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Subcritical Water Extraction (SWE) and Ultrasonic-Assisted Extraction (UAE) of Bioactive Compounds from *Morinda citrifolia*: Comparative Evaluation of Process Efficiency

(Pengekstrakan Air Subkritikal (SWE) dan Pengekstrakan Berbantukan Ultrasonik (UAE) Sebatian Bioaktif daripada *Morinda citrifolia*: Penilaian Perbandingan Kecekapan Proses)

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ABSTRACT

Morinda citrifolia has garnered significant interest from researchers in the food and pharmaceutical industries. Despite this interest, research on extracting its beneficial components using green technologies remains limited. This study aims to investigate the effect of two extraction techniques namely subcritical water extraction (SWE) and ultrasonic-assisted extraction (UAE) on the total phenolics content and antioxidant activity of *M. citrifolia* leaf extracts using response surface methodology (RSM). A three-level factorial central composite design (CCD) was applied to determine the effect of three independent variables for each method of UAE (X_1: citric acid concentration, X_2: extraction time, X_3: ultrasonic amplitude) and SWE (X_1: operating temperature, X_2: extraction time, X_3: solid loading) on three dependent variables (Y_1: total phenolic content (TPC), Y_2: free-radical scavenging activity (DPPH), Y_3: ferric reducing antioxidant activity (FRAP)). The optimum value of TPC and DPPH by UAE are 48.25 mg GAE/g and 22.59% inhibition, respectively, which are lower than the optimal values obtained by SWE method, with respective values of 64.96 mg GAE/g and 80.75% inhibition. However, the FRAP value possessed by UAE was higher compared to SWE, with respective values of 29.63 mg GAE/g and 11.33 mg GAE/g. The results indicate that extraction temperature ($p<0.05$) was the most significant factor that enhances phenolic yield and antioxidant activity in MC leaves using SWE, while citric acid concentration notably influences UAE extracts the most. The UAE demonstrated higher process efficiency compared to SWE. However, due to the use of samples with significantly different sizes in the evaluation, further analysis is needed to make a definitive comparison of process efficiency between the two methods.

Keywords: Antioxidant activity; hydrothermal processing; medicinal plant; Morinda; total phenolic content

ABSTRAK

Morinda citrifolia telah berjaya menarik minat para penyelidik dalam industri makanan dan farmaseutikal kerana potensi pengaplikasiannya dalam bidang perubatan. Walau bagaimanapun, penyelidikan tentang pengekstrakan komponen yang bermanfaat daripada tumbuhan ini menggunakan teknologi hijau masih terhad. Penyelidikan ini bertujuan untuk mengkaji kesan dua teknik pengekstrakan yang berbeza, iaitu pengekstrakan air subkritikal (SWE) dan pengekstrakan dengan bantuan ultrasonik (UAE) terhadap kandungan total fenol dan aktiviti antioksidan ekstrak daun *M. citrifolia* menggunakan kaedah rangsangan permukaan (RSM). Satu reka bentuk komposit putaran tengah (CCD) tiga aras telah digunakan untuk menentukan kesan tiga pemboleh ubah bebas bagi setiap kaedah UAE (X_1: kepekatan asid sitrik, X_2: masa pengekstrakan, X_3: amplitud ultrasonik) dan SWE (X_1: suhu pengekstrakan, X_2: masa pengekstrakan, X_3: beban pepejal sampel) terhadap pemboleh ubah berbalas (Y_1: kandungan total fenol (TPC), Y_2: aktiviti penangkapan radikal bebas (DPPH), Y_3: aktiviti pengurangan ferik antioksidan (FRAP)). Nilai optimum TPC dan DPPH bagi UAE masing-masing ialah 48.25 mg GAE/g dan 22.59% perencatan, lebih rendah berbanding nilai optimum yang diperoleh dengan kaedah SWE, masing-masing sebanyak 64.96 mg GAE/g dan 80.75% perencatan. Walau bagaimanapun, nilai FRAP oleh UAE adalah lebih tinggi berbanding SWE, dengan nilai masing-masing 29.63 mg GAE/g dan 11.33 mg GAE/g. Hasil kajian menunjukkan bahawa

suhu pengekstrakan ($p<0.05$) adalah faktor yang paling signifikan yang meningkatkan hasil fenol dan aktiviti antioksidan dalam daun MC menggunakan SWE, manakala kepekatan asid sitrik adalah faktor paling berpengaruh untuk ekstrak UAE. UAE menunjukkan kecekapan proses yang lebih tinggi berbanding SWE. Walau bagaimanapun, disebabkan penggunaan sampel dengan saiz yang berbeza secara signifikan dalam penilaian tersebut, analisis lanjut diperlukan untuk membuat perbandingan yang lebih muktamad mengenai kecekapan proses antara kedua-dua kaedah.

Kata kunci: Aktiviti antioksidan; jumlah kandungan fenol; pemprosesan hidrotermal; Morinda; tumbuhan perubatan

INTRODUCTION

Morinda citrifolia or 'mengkudu besar', is a perennial plant of Southeast Asia that has been used for over 2000 years. It has garnered attention in the pharmaceutical and food industries due to the diversity and therapeutic suitability of its plant structures. The primary industrial products derived from this plant include beverages (juice), powder (from dried fruits), oil (from seeds), and leaf powder (Almeida, de Oliveira & Hotza 2019). Furthermore, *M. citrifolia* leaves are widely utilized in traditional medicine in several countries worldwide. It is employed as a supplement and alternative treatment due to its perceived health benefits (Lohani et al. 2019). Chemical and food analyses have identified the presence of over 200 phytochemicals and bioactive compounds in every part of *M. citrifolia* plants. These include acids, alcohols, phenols, sugars, anthraquinones, carotenoids, esters, triterpenoids, flavonoids, glycosides, lactones, iridoids, ketones, lactones, lignans, nucleosides, triterpenes, sterols, and aromatic compounds. The biological and phytotherapeutic applications of *M. citrifolia* are seen as highly beneficial. Therefore, more extensive and comprehensive studies regarding this plant and advancements in processing and standardizing products derived from it are needed (Almeida, de Oliveira & Hotza 2019). Some latest literature on the bioactive compounds extraction from *M. citrifolia* was conducted mostly using its fruits (Fontes et al. 2023; Jamaludin et al. 2021; Nuengchamnong et al. 2023) and leaves (Fontes et al. 2023; Zhu et al. 2020).

Extraction is the initial step and the most crucial step in phytochemical processing to identify, separate, and isolate desired bioactive compounds from plant materials (Belwal et al. 2018). In recent years, modern extraction methods, also considered 'green extraction methods', have garnered attention due to their high extraction yields, selectivity, extract stability, and safety during processing (Zhang, Lin & Ye 2018). These modern methods include ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and subcritical water extraction (SWE). Moreover, compared to conventional methods, these modern techniques offer benefits such as lower use of organic solvents and shorter extraction times. Therefore, extracting bioactive compounds from *M. citrifolia* leaves can be optimized by selecting appropriate extraction methods.

Two extraction methods were chosen to extract bioactive compounds from *M. citrifolia* leaves in this study which are UAE and SWE. Ultrasonic extraction, also known as sonication extraction, is a green technique that utilizes ultrasonic waves to facilitate extraction involving cavitation processes that accelerate solvent dissolution and solute absorption, as well as heat transfer, thereby enhancing extraction efficiency (Zhang, Lin & Ye 2018). It was considered green because it can operate at lower temperatures and often uses less solvent, reducing environmental impact. The latest study on the UAE of bioactive compounds from *M. citrifolia* leaves was reported by Lima et al. (2018), Pak-Dek et al. (2011) and Zhu et al. (2020). However, among the green extraction techniques invented, SWE is considered more environmentally friendly because it uses water as a solvent, avoiding the need for harmful chemicals. SWE using distilled water as a solvent utilizes the unique properties of subcritical water, which is water remains in its liquid state when heated above its boiling point at high temperatures and pressures below the critical point (374 °C, 22.1 MPa) (Zhao et al. 2019). Under subcritical conditions, the properties of water are nearly like those of organic solvents due to the reduction in its dielectric constant value when water is heated above boiling point, resembling other organic solvents' dielectric constant value. This allows water to dissolve compounds with low polarity (Cheng et al. 2021; Munir et al. 2018; Zhang et al. 2020). Although SWE operates at high temperatures and pressures, advancements in technology have made it more energy-efficient compared to traditional methods such as maceration, percolation, and reflux extraction. Hence, this study aimed to compare the effects of these two different green techniques on the total phenolic contents and antioxidant activity of *M. citrifolia* leaf extracts and evaluate their process efficiencies.

MATERIALS AND METHODS

SAMPLE PREPARATION

The *M. citrifolia* leaves, identified with voucher number 10058/2022, were sourced from Universiti Kebangsaan Malaysia, Bangi Selangor. The leaves were initially washed and dried in incubator (Memmert INBN02) at 60 °C for 48 h. Following drying, the leaves were ground into a fine powder and passed through a 250 mm sieve. The powdered leaves were then stored at -20 °C, protected from light and moisture, until further use. All chemicals

used in the study, including carboxylic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder, acetate buffer, 2,4,6-Tripyridyl-S-triazine (TPTZ) powder, hydrochloric acid, Folin reagent-Ciocalteu, and sodium carbonate, were of analytical grade and sourced from reputable suppliers such as Sigma-Aldrich (USA) and Merck (Germany).

EXPERIMENTAL DESIGN

Both UAE and SWE were conducted based on Response Surface Methodology (RSM) using a three-level factorial central composite design (CCD). Twenty experimental points consisted of 14 trials and 6 replicates at the centre point were generated by Stat-Ease Design Expert Software 13 using the experimental parameters shown in Table 1 for both method of extraction. Total phenolic content (TPC) and antioxidant activity through DPPH and FRAP method was chosen as the dependent variables in the design. The response optimizer was utilized for both graphical and numerical optimizations to determine the optimal conditions for the response variables.

SUBCRITICAL WATER EXTRACTION (SWE)

The subcritical water extraction (SWE) was conducted in a batch-type oil bath reactor (Thomas Kagaku Co. Ltd, Japan) as shown in Figures 1 and 2. Silicone oil is used for an oil-bath reactor and the heating rate transfer from the oil bath into the reactor is assumed to be constant throughout the process until reaching a steady state. The stainless-steel reactor with a 2 cm cap diameter and 17 cm length (Swagelok, Malaysia) was filled with dried *M. citrifolia* leaf powder and distilled water at a specific solid loading before being purged with argon gas for 1 min to remove the air in the reactor. The cap of the reactor was then tightly clamped before it was put in the oil bath at different process temperature and time. After the extraction process was completed, the reactor was immediately cooled down by putting it in running water to stop the reaction. The obtained hydrolysate was centrifuged (Hettich® Universal 320/320R Centrifuge) at 9000 rpm for 20 min and filtered using Whatman No. 1 filter paper to separate the aqueous phase from the solid residue of the extract. The collected aqueous phase was then kept at -4 °C temperature until further analysis.

ULTRASONIC-ASSISTED EXTRACTION (UAE)

The extraction of *M. citrifolia* leaves was conducted using an ultrasonic homogenizer (JY96-IIN/JY88-IINTen, China) equipped with a titanium probe measuring 14 mm in diameter (Figures 3 & 4). The extraction frequency was set at 30 kHz in pulse mode for three cycles. Before extraction, 5.556 g of dried *M. citrifolia* leaf powder will be added to 50 mL of distilled water mixed with specific concentration of citric acid as a catalyst in a 100 mL beaker. During sonification, the beaker will be placed inside a larger beaker filled with ice and water to maintain the temperature within the range of 15-25 °C until specific extraction time was reached. The extracts were centrifuged at 8000 rpm for 15 min using centrifuge Eppendorf 5810 R. The supernatant was filtered through Whatman no.1 paper, labeled, and stored at -18 °C until further analysis.

TOTAL PHENOLIC CONTENT (TPC) ANALYSIS

The determination of the total phenolic content of the extracts was conducted using Folin-Ciocalteu method (Mokhtar, Nordin & Morad 2018) with slight modification. A test tube containing 200 µL diluted sample mixed with 1 mL of diluted Folin-Ciocalteu reagents in distilled water (1:10 v/v) was shaken and left for 3 min. After that, the mixture was added with 0.8 mL of 7.5% sodium carbonate and incubated in a dark surrounding for 2 h. The absorbance of the mixture was then measured using the microplate spectrophotometer (Model Epoch, Biotech 259037) at 750 nm. A standard curve of gallic acid with a concentration range of 10 to 200 µg/L was prepared using the same method and the total phenolic content of the extract was then determined as milligram gallic acid equivalent (GAE) per gram of dry sample according to the Equation (1).

$$\text{Total Phenolic Content (mg GAE/g)} = \frac{\mathbf{R \times DF \times V}}{\mathbf{wt}} \quad (1)$$

where R (µg/L) is the reading from the gallic acid standard curve regression line; DF is the sample dilution factor; V (mL) is the total volume of extract; and wt (g) is the weight of dry sample used.

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) FREE RADICAL-SCAVENGING ASSAY

The free radical-scavenging activity of *M. citrifolia* leaf extracts was determined using a modified method explained by Akowuah et al. (2005). 2.95 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was dissolved in methanol to prepare 50 mL of 0.15 mM DPPH reagent. 500 µL of diluted sample and 500 µL of methanol operated as a control was each mixed with 500 µL of methanolic DPPH reagent in a test tube and the mixture was then incubated at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using the spectrophotometer (Model Epoch, Biotech 259037) after 30 min. A standard curve of ascorbic acid was prepared and the percentage of free-radical scavenging activity by DPPH was calculated by comparison with a control using Equation (2)

$$\text{DPPH (\%)} = \frac{\mathbf{A_C - A_S}}{\mathbf{A_C}} \times 100 \quad (2)$$

where A_C is the absorbance of the control; and A_S is the absorbance of the sample.

TABLE 1. Experimental parameters and coded variables of central composite design (CCD)

Independent variables	Coded variables			
	Unit	-1	0	1
Ultrasonic-assisted extraction				
Citric acid concentration (X_1)	%	0.1	1	0.55
Extraction time (X_2)	Min	1	11	6
Ultrasonic amplitude (X_3)	%	1.5	112.5	57
Subcritical water extraction				
Extraction temperature (X_1)	°C	140	160	180
Extraction time (X_2)	Min	5	10	15
Solid loading (X_3)	% (w/v)	1	2	3

FIGURE 1. Batch-type oil bath reactor

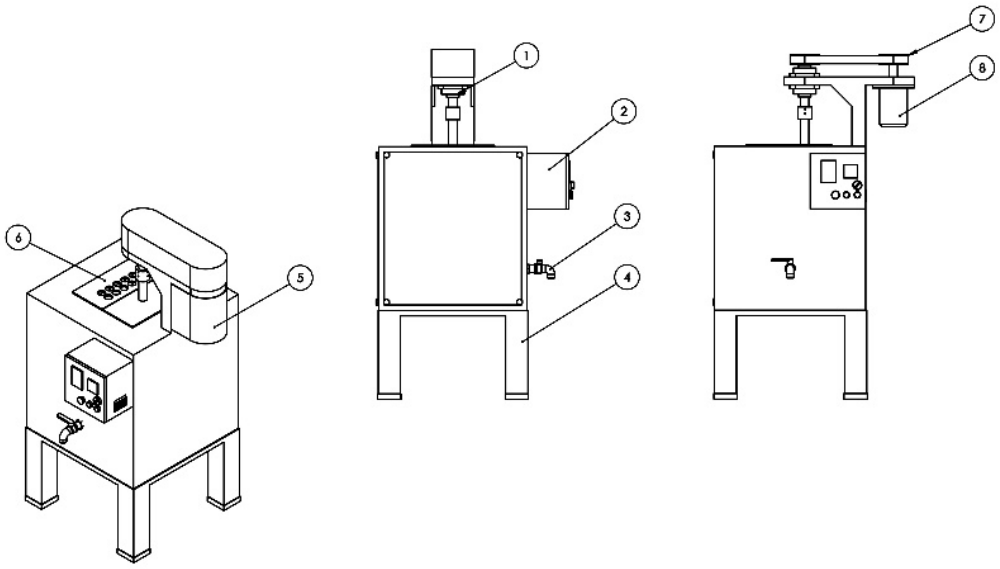

FIGURE 2. Oil bath reactor with its components; (1) Stirring bearing; (2) Operational panel control box; (3) Discharge pipe; (4) Frame; (5) Heat cover; (6) Liquid tank top cover; (7) Belting; and (8) Electric motor (Nurfatimah et al. 2022)

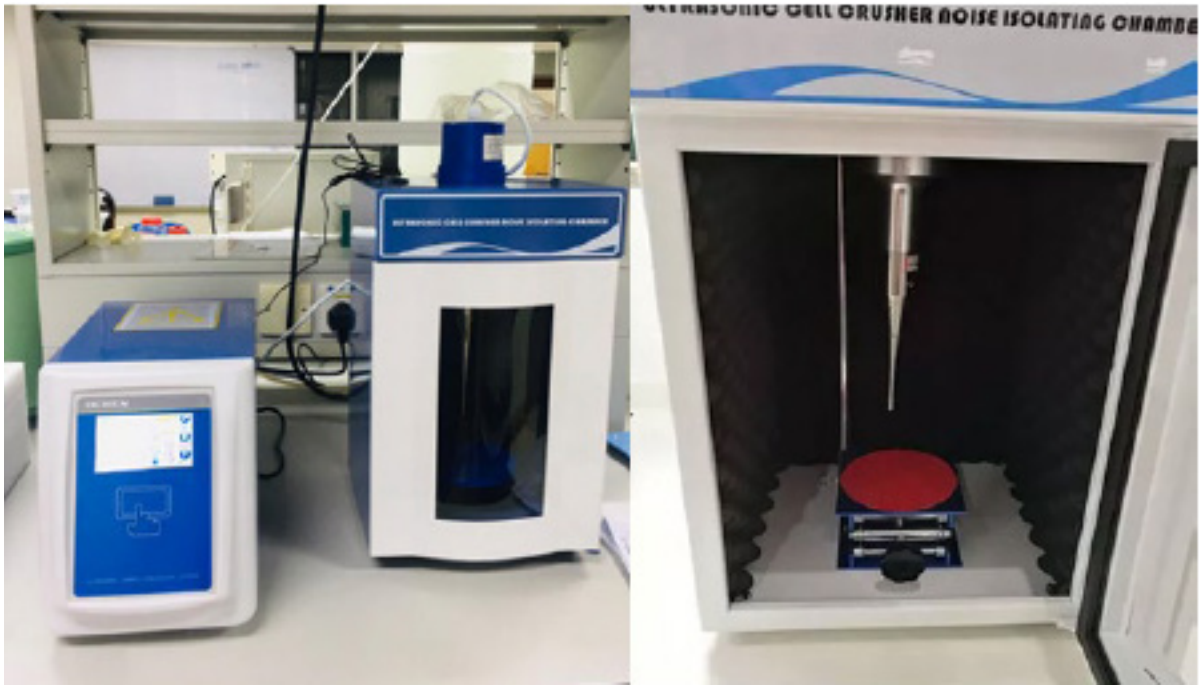

FIGURE 3. Probe-type ultrasonic extractor

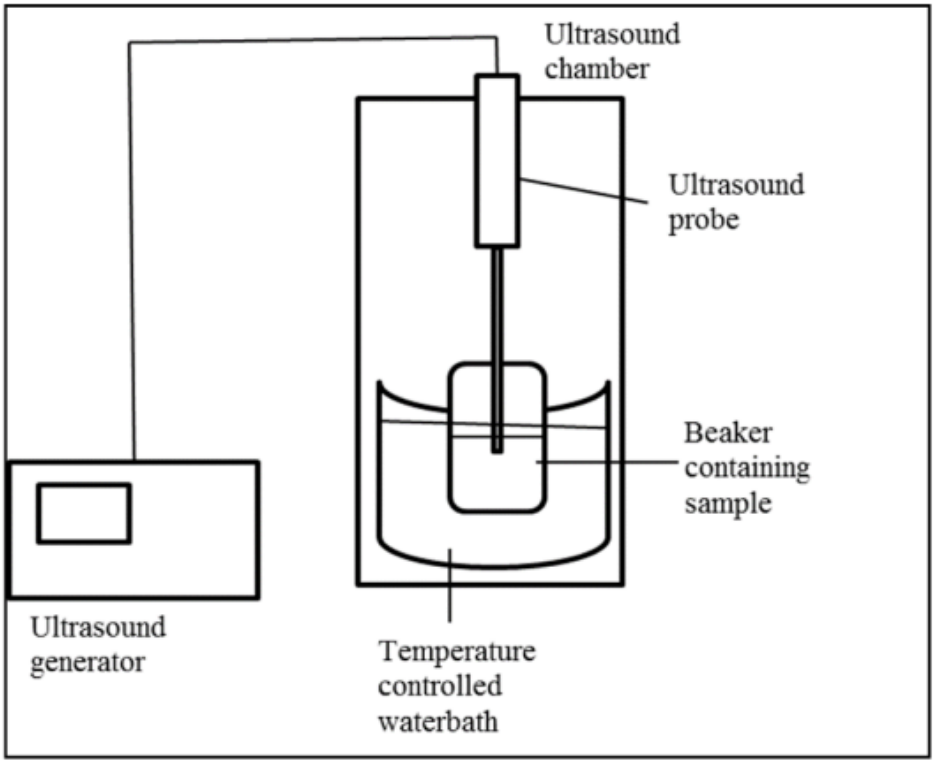

FIGURE 4. Ultrasonic assisted extraction procedure

FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP) ASSAY

The FRAP assay was conducted following procedure by Asari et al. (2021) with slight modifications. FRAP reagent (acetate buffer, TPTZ, FeCl3) was incubated at 37 °C for 10 min. 0.5 mL of extract was mixed with 0.95 mL FRAP reagent and incubated at 37 °C for 30 min. 200 µL of the mixture was then pipetted into the microplate well and the absorbance was measured at 593 nm using microplate reader (Epoch, Biotech 259037 (Vermont, USA)). Using the same method, gallic acid was used to construct the standard curve, and the results were represented in milligram gallic acid equivalent (GAE) per gram of dry sample calculated from the formula in Equation (3).

$$\text{Ferric Reducing Antioxidant Potential (mg GAE/g)} = \frac{\mathbf{R \times DF \times V}}{\mathbf{wt}} \quad (3)$$

where R (µg/L) is the reading from the gallic acid standard curve regression line; DF is the sample dilution factor; V (mL) is the total volume of extract; and wt (g) is the weight of dry sample used.

EFFICIENCY EVALUATION

The efficiency of subcritical water extraction (SWE) and ultrasound-assisted extraction (UAE) was comparatively evaluated based on its process efficiency. In this evaluation, the process efficiency was determined by the following equation:

$$\text{Process efficiency (mg/min)} = \frac{\text{Bioactive compounds extracted}}{\text{Process time}} \quad (4)$$

This metric provided a measure of the productivity of each extraction technique in terms of the amount of bioactive compounds extracted per unit of time. The calculations for process efficiency (Equation 4) was performed using the TPC, DPPH and FRAP values obtained at optimum conditions for both UAE and SWE. This comparative analysis allowed for the identification of the more efficient extraction method based on the time-based productivity of the process.

STATISTICAL ANALYSIS

The total phenolic content and antioxidant activity were analyzed using Analysis of Variance (ANOVA) and the significant differences among means were evaluated by Turkey's test. Each analysis was performed for three replications (n=3) and the results were expressed by means ± standard deviation using State-Ease Design Expert Software 13.

RESULTS AND DISCUSSION

EFFECT OF UAE ON BIOACTIVE COMPOUNDS RECOVERY

In this work, the efficiency of UAE was evaluated by the effect of citric acid concentration, extraction time, and ultrasonic amplitude on the TPC, DPPH, and FRAP values. Table 2 shows the TPC, DPPH, and FRAP values obtained from the UAE conducted at different operating conditions following RSM experimental design meanwhile Table 3 shows the Analysis of variance (ANOVA) test conducted for TPC, DPPH, and FRAP analysis. The data fit the second-order of polynomial models as the coefficients of determination, R^2 for all TPC, DPPH, and FRAP models are approaching to 1 (R^2>0.9) as stated in Table 3.

The results shown in Table 2 indicate the highest value of TPC, DPPH, and FRAP obtained in the extract was at sample run 11 (50 mg GAE/g), sample run 14 (24.68% inhibition) and sample run 19 (30.29 mg GAE/g), respectively. Based on the ANOVA test, citric acid concentration, time, amplitude, and the citric acid concentration-time interaction are significantly affects the TPC in extracts as the *p*-values obtained are less than 0.05 while the interaction of citric acid concentration-amplitude and time-amplitude are not significant. Meanwhile, for the DPPH, the models that show significant effect (p<0.05) were only citric acid concentration and the citric acid concentration-citric acid concentration interaction. Similarly, citric acid concentration again shows its significant effect towards FRAP models together with time-time and time-amplitude interaction. The results concluded that citric acid concentration are the most significant factors that affect the UAE efficiency towards MC leaf extracts followed by time and amplitude.

The combination of citric acid and water was chosen as the extraction solvents for UAE due to its environmental benefits, improved solubility, and cost-effectiveness, while delivering high yields of bioactive compounds (Şahin, Pekel & Toprakçı 2022; Vo et al. 2023). Research has shown that a mixture of citric acid and water can extract up to 90% of the phenolic content compared to traditional solvents like ethanol, while maintaining lower environmental and financial costs (Kumar, Methven & Oruna-Concha 2023). This is because acids can facilitate the breakdown of cell walls and enhance the release of phenolics from the matrix. This can be seen in Table 2, where the TPC value of sample run 13 using 1% acid concentration (47.25 mg GAE/g) was higher than sample run 1 using 0.1% acid concentration (30.58 mg GAE/g) when the UAE was performed at same extraction time and ultrasonic amplitude. This outcome was parallel with the findings reported by Mohd Zin et al. (2021) which showed that the extraction of polyphenols in *M. citrifolia* leaves extract using 0.01% hydrochloric acid in 90% methanol gave a higher extraction yield compared to 0.005%. On the other hand, the DPPH and FRAP value was

TABLE 2. RSM experimental design and the results for *Morinda citrifolia* leaves via UAE

Run	Citric acid (%) (X_1)	Time (min) (X_2)	Ultrasonic amplitude (%) (X_3)	TPC (mg GAE/g) (Y_1)	DPPH inhibition (%) (Y_2)	FRAP (mg GAE/g) (Y_3)
1	0.1	1	1.5	30.58	21.72	30.10
2	0.1	1	112.5	31.87	22.29	28.78
3*	0.55	6	57	42.15	16.64	26.39
4	0.1	11	1.5	39.53	20.17	27.27
5*	0.55	6	57	42.50	14.95	25.43
6	1	11	1.5	49.30	15.23	19.65
7	0.55	11	57	47.11	16.64	25.02
8	1	6	57	49.19	16.50	21.42
9	0.55	6	1.5	41.94	16.79	24.83
10	1	11	112.5	50.00	17.35	21.51
11*	0.55	6	57	43.93	16.22	26.14
12*	0.55	6	57	44.98	15.66	25.14
13	1	1	1.5	47.25	16.08	21.40
14	0.1	6	57	34.55	24.68	29.24
15	1	1	112.5	48.25	16.36	20.61
16*	0.55	1	57	40.93	16.08	22.18
17*	0.55	6	112.5	47.78	16.64	27.77
18*	0.55	6	57	45.45	16.22	26.02
19	0.1	11	112.5	49.19	21.58	30.29
20*	0.55	6	57	46.70	15.94	24.21

*Center points

TPC, total phenolic content; DPPH, free radical 2,2-diphenyl-1-picrylhdrazyl; FRAP, ferric reducing antioxidant potential; GAE, gallic acid equivalents

TABLE 3. Analysis of variance (ANOVA) model for the total phenolic content (TPC) and antioxidant activity through DPPH and FRAP for Morinda citrifolia leaves via UAE

Independent Variables	TPC Model				DPPH Model				FRAP Model			
	SS	*df*	*F*-value	*p*-value	SS	*df*	*F*-value	*p*-value	SS	*df*	*F*-value	*p*-value
Linear												
X_1	339.54	1	98.16	<0.001[b]	83.64	1	81.30	<0.001[b]	168.84	1	159.18	<0.001[b]
X_2	131.41	1	37.99	0.001[b]	0.2434	1	0.2366	0.6372	0.0449	1	0.0423	0.8411
X_3	34.19	1	9.88	0.0104[a]	1.79	1	1.74	0.2166	3.26	1	3.07	0.1101
Quadratic												
X_1^2	11.30	1	3.27	0.1008	35.29	1	34.30	0.0002[b]	0.0164	1	0.0155	0.9034
X_2^2	0.0414	1	0.0120	0.9150	1.15	1	1.12	0.3145	7.51	1	7.08	0.0239[a]
X_3^2	2.55	1	0.7368	0.4108	0.2356	1	0.2291	0.6425	3.02	1	2.84	0.1226
Interaction												
X_1X_2	63.11	1	18.25	0.0016[b]	0.72	1	0.6999	0.4224	0.0276	1	0.0260	0.8750
X_1X_3	10.70	1	3.09	0.1092	0.0221	1	0.0214	0.8865	0.0496	1	0.0468	0.8331
X_2X_3	8.14	1	2.35	0.1560	0.8978	1	0.8727	0.3722	6.11	1	5.76	0.0373[a]
Lack of Fit	9.65	5	1.23	0.4144	8.58	5	5.03	0.0503	7.35	5	2.26	
Pure Error	15.54	5			1.71	5			3.25	5		
Total SS	634.44	19			145.21	19			197.44	19		
R^2	0.9455				0.9292				0.9463			

[a,b] statistically significant at p <0.05 and p <0.01, respectively; X_1-citric acid concentration; X_2-time; X_3-ultrasonic amplitude; SS-sum of squares; *df*-degrees of freedom

different and it can be seen from Table 2 that increasing the concentration of citric acid from 0.1% (run 1) to 1% (run 13) caused a significant decrease of the DPPH (21.72% to 16.08%) and FRAP value (30.10 mg GAE/g to 21.40 mg GAE/g). This indicates that higher concentrations of citric acid led to lower DPPH and FRAP values. The decrease of antioxidant activity observed with increasing citric acid concentration may be due to excessively high acid conditions leading to the degradation of antioxidants in the sample during the extraction process.

Apart from that, the extraction time also plays an important role as it allows sufficient contact between the solvent and the sample to increase the frequency of cell wall disruption by ultrasonic waves, resulting in the release of phenols from the cell components, thereby increasing the phenols extraction at higher extraction times. When UAE was performed at 1.5W ultrasonic amplitude using 0.1% citric acid concentration, the TPC values significantly (p<0.05) increased with time from 30.58 mg GAE/g (run 1) for 1 min extraction to 49.30 mg GAE/g (run 6) for 11 min extraction. This is consistent with a study by Tasfiyati et al. (2022), which showed an increase in the yield of scopoletin extracted from noni leaves as the extraction time increased from 5 min to around 12 to 14 min. Meanwhile, there were fluctuating readings for free radical scavenging activity by DPPH and the antioxidant activity by FRAP when extraction time increase as shown in Table 2. Results show an insignificantly increase and decrease of DPPH and FRAP value demonstrated that time was not a significant factor for DPPH and FRAP models based on ANOVA test in Table 3. Similar to TPC, the increased value of DPPH and FRAP might be due to more extraction of antioxidants are allowed at longer extraction time. However, extended extraction times can lead to the degradation of the sensitive antioxidants contributing to the DPPH and FRAP activity, leading to a decrease value. Optimizing extraction time and conditions is crucial to achieve the highest yield and activity of bioactive compounds.

The ultrasonic amplitude applied during UAE can also influences the efficiency and effectiveness of extracting bioactive compounds from plant materials. In this study, there was an erratic pattern on DPPH, and FRAP analyses on the MC leaves extracts by UAE with increasing ultrasonic amplitude. However, the increase of ultrasonic amplitude from 1.5W to 112.5W was significantly (p<0.05) increasing the TPC value as shown by run 1 (30.58 mg GAE/g) to run 2 (31.87 mg GAE/g) and from run 13 (47.25 mg GAE/g) to run 15 (48.25 mg GAE/g) in Table 2. According to Zhang, Lin and Ye (2018), higher ultrasonic amplitude causes a 'saturation effect'. This occurs when more cavitation bubbles are produced, increasing the pressure in the sample and solvent solution. As a result, it can disrupt the cell walls, thereby facilitating mass transfer to move compounds from the sample. Consequently, more compounds can be extracted at a given time, and this explains some increase in TPC, DPPH, and FRAP values. This study is supported by Wang, Liu and Hu (2014), who examined the extraction of polysaccharides from *Trametes robiniophila* (Huaier), where the extraction yield continued to increase with rising extraction amplitude and reached a maximum amplitude. According to them, excessively high amplitude can lead to the hydrolysis and aggregation of polysaccharides, which results in higher viscosity of the polysaccharides and a decrease in extraction yield. Similarly, the decreased value of TPC, DPPH, and FRAP obtained in this study with increasing ultrasonic amplitude of UAE are maybe due to excessive amplitude that can lead to the breakdown of compounds present in the extracts.

EFFECT OF SWE ON BIOACTIVE COMPOUNDS RECOVERY

The process parameters for the SWE method were studied to improve the extraction efficiency while maximizing the extraction yield. Table 4 shows the values of TPC, DPPH and FRAP in the extract of MC leaves obtained through SWE under different operating temperatures, extraction time, and solid sample loading following the RSM experimental design meanwhile Table 5 shows the Analysis of variance (ANOVA) test conducted for TPC, DPPH and FRAP analysis. The data fit the second-order of polynomial models as the coefficients of determination, R^2 for all TPC, DPPH and FRAP models are approaching to 1 (R^2>0.9) as stated in Table 5.

The results shown in Table 4 indicate the highest value of TPC (74.94 mg GAE/g) and FRAP (30.29 mg GAE/g) obtained in the extract was at sample run 6 while the highest DPPH value obtained was at run 16 (82.18% inhibition). Based on the ANOVA test, the operating temperature and time was significantly affects the TPC, DPPH and FRAP in the extracts as the p-values obtained are less than 0.05 while the solid loading only significant to DPPH and FRAP models. No interaction of factors is significant to TPC model while temperature-solid loading interaction was significant to DPPH model and temperature-time interaction was significant to FRAP model. As for quadratic variables, temperature-temperature interaction was the significant model term to all TPC, DPPH and FRAP while solid loading-solid loading interaction was only significant to FRAP model.

The results from ANOVA test (Table 5) demonstrated that operating temperature was the most significant factor that affects the efficiency of SWE in extracting bioactive compounds from MC leaf. The significant increases of TPC, DPPH and FRAP values with increasing temperature can be observed at constant extraction time and solid loading used as shown in Table 4. The TPC values significantly (p<0.05) increased from 22.56 mg GAE/g (run 9) to 35.81 mg GAE/g (run 2) and 74.94 mg GAE/g (run 6) when the temperature used increased from 126 °C to 160 °C and 194 °C, respectively. This finding is in line with the results of the study by Gonçalves Rodrigues et al. (2019) where the TPC of papaya seeds extracted using SWE increased from

TABLE 4. RSM experimental design and the results for *Morinda citrifolia* leaves via SWE

Run	Temperature (°C) (X_1)	Time (min) (X_2)	Solid loading(%) (X_3)	TPC (mg GAE/g) (Y_1)	DPPH inhibition (%) (Y_2)	FRAP (mg GAE/g) (Y_3)
1	180	5	3	50.36	80.73	7.29
2*	160	10	2	35.81	40.20	5.93
3	180	5	1	52.92	29.07	6.40
4	140	5	1	24.44	20.57	1.20
5	180	15	1	67.22	29.68	10.55
6	194	10	2	74.94	78.92	12.67
7*	160	10	2	34.87	33.95	4.93
8*	160	10	2	34.81	39.99	5.03
9	126	10	2	22.56	25.67	2.34
10	160	18.4	2	47.78	44.32	6.97
11	140	5	3	14.23	27.30	2.54
12*	160	10	2	34.87	36.48	5.89
13	160	1.59	2	19.35	8.93	1.59
14	140	15	3	22.11	51.41	3.39
15	140	15	1	22.44	16.99	2.05
16	180	15	3	61.83	82.18	11.00
17*	160	10	2	40.68	27.26	5.46
18	160	10	3.68	33.07	77.15	4.93
19*	160	10	2	37.55	44.40	6.38
20	160	10	0.32	17.50	5.82	0.35

*Center points

TPC, total phenolic content; DPPH, free radical 2,2-diphenyl-1-picrylhdrazyl; FRAP, ferric reducing antioxidant potential; GAE, gallic acid equivalents

TABLE 5. Analysis of variance (ANOVA) model for the total phenolic content (TPC) and antioxidant activity through DPPH and FRAP for Morinda citrifolia leaves via SWE

Independent Variables	TPC Model				DPPH Model				FRAP Model			
	SS	*df*	*F*-value	*p*-value	SS	*df*	*F*-value	*p*-value	SS	*df*	*F*-value	*p*-value
Linear												
X_1	4119.90	1	118.80	<0.001[b]	2782.76	1	52.44	<0.001[b]	138.13	1	205.32	<0.001[b]
X_2	462.36	1	13.33	0.0045[b]	493.66	1	9.30	0.0123[a]	25.35	1	37.69	0.001[b]
X_3	4.34	1	0.1250	0.7310	5152.68	1	97.10	<0.001[b]	10.06	1	14.96	0.0031[b]
Quadratic												
X_1^2	377.38	1	10.88	0.0080[b]	484.13	1	9.12	0.0129[a]	10.16	1	15.10	0.0030[b]
X_2^2	0.9111	1	0.0263	0.8745	155.01	1	2.96	0.1182	1.30	1	1.94	0.1942
X_3^2	145.63	1	4.20	0.0676	56.16	1	1.06	0.3278	11.17	1	16.61	0.0022[b]
Interaction												
X_1X_2	49.45	1	1.43	0.2600	42.64	1	0.8035	0.3911	4.74	1	7.05	0.0241[a]
X_1X_3	0.8385	1	0.0242	0.8795	496.28	1	9.35	0.0121[a]	0.2244	1	0.3336	0.5763
X_2X_3	6.21	1	0.1792	0.6811	101.74	1	1.92	0.1963	0.0242	1	0.0360	0.8534
Lack of Fit	319.59	5	11.75	0.0086[b]	352.31	5	1.98	0.2365	5.13	5	3.22	0.1127
Pure Error	27.19	5			178.37	5			1.59	5		
Total SS	5567.80	19			10345.68	19			210.24	19		
R^2	0.9413				0.9574				0.9680			

[a,b] statistically significant at p <0.05 and p <0.01, respectively; X_1-temperature; X_2-time; X_3-solid loading; SS-sum of squares; *df*-degrees of freedom

34.7 mg GAE/g to 91.6 mg GAE/g when the extraction temperature used increased from 70 °C to 150 °C. This is because higher temperatures enhance the solubility of phenolic compounds in water, leading to higher extraction yields. Similarly for DPPH and FRAP analysis, there was an increase of free-radical inhibition percentage and FRAP value when 10 min SWE was conducted using 2% w/v of MC leaf powder from temperature 126 °C (25.67% and 2.34 mg GAE/g) to 160 °C (40.20% and 5.93 mg GAE/g) and 194 °C (78.92% and 12.67 mg GAE/g). A previous study by Essien, Young and Baroutian (2020) in SWE of kanuka leaves also reported the same trend with increasing DPPH and FRAP values from 218 mg TE/g and 315 mg TE/g dry sample to 225 mg TE/g and 340 mg TE/g dry sample, respectively, when the SWE temperature was increased from 150 °C to 170 °C. The results shows that the total phenolic compounds and ferric reducing antioxidant activity in the MC leaf extracts are highest at temperature 194 °C. However, the DPPH free radical inhibition was maximum at lower temperature (180 °C) with longer extraction times and higher solid loading.

Extraction time is also one of the important factors influencing the results of SWE. It was clear in Table 2 that the TPC values were gradually increased from 19.38 mg GAE/g to 34.87 mg GAE/g and 47.78 mg GAE/g with increasing extraction time from 1.6 min to 10 min and 18.4 min using 2% w/v solid sample loading at 160 °C. This is because more time allows for greater diffusion of phenolic compounds from the *M. citrifolia* leaves matrix into the solvent. Similar trends were reported by Ko, Nam and Chung (2020) where the TPC values from SWE of *Orastachys japanicus* at a temperature of 150 °C increased from 15 mg GAE/g to 18 mg GAE/g and 19 mg GAE/g when the extraction time increased from 5 min to 10 and 15 min. DPPH activity often increases with extraction time since longer extraction times will allow the extraction of more antioxidant compounds that can effectively scavenge DPPH radicals. This can be seen from the results in Table 2 where the DPPH inhibition at 1.6 min of extraction, 8.93% was increased to 33.95% for 10 min extraction and 44.32% for 18.4 min extraction when the SWE was conducted at temperature 160 °C using 2% w/v solid sample loading. Similar to DPPH, FRAP values typically show a similar trend as DPPH activity extraction time where longer extraction time generally led to higher FRAP values reflecting increased extraction of antioxidant compounds. This can be seen from the increasing values of FRAP from 1.59 mg GAE/g to 4.93 mg GAE/g and 6.97 mg GAE/g as the SWE was conducted for 1.6 min to 10 min and 18.4 min, respectively, with constant operating temperature of 160 °C using 2% w/v solid sample loading.

Another factor that can affect the SWE efficiency is solid sample loading or the ratio of solid material to the extraction solvents which is water. Generally, all TPC, DPPH, and FRAP values tend to increase with higher solid loading since more solid material will provide a higher concentration of phenolic compound available for extraction. This positive pattern was only demonstrated by the increase of free-radical inhibition (DPPH) antioxidant activity as shown in Table 4 when the solid loading used increase from 0.32% (5.82%) to 2% (33.95%) and 3.68% w/v (77.15%). Regardless, there was a significant increase of TPC and FRAP value as solid loading used increase from 0.32% w/v (17.50 mg GAE/g and 0.35 mg GAE/g, respectively) to 2% w/v (34.87 mg GAE/g and 5.89 mg GAE/g, respectively). However, the decrease of TPC and FRAP value to 33.07 mg GAE/g and 4.93 mg GAE/g was observed when the SWE operated at same temperature and time using 3.68% w/v solid sample loading. Similarly, the extraction yield of saponins, a type of phenolic compound, from *Camellia oleifera Abel* seeds in the study by Wu et al. (2018) showed a same decrease pattern from 62.56% to 59.61% when the solid content increased from 5% to 15% during subcritical water extraction at 150 °C for 30 min. The same trend was observed at 130 °C, where the extraction yield of saponins (phenolic compounds) decreased from 70.69% to 66.61% when the solid ratio increased from 5% to 15%. Based on the conducted studies and previous research, excessively high solid content reduces the density gradient formed, thereby, reducing mass transfer and subsequently decreasing the absorption rate of bioactive compounds from *M. citrifolia* leaves matrix to the water matrix (Essien, Young & Baroutian 2020).

Overall, ANOVA test analysis has shown that all three factors have a significant effect ($p<0.05$) on all TPC, DPPH, and FRAP analyses and the extraction temperature was the most significant factor affecting SWE efficiency compared to extraction time and solid sample loading. Higher temperatures enhance mass transfer kinetics by increasing the diffusion coefficients of solutes within the solvent. This facilitates faster extraction of bioactive compounds from the solid matrix into the solvent. However, prolonged extraction at high temperatures may lead to the degradation of existing sensitive compounds in the plant matrix and would limit the extraction performance.

PROCESS EFFICIENCY EVALUATION

Evaluating process efficiency is crucial when comparing different extraction techniques to provide a comprehensive understanding of how well each method extracts target compounds, such as phenolics, antioxidants, or other bioactive substances. Table 6 shows the optimum value of TPC, DPPH and FRAP in MC leaf extract obtained using optimum parameters suggested by CCD response surface for both UAE method (0.1% citric acid, 11 min, 112.5W) and SWE method (180 °C, 15 min, 3% w/v solid loading) as the value was used to calculate the process efficiency using Equation (4).

TABLE 6. Optimum value of the total phenolic content (TPC) and antioxidant activity through DPPH and FRAP of *Morinda citrifolia* leaves via UAE and SWE

Extraction	TPC (mg GAE/ g)	DPPH inhibition (%)	FRAP (mg GAE/g)
Ultrasonic-assisted extraction	48.25	22.59	29.63
Subcritical water extraction	64.96	80.75	11.33

This study demonstrated that UAE shows the highest process efficiency of 24.37 mg of phenolic compounds extracted per minute while SWE recorded the highest process efficiency of only 0.65 mg of phenolic compounds extracted per minute. The high process efficiency of UAE indicates high productivity, as UAE can extract significant amounts of compounds in a short time compared to SWE. Similarly for antioxidant activity by both extracts, the DPPH assay shows that UAE is approximately 6.68 times more efficient than SWE as UAE recorded the highest process efficiency where the antioxidants extracted by UAE can neutralize a significant amount of DPPH radicals at a rate of 11.41 mg per min compared to SWE (0.81 mg/min). Meanwhile, for FRAP assay, antioxidants extracted by UAE can reduce 14.97 mg of ferric ions per min compared to SWE with only 0.11 mg of ferric ions per min. However, these outcomes cannot conclude that UAE is more efficient than SWE solely based on its higher process efficiency values, as the sample sizes used are not the same and vary significantly where UAE (5.556 g) literally use 37.04 times the amount of dried MC leaf powder for the extraction compared to SWE (0.15 g). Future studies should use the same sample weight for both methods to accurately assess and compare the process efficiencies. This will allow for a more precise conclusion on which green extraction method is more efficient. However, the choice between the two methods should consider specific application needs, such as compound stability, desired yield, and resource availability.

Despite using a smaller sample size and having only 4 min difference in extraction time compared to UAE, the higher TPC and DPPH value possessed by SWE method along with a 2.6-fold lower in FRAP value at optimum parameter (Table 6) compared to UAE method logically suggest the effectiveness of SWE in extracting a wide range of compounds, including those sensitive to high temperatures. The lower TPC and DPPH values obtained through the UAE method can be attributed to several factors, as discussed in previous section. Most possible reason may be due to the potential degradation or structural changes of phenolic compounds at excessive ultrasonic amplitudes and duration during UAE. For example, a study on avocado leaves found that as ultrasound amplitude increased, the total phenolic content increased initially, but degradation followed due to excessive cavitation, leading to reduced antioxidant activity (Che-Galicia et al. 2020).

Both UAE and SWE offer unique advantages for improving extraction efficiency, but they operate based on different mechanisms where UAE uses ultrasound waves to create cavitation bubbles enhancing the breakdown of plant matrices and inducing acoustic streaming which improves the contact efficiency between the solvent and plant matrices leading to more efficient extraction compared to other conventional extraction method (Ampofo & Ngadi 2022). SWE, on the other hand, utilizes water near its critical point (usually around 100-374 °C) at high pressure which enhances the diffusion rate of solutes from the plant material into the solvent, leading to faster and more efficient extraction (Cheng et al. 2021). Furthermore, the elevated temperature and pressure can be tuned to optimize the extraction of specific compounds, providing some level of selectivity in the extraction process which makes this method advantageous compared to other conventional methods (Zhang et al. 2020).

Despite numerous advantages aspects possessed by UAE and SWE, there are limitations and challenges for these two methods that may impact the extraction efficiency. One significant limitation faced by UAE and SWE is both UAE and SWE involve high equipment costs since ultrasonic equipment for the UAE process can be expensive, especially for high-amplitude systems and SWE requires high temperature and pressure endurance equipment which can be very expensive to purchase and maintain. Moreover, the energy cost for both the SWE and UAE processes also can be substantial where the SWE process demands significant energy to reach high temperatures and pressure for maintaining the subcritical conditions leading to high operational costs. These challenges can be a barrier to adoption, especially for small-scale operations. Meanwhile, UAE process requires high energy to produce ultrasonic waves which can be costly and inefficient for large volumes; one of the strategies that can be applied to overcome this problem is performing the extraction in conjunction with other extraction techniques to enhance overall extraction efficiency and yield (Hoo et al. 2022). Furthermore, the technology developments and additional research into process optimization by UAE and SWE may assist in reducing some of the costs over time.

CONCLUSION

The results of this study indicate that both UAE and SWE effectively extracted a substantial amount of phenolic compounds and exhibited high antioxidant activity in *M. citrifolia* leaf extract. However, this study was unable to draw a definitive conclusion on which method is more efficient in terms of process efficiency, as the evaluation involved samples with significantly different sizes between the UAE and SWE methods. To gain a clearer understanding of the process efficiencies between these methods, further research using the same sample size is recommended. This would help identify the optimal conditions for maximizing the yield and activity of bioactive compounds. Such an approach will allow for more comprehensive optimization of both techniques, potentially enhancing efficiency and productivity in extracting valuable bioactive compounds from *M. citrifolia* leaves, with a focus on their practical applications in an industrial setting. Future studies should also explore the optimization of conditions for large-scale applications, particularly for commercialization. This includes assessing the economic viability, environmental impact, and product quality in commercial settings. Developing standardized protocols for scaling up these processes could promote broader adoption across industries, contributing to more sustainable production practices.

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REFERENCES

Akowuah, G.A., Ismail, Z., Norhayati, I. & Sadikun, A. 2005. The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chemistry* 93(2): 311-317. https://doi.org/10.1016/j.foodchem.2004.09.028

Almeida, É.S., de Oliveira, D. & Hotza, D. 2019. Properties and applications of *Morinda citrifolia* (Noni): A review. *Comprehensive Reviews in Food Science and Food Safety* 18(4): 883-909. https://doi.org/10.1111/1541-4337.12456

Ampofo, J. & Ngadi, M. 2022. Ultrasound-assisted processing: Science, technology and challenges for the plant-based protein industry. *Ultrasonics Sonochemistry* 84: 105955. https://doi.org/10.1016/j.ultsonch.2022.105955

Asari, N.A., Ab. Halim, S., Mohd Thani, N. & Hashim, H. 2021. Potensi rizom serai Acheh (*Elettariopsis smithiae*) sebagai punca tumbuhan antioksida: Kesan suhu dan masa pengekstrakan terhadap antioksida, perubahan warna dan sebatian meruap. *Sains Malaysiana* 51(8): 2681-2693. https://doi.org/10.17576/jsm-2022-5108-26

Belwal, T., Ezzat, S.M., Rastrelli, L., Bhatt, I.D., Daglia, M., Baldi, A., Devkota, H.P., Orhan, I.E., Patra, J.K., Das, G., Anandharamakrishnan, C., Gomez-Gomez, L., Nabavi, S.F., Nabavi, S.M. & Atanasov, A.G. 2018. A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies. *TrAC Trends in Analytical Chemistry* 100: 82-102. https://doi.org/10.1016/j.trac.2017.12.018

Che-Galicia, G., Váquiro-Herrera, H.A., Sampieri, Á. & Corona-Jiménez, E. 2020. Ultrasound-assisted extraction of phenolic compounds from avocado leaves (*Persea americana* Mill. var. Drymifolia): Optimization and modeling. *International Journal of Chemical Reactor Engineering* 18(7): 20200023. https://doi.org/10.1515/ijcre-2020-0023

Cheng, Y., Xue, F., Yu, S., Du, S. & Yang, Y. 2021. Subcritical water extraction of natural products. *Molecules* 26(13): 4004. https://doi.org/10.3390/molecules26134004

Essien, S., Young, B. & Baroutian, S. 2020. Subcritical water extraction for selective recovery of phenolic bioactives from Kānuka leaves. *The Journal of Supercritical Fluids* 158: 104721. https://doi.org/10.1016/j.supflu.2019.104721

Fontes, R.F., Andrade, J.K.S., Rajan, M. & Narain, N. 2023. Chemical characterization of different parts of noni (*Morinda citrifolia*) fruit and its freeze-dried pulp powder with emphasis on its bioactive compounds and antioxidant activities. *Food Science and Technology (Brazil)* 43(5): e103722. https://doi.org/10.1590/fst.103722

Gonçalves Rodrigues, L.G., Mazzutti, S., Vitali, L., Micke, G.A. & Ferreira, S.R.S. 2019. Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction. *Biocatalysis and Agricultural Biotechnology* 22: 101367. https://doi.org/10.1016/j.bcab.2019.101367

Hoo, D.Y., Low, Z.L., Low, D.Y.S., Tang, S.Y., Manickam, S., Tan, K.W. & Ban, Z.H. 2022. Ultrasonic cavitation: An effective cleaner and greener intensification technology in the extraction and surface modification of nanocellulose. *Ultrasonics Sonochemistry* 90: 106176. https://doi.org/10.1016/j.ultsonch.2022.106176

Jamaludin, R., Kim, D.S., Salleh, L.M. & Lim, S.B. 2021. Kinetic study of subcritical water extraction of scopoletin, alizarin, and rutin from *Morinda citrifolia*. *Foods* 10(10): 2260. https://doi.org/10.3390/foods10102260

Ko, M.J., Nam, H.H. & Chung, M.S. 2020. Subcritical water extraction of bioactive compounds from *Orostachys japonicus* A. Berger (Crassulaceae). *Scientific Reports* 10(1): 10890. https://doi.org/10.1038/s41598-020-67508-2

Kumar, R., Methven, L. & Oruna-Concha, M.J. 2023. A comparative study of ethanol and citric acid solutions for extracting betalains and total phenolic content from freeze-dried beetroot powder. *Molecules* 28(17): 6405. https://doi.org/10.3390/molecules28176405

Lima, D., dos Santos, A., Celestino, A., Sampaio, N., Baldez, J., Melecchi, M., Bjerk, T., Krause, L. & Caramão, E.B. 2018. Ultrasonic extracts of *Morinda citrifolia* L.: Characterization of volatile compounds by gas chromatography-mass spectrometry. *Journal of the Brazilian Chemical Society* 30(1): 132-139. https://doi.org/10.21577/0103-5053.20180162

Lohani, M., Majrashi, M., Govindarajulu, M., Patel, M., Ramesh, S., Bhattacharya, D., Joshi, S., Fadan, M., Nadar, R., Darien, B., Maurice, D.V., Kemppainen, B. & Dhanasekaran, M. 2019. Immunomodulatory actions of a Polynesian herb Noni (*Morinda citrifolia*) and its clinical applications. *Complementary Therapies in Medicine* 47: 102206. https://doi.org/10.1016/j.ctim.2019.102206

Mohd Zin, Z., Mohamad, N., Kah Hui, C., Majid, N.I. & Zainol, M.K. 2021. Effect of acidified ethanol on antioxidant properties of *Morinda citrifolia* leaf extract and its catechin derivatives. *Current Research in Nutrition and Food Science Journal* 9(1): 172-183. https://doi.org/10.12944/CRNFSJ.9.1.17

Mokhtar, N., Nordin, M.F.M. & Morad, N.A. 2018. Total phenolic content, total flavonoid content and radical scavenging activity from *Zingiber zerumbet* Rhizome using subcritical water extraction. *International Journal of Engineering* 31(8): 1421-1429. https://doi.org/10.5829/ije.2018.31.08b.34

Munir, M.T., Kheirkhah, H., Baroutian, S., Quek, S.Y. & Young, B.R. 2018. Subcritical water extraction of bioactive compounds from waste onion skin. *Journal of Cleaner Production* 183: 487-494. https://doi.org/10.1016/j.jclepro.2018.02.166

Nagalingam, M., Rajeshkumar, S., Balu, S.K., Tharani, M. & Arunachalam, K. 2022. Anticancer and antioxidant activity of *Morinda citrifolia* leaf mediated selenium nanoparticles. *Journal of Nanomaterials* 2022: 2155772. https://doi.org/10.1155/2022/2155772

Nuengchamnong, N., Saesong, T., Ingkaninan, K. & Wittaya-areekul, S. 2023. Antioxidant activity and chemical constituents identification by LC-MS/MS in bio-fermented fruit drink of *Morinda citrifolia* L. *Trends in Sciences* 20(4): 6498. https://doi.org/10.48048/tis.2023.6498

Nurfatimah Mohd Thani, Siti Mazlina Mustapa Kamal, Farah Saleena Taip, Alifdai Sulaiman, Rozita Omar & Mohd Hafizz Wondi. 2022. Risk assessment of subcritical water hydrolysis (SWH) system for sugar recovery using failure modes and effects analysis (FMEA) methods. *Sains Malaysiana* 51(10): 3333-3345.

Pak-Dek, M.S., Osman, A., Gooda Sahib, N., Saari, N., Markom, M., Hamid, A.A. & Anwar, F. 2011. Effects of extraction techniques on phenolic components and antioxidant activity of Mengkudu (*Morinda citrifolia* L.) leaf extracts. *Journal of Medicinal Plants Research* 5(20): 5050-5057. http://www.academicjournals.org/JMPR

Şahin, S., Pekel, A.G. & Toprakçı, İ. 2022. Sonication-assisted extraction of *Hibiscus sabdariffa* for the polyphenol's recovery: Application of a specially designed deep eutectic solvent. *Biomass Conversion and Biorefinery* 12(11): 4959-4969. https://doi.org/10.1007/s13399-020-00837-4

Tasfiyati, A.N., Antika, L.D., Dewi, R.T., Septama, A.W., Sabarudin, A. & Ernawati, T. 2022. An experimental design approach for the optimization of scopoletin extraction from *Morinda citrifolia* L. using accelerated solvent extraction. *Talanta* 238: 123010. https://doi.org/10.1016/j.talanta.2021.123010

Vo, T.P., Pham, T.V., Tran, T.N.H., Vo, L.T.V., Vu, T.T., Pham, N.D. & Nguyen, D.Q. 2023. Ultrasonic-assisted and microwave-assisted extraction of phenolics and terpenoids from *Abelmoschus sagittifolius* (Kurz) Merr roots using natural deep eutectic solvents. *ACS Omega* 8(32): 29704-29716. https://doi.org/10.1021/acsomega.3c03929

Wang, Y., Liu, Y. & Hu, Y. 2014. Optimization of polysaccharides extraction from *Trametes robiniophila* and its antioxidant activities. *Carbohydrate Polymers* 111: 324-332. https://doi.org/10.1016/j.carbpol.2014.03.083

Wu, H., Li, C., Li, Z., Liu, R., Zhang, A., Xiao, Z., Ma, L., Li, J. & Deng, S. 2018. Simultaneous extraction of oil and tea saponin from *Camellia oleifera* Abel. seeds under subcritical water conditions. *Fuel Processing Technology* 174: 88-94. https://doi.org/10.1016/j.fuproc.2018.02.014

Zhang, J., Wen, C., Zhang, H., Duan, Y. & Ma, H. 2020. Recent advances in the extraction of bioactive compounds with subcritical water: A review. *Trends in Food Science & Technology* 95: 183-195. https://doi.org/10.1016/j.tifs.2019.11.018

Zhang, Q.W., Lin, L.G. & Ye, W.C. 2018. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine* 13: 20. https://doi.org/10.1186/s13020-018-0177-x

Zhao, T., Luo, Y., Zhang, X., Zhang, W., Qu, H., Mao, G., Zou, Y., Wang, W., Li, Q., Chen, Y., Feng, W., Yang, L. & Wu, X. 2019. Subcritical water extraction of bioactive compounds from *Radix Puerariae* and optimization study using response surface methodology. *Chemical Engineering Communications* 206(9): 1218-1227. https://doi.org/10.1080/00986445.2018.1555529

Zhu, H., Zhang, J., Li, C., Liu, S. & Wang, L. 2020. *Morinda citrifolia* L. leaves extracts obtained by traditional and eco-friendly extraction solvents: Relation between phenolic compositions and biological properties by multivariate analysis. *Industrial Crops and Products* 153: 112586. https://doi.org/10.1016/j.indcrop.2020.112586

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