Research Article

# Valorization of Bambangan (*Mangifera pajang*) By-Products through Extraction of Mangiferin using Ultrasonic Assisted Extraction

**Ahmad Hazim Abdul Aziz[1], Aezerah Ekos[2], Norliza Julmohammad[1], Siti Faridah Mohd Amin[1], Mohd Azrie Awang[1], Hasmadi Mamat[1], Sariah Saalah[3], Suryani Saallah[4], Nicky Rahmana Putra[5], Muhammad Abbas Ahmad Zaini[6] and Jumardi Roslan[1*]**

[1]Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
[2]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
[3]Faculty of Engineering, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
[4]Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
[5]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Complex Cibinong Science Center–BRIN, Cibinong, Jawa Barat 16911, Indonesia
[6]Centre of Lipids Engineering and Applied Research (CLEAR), Ibnu-Sina Institute for Scientific and Industrial Research (ISI-SIR), Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

*jumardi@ums.edu.my

## ABSTRACT

Bambangan (*Mangifera pajang*) is a fruit belonging to the Anacardiaceae family, which is a lesser-known member of the mango family. It is native to East Malaysia, namely in Sabah and Sarawak. This fruit possesses a significant amount of dietary fibre, is rich in juice, and emits a strong smell. Typically, around 35 – 40% of the fruit's total weight, which includes the peel and seed, is discarded as waste. Mangiferin, a xanthone C-glucoside possessing notable antioxidant and antibacterial characteristics, is predominantly present in the foliage of mango plants. This study employed ultrasonic-assisted extraction (UAE) to extract mangiferin from bambangan peels and seeds. The study specifically investigated the impact of ethanol concentration (ranging from 40% to 60%), temperature (ranging from 40 °C to 60°C), extraction time (ranging from 5 to 15 minutes), and sample-solvent ratio (ranging from 1:10 to 1:30 w/v) on the concentration of mangiferin. Mangiferin was quantified using high-performance liquid chromatography (HPLC). The optimal conditions for extracting mangiferin using UAE were determined to be 50% ethanol concentration, temperature of 50°C, extraction time of 10 min, and a sample-solvent ratio of 1:20. The seeds of the bambangan fruit had the highest concentration of mangiferin at 264.89 ± 5.67 mg/mL, whereas the peels had a value of 94.82 ± 1.49 mg/mL. These findings indicate that the peels and seeds of bambangan have potential as alternate sources of mangiferin for the food and pharmaceutical industries.

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# 1. Introduction

Bambangan, scientifically known as *Mangifera pajang*, is a tropical fruit tree belonging to the Anacardiaceae family. It is mostly found in the lowland rainforests of Borneo, namely in regions such as Sabah, Sarawak, Brunei, and Kalimantan. In Sabah, bambangan cultivation is prevalent in Beaufort, Ranau, Sipitang, Kota Belud, and Kota Marudu (Department of Agriculture, Malaysia, 2022). The local demand for bambangan fruit has risen, with increase in production from 39.6 to 52.5 metric tons in Sabah from 2018 to 2021. However, the production fell drastically to 9.90 metric tons in 2022 (Department of Agriculture, Malaysia, 2022). This may be due to weather changes when heavy rains occurred throughout the bambangan pollination and fruiting seasons.

Bambangan tree can reach a height of over 30 meters. Its cylindrical trunk varies between a diameter of 30 and 70 cm and can yield hundreds of fruits during the fruiting season. Bambangan is a seasonal fruit which is only available between October to February (Abu Bakar and Fry, 2013). The bambangan fruit has an oval shape, thick peel, rough-brown skin, big seed size and weighs between 0.5 to 1.0 kg (Prasad *et al*., 2011; Tangah *et al.,* 2017). Bambangan pulp finds diverse culinary applications. It is usually eaten fresh or processed into juice, dehydrated fruits, and pickles. Currently, bambangan pulp and bambangan juice powders are commercially used. The pulp, which makes up 50 – 67% of the fruit's overall weight, is composed of fibres, contains a lot of juice, and is suitable for consumption. According to Jahurul *et al*. (2019), the peels constitute 10 – 15% and the seeds make up 15 – 20% of the fruit's overall weight. Typically, both the peels and seeds are discarded as waste.

Discarding of bambangan peels and seeds would be a huge loss to the food industry. This is because there are many available bioactive compounds in bambangan peels and seeds including mangiferin. Mangiferin, a xanthonoid with chemical formula 1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside, is an important natural compound with extensive applications in the pharmaceutical and related industries (Kulkarni and Rathod, 2014; Zou *et al*., 2014). It exhibits antioxidant, antitumour, and antiviral properties (Dar *et al*., 2005; Duang *et al*., 2011; Das *et al*., 2012). Due to its complex structure, synthesizing mangiferin chemically is quite challenging, making extraction from natural sources the best alternative for its production. There are reports available on its medicinal uses. Mangiferin is widely distributed in different portions of the mango plant (*Mangifera indica*), and it is also discovered in bambangan (Hassan *et al*., 2011).

Mangiferin can be extracted using several conventional methods, such as maceration and reflux extraction. These traditional extraction methods are typically time consuming and require large amounts of solvent (Zou *et al*., 2013). Recently, various novel extraction techniques have been developed for extracting active compounds from plant such as ultrasound assisted extraction (UAE), subcritical and supercritical fluid extraction (SFE), microwave assisted extraction (MAE), and accelerated solvent extraction (ASE) (Zou *et al*., 2014). MAE stands out as a rapid and efficient extraction method. It utilizes microwave energy to induce molecular movement in compounds with a permanent dipole, leading to rapid heating of the solvent and sample. SFE uses fluids such as $CO_2$ at temperatures and pressures above (supercritical) or just below (subcritical); their critical point to dissolve and extract bioactive compounds. The benefit of using this method including utilization of non-toxic solvent and no solvent residue in the final product. ASE method uses elevated temperatures and pressures to increase the solubility and mass transfer rates of bioactive compounds from plant material into the solvent. This technique offers short extraction times, high extraction efficiency and reduced solvent consumption. Additionally, innovative pre-treatments such as high pressure processing and pulsed electric field are also utilized. However, these methods still have limitations and drawbacks including significant financial investment, excessive energy usage, high levels of carbon dioxide emissions, and the use of harmful organic solvents that can leave behind residues in the extract (Tiwari, 2015; Jha and Sit, 2022).

UAE has become popular because it overcomes the limitations of traditional and other modern methods. UAE is particularly efficient, as it can enhance extraction efficiency through acoustic cavitations produced in the solvent by ultrasound waves (Ghafoor *et al.*, 2009). Ultrasound also exerts a mechanical effect, allowing greater penetration of the solvent into the tissue and increasing the contact surface area between the solid and liquid phases. Consequently, the solute quickly diffuses from the solid phase into the solvent (Rostagno *et al.*, 2003). Therefore, this work specifically aims to extract mangiferin from the peels and seeds of bambangan using UAE.

## 2. Materials and Methods

### *2.1 Sample preparation*

Bambangan fruits (*Mangifera pajang*) were bought from Papar market, Sabah and brought to the laboratory. Upon arrival at laboratory, the fruits were washed, and the peel and seed were manually removed to get the pulp. The pulp maturity was evaluated according to its total soluble solid (°Brix) using refractometer (Atago, Germany). Bambangan fruit is considered as matured at approximately 15°Brix of total soluble solid (Roslan *et al.*, 2020). Bambangan peel and seed were thoroughly washed with water, and then dried in an oven at 50°C for 48 h. The dried peel and seed were subsequently ground into powder using a blender. The bambangan peel and seed powders were then stored separately in a container and kept at temperature of -18°C until further use.

### *2.2 Ultrasound assisted extraction (UAE)*

Ultrasonic water bath (SL SciLab, Korea) at constant power of 180 W and frequency of 40 kHz was used in this study. Ethanol was prepared at concentrations of 40%, 50%, and 60%. A 5 g sample of the powdered bambangan peels and seeds was placed in a flask and subjected to sonication at 40°C for 5 min, with a sample-solvent ratio of 1:10 w/v. The UAE parameters were varied to test different temperatures (40, 50, and 60°C), extraction times (5, 10, and 15 min), and sample-solvent ratios (1:10, 1:20, and 1:30 w/v).

### *2.3 Quantification of mangiferin*

High-performance liquid chromatography (HPLC) was employed to quantify the concentration of mangiferin, following the method described by Lim *et al.* (2019), with slight modifications. An Agilent 1200 HPLC system (Agilent Technologies, United States) equipped with a diode array detector (DAD) was used for the analysis. A C18 column (250 mm × 4.6 mm, 5 µm) was utilized. The detection wavelength was set at 258 nm, the column temperature at 25°C, and the injection volume at 20 µL. The mobile phase consisted of 0.1% formic acid and acetonitrile (82:18) with a flow rate of 1.0 mL/min. Standard mangiferin at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm were prepared to generate a standard calibration curve (Figure 1), yielding the equation $Y = 16.715X$ ($R^2 = 0.9996$) with a retention time of mangiferin about 2.792 min. Mangiferin was quantified based on peak area and comparison with the standard curve.

### *2.4 Statistical analysis*

The data were statistically analysed using SPSS version 28.0. Comparison of means was done using One-Way Analysis of Variance (ANOVA) and $p<0.05$ was considered statistically significant. Tukey's test was used to determine the significant difference between groups.

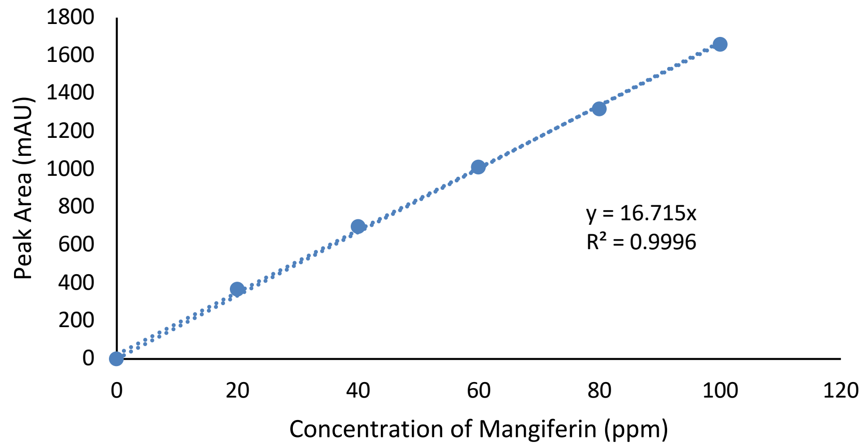


**Figure 1.** Calibration curve of mangiferin

# 3. Results and Discussion

## *3.1 Effect of ethanol concentration on the mangiferin content*

Methanol, ethanol, and water are commonly used extraction solvents. However, methanol is more toxic to humans and the environment compared to ethanol. Water is a nontoxic and inexpensive solvent widely used for extracting bioactive compounds, but its extraction efficiency is generally lower than that of an ethanol-water mixture (Zou *et al.*, 2013). Thus, aqueous ethanol was used in this study. Figure 2 illustrates the effect of different ethanol concentrations (40%, 50%, and 60%) on the mangiferin content extracted from the bambangan peel and seed using UAE. The other parameters such as sample-solvent ratio, temperature, and extraction time were kept constant at 1:10 w/v, 40 °C, and 5 min, respectively.

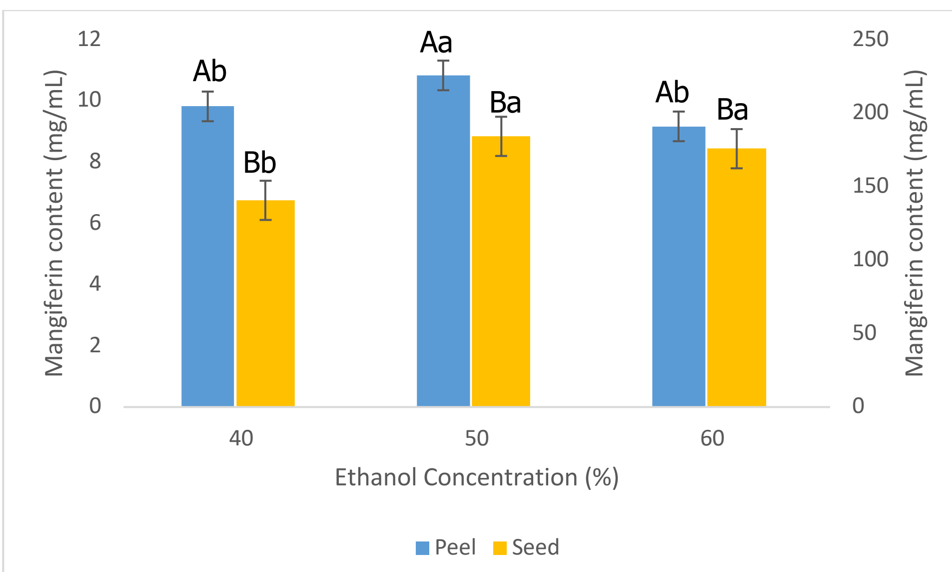


**Figure 2**. Effect of different concentration of ethanol on the mangiferin content from the peel and seed of bambangan. Lowercase letters indicate statistically different between values within the parameter, while uppercase letters indicate statistically different between peel and seed sample. The left Y axis refers to mangiferin content for peel, while the right Y axis refers to mangiferin content for seed

It was found that when 40% concentration of ethanol was used, a statistically different mangiferin content was obtained for both bambangan peel and seed with value of 9.81 ± 0.24 mg/mL and 140.58 ± 1.59 mg/mL, respectively. The mangiferin content reached the maximum values at 50% of ethanol with values of 10.83 ± 0.15 mg/mL (peel) and 184.11 ± 8.08 mg/mL (seed). However, further increasing the ethanol concentration to 60% led to a significant decrease in mangiferin content to 9.16 ± 0.16 mg/mL and 175.70 ± 1.21 mg/mL of peel and seed, respectively. The results showed that 50% ethanol was effective for extracting mangiferin from bambangan peel and seed. Also, the yield of mangiferin was higher in the bambangan seed compared to the peel. These findings align with previous research suggesting that a mixture of ethanol and water is the optimal solvent for extracting bioactive components (Myo and Khatudomkiri, 2024).

These findings align with findings from previous research suggesting that a mixture of ethanol and water is the optimal solvent for extracting bioactive components. These observations can be attributed to the increased solubility and diffusivity of the phenolic compound as the solvent's dielectric constant decreases with higher ethanol concentration. Near 100% ethanol, the highly pure solvent dehydrates plant tissue and denatures proteins, resulting in decreased yield at such high concentrations (Kumar *et al.,* 2021). Similar observations have been reported by Lim *et al.* (2019), who found that mangiferin content increased with ethanol concentrations from 20% to 80%, but decreased when the concentration was further increased to 100%. Zou *et al.* (2014) also investigated the effect of ethanol concentration (0 – 80%) on mangiferin extraction from mango leaves using UAE, noting that mangiferin content increased up to 40% ethanol before decreasing as ethanol concentration continued to rise to 80%. Based on these findings, 50% ethanol concentration was selected for the subsequent experiments.

### *3.2 Effect of temperature on the mangiferin content*

The effect of different temperatures (40, 50 and 60°C) on the concentration of mangiferin in the peel and seed of bambangan is presented in Figure 3. The ethanol concentration was maintained at 50%, with a sample-solvent ratio of 1:10 w/v and an extraction time of 5 minutes. The highest mangiferin content was observed at 50°C, yielding approximately 19.80 ± 0.53 mg/mL in the peel and 182.63 ± 0.84 mg/mL in the seed.

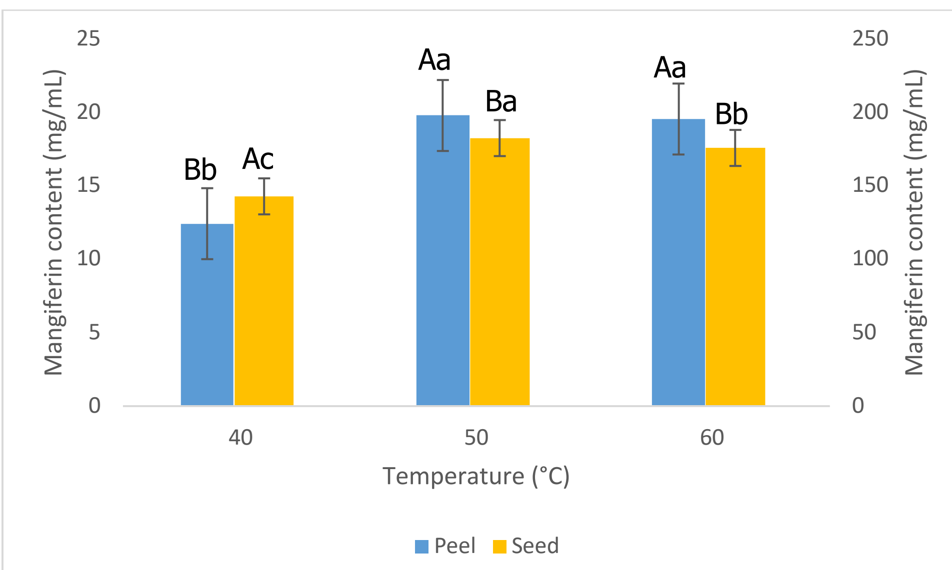


**Figure 3**. Effect of different extraction temperature on the mangiferin content from the peel and seed of bambangan. Lowercase letters indicate statistically different between values within the parameter, while uppercase letters indicate statistically different between peel and seed sample. The left Y axis refers to mangiferin content for peel, while the right Y axis refers to mangiferin content for seed

The study showed that increasing the extraction temperature from 40 to 50°C enhanced the mangiferin content in both the peels and seeds of bambangan, followed by a slight decrease at 60°C. These observations can be attributed to the fact that higher extraction temperatures improve solvent diffusion into cells and increase the solubility of compounds, facilitating the breakdown of mangiferin components. However, excessive temperatures can lead to further chemical decomposition, reducing the mangiferin yield (Carrera *et al.,* 2012; Aziz *et al.,* 2024). Almost similar findings were reported on the extraction of mangiferin from mango leaves where the optimal temperature was achieved at 60°C (Zou *et al.,* 2014). Thus, the temperature of 50°C was selected for subsequent experiments.

### *3.3 Effect of extraction time on the mangiferin content*

The effect of extraction time on the mangiferin content in bambangan peels and seeds was investigated at intervals of 5, 10, and 15 min, while maintaining constant parameters for sample-solvent ratio (1:10 w/v), ethanol concentration (50%), and extraction temperature (50°C). As shown in Figure 4, the highest mangiferin concentrations were achieved with a 10-min extraction time, yielding 68.92 ± 2.11 mg/mL for the peels and 201.65 ± 3.96 mg/mL for the seeds.

The initial increase in extraction time enhances the cavitation effect of ultrasound, which improves the swelling, hydration, fragmentation, and pore formation of the plant tissue matrix, facilitating the release of the solute into the solvent (Kumar *et al.,* 2021; Suhaimi *et al.,* 2019). These factors increase the exposure of the solute to the extraction medium, improving extraction efficiency. However, prolonged ultrasound exposure can cause structural damage to the solute, reducing the extraction yield. This finding is consistent with Morales *et al.* (2020), who studied the extraction of mangiferin from *Mangifera indica* L. and found that a 10-min extraction time was optimal for UAE. Therefore, a 10-min extraction time was selected for subsequent parameter studies.

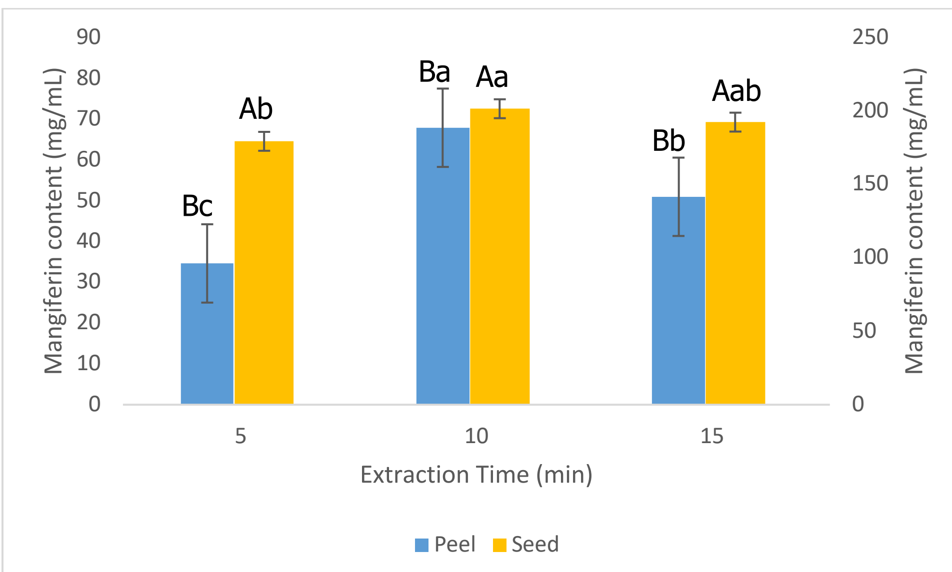


**Figure 4.** Effect of extraction time on the mangiferin content from the peel and seed of bambangan. Lowercase letters indicate statistically different between values within the parameter, while uppercase letters indicate statistically different between peel and seed sample. The left Y axis refers to mangiferin content for peel, while the right Y axis refers to mangiferin content for seed

### *3.4 Effect of sample-solvent ratio on the mangiferin content*

The effect of the sample-solvent ratio on the mangiferin content in bambangan peels and seeds was investigated at ratios of 1:10, 1:20, and 1:30 w/v, while keeping other parameters constant: 50% ethanol concentration, 50°C extraction temperature, and 10 min of extraction, as shown in Figure 5.

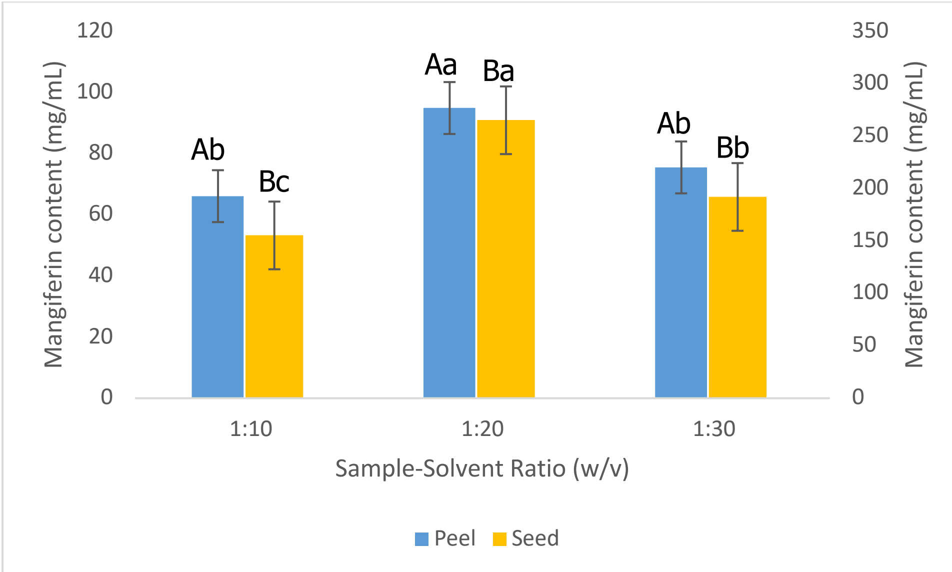


**Figure 5.** Effect of sample-solvent ratio on the mangiferin concentration from the peel and seed of bambangan. Lowercase letters indicate statistically different between values within the parameter, while uppercase letters indicate statistically different between peel and seed sample. The left Y axis refers to mangiferin content for peel, while the right Y axis refers to mangiferin content for seed

The optimal sample-solvent ratio was found to be 1:20, yielding 94.82 ± 1.49 mg/mL of mangiferin in the peel extract and 264.89 ± 5.67 mg/mL in the seed extract. Increasing the sample-solvent ratio reduces the viscosity and concentration of the extraction medium, which enhances the cavitation effect. The greater concentration gradient of the solute improves diffusivity and dissolution, augmenting the extraction process. However, at higher sample-solvent ratios, the enhanced cavitation effect can degrade the solute, reducing the yield (Kumar *et al.,* 2021; Samaram *et al.,* 2020). The study showed that increasing the sample-solvent ratio from 1:10 to 1:20 could improve the yield of mangiferin. However, the yield of mangiferin significantly decreased when the sample-solvent ratio increased to 1:30. These findings are consistent with the findings reported by Loan *et al.,* (2021) and Anbalangan *et al.* (2019). Loan *et al.* (2021) observed a high mangiferin yield at low sample-solvent ratio (1:5 to 1:10), followed by a slight decrease of mangiferin yield at high ratio (1:10 to 1:50). Anbalangan *et al.* (2019) reported similar findings, noting that the yield of mangiferin increased from a ratio of 1:5 to 1:15, but then decreased when the ratio was further increased to 1:25 during the preliminary scale extraction of mangiferin from mango leaves.

# Conclusion

In conclusion, the valorization of bambangan by-products was successfully achieved through extraction of mangiferin using ultrasonic assisted extraction at the parameters of 50% ethanol, temperature of 50°C, 10 min of extraction, and a sample-solvent ratio of 1:20. The study demonstrated that the bambangan seed contain significantly more mangiferin (264.89 ± 5.67 mg/mL) as compared to bambangan peel (94.82 ± 1.49 mg/mL). These findings indicated that bambangan by-products can be an alternative source of mangiferin which has great potential applications in the food and pharmaceutical industries. In addition, the findings also confirmed that ultrasonic-assisted extraction is a powerful tool for the extraction of mangiferin from bambangan by-products which can be very useful for scaling up the UAE process for industrial applications.

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