Ultrasound-assisted deep eutectic solvent extraction of phenolic compounds from *Rose myrtle*

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ABSTRACT Rose myrtle fruits contain high levels of phenolics and other bioactive compounds. To enhance the extraction efficiency of these phenolic compounds, ultrasound-assisted deep eutectic solvent (UA-DESs) extraction has been proposed. Initially, six distinct DESs were utilized in this study and compared with traditional solvents (ethanol, methanol, and hexane). The most suitable solvent for extracting phenolic compounds from rose myrtle fruits was determined to be choline chloride: urea (with a molar ratio of 1:2). Following that, the Box-Behnken design was employed to identify the optimal extraction conditions (ultrasonic power (X_1), solid to liquid ratio (X_2), and extraction time (X_3)) for achieving high total phenolic content (TPC) and antioxidant activity (IC50). Results demonstrated that the optimum ultrasound-assisted DES extraction parameters for attaining the highest TPC and antioxidant activity (IC50) (85.19 mgGAE/g fresh fruit and 2.31 mg/g, respectively) were X_1 of 233 W, X_2 of 440 mg/mL, and X_3 of 55 minutes. In comparison to traditional maceration extraction, extracts obtained through UA-DES extraction exhibited higher TPC and antioxidant activity (IC50), while requiring less extraction time. Scanning electron microscopy images (SEM) disclosed that the dissolution of bioactive components was enhanced due to cell disruption after ultrasound-assisted DES extraction. Consequently, the amalgamation of UA-DESs offers an exceptional extraction technique for the efficient retrieval of bioactive components from rose myrtle fruit.

KEYWORDS Rose myrtle fruits; Deep eutectic solvents (DESs); Ultrasound-assisted DESs extraction; Phenolic compounds

1. Introduction

The rose myrtle fruit (Rhodomyrtus tomentosa, Myrtaceae) is a shrub belonging to the Myrtaceae family, a widely spread plant throughout Asian countries. This fruit is abundant in antioxidant compounds, with 19 phenolic and triterpenoid compounds identified in it (Vo and Ngo, 2019; Ismandari et al., 2020; Lai et al., 2014). These compounds exhibit numerous biological properties, such as anti-inflammatory, anti-cancer, and antitumor effects (Vo and Ngo, 2019; Jeong et al., 2013). Some previous studies showed that rose myrtle fruit can be used to make traditional jams, wine, and beverages (Pham et al., 2022; Ismandari et al., 2020).

In order to recover phenolic components from natural sources, particularly rose myrtle fruit, extraction is a crucial step (Vo and Ngo, 2019; Ismandari et al., 2020; Lai et al., 2014; Lavanya et al., 2012). Ultrasound-assisted extraction (UAE), supercritical fluid CO_2 extraction (SC-CO_2), microwave-assisted extraction (MAE), and other advanced extraction techniques have recently been developed to extract bioactive components from rose myrtle fruit (Pham et al., 2022; Vo and Le, 2014; Zhang et al., 2018). Due to its simplicity, low equipment cost, quick extraction time, and low-temperature requirements, ultrasound-assisted extraction has attracted the most attention of these techniques. Additionally, ultrasound-assisted extraction's shortened extraction time and low-temperature work prevent bioactive chemicals from deteriorating, which enhances the recovery of these priceless substances (Xue et al., 2020).

Volatile organic solvents, including methanol, hexane, and ethanol, are frequently utilized to extract bioactive compounds from rose myrtle fruits (Lavanya et al., 2012; Lai et al., 2014; Liu et al., 2012). These conventional solvents boast impressive extraction power and dissolution capabilities. However, they also present drawbacks such as flammability, toxicity, and high volatility (Ozturk et al., 2018). In recent years, researchers have explored alternative solvent extractions using ionic liquids (ILs) and deep eutectic solvents (DESs) for phenolic extraction from natural resources (Lim et al., 2022; Wu et al., 2020; Hao et al., 2020). Unfortunately, ILs come with their own set of challenges, such as high cost, toxicity, and complex synthesis processes, which limit their practical applications (Lim et al., 2022; Pinkert et al., 2009). On the other hand, green solvents like deep eutectic solvents (DESs) are being considered as potential replacements for conventional solvents, as they may help reduce environmental impact and enhance extraction efficiency (Wu et al., 2020; Hao et al., 2020). DES is a mixture of two or more components, including a hydrogen bond acceptor (HBA) and

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a hydrogen bond donor (HBD), which have a significantly lower melting point than any of their components (Wang et al., 2018; Xue et al., 2020). Notably, choline chloride, glycerol, and urea are the most common hydrogen bond donors used in the extraction of phenolic compounds in various molar ratios (Wu et al., 2020; Wang et al., 2018; Xue et al., 2020).

Numerous studies have focused on optimizing the extraction of bioactive compounds from natural plants using ultrasound-assisted DES solvent extraction. However, little is known about the effect of ultrasound-assisted DES extraction on the recovery of phenolic compounds and the antioxidant activity of extracts from rose myrtle fruits. Therefore, the objectives of this study were: (i) to determine the impact of different DESs (choline chloride: glycerol, and choline chloride: urea) on the recovery of phenolic compounds and antioxidant activity; (ii) to optimize the ultrasound-assisted DES extraction conditions for maximum recovery of phenolics and antioxidant properties from rose myrtle fruit.

2. Methods

2.1 Materials and chemicals

The fruits of the rose myrtle were bought from a farm in Phu Quoc, Vietnam. These fruits were washed in tap water to remove any dust before being pureed in a blender (Panasonic, Malaysia) until smooth. The mash was stored at -20 °C, before use it was defrosted and extracted using different extraction solvents.

The analytical grade choline chloride, glycerol, urea, ethanol, methanol, hexane, 2,2-diphenyl-1 picrylhydrazyl, trolox, sodium carbonate (Na_2CO_3), and gallic acid were purchased from Merck (Darmstadt, Germany).

2.2 Preparation of deep eutectic solvents (DESs)

In this work, Choline chloride (ChCl) was used in this study as a hydrogen bond acceptor of the DESs type, and glycerol (Gly) and urea as hydrogen bond donors. Minor modifications were made to the process described in our earlier work to prepare DESs (Ozbek Yazici and Ozmen, 2022). As seen in Table 1, the ChCl, Urea, and Gly were initially weighed at various molar ratios.

Table 1: TPC and antioxidant activity of rose myrtle fruits extracted with different extraction solvents.

Extraction solvent*	Viscosity (Pa·s)	TPC (mg GAE/g dm)	DPPH (IC50; mgTE/g)
Methanol	0.50	34.15±1.14	0.56±0.02
Ethanol	0.48	42.15±1.19	0.80±0.01
Hexane	0.69	31.65±2.01	0.41±0.01
DES-1	0.12	54.14±1.09	1.33±0.04
DES-2	0.13	70.34±1.75	1.58±0.02
DES-3	0.21	68.15±1.81	1.22±0.02
DES-4	0.29	31.21±1.67	0.47±0.02
DES-5	0.55	30.54±1.14	0.41±0.01
DES-6	0.61	28.67±1.21	0.30±0.01

* The molar ratios are 1:2, 1:3, and 1:4 for ChlCl:Urea (DES-1, DES-2, and DES-3) and ChlCl:Gly (DES-3, DES-4, and DES-5), respectively.

The high viscosity is the biggest problem of DES extraction, which leads to slow mass transfer in extractions and time-consuming solvent transfer operations (Vo and Ngo, 2019; Ozbek Yazici and Ozmen, 2022). Therefore in this work, DES extraction was prepared with a final percentage of water of 30 % (w/w). The mixtures were then stirred and heated on IKA magnetic hotplate at 55 *pm* 0.5 °C, until a homogeneous transparent liquid was formed (Vo and Ngo, 2019; Wu et al., 2020). The viscosity of all DES was analyzed by DVII-Brookfield viscometer (Middleboro, USA) with S52 adapter at 35 *pm* 2 °C (New York, USA) with the rotation speed of 50 rpm, for 10 s at room temperature (24 °C).

2.3 Design of experiment

Response surface methodology (RSM) was used to optimize the recovery of phenolic content and antioxidant activity (IC50) from the rose myrtle fruit. A three-level, three factor Box–Behnken design was employed to evaluate the extraction parameters on the recovery of phenolic compounds with high antioxidant activity from fresh rose myrtle fruits. The design consisted of 17 experiments, and each parameter was varied at three levels (low, moderate, and high) coded as -1, 0, and +1 (Table 2). The independent variables were ultrasound power (X_1: 100, 300, and 300 W), sample:solvent ratio (X_2: 150, 300, and 450 mg/mL), and sonication time (X_3: 10, 45, and 80 min). The extraction temperature was set to 40 °C *pm* 1, based on the preliminary experiments showing a suitable extraction condition. The total phenolic content (Y_1) and antioxidant activity (DPPH: IC50) (Y_2) were chosen as the responses. A second-order polynomial was used to establish the mathematical relationship between independent variables and response and expressed as Eq. 1.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X^2 \quad (1)$$

where Y is the response variable; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients; X_i and X_j are the different independent variables ($i \neq j$).

The ANOVA was performed to evaluate the adjustment of the model. The lack of fit (LOF), an F-test, and the coefficient of determination (R^2) were used to evalutate the model adequacy. All data were processed using Modde software (version 10.0; Umetri, Umeå, Sweden).

2.4 Conventional extraction

The maceration method was used to compare the efficacy of the DES-based ultrasound-assisted extraction technique. In a 500 mL Erlen-meyer flask, 30 mL of ethanol, methanol, or hexane were added to one gram of rose myrtle fruit. The mixture was then centrifuged at 3000 rpm for 10 minutes to collect the supernatant after being extracted for 120 minutes at 100 rpm at 50 °C

2.5 Determination of phenolic content (TPC)

TPC were determined according to the previous study described by Zhao et al. (2014) with slightly modifications. Initially, 40 µL of the extract was combined with 1,560 µL of water. This mixture was then blended with 100 µL of Folin-Ciocalteu reagent and vigorously shaken

Table 2: Box–Behnken design of experimental conditions and observed responses for TPC and antioxidant capacity of rose myrtle extracts.

Run	Independent Variables			Responses	
	X_1 Ultrasonic Power (W)	X_2 Solid to liquid ratio (mg/mL)	X_3 Extraction Time (min)	Y_1 TPC (mg GAE/g)	Y_2 Antioxidant Activity (IC50; mgTE/g)
1	200 (0)	300 (0)	45 (0)	79.96±1.15	2.07±0.06
2	100 (-1)	450 (+1)	45 (0)	65.8±1.47	1.05±0.01
3	300 (+1)	300 (0)	10 (-1)	51.67±1.34	1.56±0.10
4	200 (0)	150 (-1)	10 (-1)	49.97±1.58	1.09±0.08
5	300 (+1)	300 (0)	80 (+1)	73.56±1.15	1.68±0.10
6	200 (0)	300 (0)	45 (0)	79.96±2.01	2.07±0.04
7	300 (+1)	450 (+1)	45 (0)	75.76±1.65	1.97±0.02
8	200 (0)	450 (+1)	10 (-1)	59.65±2.15	1.77±0.08
9	200 (0)	150 (-1)	80 (+1)	61.03±1.79	1.03±0.20
10	200 (0)	300 (0)	45 (0)	79.96±1.35	2.06±0.18
11	100 (-1)	300 (0)	10 (-1)	48.65±1.91	0.87±0.05
12	100 (-1)	300 (0)	80 (+1)	39.87±1.15	0.97±0.09
13	300 (+1)	150 (-1)	45 (0)	63.33±1.15	1.75±0.06
14	200 (0)	300 (0)	45 (0)	79.56±1.78	2.08±0.10
15	100 (-1)	150 (-1)	45 (0)	45.89±2.15	0.85±0.05
16	200 (0)	300 (0)	45 (0)	79.56±1.81	2.07±0.03
17	200 (0)	450 (+1)	80 (+1)	76.76±2.43	1.97±0.05

for 6 minutes. Following this, 300 µL of 10 % (w/v) sodium carbonate was added, and the solution was incubated in the dark for 2 hours. Absorbance was measured at a wavelength of 765 nm. Gallic acid (GAE) served as a positive control, and the TPC of the samples was expressed as milligrams of GAE per gram of fresh fruit (mg GAE/g), as determined using a gallic acid standard curve (0.004 - 0.5 mM).

2.6 Antioxidant activity

DPPH free-radical scavenging potential was carried out according to the method of Ismandari et al. (2020) with some modifications. A 100 µL sample was combined with 3 mL of 0.1 mol/L DPPH solution. This mixture was then kept in the dark for 30 minutes at room temperature before measuring its absorbance at 517 nm. Trolox, ranging from 0.015 to 0.250 µg TE/g, was employed for creating the standard curve, and the findings were presented in terms of milligrams of Trolox equivalents per gram of sample weight (mg TE/g). The IC50 value was determined by identifying the concentration necessary for the sample to inhibit 50 % of free radicals.

2.7 Scanning electron morphology (SEM)

The rose myrtle fruits were dehydrated in 99.5 % ethanol and then dried at 60 °C for 5 hours. The dried samples were coated with a thin gold film using a sputter coater to improve the sample conductivity. The coated samples were then observed in a scanning electron microscope (SEM; model KYKY-32000), under an accelerating potential of 30 kV.

2.8 Statistical Analysis

All experimental results were expressed as the mean of three replicates ±SD. The Modde software (version 10.0; Umetri, Umeå, Sweden) was used to optimize the experimental extraction condition. The statistical analyses were performed using Stagraphic Centrution XV (Statsoft Inc., Umeå, Sweden).

3. Results and discussion

3.1 Effect of the type of DESs on the extraction efficiency

In this study, six kinds of synthetic eutectic solvents were used to extract phenolics from fresh rose myrtle fruits and were contrasted with conventional organic solvents. According to Tranquilino-Rodríguez et al. (2021), the rose myrtle mash (1 g) was combined with 30 mL of the various DESs systems and extracted at 50 °C for 120 min. Table 1 demonstrates that DES extraction (ChCl:urea) was more effective than conventional solvents (ethanol, methanol, and hexane), particularly DES-2. The DES-2 compound produced the highest levels of TPC and antioxidant activity (IC50) (70.34 mg GAE/g and 1.76 mgTE/g), followed by DES-3 (68.15 mg GAE/g and 1.54 mg TE/g, respectively) and DES-1 (54.14 mg GAE/g and 1.05 mgTE/g). The least effective DESs, however, were DESs that combined ChCl with Gly (DES-4, DES-5, and DES-6; 28.67 – 31.21 mg GAE/g and 0.26 – 0.41 mgTE/g). The numerous hydrogen-bonding interactions with phenolic compounds that increased the solubility in the solvents may be responsible for the observed effects. Additionally, the viscosity of ChCl:urea (DES-1, DES-2, and DES-3) is lower than that of hexane, ethanol, and methanol, making it simpler to penetrate cell walls, and increase the phenolic extraction (Vo and Ngo, 2019). The finding was consistent with the previous study of Kovač et al. (2022), who found that the system of DES-2 (the molar ratio of Chcl:urea of 1:3) was the most efficient solvent for phenolic extraction from *Alchemilla vulgaris L.* In summary, DES-2 was selected as an extraction solvent to optimize the ultrasound-assisted DES extraction process parameters.

3.2 Optimization of Extraction Conditions for phenolics by RSM

3.2.1 Testing model

To examine the impact of several independent factors on the recovery of phenolics and antioxidant activity (IC50), the Box-Behnken design was used. Three independent variables such as ultrasonic power (X_1), solid-to-liquid ratio (X_2), and irradiation time (X_3) were determined and encoded at three levels (-1: low, 0: moderate, and +1: high). As presented in Table 2, it can be seen that the total phenolic contents and antioxidant activity (IC50) varied from 39.87 to 79.96 mg GAE/g fresh fruit and 0.85 to 2.07 mg TE/g, respectively. The sufficiency and fitness of the models were assessed using the ANOVA results (Table 3). Due to the extremely low probability value (p 0.05), the results showed that the models were considerably established for TPC and antioxidant activity (IC50).

The fitness and predictive ability of the model were evaluated based on the coefficient of determination (R^2), and the adjusted R^2. This study found that R^2 and adj-R^2 for TPC and antioxidant activity were 0.985-0.988 and 0.967-0.974, respectively, confirming the reliability of the models for the prediction of the responses (Lee and Yoon, 2021; Aboulghazi et al., 2022). Moreover, the lack of fit ($p > 0.05$) values were not significant (Table 3), and it was concluded that the models were the appropriate models for the response of TPC and antioxidant activity. Thus, RSM models can be used for analyzing the effects of the ultrasound-assisted DES parameters on TPC and antioxidant activity for the prediction of the optimal extraction condition.

Table 3: Regression coefficient and analysis of variance (ANOVA) of the predicted second-order polynomial models for the total polyphenol content (TPC) and antioxidant activity of rose myrtle juice.

Factor	Coefficient estimate	
	TPC	DPPH
Intercept	79.8	2.07
X_1-Ultrasonic power	8.01	0.2937
X_2-Solid to liquid ratio	7.22	0.2638
X_3-Extraction time	5.16	0.15
$X_1 \times X_2$	-1.87*	-0.0825*
$X_1 \times X_3$	7.67	0.175
$X_2 \times X_3$	1.51*	0.135
X_1^2	-12.76	-0.3832
X_2^2	-4.34	-0.1482
X_3^2	-13.6	-0.5907
F-value (model)	52.97	66.41
F-value (lack of fit)	313.81	521.39
R^2	0.9855	0.9884
Adjusted R^2	0.9669	0.9735
Predicted R^2	0.7694	0.8152

*stands for insignificant differences ($p < 0.05$).

3.2.2 Influence of the extraction conditions on TPC

The TPC in rose myrtle extract varied from 39.87 to 79.96 mg GAE/g fresh fruit (Table 2). The lowest TPC was observed at X_1 (100 W) and X_2 (300 mg/mL) after 80 min, while the highest TPC was obtained at X_1 (300 W) and X_2 (300 mg/mL) after 45 min. Table 3's findings reveal that TPC under ultrasound-assisted DES is significantly positively influenced by X_1, X_2, and X_3, with X_1 being the most important element. Additionally, the three quadratic terms (X_1^2, X_2^2, and X_3^2) significantly affected TPC ($p < 0.05$). After removing all of the irrelevant terms (X_2X_3 and X_1X_2), the second-order polynomial equation for TPC was written as:

$$Y_1 = 79.80 + 8.01X_1 + 7.22X_2 + 5.12X_3 + 7.67X_1X_3 - 2.76X_1^2 - 4.34X_2^2 - 13.60X_3^2 \quad (2)$$

Based on Eq. 2, the three-dimensional (3D) plots were generated to explain the influences and mutual interactions of the UAE variables on the TPC (Fig. 1a-c). Figure 1a shows that ultrasonic power (X_1) and the ratio of solid to liquid (X_2) had a greater impact on TPC. The maximum TPC obtained with intermediate ultrasonic powers (X_1) and a solid to liquid ratio (X_2) of 236 W and 420 mg/mL, respectively, fixed the extraction time (X_3) at 45 minutes. At ultrasonic powers larger than 240 W and solid to liquid ratios greater than 420 mg/mL, TPC extraction declined. Lower recoveries were obtained with a considerable amount of solvent, which may be related to a decrease in the dispersion of ultrasonic energy density when the treatment was administered in larger volumes (Chen et al., 2022). A similar finding was found by Wang et al. (2018), who concluded that a low liquid to solid ratio has a higher concentration gradient, resulting in higher diffusion and extraction yield.

Figure 1b shows the response surface. At a constant solid to liquid ratio (X_2) of 300 mg/mL, the recovery of TPC increased with increasing sonication power (X_1) and extraction duration (X_3), and it is maximized at 235 W for 45 min. Cell deformation is induced by increased sonication power and extended irradiation time, which enhances solvent penetration into the cell (Xue et al., 2020). However, phenolic compounds may be broken down by reactive hydroxyl radicals at high ultrasonic power (300 W), increasing the irradiation period after 45 min, and reducing the recovery of TPC.

Figure 1c shows that the liquid to solid ratio (X_2) and extraction time (X_3) both significantly improved TPC. However, the interaction of extraction time and liquid to solid ratio were insignificant ($p < 0.05$). As can be observed in Fig. 1c, the extraction of TPC rose when the solid to liquid ratio was increased to approximately 360 mg/mL and the sonication period was extended to 45 min at a fixed ultrasonic power. Subsequently, it stabilized and then began to drop. The breakdown of certain phenolic compounds increased with increased exposure

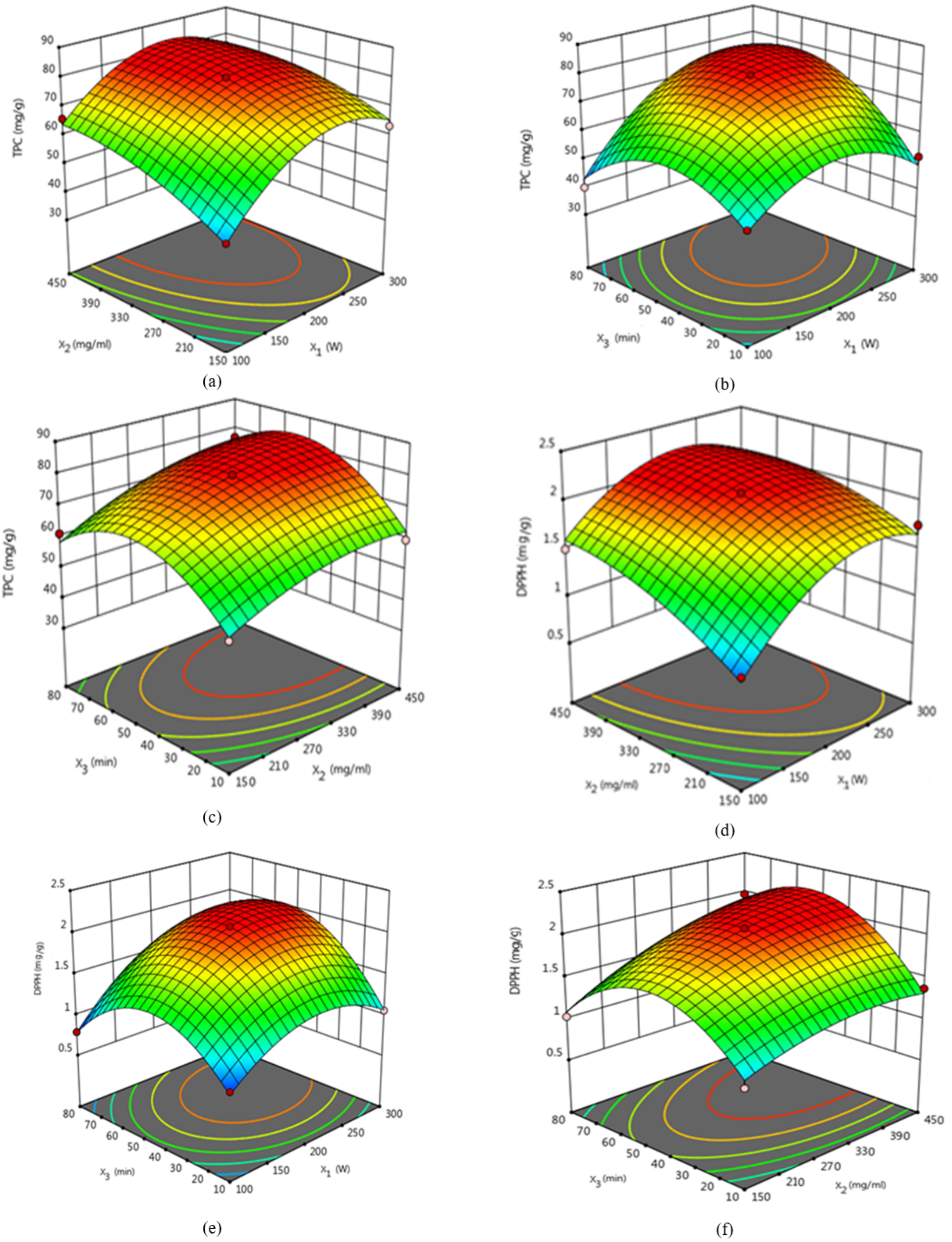

Figure 1: 3D surface plot of the effects of extraction parameters: (a-d) ultrasonic power and solid to solvent ratio; (b-e) ultrasonic power and extraction time; (c-f) solid to liquid ratio and extraction time on the total phenolic compounds (TPC) and DPPH (IC50).

to light and oxygen, which resulted in a decrease in TPC (Zhao et al., 2014; Aboulghazi et al., 2022). The findings showed that 45 minutes were needed to reach the highest TPC.

3.2.3 Influence of the extraction conditions on antioxidant activity (IC50)

The antioxidant activity (IC50) of rose myrtle extracts was determined using the DPPH method. The results showed that all studied variables were significantly influenced by antioxidant activity ($p < 0.05$) by all three linear (X_1, X_2, X_3) and quadratic parameters (X_1^2, X_2^2, X_3^2). The interaction parameters (X_2X_3) were also removed because they had no significant impact on the antioxidant activity (IC50). The RSM models for antioxidant properties (IC50) is then expressed by the following polynomial equation:

$$Y_2 = 2.07 + 0.2937X_1 + 0.2638X_2 + 0.15X_3 + 0.175X_1X_3 + 0.135X_2X_3 - 0.3832X_1^2 - 0.1428X_2^2 - 0.5907X_3^2 \quad (3)$$

Table 4: Comparison the different extraction methods (UA-DES extraction, ME extraction) on the recovery of TPC and antioxidant activity.

Extraction method*	Extraction condition	TPC (mg GAE/g dm)	DPPH (IC50; mgTE/g)
UA-DES	233 W, 55 min	85.19±1.65	2.31±0.05
ME-Ethanol		35.01±1.54	0.60±0.03
ME-Methanol	50 °C for 120 min	42.27±1.73	0.83±0.03
ME-Hexane		31.69±2.10	0.44±0.05

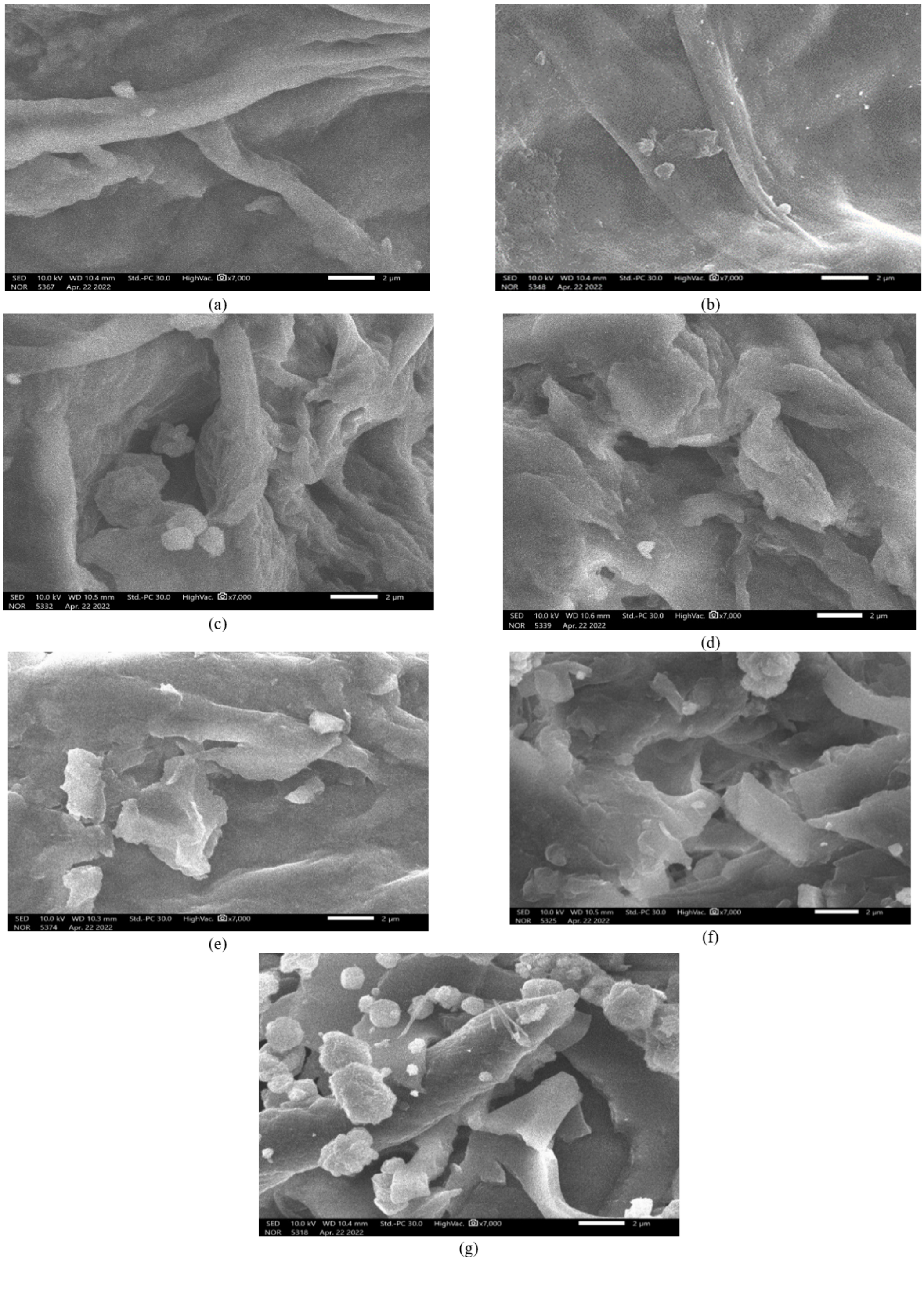

Figure 2: SEM images of rose myrtle fruits. (a) Raw materials; (b, c, d) DSE based ME (ethanol, methanol, and hexane); (e) DES-based UAE at lower ultrasound parameters (100 W, 150 mg/mL, 10 min); (f) DES-based UAE at optimal extraction conditions; (g) DES-based UAE at extremely ultrasonic parameters (300 W, 450 mg/mL, and 80 min).

The response surface plots of antioxidant activity can be seen in Fig. 1d-f. The red color of 3D surface indicated the highest value of antioxidant activity response (2.06 mg TE/g), and the blue color showed the lowest response value of 0.95 mg TE/g.

As presented in Fig. 1d-f, it can be seen that antioxidant activity rapidly increased with increasing ultrasonic power (X_1) in the low liquid to solid ratio (X_2: 150-350 mg/mL) at long irradiation times (X_3: 40-55 min). The highest the antioxidant activity value was observed at 233 W, 350 mg/mL, and 54.8 min. However, the antioxidant activity decreased at ultrasonic powers greater than 240 W, liquid to solid ratio above 350 mg/mL, and extraction time longer than 55 min. Our findings are in agreement with Saifullah et al. (2020), who reported that the antioxidant activity declined with increasing extraction time at around 60 min and sonication power above 200 W.

3.3 Optimization of extraction parameters for TPC and antioxidant activity (IC50)

According to the RSM results, the optimal conditions were ultrasonic power (X_1) of 233 W, solid-liquid ratio (X_2) of 440.03 mg/mL, and extraction time (X_3) of 54.812 min. Under these optimal conditions, the predicted TPC and antioxidant activity (IC50) values are 84.91 mgGAE/g fresh fruit and 2.26 mg TE/g, respectively. The validation of the ideal condition was changed to include the following adjustments for operability in actual production: extraction time (X_1) of 233W, solid to liquid ratio (X_2) of 440 mg/mL, and extraction duration (X_3) of 55 min. The experimental values of TPC and antioxidant activity (IC50) were 85.19 mgGAE/g fresh fruit and 2.31 mg TE/g, which was consistent with the predicted value. The results confirmed that the RSM model is reasonable and had good predictability.

3.4 Comparison of ultrasound-assisted DES extraction with ME method

The phenol-rich extract obtained under optimal extraction conditions was compared with maceration extraction (ME) in order to demonstrate the extraction effectiveness and antioxidant activity (IC50) of the ultrasound-assisted DES extraction. The ultrasonic-assisted DES extraction (UA-DES) significantly outperformed the ME approach in terms of extraction effectiveness and antioxidative activity, as can be observed in Table 4. This may be attributed to the degree of cell wall plant changes observed by SEM. As shown in Fig. 2a, the surface structure of the original rose myrtle was completed and smooth. After extraction with organic solvent, the rose myrtle cell was slightly wrinkled but the surface was intact (Fig. 2b-d). The surface structure and organization of rose myrtle treated with ultrasound combined DES extraction (Fig. 2e-g) showed obvious shrinkage and became loose and porous. In the UA-DES (low ultrasonic powers and short extraction times), the plant cells have several holes and a few cells were broken (Fig. 2e). Meanwhile, in the UA-DES extraction (at optimal extraction condition and extreme extraction condition) the extracted rose myrtle was seriously destroyed with small fragments, which enhanced the dissolution of bioactive compounds into the solvent extraction. It could be due to the synergistic impact of ultrasonic cavitation and penetration of ChCl:Urea being incorporated into the internal structure of plants. The more ruptured plant cells resulted in a more efficient extraction. In addition, the consumption of solvent and extraction time were significantly reduced by UAE in comparison with ME. The finding is consistent with Zhao et al. (2014), who reported that the ultrasonic treatment induced structural changes, fissures, and cavities. Therefore, the DES-based UAE was a new alternative for the extraction of phenolic from rose myrtle fresh fruits.

4. Conclusions

The effects of different extraction solvents on the recovery of TPC and antioxidant activity (IC50) were studied, and DES solvent (ChlCh:urea at a 1:2 ratio) was found to be the most efficient solvent for rose myrtle fruits. The Box-Behnken design was used to optimize the extraction conditions of TPC from rose myrtle fruits by ultrasound-assisted DES extraction. The optimal extraction conditions of TPC and antioxidant activity (IC50) were ultrasonic power of 233 W, irradiation time of 55 min, and a liquid-solid ratio of 440 mg/mL. Under these conditions, the TPC and anti-oxidant activity were 85.19 mg GAE/g fresh fruit and 2.31 mg TE/g, respectively, which agreed closely with the predicted values. The ultrasound-assisted DES is more efficient than the traditional procedure (maceration). Furthermore, the DESs-based UAE method has a shorter extraction time, is highly efficient, and is a green extraction method. Therefore, the combination of ultrasound-assisted DESs has an excellent alternative extraction of phenolic compounds from rose myrtle juice.

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