

Microwave-assisted extraction of phenolic and flavonoid from *Morinda citrifolia* L. fruit

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Abstract

Morinda citrifolia L. has been used as a traditional medicine and health supplement. *Morinda citrifolia* L. is a tropical fruit with various bioactive components, including flavonoids and s, antimicrobials, anti-inflammatories, and anticancer agents. Bioactive materials can be extracted to obtain the desired product purity and properties. *Morinda citrifolia* L. fruit extraction using microwave-assisted extraction (MAE) can reduce the extraction time, heat the material quickly, and increase the extraction yield and product quality. Furthermore, MAE can minimize the use of solvents and, therefore, can be called a green process. The extraction of bioactive compounds from *M. citrifolia* L. fruit is essential to isolate specific antioxidants in high concentrations and to separate the targeted bioactive compounds from unwanted materials, such as fiber. This study aimed to examine how the extraction process impacts the total flavonoid and phenolic contents of the extract and to determine the process that yields the highest values for total flavonoid content (TFC) and total phenolic content (TPC). Experiments performed using *M. citrifolia* L. fruit extract indicated that the TPC and TFC values of the extract were affected by the ratio of fruit pulp and solvent, the type of solvent used for extraction, the duration of mixing needed to blend the fruit pulp and the solvent, and the length of microwave irradiation. The optimal conditions for extraction involved using a ratio of 5 parts fruit to 10 parts solvent, a solvent system comprising 70% ethanol in distilled water, a mixing time of 45 min, and an irradiation duration of 90 s. Under these optimal extraction conditions, the TFC was 16.1 ± 0.044 mg QE/g of solids, and the TPC was 21.04 ± 0.886 mg gallic acid equivalents/g of solids.

1. Introduction

Morinda citrifolia L. was designated as a food supplement, functional food ingredient, or natural health supplement as it contains phytochemicals with antioxidant, anticancer, antimicrobial (antibacterial and antifungal), antiviral, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing properties based on the study by López and Yahia (2011) and Assi *et al.* (2017). Furthermore, human preclinical and clinical safety trials investigating the consumption of *M. citrifolia* L. fruit found no adverse health effects, even at high doses (West *et al.*, 2006).

Phytochemicals can be extracted from *M. citrifolia* L. fruit to obtain and purify active substances, such as

antioxidants, which are beneficial for health. Antioxidants are chemical compounds that inhibit, stop, or control the oxidation of a substrate (Zeb, 2021). *Morinda citrifolia* L. fruit can be extracted using microwave-assisted extraction (MAE). The use of MAE can significantly reduce the extraction time and provide higher extraction results than ultrasound-assisted extraction, batch extraction, and conventional solvent extraction (Vetal *et al.*, 2014). MAE is used in the extraction of biomaterials because it shortens the extraction time, reduces solvent consumption, and minimizes environmental pollution owing to the transfer of clean energy to the matrix in a short time. In addition, MAE can increase the quantity and quality of the extraction results because the material can be heated

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quickly and processed at lower temperatures. In addition, the process delivers energy savings of up to 70% compared with conventional forms of energy derived from high energy density and direct absorption of energy by materials. Furthermore, it facilitates selective energy absorption owing to the dielectric properties of the material and applicator design (Veggi *et al.*, 2013).

The application of an electromagnetic field in MAE directly impacts the sample by boosting cell breakdown and subsequently leading to the release of substances into the solvent under milder extraction conditions, thereby reducing the degradation of sensitive compounds. Solvents that are compatible with MAE, including ethanol, water, or their mixtures, are all Generally Recognized as Safe (Figueroa *et al.*, 2021). The principle of MAE involves the heating of moisture within cells because of the direct impact of microwaves on water molecules via dipole polarization and ionic conduction. This process enhances the porosity or breakdown of the cell wall, which leads to the increased extraction of compounds (Araujo *et al.*, 2021).

Microwave-assisted solvent extraction involves using microwave energy to treat organic solvents (extractants) in contact with either dry or wet samples. The separation of the analytes from the sample matrix relies on the temperature and polarity of the solvent. The solvent absorbs the microwave energy, causing polar solvents such as ethanol, methanol, and water to heat up and boil. Subsequently, the solvents diffuse into the sample matrix, thereby dissolving the analyte. Heat transfer within the solid matrix occurs via conduction from the solvent. In this scenario, the mechanism of MAE is similar to that of conventional solid–liquid extraction. However, microwaves serve as a controllable and quickly adjustable energy source (Li *et al.*, 2013).

The drawbacks of MAE include (Ekezie *et al.*, 2017): the necessity of solvent removal from the material once the process is complete; MAE's restriction to polar extractants, which may limit its usage; the susceptibility of closed MAE systems to the thermal degradation of heat-sensitive compounds. Additionally, large volumes of solvent can lower recoveries and hinder microwave absorption by the material, as too much energy is absorbed by the solvent, resulting in reduced mass transfer.

In this research, various factors affecting MAE were examined, including different ratios of fruits and solvents, variations in mixing time, adjustments in ethanol as a solvent, and changes in the duration of microwave irradiation. The different variations tested were based on earlier studies by Hemwimon *et al.* (2007) on *M. citrifolia* roots, Kaderides *et al.* (2019) involving

pomegranate peels, Sonar and Rathod (2020) focusing on Aegle marmelos fruit, Baltacıoğlu *et al.* (2021) examining tomatoes, and Figueroa *et al.* (2021) studying avocado peel samples. Additionally, the study by Tasfiyati *et al.* (2022) using *M. citrifolia* L. powder samples also informed the choice of solvent.

This study investigated how the extraction process affects the total flavonoid content (TFC) and total phenolic content (TPC) of the extract. Additionally, it identified the extraction process that generates the highest TFC and TPC values.

2. Materials and methods

2.1 Chemicals and materials

Morinda citrifolia L. fruit, also known as noni fruit, is characterized by its fresh and ripe state, with a soft white skin emitting a distinct fruit odor. The fruit was collected from Vocational College, UGM, Yogyakarta, Indonesia. Absolute ethanol from the Supelco brand and distilled water were utilized as solvents. The analysis of TFC required aluminum trichloride, sodium nitrite, sodium hydroxide, and quercetin. The analysis of TPC involved the use of Folin–Ciocalteu's reagent, sodium carbonate, and gallic acid.

The *M. citrifolia* L. fruit extraction utilized a 1000 W microwave oven, specifically the Panasonic NN-GF560M (Shanghai). The *M. citrifolia* L. fruit was processed using a Philips Chopper Blender HR2939 to break down the fruit and prepare fruit pulp. The extracts were filtered using Whatman filter paper No. 41 (Whatman International Ltd., England) to obtain an extract free of solids. Mixing the fruit pulp with a solvent involved the use of a heating magnetic stirrer (Arec, Velp Scientifica). Furthermore, a visible spectrophotometer, specifically the Thermo Scientific Genesys 20, was employed for the TPC and TFC tests.

2.2 Sample preparation and extraction

The raw material used was ripened *M. citrifolia* L. fruit with white skin, soft texture, and a distinctive smell. The fruit was initially washed, then cut into 6 parts, and finally crushed using a chopper blender for 5 min. The resulting fruit pulp from this process was then stored in the freezer until the next step.

The *M. citrifolia* L. fruit was extracted using the MAE technique, which was based on and adapted from the method utilized by Sonar and Rathod (2020) and Baltacıoğlu *et al.* (2021). The extraction of *M. citrifolia* L. fruit was done using a microwave oven with a maximum power level of 1000 W and irradiation time as per the experimental design. The *M. citrifolia* L. fruit

pulp was dissolved in ethanol–distilled water in varying ratios (v/v) according to the experimental design and placed in a beaker. The ratio of fruit pulp/solvent (w/v) and the duration of mixing the fruit pulp with the solvent were varied based on the experimental design. The resulting extract was filtered using Whatman filter paper no. 41. The results of the extraction were then kept in a sealed container in the fridge until the next stage was executed.

2.3 Experimental design

2.3.1 Impact of the extraction process on the total flavonoid content and total phenolic content

Four variation factors in the extraction process were tested on the samples to determine their effect on the TFC and TPC values of the samples. The four variation factors in the extraction process were the ratio of fruit and solvent, use of solvent, mixing time of fruit and solvent, and microwave irradiation duration. When one extraction factor was adjusted, the remaining factors remained constant. The constants in the extraction factors included a fruit pulp to solvent ratio of 5 g to 10 mL, a mixing duration of 45 min, a solvent concentration of 70% ethanol, and a microwave irradiation period of 90 min.

2.3.2 Proportion of *Morinda citrifolia* L. fruit and solvent

A comparison was made between the *M. citrifolia* L. fruit pulp and the solvent, considering the weight (g) per volume (mL) ratios of the *M. citrifolia* L. fruit pulp to be extracted: 1:20, 1:10, 3:10, and 5:10. The best proportion was selected based on the quantities of TFC and TPC extracted. The change in the proportion of fruit to solvent was based on the study by Sonar and Rathod (2020), which involves the extraction of *Aegle marmelos* fruit using MAE, as well as the research conducted by Figueroa et al. (2021), specifically focusing on MAE applied to avocado peel.

2.3.3 Mixing time

Before the microwave extraction process, the fruit pulp and solvent were agitated using a magnetic stirrer at 1,000 rpm for 15, 30, 45, and 60 min. There was no heat application during the mixing process. The mixing occurred at a typical room temperature. The optimal mixing time was determined based on the TFC and TPC extracted. The variation of mixing time between fruit pulp and solvent was adapted from Sonar and Rathod's (2020) research on *Aegle marmelos* fruit.

2.3.4 Solvent

In MAE, the solvent used was a blend of ethanol and

distilled water, with varying ratios of 75:25, 70:30, 65:35, 60:40, 55:50, and 50:50. The optimal solvent variation was selected based on the extracted TFC and TPC amounts. Variations in the ratio of ethanol and distilled water used as MAE solvents were adopted from MAE conducted by several researchers, namely Hemwimon et al. (2007) on *M. citrifolia* roots, Kaderides et al. (2019) on pomegranate peels, Sonar and Rathod (2020) on *Aegle marmelos*, and Figueroa et al. (2021) on avocado peels. Water and organic solvents, like ethanol, are commonly utilized in evaluating the extraction efficiency of phenolic compounds (Zhu et al., 2020). Tasfiyati et al. (2022) also used 70% ethanol as a solvent to extract *Morinda citrifolia* L. powder.

2.3.5 Duration of microwave irradiation

The duration of irradiation refers to the period for which the sample was subjected to microwave heating. The options considered were 60, 90, and 120 s. Once irradiation was finished, the sample's temperature was measured. Based on the quantities of TFC and TPC obtained, the most favorable outcomes from the extraction duration were chosen. The changes in microwave irradiation duration were based on the studies conducted by Sonar and Rathod (2020) regarding the MAE of *Aegle marmelos* and by Baltacıoğlu et al. (2021) regarding the MAE of tomato.

2.3.6 Total flavonoid content measurement

The aluminium chloride method was used to analyze the TFC (Koley et al., 2019). In a 10 mL volumetric flask, 1 mL of the sample extract was combined with 4 mL of distilled water, 0.3 mL of 5% NaNO₂, and 0.3 mL of 10% AlCl₃.6H₂O. After allowing the mixture to stand for 6 min at room temperature, 2 mL of 1 mol NaOH was added, and the solution was diluted to 10 mL with distilled water. The absorbance of the solution was measured at 510 nm against a reagent blank. The reagent without a sample served as the blank.

2.3.7 Total phenolic content measurement

The Folin–Ciocalteu method was used to determine the TPC of the samples. This method was derived from the technique employed by Kaderides et al. (2019). After adding 0.2 mL of the sample to a 25-mL volumetric flask, distilled water was added to reach a final volume of 10 mL. Subsequently, 0.5 mL of Folin–Ciocalteu phenol reagent was vigorously mixed with the mixture. Then, 5 mL of the 5% Na₂CO₃ solution was gradually added while stirring, and the solution was further diluted to 25 mL with distilled water. After thorough stirring, the solution was left for 90 min. The absorbance was then measured at 750 nm using a UV-Vis spectrophotometer, specifically the Genesys 20 by Thermo Scientific. Blank

samples contained distilled water. The TPC of the samples was quantified in terms of mg/mL gallic acid equivalents (GAE).

The calibration standard for gallic acid was altered using the method described by Waterhouse (2001). To prepare the calibration standard, 0.5 g of gallic acid was dissolved in 10 mL of ethanol, and then the solution was diluted to 100 mL with distilled water to achieve a concentration of 5 g/L. Standards with concentrations of 50, 100, 250, and 500 mg/L were prepared by diluting 1, 2, 5, and 10 mL, respectively, to 100 mL with distilled water.

2.3.8 Statistical analysis

Statistical analysis was performed using the SPSS 27.0 software by IBM. The analysis involved a one-way analysis of variance (ANOVA) and Duncan's multiple range test (P -value < 0.05) to assess the differences among the tested samples (Chen *et al.*, 2020).

3. Results and discussion

The recovery of target compounds from the sample matrix depends on the extraction process. Several factors, including the solvent composition, irradiation time, and solvent-sample ratio, affect the efficiency of this process, and their effects can be either independent or interactive. The research utilized fresh, ripened fruit as the raw material. *Morinda citrifolia* L. fruit juice, which was obtained by squeezing *M. citrifolia* L. pulp, yielded a TFC value of 0.503 ± 0.006 mg QE/mL or 1.01 ± 0.012 mg QE/g solids and a TPC of 1.55 ± 0.045 mg GAE/mL or 1.19 ± 0.019 mg QE/g solids. Here, solid refers to *M. citrifolia* L. fruit pulp. Singh (2013) observed that the TPC in the raw fruit was 213.4 ± 3.7 mg GAE/100 g and that in the ripened fruit was 250.7 ± 7.2 mg GAE/100 g. An investigation by Dussossoy *et al.* (2011) in *M. citrifolia* L. fruit juice revealed a total polyphenol content of 47.6 ± 2.0 mg GAE/100 g fresh weight. In a study conducted by Lin *et al.* (2013), fermented and pasteurized *M. citrifolia* L. fruit juice was analyzed using high-performance liquid chromatography. The analysis revealed a phenolic acid quantity of 59.41 ± 1.22 mg/100 mL of fruit juice and a flavonoid quantity of 10.61 ± 0.04 mg/100 mL of fruit juice. After reviewing the TPC and TFC test results of the raw materials utilized in this study and comparing them with findings from other researchers' work, the *M. citrifolia* L. fruit employed was determined to be of sufficient quality for extraction.

3.1 Effect of the extraction process on total flavonoid content

The TFC of the *M. citrifolia* L. extract was determined using quercetin as the standard. A linear

correlation was noted between the quercetin concentration (ppm) and its absorbance when measured on a 510 nm UV-Vis spectrophotometer. For quercetin concentrations of 100–500 ppm, the linear regression equation was $y = 0.001064x - 0.01113$, with an R^2 of 0.9860, within the absorbance range of 0.105–0.537. With quercetin levels of 5–100 ppm, the linear regression formula was $y = 0.0008125x + 0.0006369$, with an R^2 of 0.9952, within the absorbance range of 0.004–0.083. The TFC in the extraction results in mg quercetin equivalent/mL was determined using the linear regression formula. The formula was adjusted to the sample absorbance results obtained using UV-Vis spectrophotometry at 510 nm.

3.2 Effect of fruit pulp and solvent ratio on total flavonoid content

The fruit was mashed into a pulp to increase the surface area available for reacting with the solvent in the extraction process. The ratio of fruit pulp (in g) to solvent (in mL) was investigated to obtain the best ratio for extracting fresh noni fruit. Four fruit pulp-to-solvent ratio variations were selected, namely, 1 g:20 mL, 1 g:10 mL, 3 g:10 mL, and 5 g:10 mL. The highest TFC value of 0.351 ± 0.002 mg QE/mL was achieved when comparing fruit pulp and extraction solvent at a ratio of 5 g:10 mL solvent, and this result was significantly different from the other ratios. For this experimental design, the extraction conditions were as follows: the solvent used was 70% ethanol in distilled water, the mixing time using a magnetic stirrer was 45 min, and the irradiation time was 90 s. Sonar and Rathod (2020) documented that bioactive substances are likely to move from the plant matrix (feed) into the solvent via dissolution and diffusion. Using too much solvent hinders the ability of the microwaves to penetrate deeply into the solvent, as the energy is absorbed in the outer layer of the solvent. Figure 1 displays the TFC value result of another ratio. Each treatment variation was tested in duplicate, and different letter notations indicate significant differences at the 0.05 significance level.

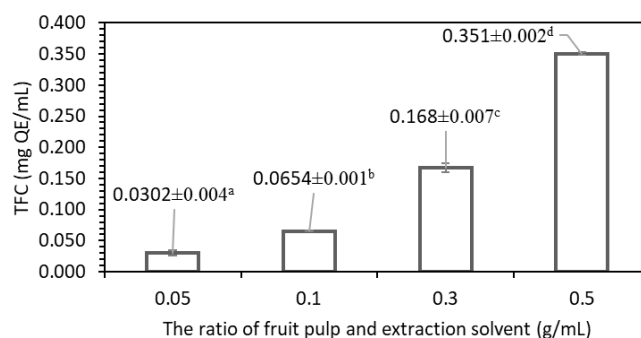


Figure 1. Effect of the ratio of fruit pulp to solvent on the TFC. Values are presented as mean $\pm$ SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

Figure 2 shows the influence of the ratio of fruit pulp and solvent on the TFC in the extracted solids. The highest TFC was obtained when using a ratio of 5 parts fruit pulp (5 g) to 10 parts solvent (10 mL), which amounted to 0.701 ± 0.005 mg QE/g of the extracted solids. Duplicate testing was performed for each treatment variation. Statistically, it exhibited a significance level of 0.123 (Figure 2).

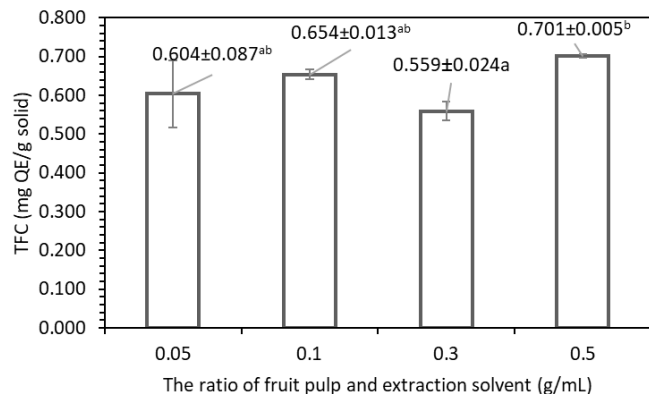


Figure 2. Influence of the ratio of fruit pulp to solvent on the TFC. Values are presented as mean $\pm$ SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

The highest TFC value was obtained when using a fruit pulp and solvent ratio of 5:10; hence, this ratio was applied during extraction while varying the irradiation time, mixing time of the fruit pulp and solvent, and identifying the solvent combination. A large volume of solvent will reduce the adsorption of materials against microwaves because more energy must be absorbed by the solvent (Kaderides et al., 2019).

3.3 Influence of the duration of mixing on the total flavonoid content

The time required to blend the fruit pulp with the solvent using a magnetic stirrer is known as the mixing time. Optimal TFC was observed at stirring times of 45 and 60 min, with no significant difference between them. Specifically, the levels were 0.342 ± 0.004 and 0.342 ± 0.017 mg QE/mL, respectively. Various letter notations were used to indicate considerable differences at the 0.05 significance level. The trial involved conducting two tests for every type of treatment. Figure 3 suggests that the TFC will increase as the mixing time is extended. Soaking the plant material for a specific duration before microwaving leads to sufficient swelling of the plant matrix, causing an increase in the water content within the cell. This, in turn, aids in rupturing the cell walls owing to thermal stress and enlarges the cellular pores, which allows the leaching of the target analytes and ultimately improves extraction efficiency (Sonar and Rathod, 2020).

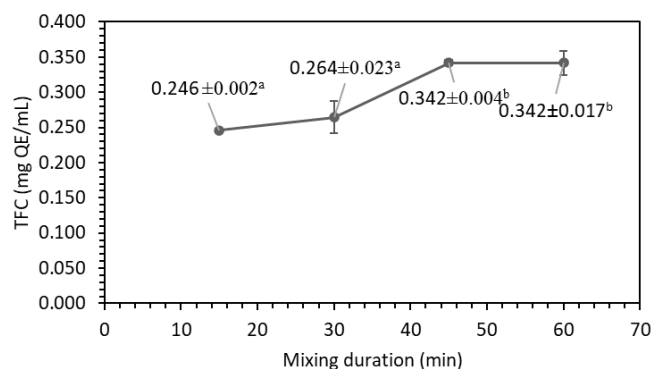


Figure 3. Influence of the duration of mixing on the TFC. Values are presented as mean $\pm$ SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

3.4 Influence of solvent concentration on total flavonoid content

The TFC in *M. citrifolia* L. fruit extract was affected by the concentration of the solvent, which was a mixture of ethanol and water. When 75% ethanol was used as the solvent, the highest TFC of 0.296 ± 0 mg QE/mL was obtained. Nonetheless, there was no statistically significant difference in the TFC value compared with the use of 70% ethanol, which resulted in a TFC of 0.283 ± 0.001 mg QE/mL. In the study by Tasfiyati et al. (2022), 70% ethanol in distilled water was used for the extraction of scopoletin from *M. citrifolia* fruit. Figure 4 shows that increasing the amount of ethanol resulted in a higher value of TFC. The efficiency of the extraction increased with the addition of small amounts of ethanol owing to the increase in polarity. Enhancing the polarity of ethanol leads to greater mass transfer and extraction yield (Sonar and Rathod, 2020). Distinct letter symbols were used to signify noticeable variances at the 0.05 significance level. The experiment included two tests for each treatment variation. The irradiation time used in this experiment was 90 s, the stirring time was 45 min, and the ratio of the fruit pulp to the solvent was 5:10.

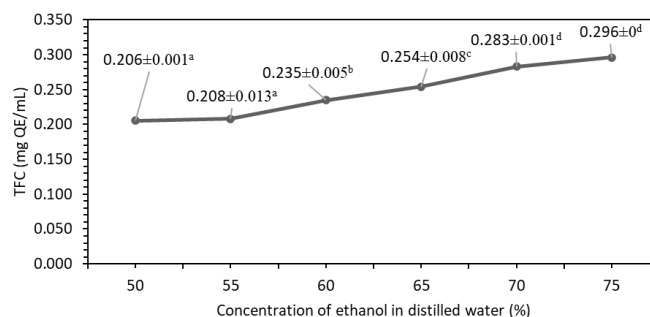


Figure 4. Influence of ethanol concentration in distilled water as the extraction solvent on the TFC. Values are presented as mean $\pm$ SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

3.5 Effect of the duration of exposure to microwave irradiation on the total flavonoid content

The time of microwave irradiation refers to the duration of heating applied to a sample in a solvent. The TFC value was the highest at 90 s of irradiation, specifically 0.357 ± 0.002 mg QE/mL, and it showed a statistically significant difference compared with the values at 60 and 120 s of irradiation. Each treatment variation was tested in duplicate. At the 0.05 significance level, various letter notations showed notable differences (Figure 5). The solvent used for extraction was 70% ethanol in distilled water, with a stirring time of 45 min, and the ratio of fruit pulp and solvent was 5:10. These findings agree with those of Odabaş and Koca (2021), who observed that excessive heating with prolonged extraction times could degrade the flavonoids. The temperature range detected after microwave irradiation was 75–76°C. As noted by Motshakeri and Ghazali (2015), around 15–20% of the original total polyphenol content in Noni juice was diminished following heating at 65 or 75°C for 24 h. Additionally, higher heating temperatures during processing may cause the conversion of flavonoid glycosides into various forms, which could decrease their antioxidant effectiveness. As a result, the temperature attained after irradiating the sample in the microwave will not harm the phenols or flavonoids present, since the changes in irradiation duration in this research were conducted in seconds.

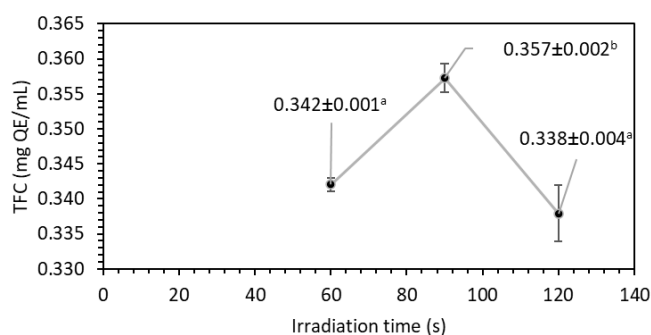


Figure 5. Effect of microwave irradiation time on the TFC. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

3.6 Effect of the extraction process on total phenolic content

The UV-Vis spectrophotometer was used to determine the TPC in *M. citrifolia* L. fruit extract at 750 nm. A standard gallic acid content curve was used to assess the TPC in the *M. citrifolia* L. fruit. The equation $y = 0.002617x + 0.009333$ ($R^2 = 0.9961$) was derived from gallic acid concentrations of 10–60 ppm, and the absorbance ranged from 0.036 to 0.138. For gallic acid concentrations of 50–2500 ppm, the equation $y = 0.0007502x + 0.07428$ ($R^2 = 0.9997$) was found, with an

absorbance range of 0.139–1.939.

3.7 Effect of the ratio of fruit pulp and solvent on the total phenolic content

The TPC increased as the ratio of fruit pulp to solvent increased, which indicated a positive relationship between the two variables (Figure 6). The operating conditions used were 70% ethanol in distilled water, an irradiation time of 90 s, and a mixing time of 45 min. If the letters were not identical, it indicates that there were important variations at the 0.05 significance level. Testing was conducted in duplicate for each treatment. The viscosity of the extraction medium decreased as the liquid-to-solid ratio increased, leading to an improved diffusion rate because of the greater concentration gradient between the solvent and the solid (Odabaş and Koca, 2021).

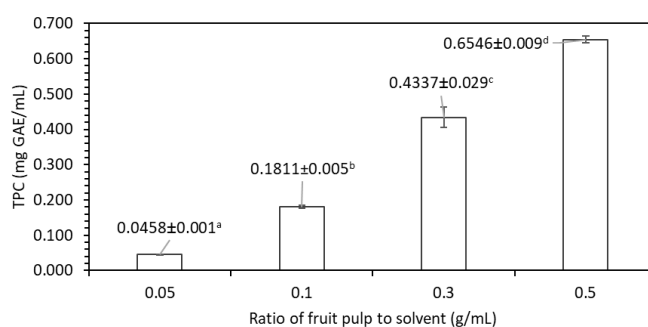


Figure 6. Effect of the ratio of fruit pulp to solvent on the TPC. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

The effect of the ratio of fruit pulp and solvent on the TPC of the extracted solids is illustrated in Figure 7. The TPC of the extracted solids was the highest when the ratio of fruit pulp to solvent was 1:10, specifically 1.811 ± 0.047 mg GAE/g extracted solids. Based on the statistical analysis, there was a significant difference between the 1:10 ratio and the 1:20, 3:10, and 5:10 ratios (Figure 7). Thoo *et al.* (2010) also used a ratio of 1 part *M. citrifolia* L. fruit to 10 parts solvent in their study. The dried fruits of *M. citrifolia* L. (2 g) were extracted with solvent (20 mL). The values obtained were 919.95 mg GAE/100 g dry weight for TPC and 472.73 mg CE/100 g dry weight for TFC. In this study, the TPC value at a fruit and solvent ratio of 1:10 was 1.811 mg GAE/g solids or 181.1 mg GAE/100 g solids and showed the highest results among the various ratio variations. The increase in the extraction yield depends on the mass transfer phenomenon that occurs during the process. When more solvent is used, the concentration difference between the solvent and the solid increases, which drives the mass transfer (Sonar and Rathod, 2020). Distinct letters denote notable variations at the 0.05 significance level (Figure 7). The experiment was performed twice for each treatment.

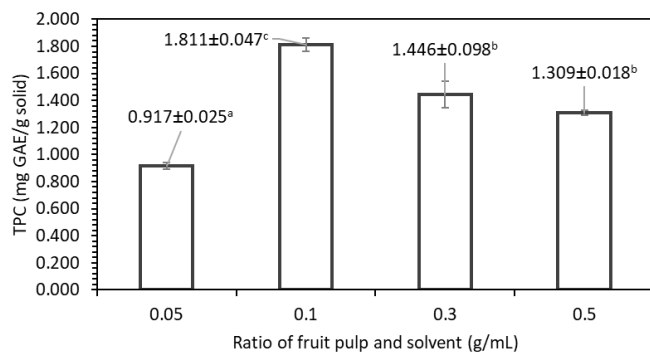


Figure 7. Effect of the ratio of fruit pulp to solvent on the TPC. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

3.8 Influence of mixing time on total phenolic content

Mixing was performed to mix the solvent with *M. citrifolia* L. fruit. The *M. citrifolia* L. fruit was first ground using a blender to increase the surface area of the fruit in contact with the solvent. Mixing was carried out at normal room temperature. Mixing was done using a magnetic stirrer before irradiation using a microwave (90 s). The solvent used for extraction was 70% ethanol, with variations in fruit pulp (g) and solvent (mL) of 5:10. The differences in mixing time revealed that a mixing time of 45 min for the fruit slurry mixed with the solvent resulted in the highest TPC, which was 0.703 ± 0 mg GAE/mL. Statistically, the ANOVA test indicated that at 45 min of mixing, the TPC significantly differed from those at stirring times of 15, 30, and 60 min. Distinct letters indicate substantial variances at the 0.05 significance level (Figure 8). The treatment was performed twice for each experiment. Thoo *et al.* (2010), through their studies, have concluded that the length of the extraction period was crucial in extracting phenolic compounds, as the concentrations of these compounds at equilibrium may influence their apparent reduction. Consequently, prolonged extraction times could lead to a decrease in the yield of phenolic compounds. The differences in optimal extraction times for TPC and TFC may be due to varying degrees of phenolic polymerization, the solubility of the phenolic compounds, and their interactions with other nutrients, which ultimately impact the time necessary to reach equilibrium between the solution within the sample and the surrounding solution (solvent).

3.9 Influence of extraction solvent concentration on total phenolic content

A mixture of ethanol and distilled water was the solvent employed for *M. citrifolia* L. fruit extraction. The highest value for the TPC was obtained with a 70% ethanol concentration, specifically 0.7248 mg GAE/mL. The extraction conditions used were a mixing time of 45 min, a microwave irradiation time of 90 s, and a ratio of

fruit pulp to solvent of 5:10. Different letter notations indicate significant differences at a significance level of 0.10 (Figure 9). Including water in a solvent typically results in a more polar environment, enhancing the extraction of polyphenols (Kaderides *et al.*, 2019). Kaderides *et al.* (2019) and Sonar and Rathod (2020) reported that the high dielectric constant of water and dielectric loss factor make it better at absorbing microwave energy compared with ethanol. When subjected to the same microwave output power intensity, water could potentially absorb more thermal energy than ethanol, thus facilitating the diffusion of the target component. Furthermore, it enhances the moisture content, causing the plant material to swell adequately, which leads to the bursting of cells under increased thermal stress, thereby releasing phytochemicals into the extracting solvent.

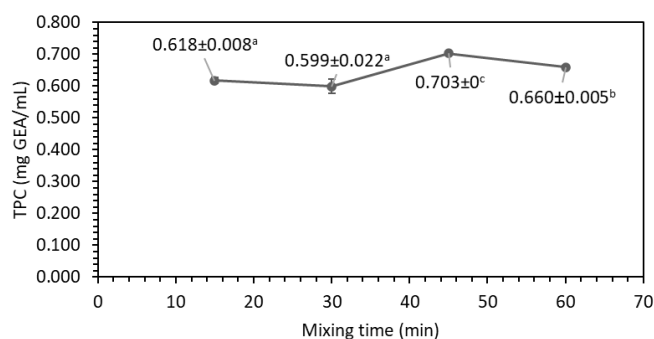


Figure 8. Influence of the duration of mixing on the TPC of the extract. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

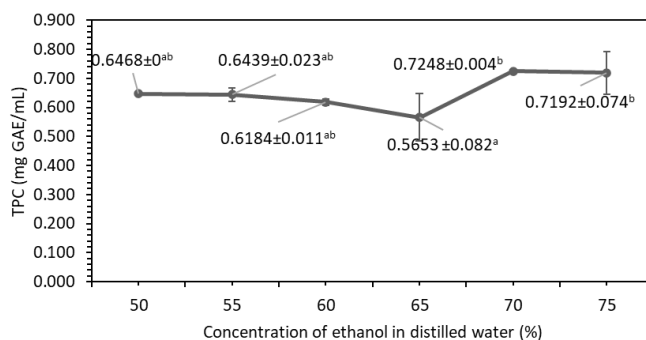


Figure 9. Influence of varying the concentration of ethanol in distilled water on the TPC. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

3.10 Effect of microwave irradiation duration on the total phenolic content

The highest TPC value of 0.666 ± 0.009 mg GAE/mL was observed at a microwave irradiation duration of 90 s. A maximum microwave power of 1000 W was utilized. In this experiment, 70% ethanol in distilled water was used, the duration of mixing was 45 min, and the ratio of fruit pulp and solvent was 5:10. According to the

ANOVA test, there were significant differences in the duration variations of 90 s and 120 s. In contrast, 60 s and 90 s did not show significant differences. The extracting solvent heats up as it absorbs the microwave energy. Molecular interactions efficiently transfer this energy to the plant material, which ultimately improves the dissolution of the sample. The gradual reduction in the yield could be attributed to the degradation of the compound because of prolonged exposure to microwave irradiation (Sonar and Rathod, 2020). The use of the same notation indicates that the values were not statistically different at the 0.05 significance level (Figure 10). Duplicate testing was done for each treatment.

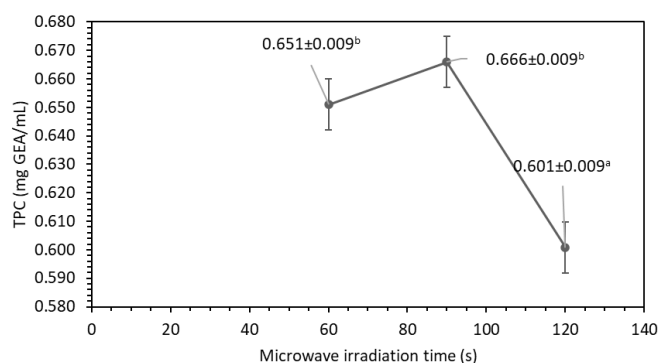


Figure 10. Effect of microwave irradiation time on TPC. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

3.11 Optimal extraction process

The extraction process was selected based on the measurement results for the TFC and TPC. The fruit pulp and solvent were in a proportion of 5:10, which indicates that there were 5 g of fruit pulp in 10 mL of solvent. The solvent used for extraction was a mixture of 70% ethanol and distilled water. Mixing the fruit pulp and solvent took 45 min, and the fruit pulp was subjected to microwave irradiation for 90 s.

The *M. citrifolia* L. fruit extract obtained using optimized methods contained 0.2227 ± 0.009 mg QE/mL of TFC or 0.4453 mg QE/g of solids and 0.596 ± 0.009 mg GAE/mL of TPC, which was equivalent to 1.19 ± 0.019 mg GAE/g of solids. After evaporating the solvent using a rotary evaporator, the extract exhibited a TFC of 8.06 ± 0.022 mg QE/mL or 16.1 ± 0.044 mg QE/g of solids and a TPC of 10.29 ± 0.276 mg GAE/mL or 21.04 ± 0.886 mg GAE/g of solids.

The obtained TFC and TPC values are closely aligned with those from the extraction of *M. citrifolia* L. fruit by Thoo et al. (2010) and Krishnaiah et al. (2015). For instance, the TPC of the *M. citrifolia* L. extract prepared by Krishnaiah et al. (2015) via high-pressure extraction was 43.18 mg GAE/10 g. The research conducted by Thoo et al. (2010) determined that the

optimal conditions for extracting dried *M. citrifolia* L. fruit powder included a powder-to-solvent ratio of 2 g to 20 mL, 40% ethanol solvent, an extraction time of 80 min, and a temperature of 65°C in a water bath shaker. This process resulted in a TFC of 472.73 mg CE/100 g dry weight and a TPC of 919.95 mg GAE/100 g dry weight.

Chen et al. (2009) and Ruhomally et al. (2016) conducted a comparative study. In the study of Chen et al. (2009), *M. citrifolia* L. fruit was extracted using the near supercritical fluid carbon dioxide extraction method, which resulted in an extract with the highest TPC value of 14.82 ± 0.83 mg/g. Ruhomally et al. (2016) reported that the TPC of ripened *M. citrifolia* L. fruit extract was 748.40 ± 8.85 µg GAE/g fresh weight and that of the raw fruit was 770.34 ± 2.27 µg GAE/g fresh weight.

MAE serves as a favorable substitute for conventional solvent extraction because it induces morphological changes that enhance mass transfer, resulting in the extraction of a greater quantity of phenolic compounds in a shorter timeframe (Baltacıoğlu et al., 2021). Kaderides et al. (2019) explained that in MAE, heat and mass transfer both happen from the inside to the outside, which hastens the solubilization of the solutes. The extraction system absorbs microwave energy, leading to the accumulation of heat in the extraction solution, which causes the phenolics to dissolve in the solution.

According to the research conducted, the MAE method can be utilized to extract phenolic and flavonoid compounds from *M. citrifolia* L. fruit. One of the benefits of employing MAE is that it shortens extraction time and reduces the amount of solvent used. However, the drawbacks of MAE include the susceptibility of closed MAE systems to the thermal degradation of sensitive compounds; it can only use polar extractants; and there is a need to remove the solvent from the substrate once the process is complete.

4. Conclusion

Based on the findings of the research, the MAE technique can be used to extract phenolic and flavonoid compounds from the fruit of *M. citrifolia* L. The benefits of using MAE include a considerable decrease in extraction duration and a reduction in the amount of solvent needed. Conversely, the drawbacks of MAE involve the susceptibility of closed MAE systems to the thermal degradation of sensitive compounds; the restriction to polar solvents; and the requirement to remove the solvent from the substrate once the process is complete. The tests conducted on *M. citrifolia* L. fruit

extract demonstrate that the TPC and TFC values of the extract are influenced by the ratio of fruit pulp and solvent, the mixing time required to mix the fruit pulp and solvent, the composition of the solvent used for extraction, and the microwave irradiation duration. The optimal extraction process was determined based on the results of TPC and TFC, wherein the ratio of the fruit pulp to the solvent was 5:10, the solvent used for extraction was 70% ethanol in distilled water, the time taken to combine the fruit pulp and the solvent was 45 min, and the duration of exposure of the fruit pulp and solvent to microwave irradiation was 90 s. After removing the solvent with a rotary evaporator, the resulting extract showed a TFC of 8.06 ± 0.022 mg QE/mL or 16.1 ± 0.044 mg QE/g of solids and a TPC of 10.29 ± 0.276 mg GAE/mL or 21.04 ± 0.886 mg GAE/g of solids.

Conflict of interest

The authors declare no conflict of interest.

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