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Enhancement Extraction of Quercetin from *Suaeda glauca* Bge. Using Ionic Liquids as Solvent

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The extraction of quercetin from *Suaeda glauca* leaf was firstly investigated by the ultrasonic-assisted extraction. Experiment of single factor (ethanol concentration, reaction temperature, ratio of solid to liquid) was preliminary performed for the parameters of quercetin extraction. Based on the experiments of single factor results, the optimal extraction parameters of quercetin from *Suaeda glauca* leaf were obtained by response surface methodology, which established a mathematical model using quercetin yield as target variable. The optimal parametres were ratio of solid to liquid 1:10 (g/mL), extraction temperature 60 ºC, ethanol concentration 72.69 %. Under optimal conditions, validation experiments result was 0.782 mg/g, which was close to the model results and showed that the model was reasonable and reliable. Enhancemen extraction experiment using ionic liquids as solvent showed the ionic liquid with highest extraction yield of quercetin was [EMIm]Br with the concentration of 0.5 mol/L, ratio of solid to liquid ratio 1:15 and the highest yield of quercetin was up to 1.076 mg/g.

Keywords: *Suaeda glauca* Bge., Quercetin, Response surface methodology, Ultrasonic extraction, Ionic liquids.

INTRODUCTION

Suaeda glauca Bge. (Chenopodiaceae, Suaeda). is a kind plant of liking salt wet with stem succulent leaves. It can tolerate temporary drought[1]. The dormancy period of seeds is very short and under appropriate conditions it can rapidly germinate seeds. Around the alkaline lake and on the saline-alkali spots scattered or cluster growth, *Suaeda glauca* Bge. can form pure community and was also associated species with other halophyte communities. At present, the research around Suaeda mainly concentrated on extraction of pigment from Suaeda[2] and biochemical activity of extracts[3]. The agricultural use of Suaeda seems to be more popular. Due to generally grown in saline-alkali soil, Suaeda plays a crucial role in beach green[4,5]. Another use of Suaeda is mainly as the green feed of livestock, which can reduce the burden of farmers. Some species of Suaeda is edible, even it was reported that Suaeda has become one of the export sales of vegetable products[6]. But preliminary study proved that flavonoids is one of the effective components. However, the extraction and the content of the active component has not reported in detail. Quercetin is one of flavonoids[1], it has the prominent biological activity and important pharmacological activities[7,8]. There were the evidences that these activities had the ability of protection against damage[9,10] .

In addition, ionic liquid with its strong extraction had received wide attention and application[11-13]. The purpose of this paper was the extraction of quercetin finished by ultrasonic assisted extraction using *Suaeda glauca* leaf on DaFeng's seawall (Yancheng,china) as the object of study, to determine the optimum extraction parametres, followed by enhancemen experiment in order to get a higher extraction efficiency of quercetin to develop application on beaches and Suaeda salsa industry to provide useful data and reference. This may provide the seful data and reference basis for the development and application of Suaeda agriculture, industry and medicine industry.

EXPERIMENTAL

Quercetin standard was purchased from Beijing Yingze New Chemical Technology Research Institute (National Standard Samples website). 1-Butyl-3-methylimidazolium chloride ([BMIm]Cl) 1-butyl-3-methylimidazolium bromide ([BMIm]Br), 1-butyl-3-methylimidazolium nitrate ([BMIm]NO_3), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIm]PF_6), 1-ethyl-3-methylimidazolium bromide ([EMIm]Br), 1-hexyl-3-methylimidazolium bromide ([HMIm]Br), 1-octyl-3-methylimidazolium bromide ([OMIm]Br) were provided by Lanzhou Greenchem ILS, LICP. CAS. China. The purity > 99 %. Ethanol was bought from Jiangsu Tong Sheng Chemical Reagent Co., Ltd. All chemicals were analytical grade. *Suaeda glauca* leaf was picked from Dafeng seawall (YanCheng, JiangSu, China) in 2013 March. It was cleaned and dried at 60 ºC to constant weight, then sealed for further use after 60 mesh sieving. Analysis of quercetin content was determined by UV-visible spectrophotometer (SPECORD-50, Germany jena), quercetin was extracted by Ultrasonic cleaner (KQ-300E, Kunshan ultrasonic instruments Co., Ltd, China), Centrifuge (800, Hong Sheng Instrument Factory, Jintan,China) was used. Rotary evaporator (R201D-II, Zhengzhou Great Wall Science and Industry Co. Ltd.).

General procedure: *Suaeda glauca* leaf powder was weighed accurately with 1 g into the flask, added 50 mL of the solvent into the flask again, which was placed into the ultrasonic cleaner at a certain temperature for a certain time, followed by centrifugation and take the supernatant for the quantitative determination of quercetin content after dilution.

Detection method: Quercetin standard curve was drawn by the following method. 0.0300 g quercetin standard was weighed and dissolved with 95 % alcohol into a 100 mL volumetric flask, dilute to the constant volum with 95 % ethanol, so 1×10^{-5} mol/L stock solution of quercetin standard was obtained.

Determination of quercetin content was using aluminum chloride method[14]. The anhydrous aluminum chloride 0.0667 g was dissolved with 95 % ethanol and transferred to a 250 mL volumetric flask, then diluted with distilled water to constant volume. The standard solution of 2×10^{-3} mol/L aluminum chloride was obtained. A certain amount of quercetin standard solution was taken into 10 mL volumetric flask, so their concentration, respectively were 0.025, 0.075, 0.15, 0.2, 0.25, 0.3 mg/L and mixed with 1 mL aluminum chloride standard solution, which was diluted to volume standing for 50 min. The above solution was scanned by UV-VIS spectrophotometer at wavelength of 273 nm. According to the results, the concentration of quercetin with absorbance relationship curve can be drawn and the standard curve equation was obtained: $y = 0.2375x + 0.0189$, $R^2 = 0.9903$, where x represents the absorbance of solution (A), y represents the quercetin solution (mg/L).

According to the above method, the extraction yield of quercetin from *Suaeda glauca* leaf can be calculated by the following formula:

$$\text{Extraction yield (mg/g)} = \frac{(0.2375\times A-0.0189)\times V\times M\times n\times 0.01}{m}$$

where A represents the absorbance of extracts solution, V represents extract volume (mL), M is the molar mass of quercetin (g/mol), n represents the diluted multiples of original solution, m is the dry weight of *Suaeda glauca* leaf (g).

RESULTS AND DISCUSSION

Effect of concentration of ethanol on the yield of quercetin in *Suaeda glauca* leaf: At the same condition temperature 40 ºC, solid-liquid ratio 1:20 g/mL, extraction time 20 min, the effect of ethanol concentration on the yield of quercetin was shown results in Fig. 1. The result showed that the yield of quercetin was increased with the increase of ethanol concentration. When the ethanol concentration reached 70 %, the yield of quercetin was decreased. This can be interpreted as the increasing solubility of quercetin in ethanol with increasing of ethanol concentration. But when ethanol concenteation was higher than 70 %, the extraction rate of quercetin was decreased with gradually increase of polarity of the solution leading to the solubility decreases. The suitable ethanol concentration was around 70 %.

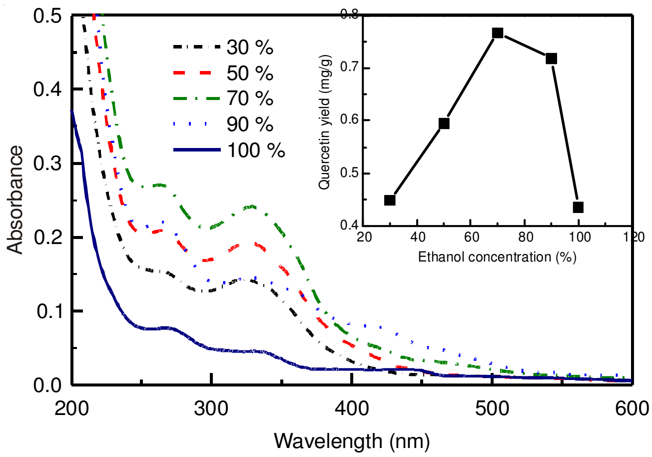

Fig. 1. Effect of ethanol concentration on the yield of quercetin from *Suaeda glauca* leaf

Effect of extraction temperature on the yield of quercetin in *Suaeda glauca* leaf: With ethanol concentration 70 %, ratio of solid to liquid 1:20 g/mL, time of 20 min, the effects of temperature on the yield of the extraction of quercetin was shown in Fig. 2. The yield of quercetin significantly increased with the increase of extraction temperature. This is the reason that the increasing extraction temperature will increase the diffusion effect of solvent and solute molecular, which helps to improve the extraction efficiency. With the increasing temperature, the yield of quercetin was decreased. This may be because the high temperature caused the leaching rate of active components increased, resulting in increased solution viscosity. The increasing viscosity hinders the active ingredient leaching, so the yield of quercetin declines. Fig. 1 showed

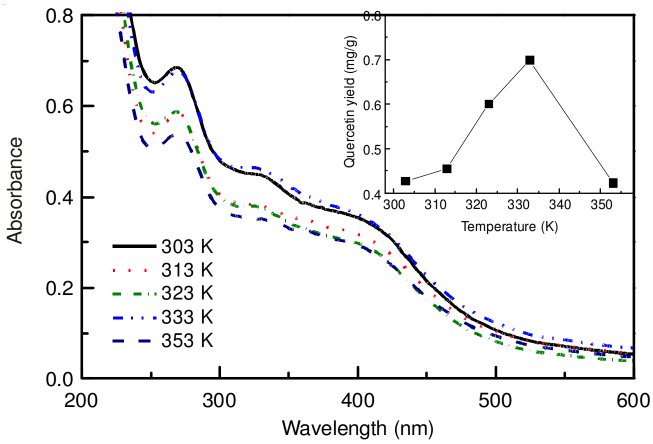

Fig. 2. Effect of extraction temperature on the yield of quercetin from *Suaeda glauca* leaf

that when the temperature is 50 ºC, there was the higher yield of quercetin. So 40-60 ºC temperature range was selected for next experiment optimization.

Effect of solid-liquid ratio on the yield of quercetin in *Suaeda glauca* leaf: The effect of solid-liquid ratio on the yield of quercetin extraction was investigated at ethanol concentration 70 %, temperature 40 ºC and time of 20 min. The result was shown in Fig. 3. With the increase of solid-liquid ratio, the yield of quercetin became gradually high and at the solid-liquid ratio 1:20 g/mL, the yield of quercetin was up to the highest value, which may be less solvent, solution is easy to reach saturation so that it was difficult to fully extract active components and that the solvent amount was too small, will cause the cost increasing. Fig. 1 showed that the yield of quercetin was relatively higher at solid-liquid ratio 1:20 g/mL.

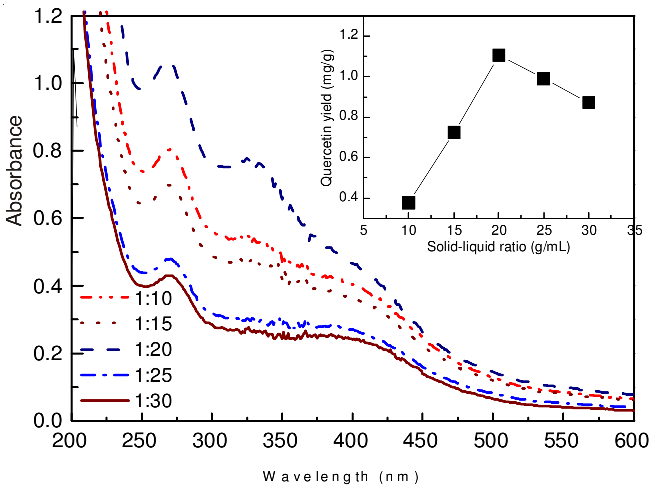

Fig. 3. Effect of solid-liquid ratio on the yield of quercetin from *Suaeda glauca* leaf

Optimization design by response surface methodology

Facors and levels of Box Behnken Design: In the single factors, thanol concentration, extraction temperature and solid-liquid ratio were all found to have a significant influence on the the yield of quercetin (R1). So three independent variable were selected for further investagation by response surface methodlolgy[15], using a Box Behnken Design (BBD) in Design-Expert v7.1.6 software. The complete design consisted of 17 experimental runs including 5 replications of the experiments, 12 factorial experiments. Table-1 listed the range of independent variables and their levels.

Fitting the model: The regression model was expressed by the following equation:

$$R1 = -1.931 + 0.0391A + 0.0141B + 15.07C - 0.000075AB - 0.04AC + 0.45BC - 0.00021A^2 - 0.00041B^2 - 194.8C^2$$

The mathematical model coefficients of intercept, linear, quadratic and interaction terms in the model were caculated. The ANOVA parameter estimation and significance of each coefficient of response were analyzed by SAS procedure in Table-2. The significance of each coefficient was determined using p value in Table-2. The p value was used a tool to check the significance of each coefficient and the interaction strength between each variable. The smaller p value, the more significant. According to the coefficient of equation, the order of the three factors can also be obtained: C > A > B. The model has shown a good fit with the experimental data, since the F-value of model 5.20 implied the model is significant and there was only a 2.05 % chance, which meant that the model was acceptable. Values of "Prob > F" less than 0.05 indicated that model terms were significant; in this case, C, BC and C^2 were significant model terms.The "Lack of Fit F-value" of 4.05 implied the 'Lack of Fit' was not significant, which was good and was used to fit the model for further validates the model. Based on these statistical tests above, the model was accepted to be adequate in Interpretating the relationship between the variables and extraction efficiency.

Model verification: The optimal parameters of quercetin extraction from *Suaeda glauca* leaf were obtained, which were ethanol concentration of 72.69 %, temperature of 60 ºC, solid-liquid ratio of 1:10 (g/mL). Considering the accuracy and operability of the experimental instrument, the correction for the optimal process were ethanol concentration of 72.69 %, temperature of 60 ºC, solid-liquid ratio of 1:10 g/mL. The experimantal value 0.782 mg/g (RSD 2.7 %) of quercetin yield

TABLE-1
RSM AND RESULTS FOR QUERCETIN YIELD FROM *Suaeda glauca* LEAF

Run	Coded levels and actual value			R1: Yield of quercetin (mg/g)	
	(A) Ethanol concentration (%)	(B) Temperature (ºC)	(C) Solid-liquidratio (g/mL)	Response value	Predicted value
1	-1(50)	-1(40)	0(1:20)	0.5	0.48
2	1(90)	-1	0	0.7	0.62
3	-1	1(60)	0	0.45	0.53
4	1	1	0	0.59	0.61
5	-1	0(50)	-1(1:10)	0.25	0.26
6	1	0	-1	0.35	0.42
7	-1	0	1(1:30)	0.65	0.58
8	1	0	1	0.67	0.66
9	0(70)	-1	-1	0.48	0.49
10	0	1	-1	0.37	0.28
11	0	-1	1	0.45	0.54
12	0	1	1	0.79	0.78
13	0	0	0	0.72	0.69
14	0	0	0	0.68	0.69
15	0	0	0	0.59	0.69
16	0	0	0	0.72	0.69
17	0	0	0	0.72	0.69

TABLE-2
VARIANCE ANALYSIS OF REGRESSION MODEL FOR QUERCETIN YIELD

Source	Squares	df	Square	F-Value	*p*-Value
A	0.026	1	0.026	3.6	0.0995
B	0.00061	1	0.00061	0.083	0.7811
C	0.15	1	0.15	20.96	0.0025
AB	0.0009	1	0.0009	0.12	0.7366
AC	0.00163	1	0.00163	0.22	0.6548
BC	0.051	1	0.051	6.90	0.0341
A^2	0.030	1	0.030	4.07	0.0834
B^2	0.00739	1	0.00739	1.00	0.3507
C^2	0.062	1	0.062	8.50	0.0225
Model	0.34	9	0.038	5.20	0.0205
Residual	0.051	7	0.00734	–	–
Lack of fit	0.039	3	0.013	4.05	0.1049
Pure error	0.013	4	0.00318	–	–
Cor total	0.39	16	–	–	–

R^2 = 0.8696; Adj R^2 = 0.7064; Pre R^2 = 0.6182; Adeq precision = 7.894.

was in agreement with the predicted value 0.784 mg/g. This indicated that the mathematical model for the optimization of quercetin extraction was reasonable and feasible.

Effect of ionic liquids on extraction of quercetin: Quercetin from *Suaeda glauca* leaf was extracted using ionic liquids as extraction solvents, which were [BMIm]Cl, [BMIm]Br, [BMIm]NO_3, [BMIm]PF_6, [EMIm]Br, [HMIm]Br, [OMIm]Br. The result shown that [EMIm]Br have higher quercetin yield (Fig. 4).

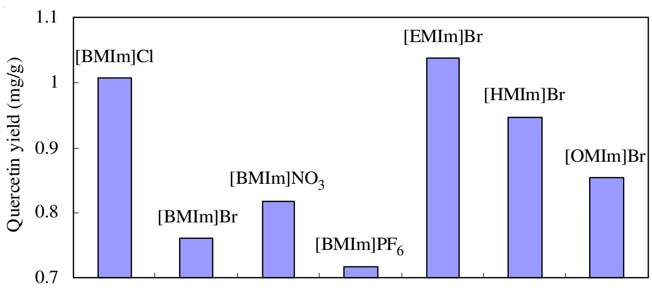

Fig. 4. Effect of type of ionic liquid on quercetin yield

Effect of [EMIm]Br concentration on extraction of quercetin: [EMIm]Br solution with different concentrations of 0.25, 0.5, 0.75, 1.00, 1.25 mol/L were accurately preparated. On basis of the optimal condition, the experimental was carried out and the results were shown in Fig. 5. There was the highest quercetin yields (1.0367 mg/g) when the concentration of ionic liquid was 0.5 mol/L. Properties of water soluble ionic liquid were similar with ones of the short chain surfactant. Its concentration in the aqueous solution higher than the critical micelle concentration (CMC) can form micelles by associating molecules. So when its concentration was greater than CMC, the ionic liquid should have better extraction efficiency of organic matter. So the concentration of ionic liquid was the key factor influence on the extraction efficiency. With the increase of ion concentration, there was a promoting effect on the yield of quercetin, but the viscosity of solution system also increased, which hindered the active ingredient stripping. Therefore, the quercetin yield had gradually decreased.

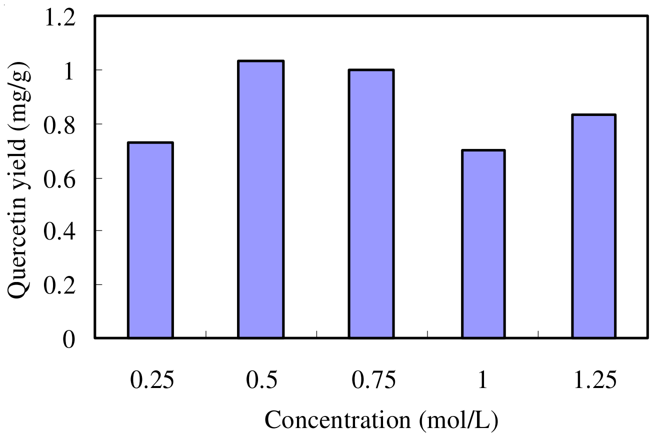

Fig. 5. Effect of concentration of [EMIm]Br on quercetin yield

Effect of solid-liquid ratio of [EMIm]Br on extraction of quercetin: The ratio of solid-liquid was 1:5, 1:10, 1:15, 1:20, 1:25 g/mL with the other unchanged conditions. The experimental results were shown in Fig. 6. It can be seen that quercetin yield was up to 1.076 mg/g when the solid-liquid ratio was 1:15 g/mL and 37.6 % higher than one of ethanol as solvent, 0.782 mg/g. The quercetin yield gradually decreased with the increasing of solid-liquid ratio, which increased the amount of solvent and improved the quercetin solubility.

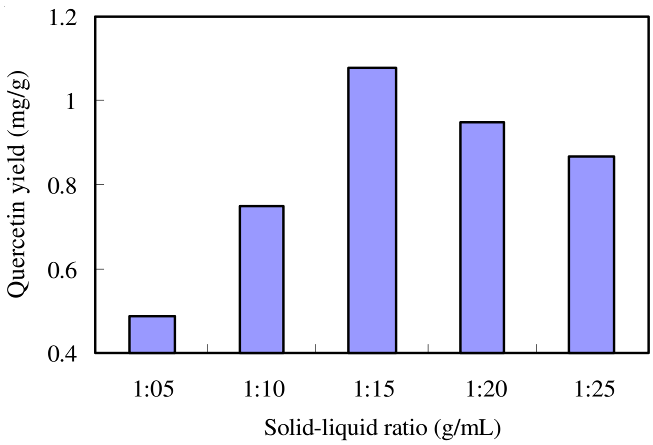

Fig. 6. Effect of solid-liquid ratio of [EMIm]Br on quercetin yield

Recovery and recycling experiment of ionic liquids: With the advantages of the low saturated vapour pressure, high boiling point and good thermal stability, ionic liquid plays a major role in the extraction and purification field.

Although ionic liquids were recognized as green solvents and relatively security for the operator, they must be recycled to ensure that does not cause pollution to the environment in the development and use because of its certain toxicity. Secondly, the complex synthesis of ionic liquids and the complicated purification steps resulted in higher prices of ionic liquids. Therefore, the ionic liquid should be economicly recycled for use again. The seven kinds of two-substituted imidazole ionic liquids were performed preliminary recovery and reuse of experiment, the experimental results are given in Table-3.

TABLE-3
RECOVERY RATE OF IONIC LIQUID USED IN THE TEST

Ionic liquids	Amount used (g)	Recovery amount (g)	Recovery rate (%)	Quercetin yield (mg/g)
[BMIm]Cl	4.3668	4.1723	95.54	1.00
[BMIm]Br	5.4780	5.3246	97.20	0.76
[BMIm]NO_3	5.0293	4.9901	99.22	0.81
[BMIm]PF_6	7.1045	7.0836	99.71	0.69
[EMIm]Br	81.7613	76.3257	93.35	1.04
[HMIm]Br	6.1795	6.1023	98.75	0.95
[OMIm]Br	6.8808	6.6813	97.10	0.85

Conclusion

[EMIm]Br as an excellent solvent can increase the extraction yield of quercetin in seven kinds of ionic liquids se-lected in this experiment. The results agreed with many results from the literature[16-18], proved that the ionic liquid had the capacity of effective extraction of active ingredients in the plants. The main mechanism probably lies in the existence of some interaction between the molecular of ionic liquids and one of the target substance. A number of studies suggested that the extraction efficiency had decided by the length of alkyl chain of ionic liquids and water-soluble anionic together. When the alkyl chain of ion liquid more than 12 carbon atoms of the cation, its water solubility is relatively poor and the length of the alkyl chain and hydrophobic had positively correlated, hydrophobic was the driving force extraction[19,20].

In this experiment, the hydrophilic anion were Br^-, NO_3^-, Cl^- (except PF_6^-), which were miscible with water in arbitrary proportion. Anion effect and easily formation of strong hydrogen bonds were considered to be consistent from the mechanism perspective and played very important role in the extraction. The molecular structure of quercetin containing multiple hydroxyl groups, including hydroxyl and adjacent hydroxyl, which more easily form hydrogen bond with ionic liquid anions. The stronger hydrogen bond, the higher extraction efficiency of quercetin. In addition, the dispersion force between imidazole ring of ionic liquid and aromatic ring of quercetin structure, was weaker than hydrogen bonds, but contribute to the extraction of quercetin.

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