



Hydrolysis of the Extract of *Rosa roxburghii* Tratt Catalyzed by Imidazolium Ionic Liquids

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Rosa roxburghii tratt (Cili) is a kind of fruit and medicinal plant in China, which is rich in vitamins and flavonoids. In the study, nine Bronsted acidic ionic liquids were first used as catalysts in the preparation of quercetin directly from the extract of *rosa roxburghii* tratt. Through rapid screening under the same reaction conditions, [PSMIM][HSO₄] was found to be the best candidate catalyst. Then the sequential experiments were carried out with [PSMIM][HSO₄] as catalyst. The reaction conditions were further optimized by response surface methodology and the hydrolytic dynamics was also investigated. Reusing and recovering of the ionic liquid were also studied with fairly good results. It is proved that this green catalyst is ideal for developing new hydrolysis process for crude extract of total natural flavonoids as the substitute of traditional inorganic acids.

Key Words: Ionic liquids, *Rosa roxburghii* tratt, Catalysis, Flavonoids, Quercetin.

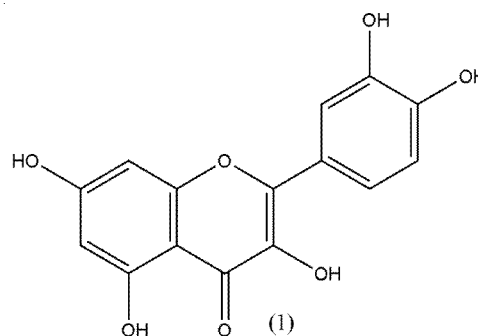
INTRODUCTION

In recent years, more and more new resources of medicinal plants are attracting the attention from relative researchers. *Rosa roxburghii* tratt (Cili), also known as sweet chestnut rose and the new king of vitamin C, is a wild plant found only in certain high mountain ranges of Mainland China (Fig. 1) and used as traditional Chinese medicine from ancient to nowadays. It is also widely used as a natural food for humans and animals alike and has been found effective in treatment of the longevity, cancer, immunity and atherosclerosis¹. Cili contains a lot of components with antioxidant activity, which may contribute to its health benefits. The major antioxidant constituents includes rutin (Fig. 2) also known as vitamin P, 59.8-128.9 mg per gram fresh weight and vitamin C (7.9-23.9 mg per gram fresh weight) and a few methods were developed to extract total flavonoids which was mainly composed of rutin². Quercetin (Fig. 2), 3,3',4',5,7-pentahydroxyflavone, is the aglycone of rutin and is used as an important bioactive ingredient in supplements, beverages drugs and foods. Recent researches have revealed that it can be used as efficient anti-tumor drug and platelet aggregation inhibitor³ and its effects of antioxidation, vasodilatation and antitumor are all stronger than rutin. However, as a matter of fact, the studies on its preparation from natural resources have fallen behind bioactivity researches and applications of quercetin in recent years.

On the other hand, the study on ionic liquids (ILs) is a globally hot field in these years. Ionic liquids are molten salts



Fig. 1. Cili and its distribution in China



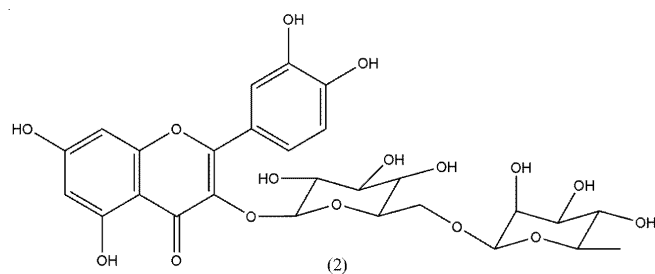


Fig. 2. Structure of quercetin (1) and rutin (2)

which are consisted of bulky organic cations with organic/inorganic anions. Most of them are liquid around room temperature. Nowadays, they are widely used in chemical synthesis as solvents and/or catalysts. As catalysts, they are promising to replace many catalysts worked in traditional reactions such as alkylation, esterification, Michael addition, oligomerization and rearrangement⁴⁻⁸. Brønsted acidic ionic liquids give great promise of using ionic liquids as green catalysts in hydrolysis reactions for their strong acidity and water solubility. Their application in acid-catalyzed hydrolysis would overcome the shortcomings in traditional hydrolysis process catalyzed by inorganic acids to some extent, such as equipment corrosion, long reaction time, sewage emission and carbonization of the product under high temperature. But it has not been reported about its application in direct hydrolysis of natural extract.

Based on the above background, there should be a new way to utilize natural resource of Cili besides the direct extraction of rutin, which is the preparation of quercetin from the crude extract by hydrolysis with the new and environment-friendly catalyst. Meanwhile, the combination of green chemistry and medicinal phytochemistry is desirable. So in the following research, nine Brønsted acidic ionic liquids replaced common inorganic acids were first used to prepare quercetin from the crude extract of Cili. Through the comparison of conversion ratios of the hydrolysis reaction, the best catalyst was selected for further study. Then the optimization of conditions was performed with the response surface method and the dynamical property under the best reaction condition was also studied. Finally, recovering and reusing of ionic liquid were investigated and obtained fairly good results.

EXPERIMENTAL

Rosa roxburghii tratt (Cili) was collected from suburbs of Liangshan Yi Autonomous Prefecture, Sichuan Province, China. For collection at the initial stage of maturity, only full yellow fruits were picked up in October, 2009. Then they were cut into pieces and dried in a ventilated drying oven and stored in plastic bags at low temperature and protected from light before their extraction.

Rutin (purity of 99.3 %) was purchased from HongYi Bio-engineering Co. (Chengdu, Sichuan, China). Quercetin (purity of 98.1 %) was purchased from the National Institute of Food and Drug Control (Beijing, China). Methanol, ethanol, *n*-butanol and trifluoroacetic acid (TFA) were purchased from Chengdu KeLong chemical reagent factory (Chengdu, Sichuan, China) with the purities up to 99.5 %. Water is redistilled. Ionic liquids were synthesized referred to the previous reports⁹⁻¹⁴, their purities were determined by HPLC and were all greater than 96 %.

HPLC analysis was performed with an LC-20AT pump (Shimadzu, Kyoto, Japan), a Welchrom C₁₈ column, 5 μ m, 250 mm $\times$ 4.6 mm i.d. (Welch Materials, Maryland, USA), an HCT-360 LC column cooler/heater (Hengao Tech & Dev, Tianjin, China) and an SPD-M20A PDA detector (Shimadzu, Kyoto, Japan). A Class-VP workstation (Shimadzu, Kyoto, Japan) was used for data acquisition. Using methanol/water/TFA (70:30:0.5 v/v/v) as mobile phase at the flow rate of 0.4 mL/min with the column temperature was set at 35 $^{\circ}$ C. The injection volume was 10 μ L and the samples were detected at 254 nm. The pH meter was provided by Shanghai Shinuo Physical Optical Instruments Co., Ltd. (Shanghai, China). Sonication was performed on the KQ2200DE model numerical controlled ultrasonic cleaner provided by Kun Shan Ultrasonic Instruments Co., Ltd. (Jiangsu, China).

Physical and structure characterization instruments used are listed as follows: XRC-1 Micro melting point apparatus (uncalibrated, Sichuan University, China); AVATAR 360-FT infrared spectrometer (Nicolet, USA); Varian Mercury 400 NMR (Varian, USA); Finnigan-TRANCE MS (Finnigan, USA).

Extraction of total flavonoids: The pieces of Cili (200 g) were powdered and extracted with 65 % ethanol (2 L) three times at 60 $^{\circ}$ C. Then the extract was evaporated to form the syrup. The syrup was then dissolved in water by sonication and partitioned with *n*-butanol of equal volume three times. The combined *n*-butanol solution was vacuum evaporated at 65 $^{\circ}$ C and *ca.* 6.8 g of residue was obtained.

Preparation and identification of quercetin: The 100 mL three neck flask was charged with aqueous ionic liquid (50 mL) and then it was heated to the temperature set before hand. Then the flavonoids extract (0.78 g) was added in. During the reaction, the reaction mixture was sampled at regular intervals and the samples were concentrated to obtain the solid substances for HPLC analysis. The mixture reacted under mild stirring for hours at constant temperature and finally it was cooled down to room temperature. The suspension was filtered and the remaining yellow solid was washed with 30 mL water. Further purification was performed by recrystallization in 50 % ethanol. Finally the crystallized product was dried at 100 $^{\circ}$ C. Physical and structure characterization data about the crystal are given below:

Yellow needle crystal. m.p. 313-314 $^{\circ}$ C. EI-MS (rel. int.): m/z 302 [M]⁺, 274 (M-28), 273 (M-29), 245 (M-57), 153, 152, 142, 137, 124, 123, 109, 77. IR (KBr, $\nu_{\max}$, cm⁻¹): 3500-3000, 1672, 1618, 1520, 1513, 1430, 1361, 1316; ¹H NMR (300 MHz, acetone-*d*₆): δ (ppm): 6.27 (1H, d, *J* = 2 Hz, H-6), 6.53 (1H, d, *J* = 2 Hz, H-8), 7.00 (1H, d, *J* = 8.5 Hz, H-5'), 7.70 (1H, dd, *J* = 8.5 Hz, 2.1 Hz, H-6'), 7.81 (1H, d, *J* = 2.1 Hz, H-2').

Analysis of the samples: First of all, the standard curves of rutin and quercetin were established on the basis of a series of methanol solution of rutin and quercetin with the concentrates of 1.000, 0.800, 0.500, 0.200, 0.100, 0.050, 0.020, 0.010 mg/mL and 0.500, 0.250, 0.100, 0.050, 0.025, 0.010 mg/mL, respectively. The obtained equation for the standard curve of rutin was

$$Y = 405908x - 47392 \quad (1)$$

$R^2 = 0.9997$, the linear scope was 0.1-8.0 μg ; the equation for the standard curve of quercetin was

$$y = 913183x - 629557 \quad (2)$$

$R^2 = 0.9996$, the linear scope was 0.1-5.0 μg .

where y was the integral area of relative peak of rutin or quercetin and x was the corresponding amount of rutin or quercetin. Then we analyzed the samples. They were analyzed under the same conditions stated above with the reference substances. The dry solid samples were dissolved in methanol and diluted to suitable concentrates and then they were filtered through a 0.2 μm film and injected into HPLC. The conversion ratios of the samples were calculated by eqns. 1 and 2 given above and the formula of conversion ratio according to the integral areas of relative peaks.

RESULTS AND DISCUSSION

Comparison of catalytic performance of ionic liquids:

Since acidity is one of the most important factors that influence the conversion ratio in the acid-catalyzed hydrolysis of rutin with traditional methods, we chose nine Brønsted acidic ionic liquids as our experiment objects. Table-1 shows their structures and pH values.

The catalytic activity of the selected ionic liquids was compared under the same molar concentrations (20 mmol/100 mL aqueous solution) by the conversion ratios of rutin at 100 °C for 4 h. Fig. 3 shows the curves of the conversion ratios of different ionic liquids. It was obvious that ionic liquids [HMIM][HSO₄], [HMIM][BF₄], [PSMIM][H₂PO₄] and [PSMIM][HSO₄] successfully promoted the reaction with relatively high efficiency and the yields were all up to 90 %. They were much higher than those reported values that were lower than 80 %¹⁵. This proved that acidity played a key role in this reaction, which was determined by both anions and cations. [H₂PO₄]⁻ is a weaker acidic group with nearly no secondary ionization compared to [HSO₄]⁻, so it could produce much less H⁺ that worked as catalyst in the reaction. The acidity of the cations follows the order: [PSMIM]⁺ > [HMIM]⁺ > [BMIM]⁺, for [PSMIM]⁺ has a sulfonic group which may cooperate with the anion to provide a stronger acidity and [BMIM]⁺ cannot ionize while [HMIM]⁺ can.

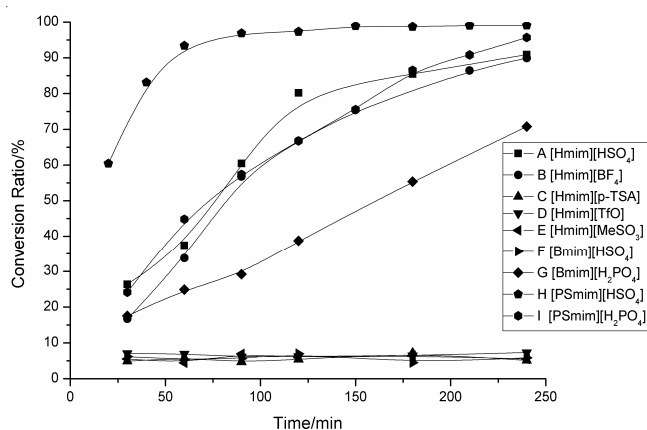


Fig. 3. Catalytic performance of ionic liquids under the standard reaction

TABLE-1
STRUCTURES AND pH VALUES OF THE IONIC LIQUIDS

No	IL	Cation	Anion	pH value*
A	[HMIM][HSO ₄]		HSO ₄ ⁻	1.2
B	[HMIM][BF ₄]		BF ₄ ⁻	1.6
C	[HMIM][p-TSA]		p-TSA ⁻	5.6
D	[HMIM][TfO]		CF ₃ SO ₃ ⁻	5.7
E	[HMIM][MeSO ₃]		MeSO ₃ ⁻	4.9
F	[BMIM][H ₂ PO ₄]		H ₂ PO ₄ ⁻	6.1
G	[PSMIM][HSO ₄]		HSO ₄ ⁻	1.6
H	[PSMIM][HSO ₄]		HSO ₄ ⁻	1.0
I	[PSMIM][H ₂ PO ₄]		H ₂ PO ₄ ⁻	1.5

*pH values were measured in the 20 mmol/100 g aqueous solution at 30 °C by pH meter.

Optimization of reaction conditions: After [PSMIM][HSO₄] was chosen as the candidate ionic liquid for further study, further investigation was carried out about the influence of temperature, time and the amount of ionic liquid on the reaction. It was found that temperature was the most important factor among them. When the temperature was lower than 80 °C, no product was detected even if the reaction time was extended. Secondly, a certain amount of ionic liquid is necessary for the reaction. The hydrolysis reaction did not occur without the addition of ionic liquids. Finally, the conversion ratios were time-dependent which could be concluded from Fig. 3. These three factors were all considered and optimized in the optimizing experiments.

Response surface methodology (RSM) is a collection of mathematical and statistical technique, which becomes an effective tool to optimize the experimental conditions with the least experiment time in the research of medicinal plants¹⁶. The following experiments were designed in Box-Behnken Design (BBD) mode of RSM. The data were processed with Design-expert 7.0.0. The predicted optimal reaction conditions were as follows: $T = 98.5\text{ }^{\circ}\text{C}$, $m_{\text{IL}} = 0.19\text{ mol/L}$, $t = 1.05\text{ h}$. The conditions were no severer than the best hydrolysis conditions in the reported method in which the reaction mixture was refluxed in 0.5 % (weight percentage) of aqueous H_2SO_4 for 1 h¹⁷. And they were validated by the corresponding experiments. Table-2 shows the designed experiments and the corresponding results. The analysis of variance is valid. And the final equation in terms of coded factors was

Run	Time (h)	Temp. ($^{\circ}\text{C}$)	Molar fraction of [PSMIM] [HSO ₄] (mol/L)	Response value
1	1.25	100	0.10	0.9075
2	1.25	80	0.20	0.1131
3	2.00	90	0.10	0.6257
4	1.25	90	0.15	0.5464
5	0.50	90	0.20	0.3555
6	0.50	100	0.15	0.8263
7	2.00	80	0.15	0.1537
8	1.25	90	0.15	0.5501
9	2.00	90	0.20	0.8848
10	2.00	100	0.15	0.9344
11	0.50	90	0.10	0.1120
12	0.50	80	0.15	0.0339
13	1.25	90	0.15	0.5474
14	1.25	100	0.20	0.9416
15	1.25	90	0.15	0.5528
16	1.25	80	0.10	0.0933
17	1.25	90	0.15	0.5481

$$\ln(R1) = -0.63 + 0.53A + 1.17B + 0.22C - 0.35AB - 0.20AC - 0.28A^2 - 0.51B^2 \quad (3)$$

where R1 was the conversion ratio of rutin, A was time, B was temperature and C was the amount of ionic liquid. The important parameters met the required standard and Table-3 shows the analysis results of variance. The response surface is depicted in Fig. 4. Since temperature and time have much more effect on the conversion ratio than that of the amount of ionic liquid, they were chosen as x and y axis in the response surface. The diagram showed that the natural logarithm of conversion ratio increased with the increase of temperature and time. The conversion ratio was prone to be constant when the temperature was close to $100\text{ }^{\circ}\text{C}$ and the time used was around 2 h. While extending of reaction time and increasing temperature are at least not energy-efficient.

The chemical reaction dynamics was also investigated under the optimal hydrolysis conditions. As we all know, in a first order reaction:

$$\ln(x_0 - x) = -k_1t + C_1 \quad (4)$$

While in a second order reaction:

$$\frac{1}{x_0 - x} = k_2t + C_2 \quad (5)$$

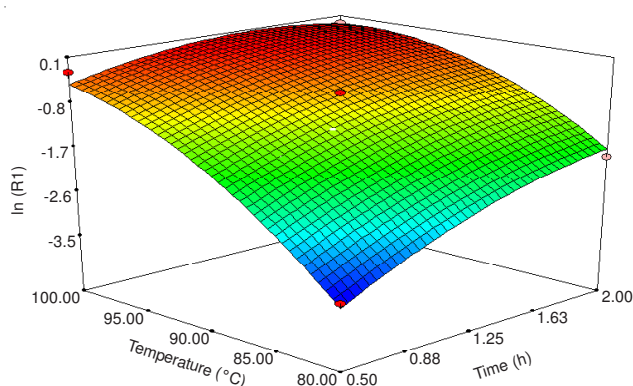


Fig. 4. Response surface of the optimizing experiments (R1 is the conversion ratio)

where x_0 is the initial concentrate of rutin, x is the concentrate of rutin at time t , k_1 and k_2 are the rate constants, C_1 and C_2 are constants. Through simple calculation on the basis of the HPLC data, the values of $\ln(x_0 - x)$ were correlated with time by a linear mode and the equation was obtained as

$$y = -1.678x - 4.400 \quad (6)$$

where y was equal to $\ln(x_0 - x)$ and x was the reaction time and the correlation coefficient (R) was 0.9953. It showed that the hydrolysis reaction was a first order reaction in which the rate constant (k_1) was 1.678 h^{-1} .

Reusing and recovering of [PSMIM][HSO₄]: In the study of reusing, the filtrate was reused directly for another nine times under the optimized conditions. The results were satisfied in terms of conversion ratio as shown in Fig. 5. The conversion ratio did not decrease severely and the total reaction time needed for full conversion also did not change.

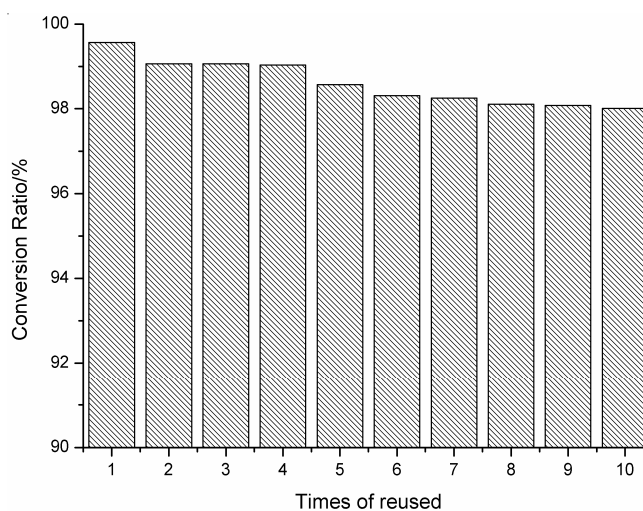


Fig. 5. Conversion ratio for the reuse of [PSMIM][HSO₄]

Then in the study of recovering, the filtrate that came from the post-process of the reaction mixture was firstly distilled under vacuum to remove water. The residual was diluted by anhydrous ethanol and filtrated to remove sugars. Then the filtrate was concentrated in vacuum and washed with three portions of acetone and subsequently disposed of the residual acetone in vacuum. Finally a brown viscous liquid was obtained. Then it was used for the next reaction and the reaction rate did not decrease.

TABLE-3
ANALYSIS OF VARIANCE

Source	Sum of squares	Df	Mean square	F Value	P-value/Prob > F
Model	15.81	7	2.26	50.45	< 0.0001
A	2.28	1	2.28	50.84	< 0.0001
B	11.03	1	11.03	246.35	< 0.0001
C	0.37	1	0.37	8.37	0.0178
AB	0.48	1	0.48	10.77	0.0095
AC	0.16	1	0.16	3.65	0.0884
A ²	0.33	1	0.34	7.49	0.0229
B ²	1.08	1	1.08	24.09	0.0008
Residual	0.40	9	0.045	—	—
Lack of fit	0.40	5	0.081	3777.71	< 0.0001
Pure error	0.0000853	4	0.00002132	—	—
Cor total	16.21	16	—	—	—

Note: A for time, B for temperature and C for amount of [Psmim]HSO₄.

Conclusion

In this study, [PSMIM][HSO₄] was found to be a promising catalyst for the hydrolysis of crude extract from *Rosa roxburghii* tratt. The hydrolysis conditions of rutin with [PSMIM][HSO₄] as catalyst were optimized by response surface methodology. With the participation of green chemical solvent, the best reaction conditions were no severer than that of the traditional methods and the yield was higher than that of the traditional methods. The reusability and recyclability were validated with fairly good results. As ionic liquids possess so many advantages compared to traditional catalysts is believed that this preparative method has great potential and broad prospects in the chemical study of fruit and natural functional foods.

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