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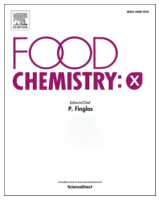

Valorization of pawpaw (*Carica papaya* L.) leaves as a source of polyphenols by ionic liquid-based microwave-assisted extraction: Comparison with other extraction methods and bioactivity evaluation

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[HMIM]Br (PubChem CID: 2734237)
[OMIM]Br (PubChem CID: 10849985)
[BMIM]BF_4 (PubChem CID: 2734178)

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ABSTRACT

This study aimed to valorize pawpaw (*Carica papaya* L.) leaves as a rich source of polyphenols through the application of ionic liquid-based microwave-assisted extraction (ILMAE). Initially, the ILMAE process was optimized using response surface methodology (RSM), resulting in a total polyphenols yield of 27.84 ± 0.33 mg GAE/g DW under the optimal conditions: [BMIM]Br concentration of 0.57 mol/L, extraction time of 14 min, microwave power of 460 W, extraction temperature of 77 °C, solvent-to-material ratio of 30 mL/g, and three extraction cycles. Compared to conventional methods such as maceration extraction (ME), heat reflux extraction (HRE), and microwave-assisted extraction (MAE), the ILMAE method exhibited a significantly higher PLTP yield. Furthermore, the PLTP extracts demonstrated strong antioxidant activity against DPPH• and $ABTS^{+}$• radicals, as well as a significant inhibitory effect on α-glucosidase activity. This work demonstrates that ILMAE is a green and efficient strategy for the valorization of pawpaw leaves.

1. Introduction

Pawpaw (*Carica papaya* L.), an evergreen perennial fruit crop, is extensively cultivated in tropical and subtropical countries because of its great economic value (Pandey, Cabot, Shaw, & Hewavitharana, 2016). Pawpaw fruit is the most important tree product and is increasingly popular with consumers due to its excellent taste and high nutritional value. In addition to the fruit, other parts of the tree, including the leaves, are considered low-value agricultural by-products (Vuong et al., 2013). Pawpaw leaves have been reported to have many pharmacological activities, such as antioxidant (Zunjar, Mammen, & Trivedi, 2015), antimalarial (Teng et al., 2019), antitumor (Nguyen et al., 2016), hypoglycemic (Juárez-Rojop et al., 2012), and immunomodulatory (Anjum, Arora, Ansari, Najmi, & Ahmad, 2017) effects. Currently, only a small portion of pawpaw leaves are utilized for tea and vegetable consumption, while the majority of pawpaw leaves remain largely unexploited and are typically discarded (Nugroho, Heryani, Choi, & Park, 2017). Previous studies have indicated that pawpaw leaves are rich in polyphenols, including chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, 4-hydroxycinnamic acid, kaempferol, quercetin, rutin and afzelin (Canini, Alesiani, D'Arcangelo, & Tagliatesta, 2007; Nugroho et al., 2017), and their chemical structures are shown in **Fig. S1**. These compounds are essential contributors to the pharmacological activity of pawpaw leaves. Hence, the extraction of bioactive polyphenols from pawpaw leaves, with their potential for development into valuable products, represents a highly effective strategy for maximizing the valorization of this natural resource.

As a first step toward the development of natural products, the extraction of bioactive ingredients is highly important for subsequent research. Vuong et al. (2013) extracted polyphenols from pawpaw leaves by maceration extraction (ME) and reported that the extraction process parameters strongly influence the yield and antioxidant activity of phenolic compounds. Other extraction techniques, including heat reflux extraction (HRE), diacolation and Soxhlet extraction, are widely used for the extraction of polyphenols. However, these traditional extraction techniques consume considerable energy, time, and organic solvents, leading to relatively low extraction yield (Proestos & Komaitis, 2008). Currently, microwave-assisted extraction (MAE) has attracted tremendous attention for the recovery of polyphenols from plant materials due to its excellent extraction performance, including high

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efficiency, ease of operation, low solvent consumption, low cost, and protective effects on thermolabile components (Kala, Mehta, Sen, Tandey, & Mandal, 2016). The MAE utilizes microwave energy in the extraction system, allowing for the dissipation of heat throughout the entire volume of the irradiated medium. MAE enhanced the release of intracellular substances into the extraction solvent due to the destruction of the plant cell wall, which thereby resulted in an increase in the extraction yield of target constituents (Mason, Chemat, & Vinatoru, 2011). In addition, ionic liquids (ILs), a class of organic salts that are usually liquid at room temperature, consist of an organic cation with a relatively large structure and an organic or inorganic anion (Aguilera-Herrador, Lucena, Cárdenas, & Valcárcel, 2010). The diversity of anions and cations is so staggering that researchers can choose ILs with specific properties according to the actual application. ILs are considered to be excellent substitutes for traditional organic extraction solvents because of their superior performance, including good solubility for various organic compounds, good thermal and chemical stability, tunable viscosity, nonflammability, eco-friendliness and recyclability (Fan et al., 2012). In recent years, an innovative ionic-liquid-based microwave-assisted extraction (ILMAE) technique that combines the beneficial properties of both approaches has been proposed. ILMAE is considered a promising green extraction method for recovering bioactive ingredients from plant materials (Fan et al., 2018; Liang et al., 2017). However, the recovery of polyphenols from pawpaw leaves using ILMAE has not been reported.

Therefore, for the valorization of pawpaw leaves, the ILMAE approach was used to recover pawpaw leaves total polyphenols (PLTP) in this work, and the ILMAE process was optimized with response surface methodology (RSM). Then, ME and HRE were carried out to validate the effectiveness of the developed ILMAE method. Furthermore, the antioxidant and anti-hyperglycemic effects of the PLTP extracts were evaluated *in vitro*.

2. Materials and methods

2.1. Chemicals and apparatus

A gallic acid standard (HPLC ≥98%) was obtained from Alfa Biotechnology Co., Ltd. (Chengdu, China). Trolox, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), *p*-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, Folin-Ciocalteu reagent, and α-glucosidase (from *Saccharomyces cerevisiae*) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Four ionic liquids (ILs), namely [BMIM]Br, [HMIM]Br, [OMIM]Br, and [BMIM]BF_4 (as shown in **Fig. S2**), were obtained from Cheng Jie Chemical Co., Ltd. (Shanghai, China).

An MAS-II microwave apparatus (2450 MHz, 0–1000 W, Sineo Microwave Chemistry Technology Co., Ltd., Shanghai, China) was used as the source of microwave energy. The absorbance was detected uisng a UV-2600 spectrometer (Shimadzu Co., Kyoto, Japan). BWZ-10 Water Bath Shaker (Being Technology Co., Ltd., Shanghai, China) was used to maceration extraction.

2.2. Plant material

The papaya leaves used in this study, harvested in July 2023 in Nanning City, Guangxi Province, China, were obtained from artificially cultivated, three-year-old *Carica papaya* L., belonging to the Caricaceae family. The leaves underwent a series of preparation steps, including washing, freeze-drying, crushing, sieving through a 50-mesh sieve, and storage at 4 °C before being subjected to further analysis.

2.3. Optimization of ILMAE process

2.3.1. Screening of ILs

An exact quantity of pawpaw leaf powder (1.0 g) was added to 30 mL of a 0.5 mol/L solution of [BMIM]Br (1-butyl-3-methylimidazolium bromide), [HMIM]Br (1-hexyl-3-methylimidazolium bromide), [OMIM]Br (1-methyl-3-n-octylimidazolium bromide), and [BMIM]BF_4 (1-butyl-3-methylimidazolium tetrafluoroborate), respectively. The ILMAE extraction was performed three times, each cycle for 15 min at 70 °C and 500 W. After the extraction was complete, the combined extract solutions were filtered through Whatman No. 1 paper for measurement of the PLTP content.

2.3.2. Single-factor test

Pawpaw leaves powder (0.6–3.0 g) was extracted with [BMIM]Br aqueous solution. Six process parameters, namely, [BMIM]Br concentration (0.1–0.9 mol/L), microwave power (200–600 W), time (5–min), temperature (40–80 °C), solvent-to-material ratio (10–50 mL/g) and number of extraction cycles (1–4), were selected as independent variables, and the PLTP yield was taken as the dependent variable. Subsequently, the extracts were processed in accordance with Section 2.3.1.

2.3.3. Response surface experimental design

The following study was designed to optimize the ILMAE process using RSM, referring to our previous work (Hou et al., 2019). In detail, a three-level Box-Behnken design with four major variables, [BMIM]Br concentration (X_1), microwave power (X_2), extraction time (X_3) and extraction temperature (X_4), was implemented to achieve the maximum PLTP yield (Y). A total of 29 experimental runs were conducted in a randomized order (Table 1), and the data were elucidated using the following polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i>j}^{n} \sum_{j}^{n} \beta_{ij} X_i X_j \quad (1)$$

where Y refers to the dependent variable; X_i and X_j refer to the independent variables; β_0 refers to the intercept; β_i, β_{ii} and β_{ij} refer to the linear, quadratic and interactive coefficients, respectively; and n represent number of factors analyzed.

Table 1
Box-Behnken design and results.

Run	X_1 (mol/L)	X_2 (W)	X_3 (min)	X_4 (°C)	Y (mg GAE/g DW)
1	0.9	300	15	70	23.65
2	0.9	400	20	70	26.06
3	0.9	400	15	80	24.38
4	0.5	400	15	80	26.18
5	0.7	400	20	60	25.31
6	0.9	400	10	70	23.78
7	0.7	500	15	80	26.9
8	0.5	400	10	70	26.73
9	0.7	300	15	60	24.19
10	0.5	400	15	60	24.54
11	0.7	300	20	70	22.19
12	0.7	500	15	60	24.75
13	0.7	400	15	70	27.42
14	0.7	300	15	80	22.46
15	0.7	400	10	80	26.34
16	0.7	400	15	70	27.68
17	0.7	400	15	70	27.48
18	0.7	400	15	70	27.25
19	0.7	400	15	70	27.56
20	0.5	300	15	70	22.17
21	0.7	400	10	60	25.98
22	0.5	400	20	70	24.56
23	0.5	500	15	70	26.97
24	0.9	500	15	70	24.89
25	0.9	400	15	60	25.74
26	0.7	300	10	70	24.11
27	0.7	500	20	70	26.02
28	0.7	400	20	80	25.79
29	0.7	500	10	70	25.47

2.4. Reference extraction methods

2.4.1. Maceration extraction (ME) of pawpaw leaves total polyphenols

The powdered pawpaw leaves (1.0 g) were respectively extracted using 30 mL of 70% ethanol and water in a 100 mL conical flask, which was placed in a thermostated water-bath shaker for 12 h with a fixed agitation rate of 100 r/min. After the extraction period, the solution was filtered through Whatman No. 1 paper to remove any residual sample, and the residue was subjected to two additional two rounds of extraction using the same method as before. Finally, the extract solutions were combined and kept at 4 °C for the determination of the PLTP yield.

2.4.2. Heat reflux extraction (HRE) of pawpaw leaves total polyphenols

The HRE was performed using a 100 mL round bottom flask fitted with a cooling condenser, and the round bottom flask was immersed in a thermostat water bath. Pawpaw leaf powder (1 g) was extracted three times using 30 mL of 70% ethanol for 2 h at 77 °C. After the extraction period, the extracts were further processed in accordance with Section 2.4.1.

2.4.3. Microwave-assisted extraction (MAE) of pawpaw leaves total polyphenols

Powdered pawpaw leaves (1.0 g) were extracted three times with 30 mL of 70% ethanol and water for 13.5 min at 77 °C and 460 W, after which the extracts were further processed as described in Section 2.4.1.

2.5. Determination of total polyphenols content (TPC)

The Folin-Ciocalteu colorimetric method described in a previous report was used to determine the TPC (Hou & Zhang, 2021). Briefly, 2.5 mL of Folin-Ciocalteu reagent (diluted 10-fold with H_2O) was mixed well with 0.5 mL of pawpaw leaves extract. After 5 min, 2.0 mL of Na_2CO_3 (7.5%, *w/v*) was promptly added. After incubation for one hour at 45 °C, the mixture was cooled naturally to room temperature. Afterwards, the absorbance was read at 765 nm. Gallic acid was used to construct a calibration curve ($A = 0.0099C + 0.0008$, A and C represent the absorbance and concentration, respectively) which demonstrated a strong linear relationship ($R^2 = 0.9996$) within the concentration range of 10–80 μg/mL. The PLTP yield was defined as mg gallic acid equivalent per g of dry weight (mg GAE/g DW).

2.6. Antioxidant assays

2.6.1. DPPH• scavenging activity

DPPH• scavenging activity was evaluated based on the method reported by Tsai et al. (2014). In brief, 2.4 mL of PLTP extract solution (50–300 μg/mL) was thoroughly mixed with 0.6 mL of DPPH solution (0.5 mM in ethanol). After 30 min of incubation at room temperature, the absorbance was monitored at 517 nm. Trolox was used as a positive control for this test, and the DPPH• scavenging activity was calculated using the following equation:

$$DPPH\cdot scavenging\ activity(\%) = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (2)$$

where A_1 represents the absorbance of the sample mixed with DPPH solution, A_2 represents the absorbance of the sample without DPPH solution (DPPH solution was replaced by ethanol), and A_0 represents the absorbance of the reaction mixture in the absence of sample.

2.6.2. $ABTS^+$• scavenging activity assay

For the $ABTS^+$• scavenging activity assay, the procedure reported by Luo, Peng, Liu, Wu, and Wu (2018) with a minor modification was employed. First, 7.0 mM ABTS prepared with phosphate buffer solution (PBS, pH 7.4) was blended with an equal volume of potassium persulfate solution (2.45 mM). This mixture was kept at room temperature for 16 h in the dark, after which its absorbance was adjusted to the desired value (0.68–0.72) at 734 nm using deionized water. Subsequently, 4.0 mL of the above mixed solution was blended thoroughly with 1.0 mL of various concentrations (50–300 μg/mL) of the PLTP solution prepared in ethanol. After 10 min of incubation at room temperature in the dark, the absorbance was read at 734 nm. Trolox was employed as a positive control for this test. The $ABTS^+$• scavenging activity of the PLTP extracts was calculated by the following equation:

$$ABTS^+\cdot scavenging\ activity(\%) = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (3)$$

where A_1 represents the absorbance of a mixture of sample and reaction solution, A_2 represents the absorbance of the sample without ABTS solution (the ABTS solution was replaced with PBS), and A_0 represents the absorbance of the ABTS solution without sample (the sample was replaced with PBS).

2.7. α-Glucosidase inhibitory activity

Evaluation of α-glucosidase inhibitory activity *in vitro* was performed based on the procedure described by Chen, Zhang, Huang, Fu, and Liu (2017) with minor modifications. In brief, 0.35 U/mL α-glucosidase solution and 1.5 mM pNPG solution were prepared with potassium phosphate buffer (pH 6.9). A volume of 100 μL of sample solution (50–800 μg/mL) and 200 μL of enzyme solution were thoroughly mixed and incubated for 10 min at 37 °C. Subsequently, the mixture was supplemented with 200 μL of pNPG solution and incubated for 20 min at 37 °C. After incubation, 2 mL of Na_2CO_3 (1 mol/L) was added. The absorbance was measured at 400 nm. Acarbose was used as a positive control. The percentage inhibition of α-glucosidase was measured as follows:

$$\alpha - Glucosidase\ inhibitory\ activity(\%) = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (4)$$

where A_0, A_1, and A_2 represent the absorbance values of the mixture (enzyme, pNPG reagent without sample), the mixture (sample, enzyme and pNPG reagent), and the mixture (sample, enzyme without pNPG reagent), respectively.

2.8. Statistical analysis

Data were presented as the mean ± standard deviation (SD) of triplicate independent experiments. Design-Expert® 10.0.7 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for RSM analysis. Data were subjected to one-way analysis of variance (ANOVA) and Duncan's test using SPSS 27 software (SPSS Inc., Chicago, IL, USA), and different letters represent statistical significance at $p < 0.05$.

3. Results and discussion

3.1. Screening of ILs

The choice of anions and cations influences important factors such as the viscosity, polarity, and solubility of the IL. These properties affect the interactions between the IL and the target components during the extraction process. (Harde, Lonkar, Degani, & Singhal, 2014). As presented in **Fig. S3**, the maximum PLTP yield was obtained with [BMIM]Br, while the minimum PLTP yield was obtained with [OMIM]Br. This may be because the increase in the number of alkyl groups on the cations leads to a decrease in the polarity of the ILs, thereby resulting in weakening of the electrostatic interactions. Additionally, an increase in the number of alkyl groups increased the steric hindrance (Wang, Su, & Yang, 2013). The variation in extraction yield attributable to disparate anions (Br^- and BF_4^-) could stem from the fact that different ILs engage in a spectrum of interactions with the target components. These

interactions encompass ionic or charge-charge interactions and hydrogen bonding, which collectively influence the extraction efficiency (Du, Xiao, & Li, 2007). Therefore, [BMIM]Br was selected for further extraction tests.

3.2. Analysis of single-factor experimental results

3.2.1. Effect of [BMIM]Br concentration

As shown in Fig. 1A, the PLTP yield increased visibly when the [BMIM]Br concentration increased from 0.1 to 0.7 mol/L. This was probably because an increase in the [BMIM]Br concentration could increase the transmission and absorption of microwave in the extraction system (Zeng, Wang, Kong, Nie, & Yuan, 2010). However, there was a distinct downward trend in the PLTP yield when the [BMIM]Br concentration increased to 0.9 mol/L. This was probably because a higher IL resulted in a higher viscosity, which was not conducive to the penetration of the IL into the plant tissue, resulting in the inability to effectively extract the target components (Harde et al., 2014). Therefore, a [BMIM]Br concentration of 0.9 mol/L was considered the optimal concentration for extracting PLTP.

3.2.2. Effect of microwave power

As shown in Fig. 1B, the TPLP yield increased as the microwave power increased from 200 to 400 W, whereas from 400 to 600 W of microwave power, the TPLP yield decreased. This suggested that a proper increase in microwave power for a short time was an effective method to promote the extraction of polyphenols from pawpaw leaves due to the accelerated transfer of intracellular polyphenols (Alara, Abdurahman, & Olalere, 2018). However, excessive microwave power could reduce the extraction yield. A similar trend was also observed in the ILMAE of polyphenols from *Peperomia pellucida* (L) kunth (Ahmad, Yanuar, Mulia, & Mun'im, 2017). This might be due to the fact that ILs possess a potent capacity to absorb microwave energy, and higher microwave irradiation power increases the temperature, potentially leading to the degradation of phenolics (Fan et al., 2018). Therefore, 400 W was considered to be the optimal microwave power for the extraction of

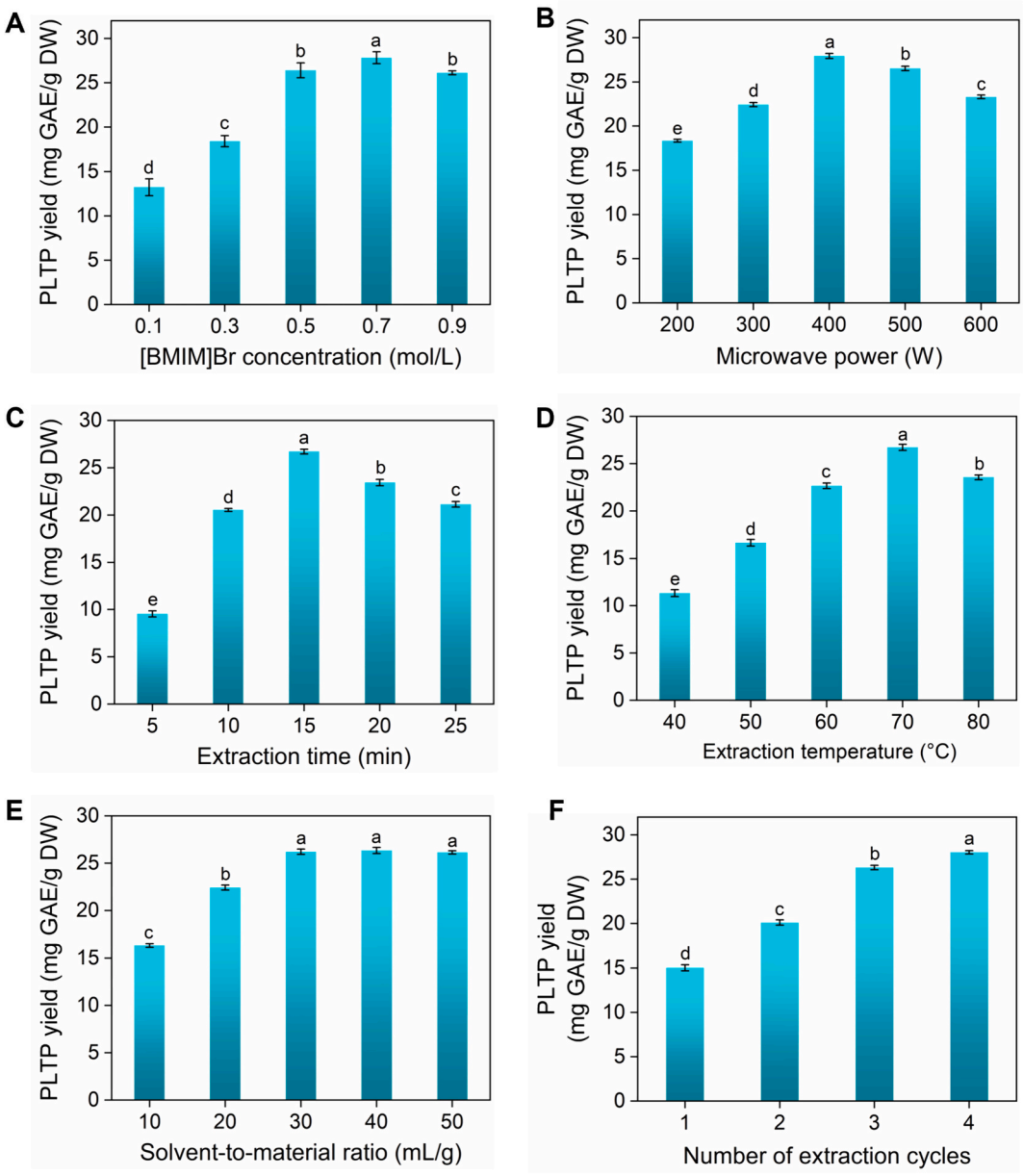

Fig. 1. Effect of independent variables on the pawpaw leaves total polyphenols yield. (A) [BMIM]Br concentration; (B) microwave power; (C) extraction time; (D) extraction temperature; (E) solvent-to-material ratio; (F) number of extraction cycles. Different letters indicate significant differences at $p < 0.05$.

TPLP.

3.2.3. Effect of extraction time

As shown in Fig. 1C, the extraction time greatly influenced the TPLP yield. When the extraction time range was 5–15 min, TPLP yield increased drastically, while the continued increase in time seemed to contradict the ILMAE of polyphenols. A proper extension of time is advantageous for improving the extraction yield. The initial stage is a heating process, and the dissolution rate of polyphenols is mainly affected by heat. However, overexposure to microwaves may lead to an increased chance of structural destruction of polyphenols, and the dissolution of other impurities may also increase (Liang et al., 2017). An analogous trend was observed in the isolation of polyphenols through ILMAE from *Peperomia pellucida* (L) Kunth (Ahmad et al., 2017). Thus, 15 min was considered to be the optimal extraction time for PLTP.

3.2.4. Effect of the extraction temperature

As shown in Fig. 1D, the TPLP yield increased when the temperature increased from 40 to 70 °C. However, there was a distinct downward trend in the TPLP yield when the extraction temperature continued to increase. A similar trend was also observed in the ILMAE of polyphenols from *Smilax china* tubers (Du, Xiao, Luo, & Li, 2009). This was probably because a higher temperature could heighten the diffusivity and solubility of polyphenols and increase the extraction efficiency. However, the overheating of plant materials can result in the thermal degradation of phenolics, which is not conducive to increasing the TPLP yield. Therefore, 70 °C was considered the optimal temperature for extracting PLTP.

3.2.5. Effect of the solvent-to-material ratio

As indicated in Fig. 1E, an obvious increase in the TPLP yield was found within the solvent-to-material ratio range of 10–30 mL/g. The reason was that the powdered sample was mixed more thoroughly with the extraction solvent at a higher solvent-to-material ratio, which enhanced the mass transmission during the extraction procedure. However, a further increase in the solvent-to-material ratio exerted no noticeable influence on the TPLP yield. A similar phenomenon has also been observed during the ILMAE of verbascoside from *Rehmannia* root (Fan et al., 2018). Considering both operational efficiency and energy consumption, a solvent-to-material ratio of 30 mL/g was determined to be the most optimal.

3.2.6. Effect of the number of extraction cycles

As indicated in Fig. 1F, as the number of extraction cycles increased from 1 to 3, the TPLP yield clearly increased. However, there was no obvious increase in the TPLP yield when the number of extraction cycles continued to increase. In the study of ILMAE of flavonoids from *Scutellaria baicalensis* Georgi, it was found that the extraction yield exceeded 90% in the first cycle and increased slightly with subsequent extraction cycles (Zhang, Zhao, Chen, & Zhang, 2015). Therefore, though it was observed that increasing the number of extraction cycles could enhance the extraction yield, it is important to consider both extraction efficiency and energy consumption. To strike a balance between these factors, three extraction cycles were employed in subsequent studies.

3.3. Modeling and validation

3.3.1. Model fitting and statistical analysis

Single-factor experimental results indicated that four variables, namely, the [BMIM]Br concentration (X_1), microwave power (X_2), extraction time (X_3), and extraction temperature (X_4), strongly influenced the TPLP yield (Y). Therefore, these parameters were chosen for further optimized through RSM. Twenty-nine experimental runs were performed in a random manner to achieve minimal influence of uncontrolled factors. Following the multiple regression analysis of the experimental data, a refined quadratic eq. (5) was derived, with all non-significant factors omitted for enhanced precision.

$$Y = \begin{array}{c} 27.48 - 0.22X_1 + 1.35X_2 - 0.21X_3 + 0.13X_4 \\ -0.89X_1X_2 + 1.11X_1X_3 - 0.75X_1X_4 + 0.62X_2X_3 \\ 0.97X_2X_4 - 1.25X_1^2 - 1.98X_2^2 - 0.91X_3^2 - 0.88X_4^2 \end{array} \quad (5)$$

The results obtained from ANOVA of the quadratic model are listed in Table 2. The F value (71.46) and p value (< 0.0001) of the model both indicated that this model was significant ($p < 0.05$). The "lack of fit" was not significant since the p value (0.1086) was >0.05, which implied that the quadratic model was appropriate for describing the experimental data (Hou et al., 2019). Two popular measures for goodness of fit in linear regression model, the coefficient of determination ($R^2 = 0.9841$) and its adjusted version (adjusted $R^2 = 0.9703$), indicated that the model was consistent with the experimental data within the experimental range of variables. A low coefficient of variance (C.V.% = 1.09) demonstrated a high level of precision and favorable reliability of the experimental data (Zhao, Meng, Zhao, & Zhao, 2020).

3.3.2. Response surface analysis

Response surface plots present intuitive information about the interaction effect of two independent variables on the dependent variable when other factors are maintained at zero. They are very helpful in obtaining the optimal level of each variable for achieving a maximum response value. Additionally, the extent of influence of the variables on the response value can be deduced from the shape of the plots (Dahmoune et al., 2014).

As shown in Fig. 2, the TPLP yield was significantly affected by the [BMIM]Br concentration, microwave power, extraction time and temperature, which was consistent with the ANOVA results. Fig. 2A–C depicts the interactions between the [BMIM]Br concentration and each of three other factors (microwave power, extraction time and temperature). Fig. 2D–F shows the interactions between microwave power and extraction time, between microwave power and extraction temperature, and between extraction time and temperature, respectively. A similar phenomenon also occurred in the optimization of ILMAE of isoflavones from *Radix puerariae* by RSM (Zhang, Liu, Li, & Chi, 2014). It was obvious that the TPLP yield increased at the beginning and then decreased with increasing variable values, which was basically consistent with the single-factor experimental results. With the help of numerical function optimization, it could be inferred that the best combination of independent variables for achieving the maximum TPLP

Table 2
ANOVA results of experiment model.

Source	Sum of Squares	DF	Mean Square	*F*-Value	*p*-Value
Model	71.32	13	5.49	71.46	< 0.0001
X_1	0.59	1	0.59	7.62	0.0146
X_1	21.95	1	21.95	285.92	< 0.0001
X_1	0.51	1	0.51	6.68	0.0208
X_1	0.20	1	0.20	2.57	0.1295
X_1X_2	3.17	1	3.17	41.27	< 0.0001
X_1X_3	4.95	1	4.95	64.48	< 0.0001
X_1X_4	2.25	1	2.25	29.31	< 0.0001
X_2X_3	1.53	1	1.53	19.87	0.0005
X_2X_4	3.76	1	3.76	49.02	< 0.0001
X_1^2	10.10	1	10.10	131.54	< 0.0001
X_2^2	25.50	1	25.50	332.15	< 0.0001
X_3^2	5.39	1	5.39	70.20	< 0.0001
X_4^2	5.07	1	5.07	66.02	< 0.0001
Residual	1.15	15	0.077		
Lack of Fit	1.05	11	0.095	3.71	0.1086
Pure Error	0.10	4	0.026		
Cor Total	72.47	28			
R^2	0.9841				
Adjusted R^2	0.9703				
C.V.%	1.09				

DF-degrees of freedom; R^2-coefficient of determination; C.V.-coefficient of variation.

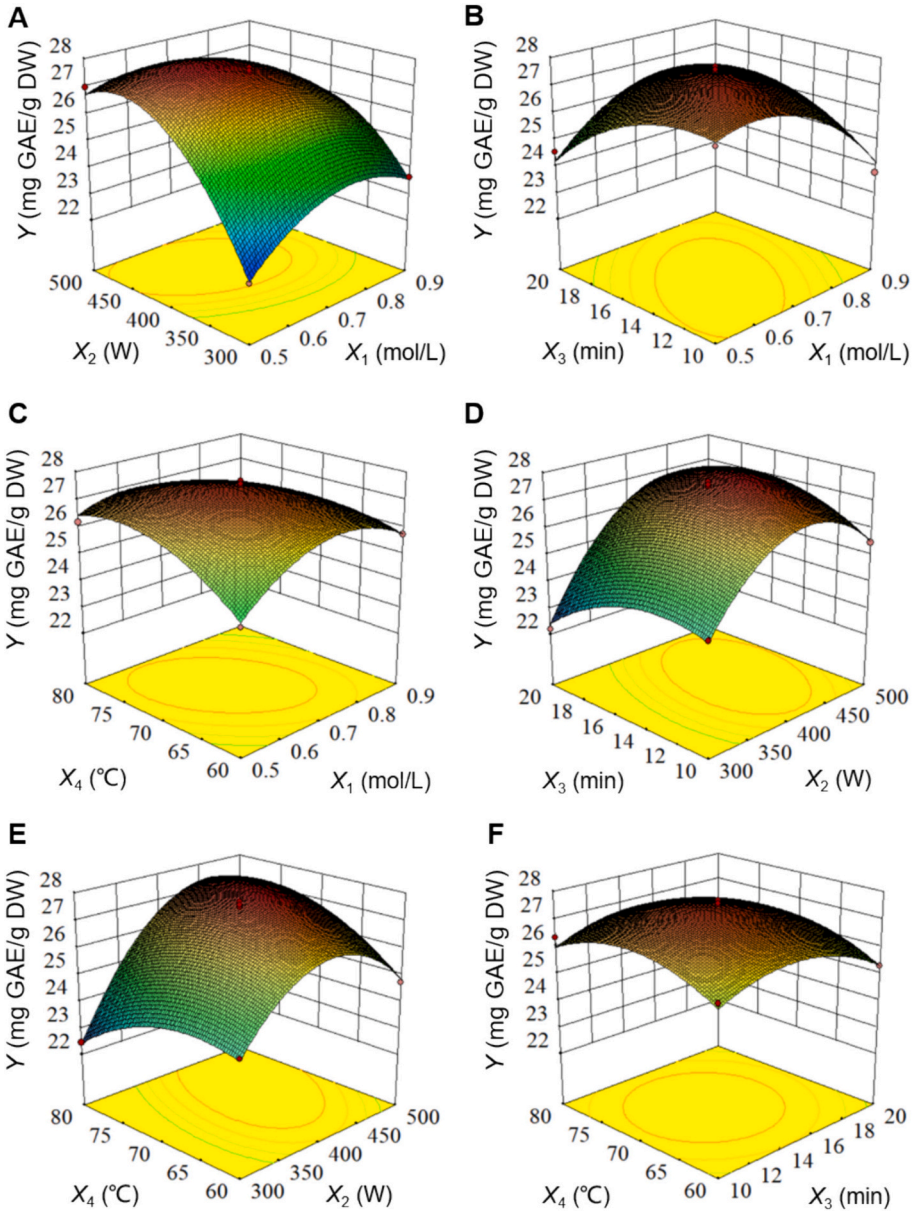

Fig. 2. Response surface graph illustrating the interactive effects of the independent factors on the pawpaw leaves total polyphenols yield.

yield was as follows: [BMIM]Br concentration of 0.57 mol/L, microwave power of 460.26 W, extraction time of 13.50 min, and extraction temperature of 76.75 °C.

3.3.3. Model verification

Considering the convenience and precision of actual operation, the suitability of the prediction model was verified under modified conditions: [BMIM]Br concentration of 0.57 mol/L, extraction time of 14 min, microwave power of 460 W, extraction temperature of 77 °C, solvent-to-material ratio of 30 mL/g and three extraction cycles. The experiment was performed three times. The TPLP yield was 27.84 ± 0.33 mg GAE/g DW, which was very close to the predicted value (28.03 mg GAE/g DW). In the ILMAE experiments, numerous verification tests have confirmed a high degree of concordance between the predictive outcomes from RSM analysis and the actual experimental results (Ahmad et al., 2017; Fan et al., 2018; Liang et al., 2017). Therefore, the prediction model proposed in this study was quite suitable for actual production.

3.4. Comparison of the ILMAE method with other extraction methods

The selection of an appropriate extraction method mainly considers the extraction yield, process complexity, environmental friendliness, energy consumption, production efficiency, safety, *etc.* (Li et al., 2010). The PLTP yields obtained by the ME, HRE, MAE, and ILMAE methods are presented in Fig. 3. When polyphenols were extracted with the same solvent, the PLTP yield of MAE was greater than that of ME and HRE. The better extraction effect of MAE could be attributed to the mechanical effects of internal heating derived from microwave irradiation (Rostagno, Palma, & Barroso, 2007). The 0.57 mol/L [BMIM]Br solution was the most suitable solvent for extracting PLTP, followed by 70% ethanol. The [BMIM]Br solution gave a higher PLTP yield than did water, possibly due to ionic/charge–charge interactions and hydrogen bonding between ions and polyphenols (Bogdanov & Svinyarov, 2013). The results indicated that the PLTP yield obtained by the ILMAE method was greater than that obtained by the other extraction methods. Hence, in light of the superior extraction capabilities demonstrated by the ILMAE method, the antioxidant properties and α-glucosidase inhibitory

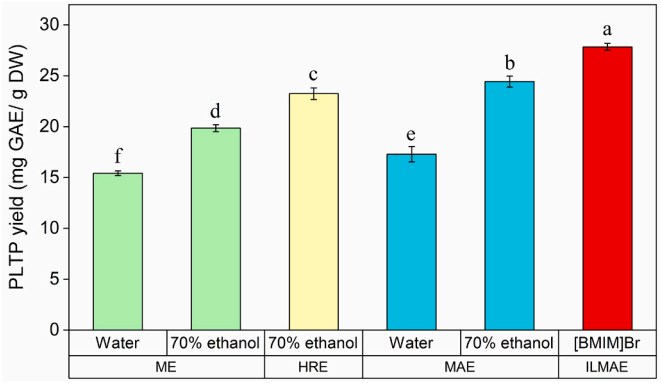

Fig. 3. Comparison of different extraction methods. Different letters indicate significant differences at $p < 0.05$.

effects of the PLTP extracts produced by ILMAE technique were further evaluated.

3.5. *In vitro antioxidant properties*

3.5.1. *DPPH• scavenging activity*

Phenolic compounds are widely recognized for their powerful ability to neutralize free radicals, a property largely ascribed to the presence of phenolic hydroxyl groups adorning their ring structures (Okawa, Kinjo, Nohara, & Ono, 2001). The DPPH• scavenging activity of the PLTP extracts is illustrated in Fig. 4A, which indicates that the DPPH• scavenging activity of the PLTP extracts increased with increasing concentration from 50 to 300 μg/mL. Although the DPPH• scavenging activity of PLTP was lower than that of AA within the tested concentration range, PLTP was able to scavenge 87.10% of DPPH• at a concentration of 300 μg/mL, and the IC_{50} value was calculated as 143.80 μg/mL. These results indicated that pawpaw leaves could be considered a good source of DPPH• radical scavengers.

3.5.2. *$ABTS^{+}$• scavenging activity*

The $ABTS^{+}$• scavenging activity assay is another method generally used for screening natural free radical scavengers. The $ABTS^{+}$• scavenging activity of PLTP was investigated in this work, and the results are shown in Fig. 4B. There was a positive correlation between the concentration of PLTP and the $ABTS^{+}$• scavenging activity. Although the $ABTS^{+}$• scavenging activity of PLTP was lower than that of Trolox, it still reached 96.25% at a concentration of 300 μg/mL. The IC_{50} value of PLTP for $ABTS^{+}$• was 128.86 μg/mL, suggesting that the PLTP extracts had a noticeable scavenging effect on $ABTS^{+}$•. Numerous phenolic compounds found in papaya leaves, such as rutin, quercetin, chlorogenic acid, protocatechuic acid, and caffeic acid have been reported to possess substantial $ABTS^{+}$• scavenging capabilities (Kędzierska-Matysek et al., 2021). Therefore, it could be speculated that these compounds have a greater contribution to the $ABTS^{+}$• scavenging activity of PLTP extracts.

3.6. *α-Glucosidase inhibitory activity*

The inhibition of α-glucosidase can effectively inhibit the absorption of glucose and thus reduce blood glucose levels. Extensive studies have highlighted the efficacy of phenolic compounds in acting as α-glucosidase inhibitors, a property that could be harnessed to significantly mitigate postprandial hyperglycemia (Swargiary, Roy, & Mahmud, 2023). As shown in Fig. 4C, the α-glucosidase inhibitory potential of the PLTP extracts improved noticeably when the concentration of the PLTP extracts increased from 50 to 800 μg/mL. Although acarbose maintained a greater α-glucosidase inhibitory potential than the PLTP extracts within the range of tested concentrations, the PLTP extracts exhibited good inhibition of α-glucosidase (86.53 ± 1.17%) at a concentration of 800 μg/mL. PLTP extracts showed good inhibitory effects on α-glucosidase, with an IC_{50} value of 157.11 μg/mL. Agada, Usman, Shehu, and Thagariki (2020) reported that *Carica papaya* seed also exhibits α-glucosidase inhibitory potential, and its anti-diabetic effects may be

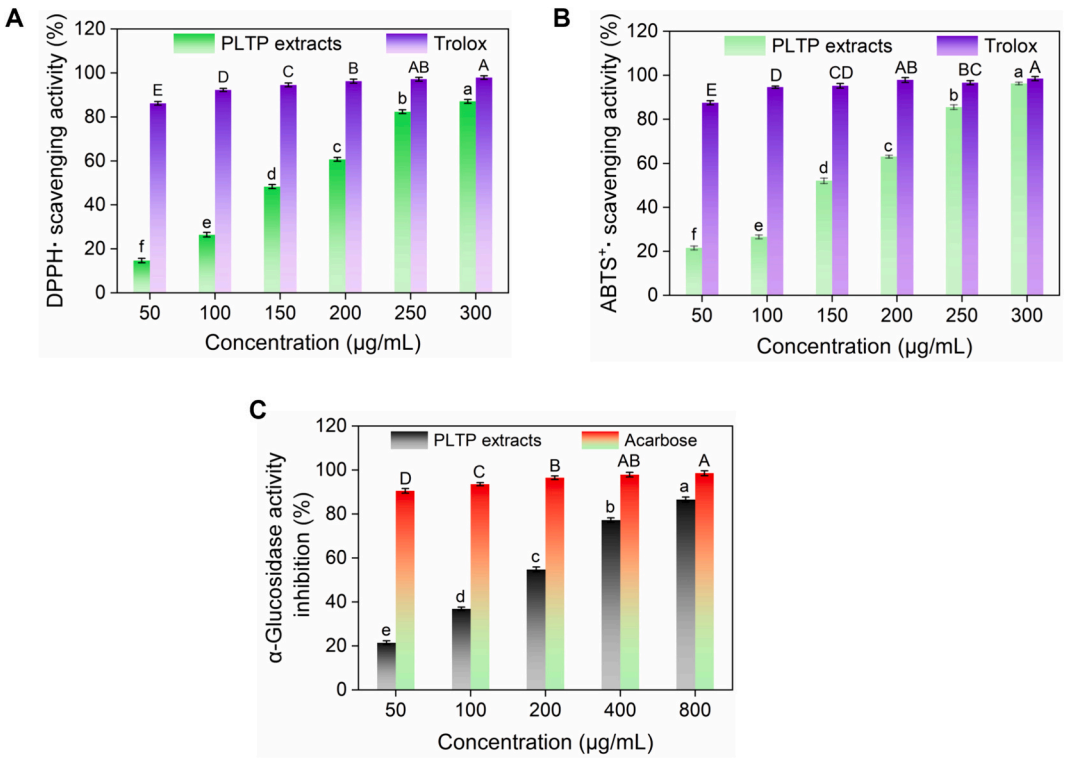

Fig. 4. Bioactivities of pawpaw leaves total polyphenols extracts. (A) DPPH• scavenging activity; (B) $ABTS^{+}$• scavenging activity; (C) α-Glucosidase inhibitory activity. Different letters indicate significant differences at $p < 0.05$.

due to the suppression of α-glucosidase enzymes, as well as the reduction of oxidative stress following meals.

4. Conclusion

This the first report on the application of the ILMAE technique for the recovery of PLTP, and a single-factor test combined with RSM was satisfactorily applied for the optimization of technological parameters. Under the optimal conditions: [BMIM]Br concentration of 0.57 mol/L, extraction time of 14 min, microwave power of 460 W, extraction temperature of 77 °C, solvent-to-material ratio of 30 mL/g, and three extraction cycles, the PLTP yield was 27.84 ± 0.33 mg GAE/g DW. Comparative research indicated that the PLTP yield provided by ILMAE was remarkably greater than that provided by other extraction methods, including ME, HRE and MAE. Furthermore, the PLTP extracts exhibited outstanding scavenging capabilities against DPPH• and $ABTS^{+}$• radicals, along with potent α-glucosidase inhibitory activity, as evidenced by their IC_{50} values of 143.80, 128.86, and 157.11 μg/mL, respectively. These findings demonstrate that the ILMAE technique serves as a green and efficient alternative to conventional extraction methods for extracting PLTP. Furthermore, pawpaw leaves show promising potential as a valuable source of antioxidants and hypoglycemic agents. Indeed, this work contributes to the development of eco-friendly and economically viable processes that maximize the potential benefits of pawpaw leaves in the food, pharmaceutical, and cosmetic industries.

CRediT authorship contribution statement

Duo Cao: Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Xiaoting Qiao:** Software, Methodology, Investigation, Data curation. **Yaqian Guo:** Methodology, Investigation, Data curation. **Pengyu Liu:** Writing – review & editing, Validation, Resources, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101500.

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