Anti-diabetic functional food with wasted litchi seed and standard of quality control

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Abstract The prevalence of diabetes has become a huge health burden in parts of quality of life and economic cost for overcoming this chronic disease. We followed a bioactivity-guided isolation using α-glucosidase inhibitory assay, four major compounds were isolated, and their structures were elucidated using nuclear magnetic resonance, mass spectrometry, and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry in litchi seed. As a result, the IC_{50} of α-glucosidase inhibitory assay of the crude extract, sugar-removed layer, pavetannin B2, procyanidin A2, and acarbose was 0.691 μM/mL, 3.686 μM/mL, 0.04 μM, 0.08 μM, and 55.845 μM/mL, respectively. With those compounds, we examined the protein tyrosine phosphatase 1B inhibitory activity. And the IC_{50} of pavetannin B2, procyanidin A2, and ursolic acid was 450.295, 338.257, and 19.686 μM, respectively. Contents analysis method for bioactive compounds, which can be used in manufacturing for extract preparations, was established. The findings of this study, litchi seed can be a cost-effective medicinal food in terms of recyclable resources in the litchi food industry and as a natural alternative medicine against type-2 diabetes.

Keywords α-Glucosidase inhibitory assay · Anti-diabetic agent · Bioactivity-guided isolation · Litchi seed · Protein tyrosine phosphatase 1B inhibitory assay · Quality control standard

Introduction

The worldwide prevalence of diabetes is a massive health burden. It impacts the quality of life and is also a significant economic cost [1]. In 2011, the number of diabetic patients was estimated at 366 million, indicating 8.3% of the global population, and this number is expected to increase to 552 million by 2030 [2]. This indicates that there is a need to prevent and control this disease. α-Glucosidase inhibitors are oral anti-hyperglycemic agents that act through competitive inhibition of α-glucosidase, delaying intestinal carbohydrate absorption and reducing postprandial increases in glucose levels [3]. Some α-glucosidase inhibitors, such as acarbose and voglibose, are used clinically, in combination with either diet or other anti-diabetic agents, to control blood glucose levels [4]. However, they often cause gastrointestinal side effects, such as gastrotympanites and diarrhea. Protein tyrosine phosphatase 1B (PTP1B) is a major regulator of stored body fat, energy balance, and insulin resistance [5]. Insulin controls various functions including gene transcription, protein translation, and enzyme activity. PTP1B is the negative regulator of insulin signaling mechanisms. Therefore, the overexpression of PTP1B can cause resistance to insulin and leptin. Thus, it is a very attractive target for the treatment of type 2 diabetes and obesity [6]. The inhibition of α-glucosidase and PTP1B is effective at preventing and treating type 2 diabetes through reducing postprandial hyperglycemia [3, 6].

The litchi fruit [(Litchi chinensis Sonnerat), which belongs to the (Sapindaceae)] family, has been widely cultivated in South-east Asia, especially China, for many years. Litchi flesh is consumed directly and can be used to manufacture juice, vinegar, jelly, wine, and ice creams.
However, after consumption and industrial processing, litchi seeds (by-products) become an environmental problem [8]. In previous studies, the litchi seed has been reported to contain high concentrations of bioactive compounds, including polyphenols, flavonoids, sterols, and saponins [9]. In addition, they can inhibit the growth of cancer cells [10] and have shown anti-platelet [11] and α-amylase inhibitory activities [12], as well as hypoglycemic effects and improvements to liver function [13]. Furthermore, there is a patent on the use of litchi seed extract in preventing or treating non-alcoholic fatty liver disease [14]. In addition, litchi seeds have been developed in Chinese clinics into a medicinal tablet to treat diabetes, especially gestational diabetes [15]. Based on these uses, we examined the anti-diabetic activity of litchi seed on α-glucosidase, which plays a major role in controlling starch digestion and lowering blood glucose, and on PTP1B, a negative regulator of insulin.

The aim of this study was to assess whether the litchi seed has any potential as an anti-diabetic agent. Litchi seeds may be an efficient, low-cost solution for the preparation of pharmaceutical or nutraceutical agents.

Materials and methods

Plant material and chemicals

Litchi seeds were obtained from Green-Life Natural Products Co., Ltd (Xi’an, China). The seeds were stored at 4 °C prior to extraction. The compounds protocatechuic acid (A), pavetamin B2 (B), procyanidin A2 (C), and 5-p-coumaroylquinic acid (D) were isolated as described in the following section. High-performance liquid chromatography (HPLC) analysis determined that the purity of these standard compounds was more than 98%. HPLC-grade acetonitrile, methanol, and water were purchased from J. T. Baker (Phillipsburg, PA, USA). HPLC-grade formic acid (99%) was obtained from Scharlau Chemical (Sant Joan Despi, Barcelona, Spain). Polyvinylidene fluoride (PVDF) membrane filters (13 mm, 0.45 μm) were purchased from Whatman (Little Chalfont, Buckinghamshire, England). Diaion HP-20, Sephadex LH-20, and MCI CHP 20P were purchased from Sigma-Aldrich (St. Louis, MO, USA). The activities were evaluated using the following regents and enzymes: dimethyl sulfoxide (DMSO), sodium phosphate monobasic, sodium phosphate dibasic, α-glucosidase from Saccharomyces cerevisiae (Saccharomycetaceae), acarbose, 4-nitrophenyl α-D-glucopyranoside (p-NPG), p-nitrophenylphosphate, uricosid acid, protein tyrosinase, and phosphatase 1B, which were purchased from Sigma-Aldrich Co.

Extract and isolation

Fresh litchi seeds (7837 g) were crushed and extracted using 50% ethanol (22 L ×3) at room temperature for 4 days, in the absence of light, in a static system. The concentration of the extraction solution, under vacuum by a rotary evaporator at 50 °C, yielded 186.51 g of a sticky brown residue. The extract was dissolved in water and then partitioned with chloroform. The chloroform soluble layer (CSL) yielded 84.90 g, and the water-soluble layer (WSL) yielded 112.50 g. To remove any sugar, the WSL was loaded onto a Diaion HP-20 column to elute the water, and then separated with methanol. The methanol fraction yielded 36.56 g of product, which was subjected to Sephadex LH-20 column chromatography using a water/methanol solvent system to increase elution power (60:40, 40:60, 30:70 vol/vol), and yielded fractions (Fr) 1–17 after pooling, according to their TLC profiles. Fr 1–5 were obtained using water/methanol solvent elution system in the ratio 60:40. Fr 6–15 and 16–17 were obtained using elution with water/methanol solvent systems at the ratios 40:60 and 30:70, respectively. For further isolation, Fr 5 (2.2543 g) was loaded onto an MCI CHP 20P column and eluted with a water/methanol solvent system with increasing methanol concentrations (80:20, 70:30, 50:50, and 30:70 vol/vol). The elution using water/methanol (70:30 vol/vol) gave subfractions 5-11 (0.1550 g of compound D). Fr 6 (0.5287 g) was separated over an MCI CHP 20P column and eluted with water/methanol (40:60 vol/vol) to yield subfractions 6-1 (0.3335 g of compound A). Fr 16 was obtained as a single compound (0.1793 g of compound B). Fr 17 (1.7566 g) was partitioned by using an MCI CHP 20P column using a water/methanol solvent system (30:70 vol/vol) to give subfraction 17-2 (0.3728 g of compound C).

HPLC analysis

To analyze the main constituents from the litchi seed, reversed-phase HPLC was performed using a YL9100 HPLC System instrument (Anyang, Gyeonggi, Korea) equipped with a UV detector. Samples were separated using a Kromasil C18 column (Phenomenex, Torrance, CA, USA, 4.6 mm × 250 mm, 5 μm inner diameter) at room temperature. Solvent A (water/formic acid = 99:1, vol/vol) and solvent B (acetonitrile) were used as gradient linear mobile phases (solvent A: solvent B = 95:5 in 0–5 min, solvent A: solvent B = 90:10 in 5–14 min, solvent A: solvent B = 85:15 in 14–18 min, solvent A: solvent B = 80:20 in 18–30 min) at a mobile phase rate of 1 mL/min. All eluents were strained using a 0.45 μm PVDF membrane filter. The injection volume was 10 μL, and compounds were measured at a wavelength of 280 nm.
Each sample was extracted, filtered, and simultaneously analyzed for protocatechuic acid (Rt 9.30 min) (A), pavetannin B2 (17.82 min) (B), procyanidin A2 (24.03 min) (C), and 5-p-coumaroylquinic acid (12.44 min) (D) using the verified HPLC–UV analysis method, in triplicate to assure reliability of results.

Establishing a quality control standard

To optimize the extraction conditions, a patent extraction method was followed [14], by using ethanol with different solvent compositions (30, 50, 70, and 100%). The average content of active components in the extract was calculated based on quantitative analysis. The contents (%) were calculated as A × (Amount of solvent)/(Amount of sample) × 100, where A is the concentration of compound, and the amount of solvent was 1 mL and the amount of sample was 10 mg. In this study, three batches containing 1 kg of litchi seed each were extracted for each solvent composition to determine the best solvent to establish a standard production method.

α-Glucosidase inhibitory activity

The enzyme inhibition was conducted using a 96-well microplate reader, measured by a spectrophotometer [16]. A total of 60 μL of reaction mixture containing 20 μL of 100 mM phosphate buffer (pH 6.8), 20 μL of 2.5 mM p-NPG in the buffer, and 20 μL of sample dissolved in 10% DMSO were added to each well, followed by the addition of 20 μL of 10 mM phosphate buffer (pH 6.8) containing 0.2 U/mL of α-glucosidase. The plate was incubated at 37 °C for 15 min, and then 80 μL of 0.2 mol/L sodium carbonate solution was added to stop the reaction. Then, the absorbance was recorded at 405 nm by using a Sunrise microplate reader, measured by a spectrophotometer [16]. The inhibition percentage was calculated with the formula:

\[
\text{Inhibition percentage} = \left( \frac{A_s - A_c}{A_s} \right) \times 100%,
\]

where A_s is the absorbance of the control and A_c is the absorbance of the sample, which is subtracted from the absorbance of the color control.

The concentration of different partitions samples was as follows: the extract, CSL, WSL, and the sugar-removed layer (SRL) were examined for their α-glucosidase inhibitory activity. The concentration of active components in the extract was calculated as A = 100, where A is the concentration of compound, and the amount of solvent was 1 mL and the amount of sample was 10 mg. In this study, three batches containing 1 kg of litchi seed each were extracted for each solvent composition to determine the best solvent to establish a standard production method.

The inhibitory activity of isolated compounds against human recombinant PTP1B was investigated by using pNPP as a substrate. To each 96-well plate (final volume 110 μL), 2 mM pNPP and PTP1B in a buffer containing 50 mM citrate buffer (pH 6), 0.1 mM NaCl, 1 mM EDTA, and 1 mM DTT was added, with or without the sample. The samples with different concentrations (127, 63.5, 31.75, and 15.88 μM) were already dissolved in 10% DMSO. The plate was pre-incubated for 10 min at 37 °C, and then 50 μL of pNPP was added. The plate was then incubated for 30 min at 37 °C. Then, 10 μL of 10 M NaOH was added to stop the reaction. The amount of p-nitrophenyl produced after the enzymatic dephosphorylation was calculated by measuring the absorbance at 405 nm. The non-enzymatic hydrolysis of 2 mM pNPP was corrected by measuring the increase in absorbance at 405 nm, which was obtained in the absence of the PTP1B enzyme. The inhibition percentage was calculated with the following formula:

\[
\text{Inhibition percentage} = \left( \frac{(A_c - A_s)/A_c} {A_c} \right) \times 100%,
\]

where A_c is the absorbance of the control and A_s is the absorbance of the sample, which is subtracted from the absorbance of the color control. In this experiment, ursolic acid was used as positive control [17]. Protocatechuic acid (A), pavetannin B2 (B), procyanidin A2 (C), and 5-p-coumaroylquinic acid (D) were examined for their PTP1B activity.

Nuclear magnetic resonance (NMR)

Samples were dissolved in deuterated methanol-d₄ (CD₄, OD) and analyzed by 1D NMR (1H NMR, 600 MHz; 13C NMR, 150 MHz) and 2D NMR (1H–1H COSY, 1H–13C HSQC) spectroscopy using a VNS NMR 600 MHz (Varian, Palo Alto, CA, USA). Chemical shifts are expressed as parts per million on the δ scale.

UPLC–Q-TOF–MS

The chromatographic separation was performed on a Waters Acquity C18 CSH column (2.1 mm × 100 mm, 1.7 μm) using an Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA). The mobile phase consisted of solvent A (0.2% aqueous formic acid) and solvent B (acetonitrile). The gradient conditions were as follows: 0–2 min, solvent B 5–10%;
2–6 min, solvent B 10–15%; 6–7.5 min, solvent B 15–20%. The flow rate was 0.2 mL/min, and the column and sampler temperature were maintained at 30 and 15 °C, respectively. The injection volume was 2.0 μL, and UV wavelength was not used. MS was performed using a Q-TOF Synapt G2 apparatus (Waters) equipped with electrospray ionization (ESI). The optimal conditions of analysis were as follows: source temperature 100 °C, desolvation gas flow 700 L/h, capillary voltage 2.5 kV, and extraction cone voltage 4.0 V.

**Statistical analysis**

The differences between the positive control and treatment groups were determined by an independent sample t test. Data are presented as mean ± standard deviation (SD).

**Result and discussion**

**Structure elucidation**

The structures of compounds A–D isolated from litchi seed extract are shown in Fig. 1. Compound A was obtained as a dark brown crystal. $^{13}$C NMR (150 MHz, CD$_3$OD) showed the presence of carboxyl carbon signals [δ 168.88 (C-7)]. $^1$H NMR (600 MHz, CD$_3$OD) showed aromatic proton signals [δ 7.43 (1H, $d$, $J$ = 2.4, H-2), 6.79 (1H, $d$, $J$ = 8.1, H-5), 7.41 (1H, $d$, $J$ = 2.4, H-6)]. The molecular weight of compound A, analyzed by using UPLC–Q-TOF–MS (negative), was confirmed at [M–H]$^-$ m/z 153.0186, and the retention time was 3.2 min (Table 1). Finally, compound A was determined to be protocatechuic acid, which was confirmed by comparing the data with the reported values from the literature [18].

Compound B was obtained as a pale brown powder. $^{13}$C NMR (150 MHz, CD$_3$OD) showed the presence of ketal carbon signals [δ 98.53 (C-2)], indicating the existence of a double-linked subunit. Aliphatic carbon signals [δ 65.76 (C-3), 77.46 (C-2’), 71.16 (C-3’), 36.86 (C-4’), 78.88 (C-2”), 66.11 (C-3”), and 28.44 (C-4”)] were present at the up-field. Phenolic carbon signals [δ 155.34 (C-5), 156.41 (C-7), 154.34 (C-8a), 144.47 (C-11), 143.91 (C-12), 150.36 (C-5’), 154.38 (C-7’), 152.73 (C-8a’), 144.04 (C-11’), 144.34 (C-12’), 154.34 (C-5”), 154.61 (C-7”), 154.14 (C-8a”), 145.18 (C-11”), and 144.86 (C-12”)] were evident at the down-field. In the $^1$H NMR (600 MHz, CD$_3$OD), the ABX coupling system at δ 3.86–4.55 was attributed to B-ring protons. In the HSQC spectrum, δ 2.82 (1H, $d$, $J$ = 4.8, 6.7, H-4") was associated with δ 28.44 (C-4”), indicating that the signals [δ 27.46 (C-4)] were directly associated with the signals [δ 4.14 (1H, $d$, $J$ = 3.54, H-4)]. The proton signals [δ 4.55 (1H, $s$, H-4")] were corre- lated with δ 36.86 (C-4”). In the $^1$H–$^1$H COSY spectrum, the forgoing protons at δ 2.82, δ 4.14, and δ 4.55 were corre- lated with the respective proton signals [δ 3.86 (1H, $s$, H-3”), 3.27 (1H, $d$, $J$ = 3.54, H-3), 4.11 (1H, $d$, $J$ = 1.32, H-3’)]. No proton was linked to C-2, indicating that the location of the C2–O–C7 linkage appeared in the upper two units. Inter-flavonoid bonding of the upper two units was indicated by the carbon signal [δ 107.42 (C-8’)]. Inter-flavonoid bonding of the lower two units was indicated by their carbon signal [δ 108.06 (C-8’’)]. The molecular weight of compound B, analyzed by using ultra-performance liquid chromatography time of flight mass spectrometry (UPLC–Q-TOF–MS, negative), was confirmed at [M–H]$^-$ m/z 863.1826, and the retention time was 7.1 min (Table 1). Finally, compound B was identified as paven-tamin B2 and confirmed by comparison to the reported values from the literature [19, 20].

Compound C was isolated as a pale brown amorphous powder. $^{13}$C NMR (150 MHz, CD$_3$OD) showed that the double-linked structure of the A-type procyanidin was evident from the characteristic ketal carbon signals [δ 98.76 (C-2)]. Another double-linked procyanidin signal involving an inter-flavonoid bond was indicated in carbon

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Fig. 1 Molecular formula of the isolated compounds from the *Litchi chinensis* seed

(A) (B) (C) (D)
signals [δ 27.85 (C-4)]. 1H NMR (600 MHz, CD3OD) revealed two ABX coupling systems at δ 6.80–7.15. UPLC–Q-TOF–MS in the negative ion mode revealed a molecular ion signal [M–H]− at m/z 575.1188, and the retention time of compound C was 9.7 min (Table 1). Consequently, compound C was identified as procyanidin A2 or epicatechin-(4β → 8, 2β → O → 7)-epicatechin by 1H NMR, 13C NMR, and FAB-MS signals and comparison to the reported values from the literature [21].

Compound D was obtained as a light yellow crystal. The 1H NMR (600 MHz, CD3OD) spectrum of D showed the indication of p-substituted benzene ring proton signals [δ 7.50 (2H, d, J = 8.4, H-2’, 6’), 6.81 (2H, d, J = 8.4, H-3’, 5’)] and olefinic double bond proton signals [δ 7.67 (1H, d, J = 16, H-7’), 6.38 (1H, d, J = 15.6, H-8’)]. Particularly, the coupling constant value (J = 16 Hz) of the olefinic double bond proton signals meant that the double bond was trans. Additionally, methylene proton signals [δ 2.15 (2H, m, H-2), 2.20 (2H, m, H-6)] and three oxygenated methane proton signals [δ 4.17 (1H, m, H-3), 3.65 (1H, m, H-4), 5.37 (1H, dd, J = 3.6, 8.16, H-5)] represented quinic acid. The molecular weight of compound D, analyzed by using UPLC–Q-TOF–MS (negative), was confirmed at [M–H]− m/z 337.0921, and the retention time of compound D was 5.2 min (Table 1). According to the above data, compound D was identified as 5-p-coumaroylquinic acid by comparing the 1H NMR spectrum and molecular weight to the literature [22].

### Quantitative analysis by HPLC

The contents of the four compounds were calculated from the regression equations based on the calibration curve. The average content of compounds A, B, C, and D was 0.152 ± 0.087, 2.608 ± 0.375, 1.060 ± 0.521, and 0.625 ± 0.149 mg/g, respectively. Contents criteria per 0.5 g of powder for compounds A, B, C, and D exceeded 0.0065, 0.2233, 0.0 539, and 0.0476 mg/g, respectively.

### Development of an extraction method

In order to establish a quality control standard, this study developed a standard extraction method. The four major compounds were extracted using different ethanol–water solvent compositions (Table 2). Considering the contents of the four compounds, 50% ethanol was the most efficient solvent and thus this was the optimized solvent condition. Extraction occurred after 10 h of maceration.

### Average yield

To guarantee the quality and stability of the raw materials, we suggested that an average mass of 1 kg raw material be used for extraction. Litchi seed (1 kg) was extracted three times, and then the average yield was calculated. The average yield (%) of 1 kg litchi seed was 9.83 ± 0.27%, i.e., more than 9.56%. Considering the 10% tolerance range, it should yield more than 8.60% in the 1 kg of litchi seed.

### Major component contents

To guarantee the quality and stability of pharmaceutical or nutraceutical preparations, we measured the amount of major bio-component contents. The contents of protocatechuic acid, pavetannin B2, procyanidin A2, and 5-p-coumaroylquinic acid were 1.4 ± 0.4, 14.9 ± 1.2, 9.0 ± 1.6, and 3.8 ± 0.3 mg/g, respectively, in 1 kg of litchi seed extract. Considering the 10% tolerance range, the contents were ≥0.911, ≥12.361, ≥6.691, and ≥3.138 mg/g, respectively, in the 1 kg of litchi seed extract for pharmaceutical preparations.

### α-Glucosidase inhibitory activity of different partitions

The litchi seed crude extract, CSL, WSL, and SRL were examined for their α-glucosidase inhibitory activity. The crude extract, CSL, WSL, SRL, Fr 6, Fr 16, and Fr 17 all showed dose-dependent inhibitory activities. The activities of different layers (In the concentrations of 15.63, 7.81, 6.25, 3.91, and 0.39 μg/mL) are shown in Fig. 2. The results indicated that the SRL markedly inhibited α-glucosidase at high doses (e.g., 15.63, 7.81, and 6.25 μg/mL). However, at a low dose (e.g., 3.91 μg/mL), its inhibition and stability were lower than that of the crude extract. For α-glucosidase inhibitory activity, the IC50 of the reference drug, acarbose, was 55.845 μg/mL, while those of the

### Table 1 Compounds identified from the litchi seed by using ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-Q-TOF–MS)

| Compound | Molecular formulas | Exact mass | Retention time (min) | MS detected ions ([M–H]− m/z) |
|----------|--------------------|------------|----------------------|-------------------------------|
| (A)      | C7H6O4             | 154.0266   | 3.2                  | 153.0186                      |
| (B)      | C22H36O18          | 864.1902   | 7.1                  | 863.1826                      |
| (C)      | C16H20O12          | 576.1268   | 9.7                  | 575.1188                      |
| (D)      | C16H18O8           | 338.1002   | 5.2                  | 337.0921                      |

A, protocatechuic acid; B, pavetannin B2; C, procyanidin A2; D, 5-p-coumaroylquinic acid
crude extract, CSL, WSL, and SRL were 0.691, 6.179, 6.707, and 3.686 \( \mu g/mL \), respectively. In comparison, oligonol, derived from a litchi fruit extract, exhibited \( \alpha \)-glucosidase inhibitory activity with an IC \(_{50} \) value of 23.14 ± 0.91 \( \mu g/mL \) [23]. This indicates that the litchi seed extract has higher inhibitory activity than the litchi fruit extract or acarbose. Litchi seeds are usually discarded as waste, and therefore these data suggest its use as a cost-effective natural, alternative medicine for the treatment of type 2 diabetes. Following the bioactivity-guided isolation, \( \alpha \)-glucosidase activity was measured on different methanol fractions. Fr 6 was isolated from a 60% MeOH solvent system, and Fr 16 and 17 were isolated from a 70% MeOH solvent system. The Fr 6, 16, and 17 showed inhibitory activities against \( \alpha \)-glucosidase with IC \(_{50} \) values of 40.339, 0.023, and 0.002 \( \mu g/mL \), respectively. This indicates that Fr 6, 16, and 17 contain efficient \( \alpha \)-glucosidase inhibitor compounds.

**\( \alpha \)-Glucosidase inhibitory activity of isolated compounds**

All samples exhibited dose-dependent inhibitory activities (Fig. 3). For \( \alpha \)-glucosidase inhibitory activity, the IC \(_{50} \) of protocatechuic acid was 131.003 \( \mu M \), pavetannin B2 was 0.041 \( \mu M \), procyanidin A2 was 0.078 \( \mu M \), and acarbose was 74.819 \( \mu M \). In comparison, polyphenol compounds isolated from barnyard millet exhibited \( \alpha \)-glucosidase inhibitory activity with an IC \(_{50} \) value of 1.3–17.8 \( \mu M \) [24]. Green tea, which is the most widely consumed natural product that reduces the risk of type 2 diabetes mellitus exhibited \( \alpha \)-glucosidase inhibitory activity with an IC \(_{50} \) value of 2.82 ± 0.23 \( \mu g/mL \) [25]. This indicates that the polyphenol compounds that are isolated from litchi seeds have much stronger inhibitory activity than barnyard millet or green tea. In this study, Pavetannin B2 and procyanidin A2 showed very high inhibitory activity and this might be related to their structure—activity relationship, including the unsaturated C ring, 3-OH, and hydroxyl substitutions on the B ring. A previous study suggests that the unsaturated C ring, 3-OH, 4-CO, the linkage of the B ring at the 3 position, and the hydroxyl substitution on the B ring on flavonoids are the source of the \( \alpha \)-glucosidase inhibitory activity [26].

**PTP1B inhibitory activity**

All samples exhibited dose-dependent inhibitory activities. Figure 4 shows the activities of the isolated compounds (127, 63.5, 31.75, and 15.88 \( \mu M \)). Protocatechuic acid and...
5-γ-coumaroylquinic acid showed no inhibitory activity against PTP1B. Moreover, the IC₅₀ values of pavetannin B2, procyanidin A2, and ursolic acid were 450.295, 338.257, and 19.686 μM, respectively. Although the mechanism behind PTP1B inhibitory activity is not known, the results showed that pavetannin B2 and procyanidin A2 showed PTP1B inhibitory activity.

In conclusion, this study utilized the bioactivity-guided isolation of major compounds from litchi seeds, which are usually discarded as a waste. The isolated bioactive compounds showed anti-diabetic activity in an α-glucosidase inhibitory assay and PTP1B inhibitory assay. Based on its anti-diabetic activity, quality control standards were suggested for guaranteeing the quality and stability of litchi seed raw materials and pharmaceutical preparations. Further research, including in vivo studies and clinical trials on the formulation of these isolated compounds, is required, particularly relating to efficacy. Furthermore, the isolated compounds may have beneficial therapeutic actions against other diseases. Our results show that the litchi seed extract has potent therapeutic efficacy and may be a natural alternative medicine for the treatment of type 2 diabetes.

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