalteu, sodium carbonate, sodium nitrate, and aluminum chloride were purchased from HmbG Chemicals while gallic acid and quercetin were purchased from Sigma Aldrich (Malaysia). All chemicals used for the experiment were of analytical grade. Harumanis pruned leaves were obtained from Harumanis Sungai Batu Pahat Plantations at Perlis, Malaysia.

2.2 Sample Preparation

The leaves were washed thoroughly to remove dirt and other impurities and then dried at 55 °C overnight in a drying oven (Thermo-Line SOV140B, China). The dried leaves were then ground using a coffee grinder (Saachi Coffee Grinder, China) and sieved into five different particle sizes: 200, 100, 60, 30, and < 30 mesh (B.S.S. standard measurement). The sieved samples were kept in the freezer (Midea R600a, China) at − 20 °C until further use.

2.3 Microwave-Assisted Extraction System

A modified microwave oven technique was used to extract the phytochemicals and antioxidants from Harumanis leaves. The oven (Sharp R357EK (China) uses a digital control system to adjust the irradiation period (from 1 s to 99 min) and microwave power (from 10 to 900W). The microwave oven was adapted to condense the vapors produced in the sample during the extraction process. Water was used as the solvent for the extraction process and was added to the ground Harumanis leaves in a round-bottom flask before irradiation. After irradiation, the sample solution containing the extracted phytochemicals and antioxidants was cooled promptly in a cooled water bath, filtered, and stored in the freezer (Midea R600a, China) until further use.

2.4 The Optimization of MAE Parameters Using One-Factor-At-A-Time (OFAT) Approach

A one-factor-at-a-time (OFAT) approach was used to optimize the MAE parameters for extracting phytochemicals and antioxidants from mango leaves. Particle size (200, 100, 60, 30, and < 30 mesh), sample-to-solvent ratio (2:50, 2:100, 2:150, 2:200, and

2:250 g/mL), and sample size (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g) were the parameters investigated. These parameters were studied one at a time, and the optimum parameter found in each study was used in subsequent studies. After the extraction process, the liquid extract was cooled in an ice bath and filtered. The filtered extract was stored at − 20 °C in a freezer (Midea R600a, China) before analysis.

The Effect of Particle Size. The effect of particle size (200, 100, 60, 30, and 30 mesh) on phytochemical content and antioxidant property of the filtered extract was studied using 2 g of sample, 50 mL of water, irradiation power of 900 W, and extraction time of 3 min.

The Effect of Sample-to-Solvent Ratio. Based on the results, a particle size of 200 mesh was selected as the optimum particle size to use. The effect of sample-to-solvent ratio (2:50, 2:100, 2:150, 2:200, and 2:250 g/mL) on phytochemical content and antioxidant property of the filtered extract was studied using 2 g of sample, irradiation power of 900 W, and extraction time of 3 min.

The Effects of Sample Size. A particle size of 200 mesh and the sample-to-solvent ratio of 2:50 g/mL were selected based on the previous results. The effect of sample sizes (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g) on phytochemical content and antioxidant property of the filtered extract was studied using irradiation power of 900 W and extraction time of 3 min.

2.5 Phytochemical Analysis

Total Phenolic Content. The total phenolic content (TPC) of the samples was determined using the Folin–Ciocalteu method [10]. Briefly, 0.1 mL of plant extract was added into 8 mL of distilled water followed by 0.2 mL of the Folin–Ciocalteu phenol reagent. The mixture was then incubated for 3 min. Afterward, 1 mL of a saturated solution of sodium carbonate (Na_2CO_3) (20%) was added to the mixture. The sample mixture was then incubated for another 30 min in the dark at room temperature for the color to develop. The absorbance value of the sample mixture was then measured at 765 nm using a UV–Vis spectrophotometer (Thermo Spectronic Genesys 20, USA). The total phenolic content in the samples is expressed as mg of gallic acid equivalent (GAE) per gram of dry sample (mg GAE/g).

Total Flavonoid Content. The flavonoid content of the samples was determined using the colorimetric method [11]. About 0.5 mL of the sample extract was mixed with 2 mL of distilled water and 0.15 mL of 50% sodium nitrate ($NaNO_3$). The mixture was then incubated for 5 min. Afterward, 0.15 mL of 10% aluminum chloride ($AlCl_3$) was added to the mixture. This mixture was incubated for another 15 min at room temperature. Finally, the absorbance value of the sample mixture was measured at 415 nm using a UV–Vis spectrophotometer (Thermo Spectronic Genesys 20, USA). The concentration of flavonoids in the samples was calculated

from a calibration plot and is expressed as mg quercetin equivalents per g of dried sample (mg QE/g).

2.6 Antioxidant Assay (DPPH)

The antioxidative property of the extracts was measured using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [12]. Briefly, 200 μL of the sample extract was added with 2.5 mL of 60 μM ethanolic DPPH. The mixture was mixed thoroughly and incubated in a dark place for 30 min. A control consisting of 200 μL of distilled water with 2.5 mL of 60 μM of ethanolic DPPH was prepared. Then, the absorbance value of the sample mixture was measured at 517 nm using a UV–Vis spectrophotometer (Thermo Spectronic Genesys 20, USA). DPPH radical scavenging activity was calculated using the equation shown as Eq. (1):

$$\text{DPPH scavenging activity}(\%) = \left(1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}\right) \times 100 \quad (1)$$

2.7 Statistical Analysis

Minitab (version 17; StataCorp LLC, TX, USA) and SigmaPlot (version 12.0; Systat Software Inc., San Jose, California, USA) were used to calculate the mean and standard deviation of all measurements.

3 Results and Discussion

3.1 The Effects of Particle Size

The effect of particle size on the phytochemical compounds yield and antioxidant properties of Harumanis mango leaves is shown in Fig. 1. The results show that the DPPH, TFC, and TPC are inversely proportional to sample particle size. The DPPH activity of Harumanis mango leaf extract is the highest when the particle size is the smallest (200 mesh) and they are 70.57 ± 0.61% (Fig. 1a) and 75.78 ± 0.48% (Fig. 1b) for matured and young leaves, respectively. As the particle size increases, the DPPH activity decreases. The high DPPH scavenging activity of the leaves is caused by the presence of high amounts of phenolics and flavonoids in the leaves [13].

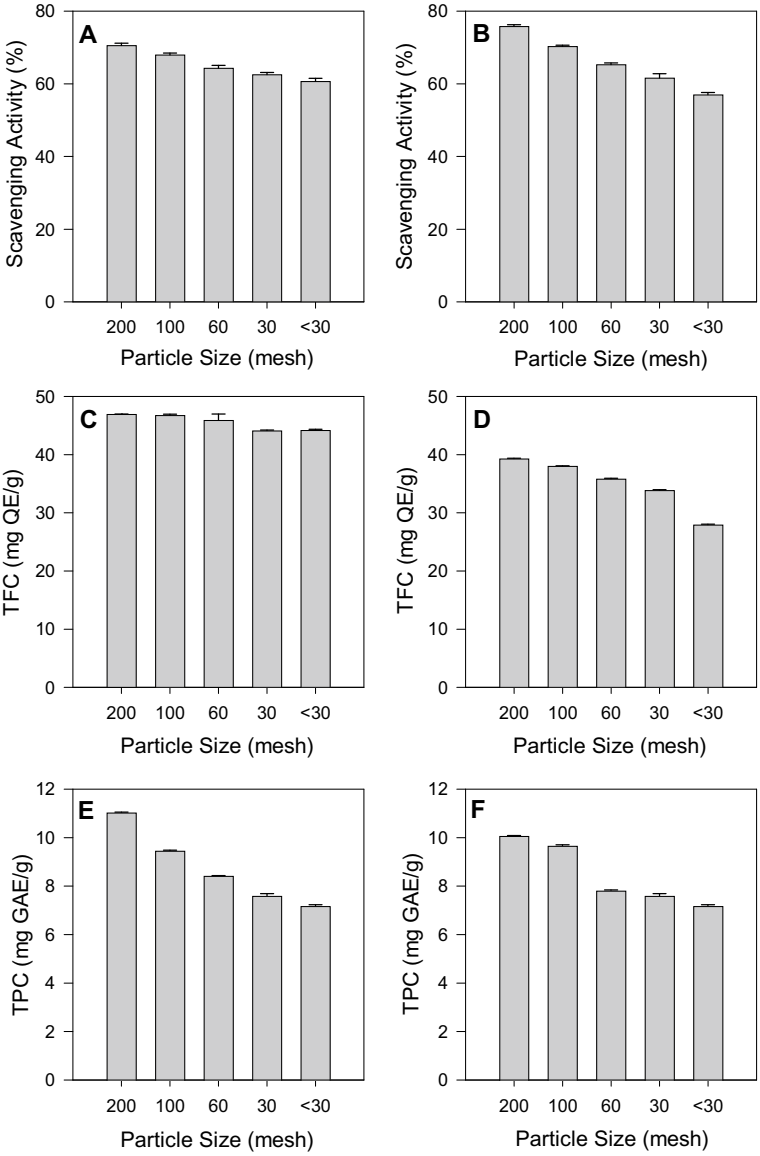

Fig. 1 The effects of particle size on the DPPH (**a** matured and **b** young leaves), TFC (**c** matured and **d** young leaves), and TPC (**e** matured and **f** young leaves)

The TFC and TPC of matured and young leaves also show a similar trend. The TFC (Fig. 1c, d) and TPC (Fig. 1e, f) of Harumanis mango leaf extracts are the highest when the particle size is the smallest and they are 46.98 ± 0.03 mg QE/g and 11.05 ± 0.002 mg GAE/g for the matured leaves; and 39.34 ± 0.05 mg QE/g and 10.08 ± 0.007 mg GAE/g for the young leaves. The matured leaves were found to have a higher phenolic and flavonoid content compared to young leaves. The maturity of the leaves plays a crucial role in influencing the photosynthesis and metabolism of the plant. A smaller particle size results in an increase in phytochemical yield due to the larger surface-to-volume ratio, which promotes greater contact between the solvent and the plant material during the extraction process [14]. The solvent will have better access to the soluble compounds within the particle. It is the most critical factor affecting the extraction efficiency of phytochemical compounds [15]. Therefore, it is better to use a small particle size to obtain the best yield of DPPH, TFC, and TPC. The particle size of 200 mesh is used in all subsequent experiments.

3.2 The Effects of Sample-To-Solvent Ratio

Figure 2 shows the effect of sample-to-solvent ratio on DPPH, TFC, and TPC values of both matured and young Harumanis mango leaves extract. The best sample-to-solvent ratio for both matured and young Harumanis mango leaves is 2:50 g/mL. The results show that the phytochemical yield and antioxidant activity are proportional to sample-to-solvent ratio. The mass transfer principle states that the higher the concentration gradient between the sample and the solvent, the stronger the driving force of mass transfer from the sample to the solvent [16]. However, when using a high sample-to-solvent ratio, the solvent becomes more rapidly saturated with phytochemicals and antioxidants, resulting in higher TFC, TPC, and antioxidant activity of the extracts [17]. A solvent volume of 50 mL is sufficient to extract the phytochemicals and antioxidants from 2 g of sample, and a larger solvent volume would only dilute the compounds of interest.

The highest DPPH activity was obtained at a sample-to-solvent ratio of 2:50 (g/mL), and it is 65.94 ± 0.87% (Fig. 2a) and 70.29 ± 0.42% (Fig. 2b) for matured and young leaves, respectively. Using matured leaves also resulted in a higher content of TFC of 44.50 ± 073 mg QE/g compared to 38.46 ± 0.38 mg QE/g for young leaves. The TPC also shows the same trend, where a greater sample-to-solvent ratio is desirable to obtain a higher concentration of phytochemicals and antioxidants. The TPC is 10.41 ± 0.03 mg GAE/g (Fig. 2e) and 10.28 ± 0.06 mg GAE/g (Fig. 2f) for matured and young leaves, respectively. Interestingly, the DPPH scavenging activity of young leaves is higher compared to that of the matured leaves. This may be explained by the excessive generation and buildup of free radicals during plant development. This process leads to a reduction in the levels of secondary metabolites and triggers the onset of senescence in the plant [18]. The sample-to-solvent ratio of 2:50 (w/v) is used in subsequent experiments.

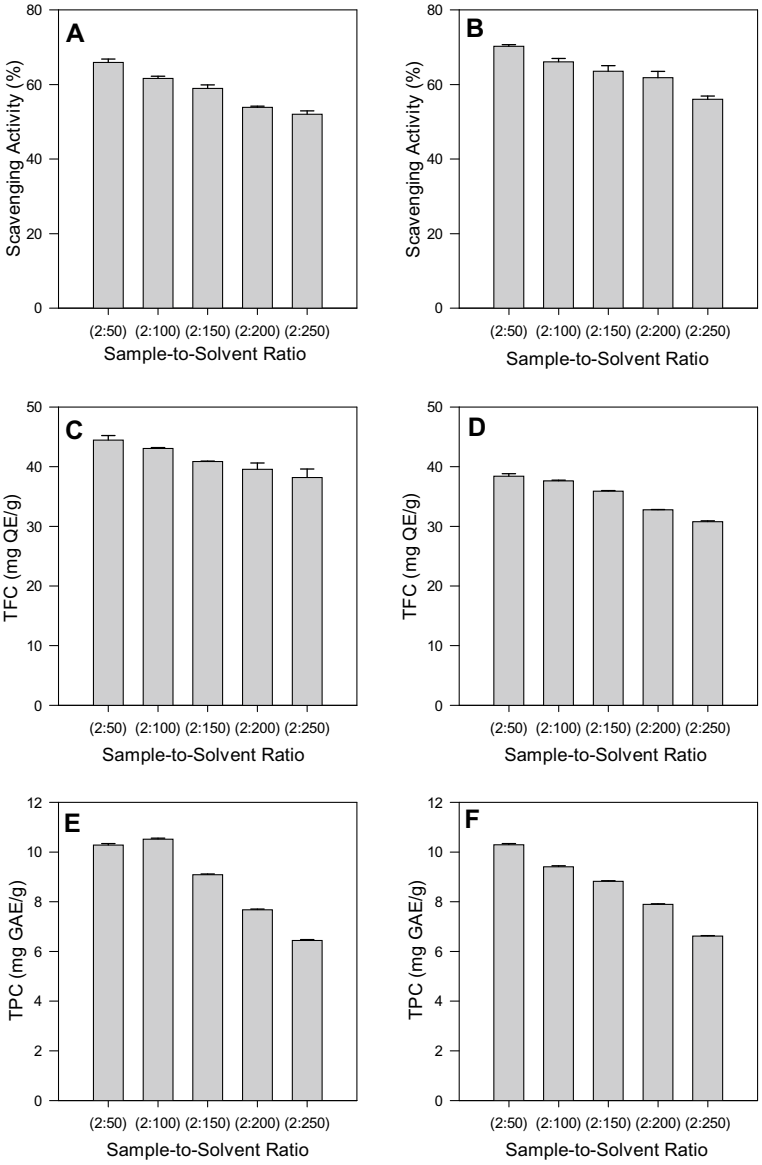

Fig. 2 The effects of sample-to-solvent ratio on the DPPH (**a** matured and **b** young leaves), TFC (**c** matured and **d** young leaves), and TPC (**e** matured and **f** young leaves)

3.3 The Effects of Sample Size

Figure 3 shows the effect of sample size on DPPH, TFC, and TPC values of Harumanis mango leaves extract. The best sample size for both leaves is 2 g. The results show that the phytochemicals compounds (TPC and TFC) and antioxidant activity (DPPH) increase with sample size, reaching a maximum at 2 g and slowly decreasing thereafter. A higher sample size simply means more material to extract the antioxidant components from [19]; however, using a sample size larger than 2 g will not increase phytochemical and antioxidant yield because the solution would already become saturated. The DPPH, TFC, and TPC contents when the sample size is 2 g are 66.30 ± 1.18%, 46.24 ± 0.02 mg QE/g, 10.99 ± 0.02 mg GAE/g, respectively, for matured leaves and 71.65 ± 0.68%, 38.30 ± 1.42 mg QE/g and 10.17 ± 0.26 mg GAE/g, respectively, for young leaves.

4 Conclusion

This study had measured the phytochemical compounds and antioxidant activities of Harumanis mango leaves extract obtained through microwave-assisted extraction (MAE). Three parameters, i.e., particle size, sample-to-solvent ratio, and sample size, have been evaluated in this study. The optimal extraction parameters to extract antioxidants from Harumanis leaves are a particle size of 200 mesh, sample-to-solvent ratio of 2:50 (g/mL), and sample size of 2 g for both matured and young leaves. These results show that Harumanis mango leaves are a promising source of natural phytochemicals and antioxidants. A significant correlation between antioxidant properties and total phenolic and flavonoid compounds were found, indicating that these compounds are a major contributor to the leaf extract's antioxidant properties. Further studies should however be conducted to understand the mechanism of extraction of phytochemical compounds and antioxidants from Harumanis mango leaves.

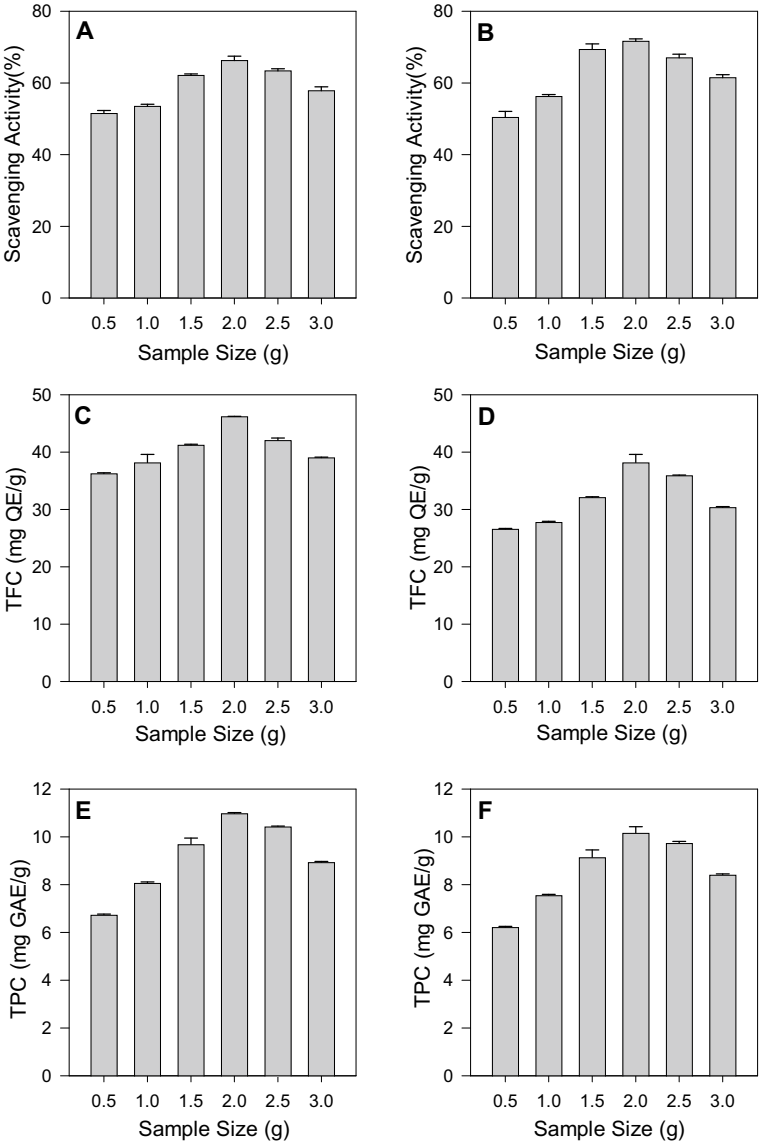

Fig. 3 The effects of sample size on the DPPH (**a** matured and **b** young leaves), TFC (**c** matured and **d** young leaves), and TPC (**e** matured and **f** young leaves)

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