Lonic Liquids-based Ultrasonic -assisted Extraction of Total Flavonoids from Manilkara Zapota Leaves

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Keywords: Manilkara zapota leaves, Ionic liquids-based ultrasonic-assisted extraction (ILs-UAE) Total flavonoids, Response surface methodology (RSM)

Abstract. In the present research, single-factor experiments and a three-variable, five-level central composite design were performed to study on the ionic liquids-based ultrasoni-assisted extraction of total flavonoids from *Manilkara zapota* leaves. The optimum parameters were: 0.95 M [C_4MIM]Br, liquid/ solid ratio 33.1:1 mL·g^{-1}, extraction duration 30.3 min. Under the optimal conditions, the average extraction yields of total flavonoids were 12.42%.

1. Introduction

Manilkara zapota is a nutritious, fleshy berry, with a scurfy brown peel and light brown, brownish yellow to reddish brown pulp, with a texture varying from gritty to smooth. The pulp has a very sweet pleasant flavor. So it is also incorporated into sherbets, milkshakes, and ice cream. The fruit and its peel contain high amounts of saponin, flavonoids, phenols and many more active compounds [1]. It had the highest oxygen radical absorbance capacity and higher reducing power [2-3]. But, there is scant report on *Manilkara zapota* leaves. Our preliminary investigation indicated flavonoids were abundant in *Manilkara zapota* leaves. Both flavonoids have great potentials to be used as clinical therapeutic agents, food additive and nutraceutical products [4]. Therefore, the investigations on efficient extracting flavonoids from *Manilkara zapota* leaves are greatly significant for the utilisation of *Manilkara zapota* resources in food and pharmaceutical industry.

The aims of the present work were to evaluate the feasibility of ionic liquids-based ultrasonic -assisted extraction (ILs-UAE) for total flavonoids from *Manilkara zapota* leaves. Meanwhile, the operating parameters were optimized using central compositedesign (CCD) combined with response surface methodology (RSM). From the economic perspective, the selected operating parameters were the important parameters involved in different extraction process were optimized by single-factor experiments. The present research would offer scientific reference for promoting the utilization and the further explore of the renewable resource leaves of *Manilkara zapota*.

2. Materials and methods

2.1 Materials and reagents

The leaves of *Manilkara zapota* collected from South Subtropical Crop Research Institute. The material was dried in the shade, powdered by a disintegrator (HX-200A, Yongkang Hardware and Medical Instrument Plant, China). ILs, including [C_4MIM]Br, [C_4MIM]Cl, [C_4MIM]H_2PO_4, [C_4MIM]BF_4和[C_4MIM]HSO_4 were purchased from Chengjie Chemical Reagents Co. (Shanghai, China). All reagents obtained from Tianjin Chemical Reagents Co. (Tianjin, China) was analytical grade. Rutin were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2 IL-UAE

Two gram of prepared *Manilkara zapota* leaves was placed into the extraction vessel. Then, a certain amount of aqueous IL solution was added into the vessel and the mixture was automatically extracted by ultrasonic with a presetting procedure.

In order to investigate the effects of total flavonoids of *Manilkara zapota* leaves on ILs-UAE, CCD and RSM was used for optimising extraction process in the present study. Extraction variables

consist of ILs, ILs concentration, liquid/solid ratio and extraction duration. The experiments of determination total flavonoids content were carried out according to the method of Jin et al [5].

3. Result and discussion

3.1 Selection of ILs for UAE

ILs were employed as solvents and cosolvents in UAE of analytes due to its strong solvent dissolving power. All of them are miscible in any proportion with water. In the experiment, 1-alkyl-3-methylimidazolium type ionic liquids with different anion were investigated to find out the optimal ionic liquid for extraction total flavonoids in *Manilkara zapota* leaves. As shown in Fig. 1, the effect of ILs on the extraction efficiency of totals flavonoids is obvious and [C4MIM]Br is more efficient than others. It was probably due to its stronger multi-interactions including π-π, ionic/charge – charge and hydrogen bonding with those compounds [6]. Thus, [C4MIM]Br was used in further experiments to investigate.

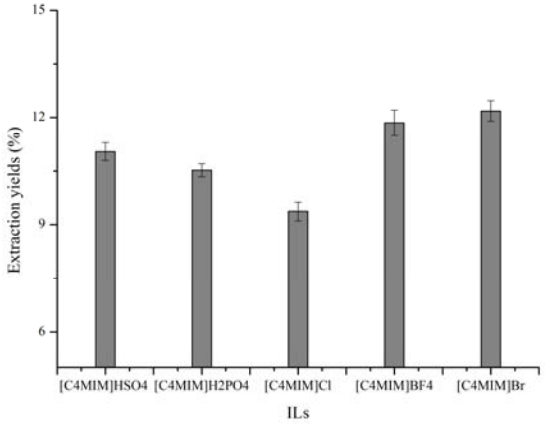

Fig. 1. Effect of different ILs on the extraction yields of total flavonoids from *Manilkara zapota* leaves.

3.2 Optimization of total flavonoids ILs-UAE using CCD

Three parameters including IL concentration, liquid/solid ratio and extraction duration supposed to affect active substances production were selected for CCD combined with RSM). All the experimental data obtained from the 20-run experiments are shown in Table 1.

Runs	Factors			Extraction yield
	X_1 (min)	X_2 (mL/g)	X_3 (M)	(%)
1	1(40)	1(40:1)	1(1.2)	11.08
2	1(40)	1(40:1)	-1(0.4)	10.75
3	1(40)	-1(20:1)	1(1.2)	10.58
4	1(40)	-120:1)	-1(0.4)	9.37
5	-1(20)	1(40:1)	1(1.2)	11.16
6	-1(20)	140:1)	-1(0.4)	10.14
7	-1(20)	-1(20:1)	1(1.2)	10.68
8	-1(20)	-1(20:1)	-1(0.4)	9.12
9	-1.682(13.18)	0(30:1)	0(0.8)	10.12
10	1.682(46.82)	0(30:1)	0(0.8)	10.34
11	0(30)	-1.682(13.18:1)	0(0.8)	10.12
12	0(30)	1.682(46.82:1)	0(0.8)	11.42
13	0(30)	0(30:1)	-1.682(1.47)	9.72
14	0(30)	0(30:1)	1.682(0.13)	11.48
15	0(30)	0(30:1)	0(0.8)	12.18
16	0(30)	0(30:1)	0(0.8)	12.34
17	0(30)	0(30:1)	0(0.8)	12.32
18	0(30)	0(30:1)	0(0.8)	12.26
19	0(30)	0(30:1)	0(0.8)	12.22
20	0(30)	0(30:1)	0(0.8)	12.21

Table 1. Experimental design and results for the CCD and the corresponding responses measured.

3.2.1 Fitting the model

In this study, a Five-level (-1, 0, +1) three-factor CCD was applied to evaluate the main and

interaction effects of the factors in the experimental region: extraction duration (X_1), liquid/solid ratio (X_2) and IL concentration (X_3) on the extraction yields of total flavonoids (Y). A second-order polynomial function was fitted to correlate the relationship of each independent variable to the response. For the three factors this equation is:

$$Y = 12.26 + 0.077X_1 + 0.41X_2 + 0.52X_3 + 0.048X_1X_2 - 0.13X_1X_3 - 0.18\ X_2X_3 - 0.73X_1^2 - 0.54X_2^2 - 0.60X_3^2$$

Analysis of variance for evaluation of the second-order model is presented in Table 2. The F test and p-value were used to determine the significance of each coefficient. The absolute F-value become larger and the p-value smaller which means the corresponding variables are more significant. From the equation and Table 2, it was observed that the factors with the largest effects on the total flavonoids yield was X_3, the second was X_2. The analysis of variance (ANOVA) of total flavonoids extraction yields showed that experimental data had determination coefficient (R^2) of 0.997.

Source	F value	P value	Significant
Model	413.34	< 0.0001	Significant
X_1	14.48	0.0035	
X_2	406.92	< 0.0001	
X_3	658.32	< 0.0001	
X_1X_2	3.24	0.1022	
X_1X_3	24.25	0.0006	
X_2X_3	45.21	< 0.0001	
X_1^2	1379.27	< 0.0001	
X_2^2	752.51	< 0.0001	
X_3^2	929.50	< 0.0001	
Lack of fit	1.74	0.2791	Not significant
R^2	0.9973		

Table 2. ANOVA of response surface quadratic model for extraction yields of total flavonoids.

3.2.2 Analysis of response surface

In order to select the optimized conditions, ILs concentration, liquid/solid ratio and extraction duration were investigated. In Fig. 2A, it was indicated that too low or high level of liquid/ solid ratio and extraction duration could decrease mass transfer development, leading to an incomplete extraction. The response surface (Fig. 2C) showed the interaction of liquid/ solid ratio and concentration of ionic liquid on the extraction yield of total flavonoids, and from the sharp of the response surface we also could see the interaction was significant. And form Fig. 2B we can see that with the ILs concentration higher and the longer extraction duration, the yield of the total flavonoids increased to the maximal value then decreased. The relation of extraction duration and concentration was that the concentration of ILs increased the viscosity of ILs solutions [7], which not only influenced the permeability of the ILs solution but also affected the formation of mass transfer, then effected the the extraction yield of the total flavonoids [8].

In summary, in order to facilitate the practical operate, the optimal condition for extracting the three compounds in the ILs-UAE process was selected as: 0.95 M [C_4MIM]Br, liquid/ solid ratio 33.1:1 mL·g^{-1}, extraction duration 30.3 min.

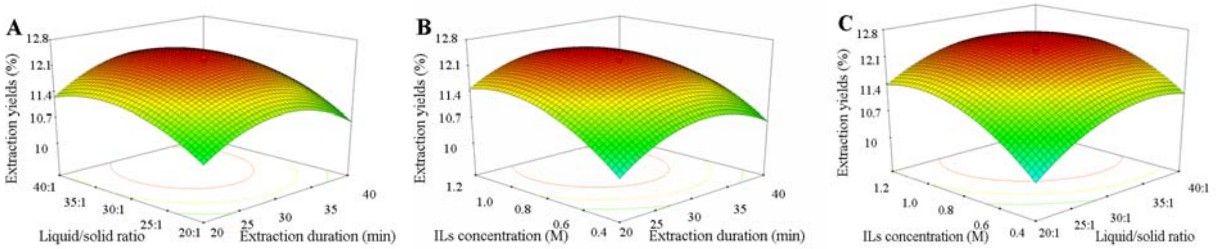

Fig. 2. Response surface plots of extraction yields affected by ILs concentration, liquid/solid ratio and extraction duration.

3.3 Validation of the models

In order to validate the suitability of the model equations for predicting the response values, a verification experiment was carried out under above optimised conditions. Under these conditions,

the extraction yields of total flavonoids reached 12.46 ± 0.12%, whereas the response values predicted by the model equations were 12.42%. The results of analysis indicated that the experimental values were in good agreement with the predicted ones, with low percentage bias, suggesting that the RSM models were reliable and reasonable.

4. Conclusion

In the present study, a green and effective ILs-UAE method was used for the extraction of total flavonoids from the leaves of *Manilkara zapota* for the first time. According to the single factors experiments and a CCD test, we chosed $[C_4MIM]Br$ as the extraction solvent and the final optimal conditions were liquid/ solid ratio 33.1:1 $mL \cdot g^{-1}$, extraction duration 30.3 min and ILs concentration 0.95 M. Therefore, the effective ILs-UAE method shows a great promising prospect to the extraction active compositions from medicinal plants. Moreover, it was proved that the optimised ILs-UAE process was scalable by pilot-scale application.

Acknowledgements

This work was financially supported by the Fund on Basic Scientific Research Project of Nonprofit Central Research Institutions (1630062015011), the National Natural Science Foundation of Hainan province (No. 20153122) and Agricultural Ecology Environmental Protection Project.

References

[1] Devatkal S. K., Kamboj R., Paul D.: J. Food Sci Technol, (February 2014) 51(2):387–391.

[2] Isabelle M, Bee L.L, Meng T.L, Woon-Puay K, Dejian H, Choon NO: Food Chem. (2010) 123:77–84.

[3] Jamuna KS, Ramesh CK, Srinivasa TR, Raghu KL: J Pharm Res (2010) 3:2378–2380.

[4] Balerdi C. F., Crane J. H., Institute of Food and Agricultural Sciences, University of Florida: Homestead, 2000.

[5] JIN S., YANG M., KONG Y., YAO X.H., WEI Z.F., ZU Y.G., FU Y.J.: Chinese Traditional and Herbal Drugs (2011) 42(11):2235-2239.

[6] Wei Z. F., Zu Y.G, Fu Y.J., Wang W., Luo M., Zhao C.J., Pan Y.Z.: Sep. Purif. Technol. (2013) 102:75–81.

[7] Guo Z., Lue B. M., Thomasen K., Meyer A. S., Xu X.B.: Green Chem. (2007) 9:1362–1373.

[8] Duan M.H., Luo M., Zhao C.J., Wang W., Zu Y.G., Zhang D.Y., Yao X.H, Fu Y.J.: Sep. Purif. Technol. (2013) 107:26–36.

