Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Extraction and isolation of shikimic acid from *Ginkgo biloba* leaves utilizing an ionic liquid that dissolves cellulose

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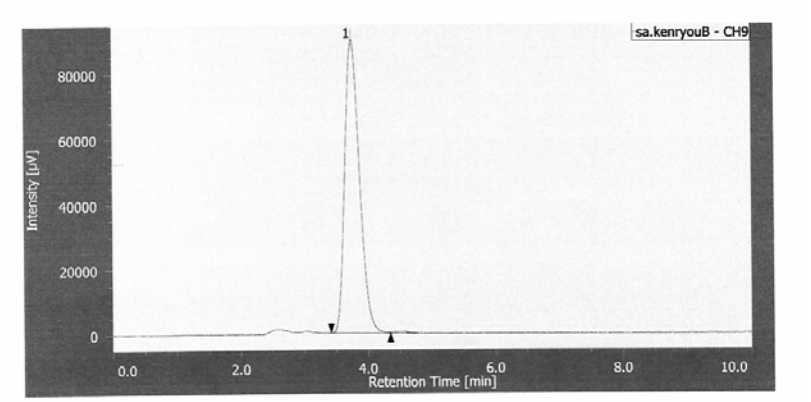
Supplementary Information

1. Calibration curve by HPLC analysis of natural shikimic acid:

In order to prepare the calibration curve for shikimic acid, HPLC analysis of natural shikimic acid (0.04 μg/mL, 0.2 μg/mL, 1.0 μg/mL, and 5.0 μg/mL) was performed as shown in **Figure S1** as the example under the condition described as main text. HPLC charts were acquired on a JASCO's instrument (MD-2010 Plus, PU-2085 Plus, CO-2065 Plus). Since the obtained peak area of shikimic acid was 54040, 272025, 1453910, and 7803301, respectively, amount of shikimic acid could be plotted as shown in **Figure S2**. Consequently, the fitting for the plots gave an equation described as $y = 2\times10^{6}x - 50967$ with $R^2 = 0.9998$ for the quantitative analysis of shikimic acid.



クロマトグラム情報

ユーザー名	usuki
更新日時	2010/08/25 18:17:41
コメント	
HPLC システム名	HPLC-1
測定日	2010/06/15 20:16:47
注入量	5.00 [μL]
サンプル#	25
プロジェクト名	HPLC-1
取込時間	10.0 [min]
測定シーケンス	sa.kenryouB
コントロールメソッド	sa0.15.10min.254nm
ピーク ID テーブル	
検量線テーブル	
追加情報	

チャンネル情報＋ピーク情報

クロマトグラム名	sa.kenryouB-CH9
サンプル名	
チャンネル名	PDA
データ取り込み間隔	800 [msec]
波形処理メソッド	sa.kenryouB
数値計算式	
判定式	

#	ピーク名	CH	NTP	分離度	シンメトリー係数	警告	補正定量値	ISTD ピーク名	ISTD 添加量	定量ファクター	ピーク・スタート	ピーク・エンド
1	Unknown	9	1232	N/A	1.204		N/A	N/A	N/A	1.0000	3.413	4.347

#	BL 開始	BL 終了	ピーク幅	S/N	分離係数	キャパシティー比	検出方法	計算値	ピークファクター	真値	重量	判定	面積 [μV·sec]
1	3.413	4.800	0.251	N/A	N/A	N/A	オートマチック	N/A	N/A	N/A	0.00000	N/A	1453910

#	tR [min]	高さ [μV]	面積%	高さ%	定量値
1	3.747	90076	100.000	100.000	N/A

Figure S1. HPLC chart of shikimic acid (1.0 μg/ml). The condition is described in main text.

Supplementary Information

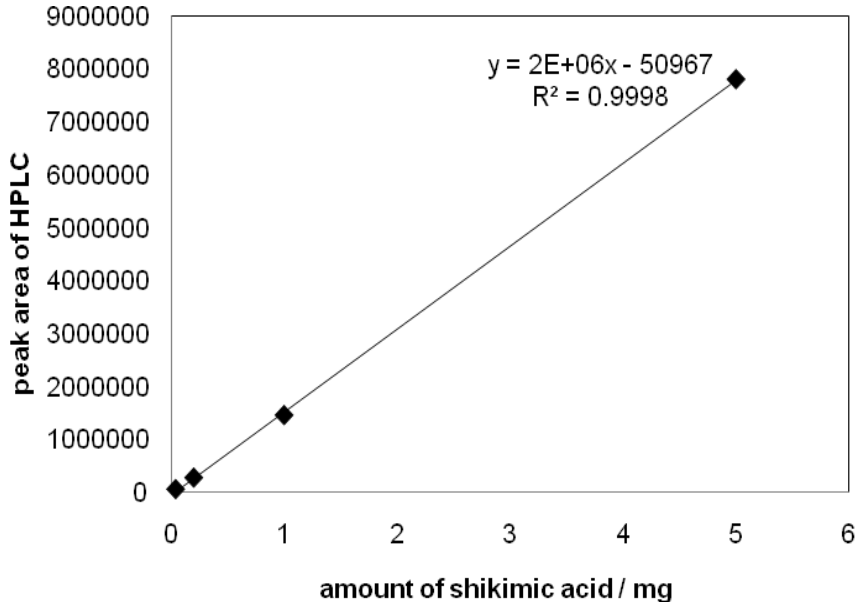
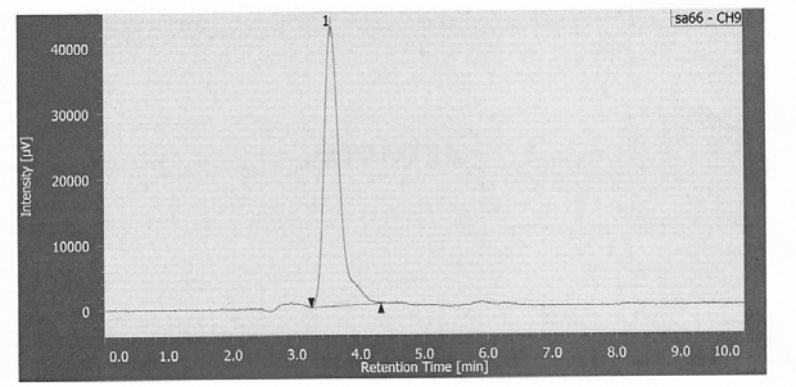


Figure S2. Calibration curve of shikimic acid based on the HPLC analysis.

2. HPLC analysis of extracted shikimic acid by MeOH:

When 0.005 mg of the extract (total: 293.9 mg) by methanol from 2.3848 g of *G. biloba* leaves was injected into the HPLC, the chart was obtained as **Figure S3** under the condition described as main text. Since the peak area of shikimic acid was found to be 718488, the value was substituted for y in the equation of $y = 2\times10^{6}\,x - 50967$, resulting in estimation of amount of shikimic acid "x" as 3.8×10^{-7} g. Ratio of contained shikimic acid in the extact was thus found to be 7.6% ($=3.8\times10^{-7} / 5\times10^{-6} \times 100$). Because total amount of shikimic acid in 293 mg of the extract was calculated to be 22 mg (= 293.9 × 7.6%), we found the extraction yield by methanol was to be 0.92% (= 0.022 g / 2.38848 × 100). Extraction yields by ethanol, H_2O, DMF, and ILs were calculated in the same procedures.



クロマトグラム情報

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更新日時	2010/11/05 17:40:05
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コントロールメソッド	sa0.15.35min.210nm
ピーク ID テーブル	
検量線テーブル	
追加情報	

チャンネル情報＋ピーク情報

クロマトグラム名	sa66-CH9
サンプル名	
チャンネル名	PDA
データ取り込み間隔	800 [msec]
波形処理メソッド	sa66-1
数値計算式	
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1	Unknown	9	1160	N/A	1.492		N/A	N/A	N/A	1.0000	3.227	4.320

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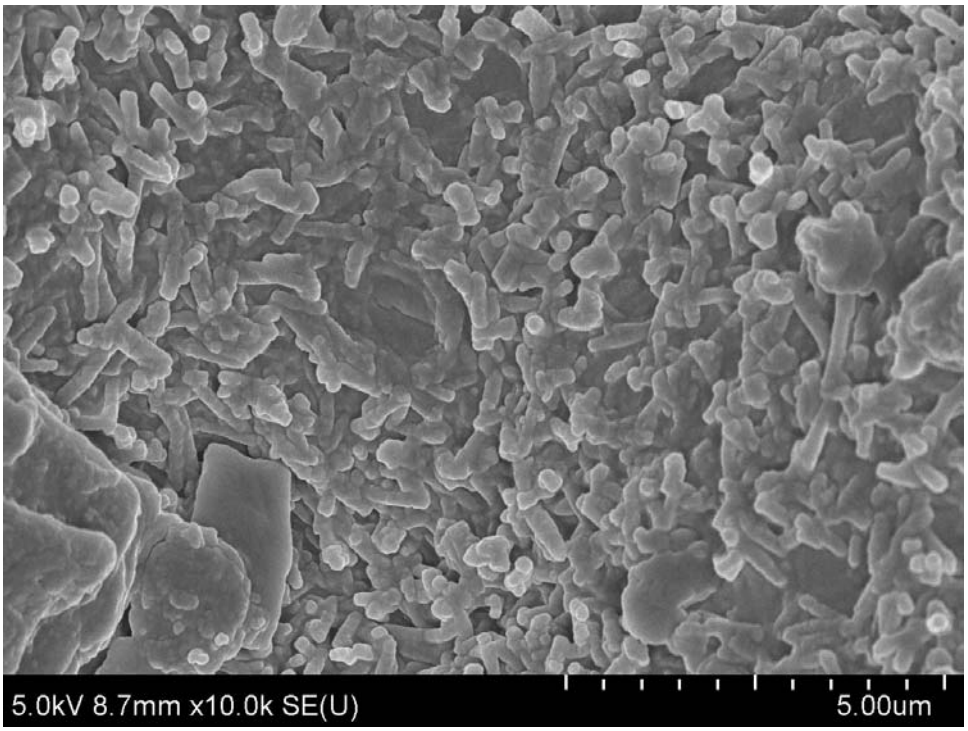
1	3.227	4.320	0.245	N/A	N/A	N/A	オートマチック	N/A	N/A	N/A	0.00000	N/A	718488

#	tR [min]	高さ [μV]	面積%	高さ%	定量値
1	3.547	42651	100.000	100.000	N/A

Figure S3. HPLC chart of extracted shikimic acid by methanol. The condition is described in main text.

3. SEM micrographs of *G. biloba* leaves:

SEM micrographs of *G. biloba* leaves before extraction (**Figure S4**), after extraction by methanol (**Figure S5**), and after IL (**Figure S6**) are shown, respectively. The micrographs were obtained by a Hitachi S-4500 equipment.



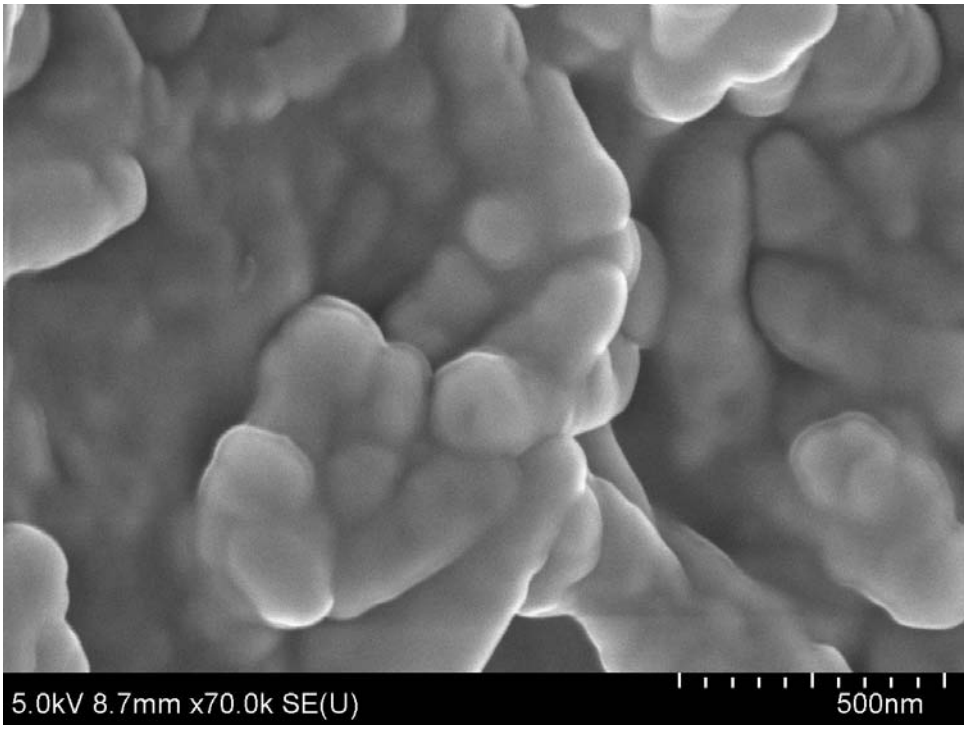
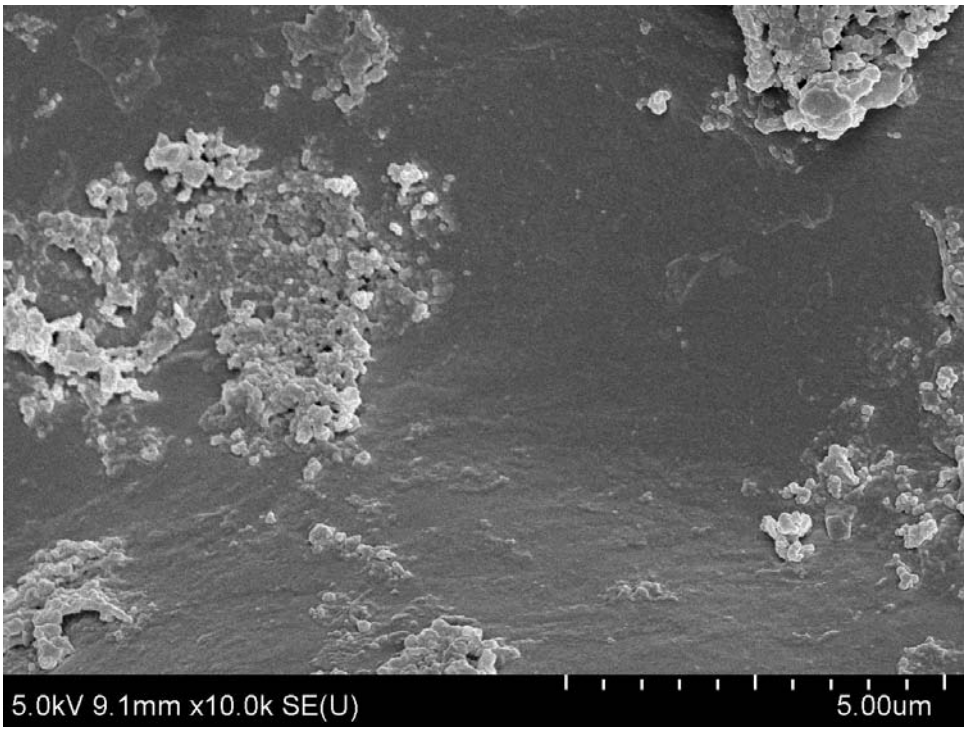


Figure S4. SEM micrographs of leaves before extraction at 10.0 k (up) and 70.0 k (bottom).



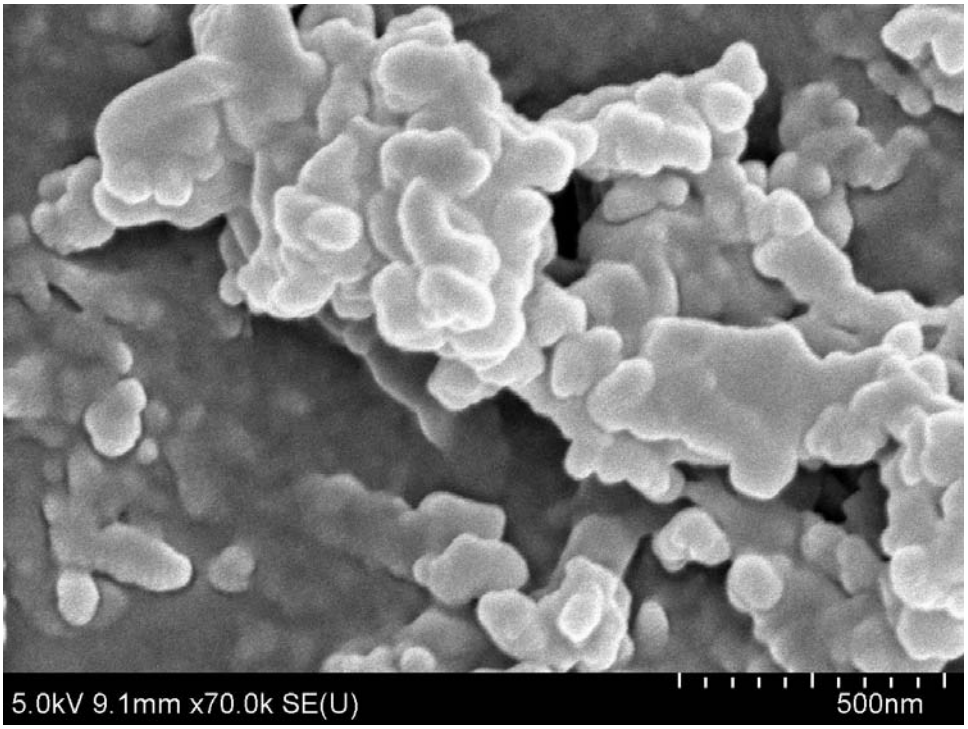
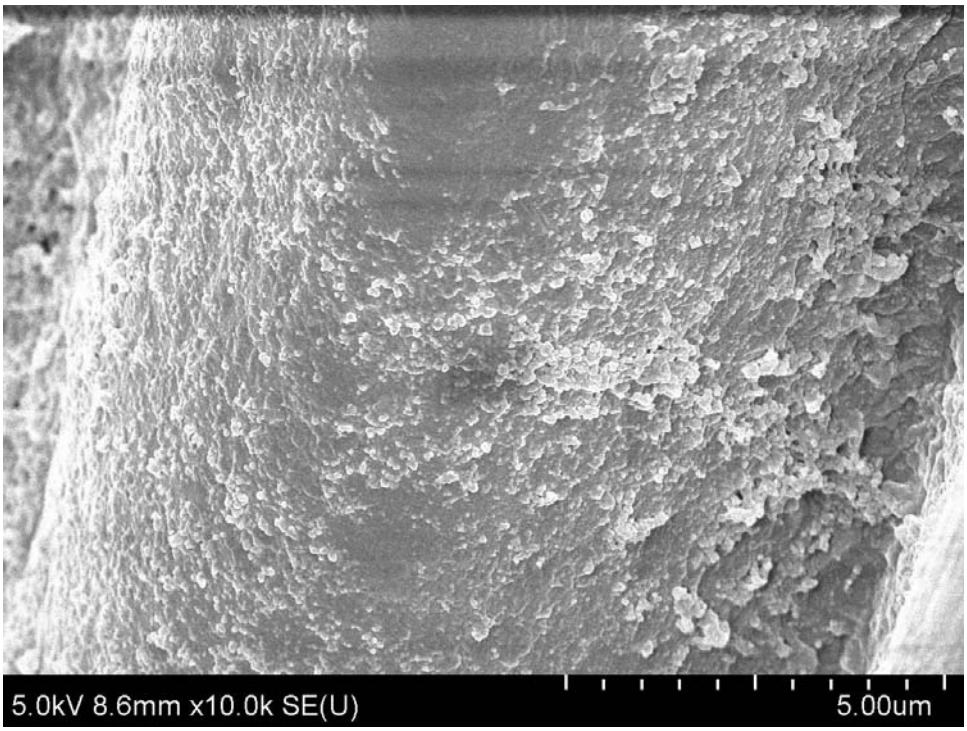


Figure S5. SEM micrographs of leaves after extraction by methanol at 10.0 k (up) and 70.0 k (bottom).



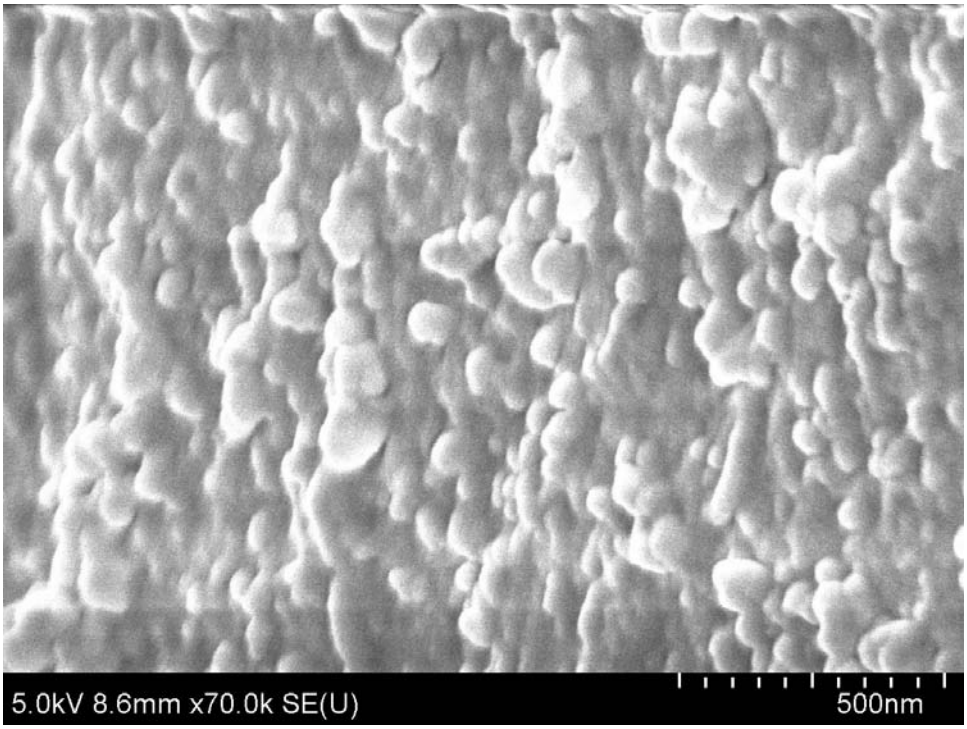


Figure S6. SEM micrographs of leaves after extraction by ionic liquid **2** at 10.0 k (up) and 70.0 k (bottom).

4. ^{1}H-NMR spectrum of the isolated shikimic acid:

Isolated shikimic acid from IL extracts by an anion-exchange resin was identified by ^{1}H-NMR measurement which was acquired on a JEOL Lambda 300 spectrometer (**Figure S7**). The isolated shikimic acid: ^{1}H-NMR (D_2O, 300 MHz) δ 2.25 (1H, m), 2.74 (1H, m), 3.80 (1H, m), 4.06 (1H, m), 4.47 (1H, m), 6.84 (1H, m).

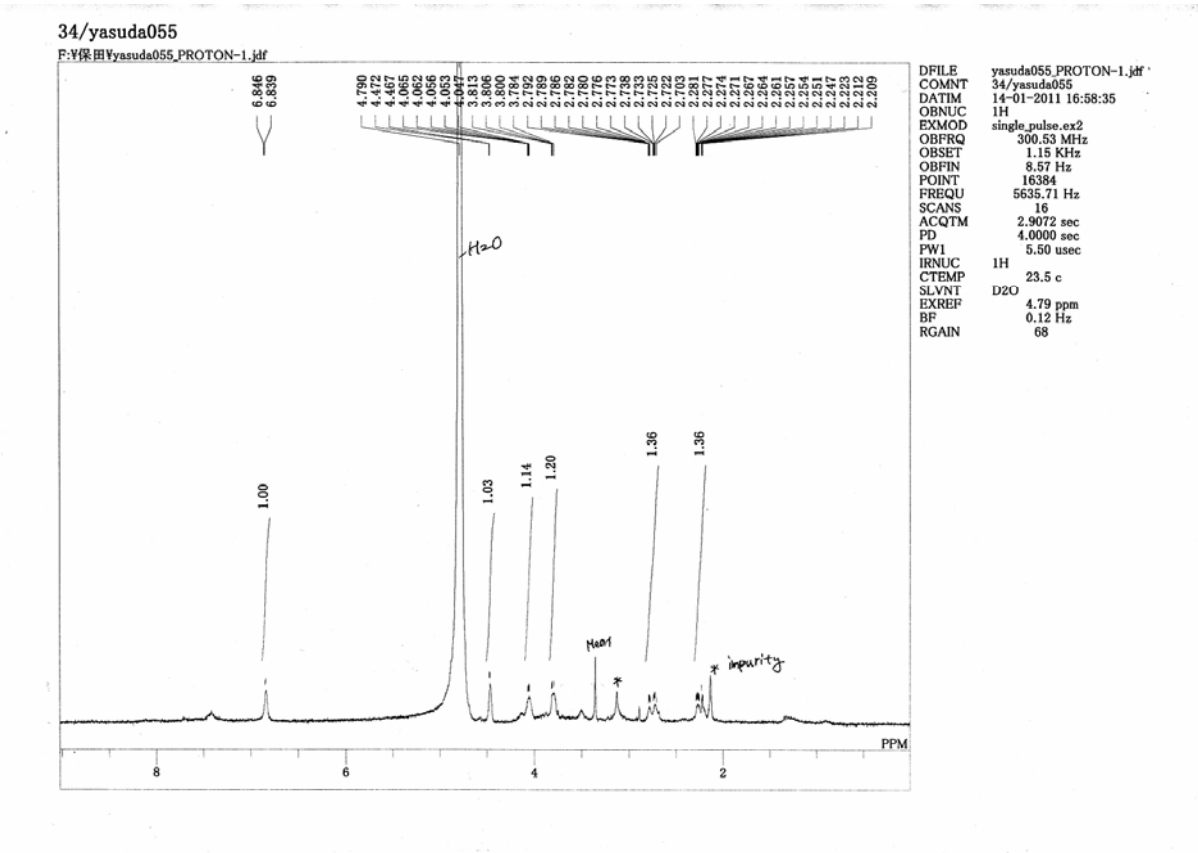


Figure S7. ^{1}H-NMR spectrum in D_2O of the isolated shikimic acid.