

ORIGINAL ARTICLE OPEN ACCESS

Inhibitory Effect of *Ilex Latifolia* Leaf Extract on Postprandial Increase in Blood Glucose Level

Hiroaki Shimada¹  | Kouichi Sasai¹ | Yoshiaki Manse² | Toshio Morikawa^{2,3}  | Shoichi Ueno⁴ | Masahiro Iwaki^{1,2,3}

¹Department of Pharmacy, Faculty of Pharmacy, Kindai University, Kowakae, Higashiosaka, Japan | ²Pharmaceutical Research and Technology Institute, Kindai University, Kowakae, Higashiosaka, Osaka, Japan | ³Antiaging Center, Kindai University, Kowakae, Higashiosaka, Osaka, Japan | ⁴Eishin Pharmaceutical Co., Ltd., Kita-ku, Osaka, Japan

Correspondence: Hiroaki Shimada (shimada@phar.kindai.ac.jp)

Received: 20 January 2025 | **Revised:** 18 April 2025 | **Accepted:** 20 April 2025

Keywords: blood glucose | caffeoylquinic acids | *ilex latifolia* | maltase

ABSTRACT

Aim: Kuding tea brewed from *Ilex latifolia* (IL) leaves has been traditionally consumed and regarded as “beauty-slimming tea” and “longevity tea” in China, albeit supporting theoretical evidence is lacking. This study aims to determine whether IL leaves can be effectively used to prevent gastrointestinal dietary glucose absorption.

Methods: Mice were administered IL leaf extract (IL-ext) with α -starch orally and measured blood glucose levels. IL-ext was partitioned with ethyl acetate-H₂O and further fractionated using reversed-phase ODS column chromatography into mono-caffeoylquinic acids (CQAs)-, di-CQAs, and triterpenoids-enriched fractions. The components contained in each fraction were quantified using HPLC, and the inhibitory effect on maltase was evaluated via in vitro assay.

Results: The increase in blood glucose level after oral α -starch administration was suppressed by treatment with 100 or 500 mg/kg of IL-ext in mice. IL-ext inhibited maltase activity, with the di-CQA-enriched fraction showing the strongest maltase inhibition. Among di-CQAs, 3,4-di-CQA showed the strongest inhibition of maltase.

Conclusion: The intake of IL leaves with meals can prevent gastrointestinal glucose absorption by inhibiting the digestion of dietary carbohydrates. The 3,4-di-CQA contained in IL leaves appears to be a major bioactive component for inhibiting postprandial increases in blood glucose levels.

1 | Introduction

Ilex latifolia (IL) leaves have been traditionally brewed and consumed as kuding tea, known as “beauty-slimming tea” and “longevity tea” in China [1]. *Ilex latifolia* grows wild in southern Japan and eastern and southern China. In China, IL is also used as folk medicine for treating indigestion, headaches, and several inflammatory diseases [2]. Various beneficial bioactivities have been reported for IL, including anti-obesity [3], anti-atherosclerotic [4], and antidiabetic activities [5].

Several constituents of IL leaves have been identified, including triterpenoid saponins, phenolic acids, flavonoids, and essential

oils [1]. Specifically, triterpenoid saponins (kudinosides and latifolosides), mono-caffeoylquinic acids (mono-CQAs; 3-CQA, 4-CQA, and 5-CQA), and di-caffeoylquinic acids (di-CQAs; 3,4-di-CQA, 3,5-di-CQA, and 4,5-di-CQA) are the major constituents of IL leaf extract (IL-ext) [6, 7]. Triterpenoid saponins from *Ilex kudingcha*, a closely related IL species, suppress the aggregated LDL-induced formation of foam cells from macrophage [8]. Moreover, kudinoside D decreases lipid accumulation in adipocyte [9]. Although triterpenoid saponins are detected in plasma after oral administration of crude extract from *Ilex kudingcha* leaves [10], their bioavailability remains unclear. CQA derivatives possess various biological effects, including neuroprotective [11] and anti-cancer [12]. However, their oral bioavailability

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Traditional & Kampo Medicine* published by John Wiley & Sons Australia, Ltd on behalf of Japan Society for Oriental Medicine and Japan Society of Medical and Pharmaceutical Sciences for Traditional Medicine.

appears quite low. For example, 5-CQA was not detected in the plasma of rats after oral administration [13]. Therefore, it is difficult for orally ingested CQA derivatives to exert such pharmacological effects directly.

In the digestive tract, dietary carbohydrates are broken down into monosaccharides by digestive enzymes (e.g., α -amylase and α -glucosidases). Compounds that inhibit these enzymes are used as medicines and functional foods for preventing diabetes mellitus and obesity [14]. Notably, 5-CQA and 3,5-diCQA inhibit α -glucosidase [15]. We hypothesized that IL leaves would inhibit postprandial increases in blood glucose levels by inhibiting the digestion of dietary carbohydrates on the luminal side of the gastrointestinal tract. However, no evidence of this mechanism has been experimentally reported *in vivo*.

Herein, we investigated the inhibitory effect of dried 60% aqueous ethanol extract of IL leaves (IL-ext) on dietary glucose absorption to empirically demonstrate that kuding tea can indeed be used as “beauty-slimming tea” and “longevity tea.” We administered IL-ext with α -starch and measured blood glucose level in mice. Moreover, we fractionated IL-ext into mono-CQAs, di-CQAs, and triterpenoid saponins-enriched fractions and investigated their inhibitory effects on maltase, a major carbohydrate digestive enzyme, *in vitro* experiments.

2 | Materials and Methods

2.1 | Materials

The α -Glucosidase Inhibitory Activity Assay Kit and LabAssay Glucose Kit were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). LAB Gluco, a blood glucose level measuring instrument, was purchased from ForeCare Japan Co. Ltd. (Tokyo, Japan). 5-CQA was purchased from Nacalai Tesque Inc. (Kyoto, Japan). 3-CQA, 4-CQA, 3,5-di-CQA, 3,4-di-CQA, and 4,5-di-CQA were purchased from Carbosynth Ltd. (Compton, UK). Kudinoside D was purchased from MedChemExpress (Monmouth Junction, NJ, USA). All other compounds and reagents used were reagent-grade commercial products.

2.2 | Preparation of IL-Ext

Harvesting of IL leaves and preparation of IL-ext were performed by Beida Cistanche (Tianjin) Biotechnology Co. Ltd. (Tianjin, China). The IL leaves were harvested from May to July 2018 at a farm in Wuzhishan City, Hainan Province, China. A voucher specimen was deposited at Eisin Pharmaceutical Co. Ltd. (Osaka, Japan; Prod. No. 1801001). IL leaves were dried in the shade under normal temperature. A sample of 1000g of dried IL leaves was crushed with a hammer mill and then extracted with 10 times the volume of a 3:2 (v/v) mixture of ethanol/water. The residue was further extracted with 8 times the volume of a 3:2 (v/v) mixture of ethanol/water. Extracts were mixed and passed through filter paper. The final filtered solution was evaporated under vacuum; 120 g of the dried extract was obtained, representing IL-ext, which was kept at room temperature until further use.

2.3 | Preparation of IL-Ext Fractions

IL-ext fractions were prepared as previously reported, with some modifications [16]. Specifically, dried IL-ext (18g) was partitioned with ethyl acetate (EtOAc)–H₂O (1:1, v/v) to furnish an EtOAc-soluble fraction (2.26 g, 12.5%) and an aqueous phase. The latter was fed into a Diaion HP-20 column chromatograph (Mitsubishi Chemical Co. Ltd.; 500g; H₂O → MeOH) to obtain H₂O-eluted (5.86 g, 32.5%) and MeOH-eluted (9.78 g, 54.3%) fractions.

An aliquot (1g) of the MeOH-eluted fraction was subjected to reversed-phase ODS column chromatography (CHROMATOREX ODS-DM1020T; Fuji Silysia Chemical Ltd., Aichi, Japan; 100–200 mesh) [10g, acetonitrile–H₂O–formic acid (80:20:0.1 v/v) → 0.1% formic acid-containing acetonitrile] to give three fractions [Fr.1 (0.38 g, 38%), Fr.2 (0.35 g, 35%), and Fr.3 (0.03 g, 3%)].

2.4 | High-Performance Liquid Chromatography (HPLC) Analysis of IL-Ext

HPLC analysis was performed with reference to the literature to detect mono-CQAs, di-CQAs, and kudinoside D [17]. The IL-ext and its fractions were diluted in 50% acetonitrile at 10–100 μ g/mL for HPLC analysis using a Shimadzu HPLC-20A series system (Shimadzu, Kyoto, Japan). The COSMOSIL Packed Column 5C₁₈-MS-II analytical column (150 × 2.0 mm inner diameter; Nacalai Tesque Inc.) was maintained at 40°C. Gradient elution was performed using a mobile phase comprising 0.1% formic acid in both water (A) and acetonitrile (B) at a flow rate of 0.4 mL/min. The gradient profile was 5%–30% B for 0–25 min, 30%–90% B for 25–45 min, 90%–90% for 45–50 min, and then, B was kept at 5% for 40 min to equilibrate the column. The components were detected via UV absorption at 325 and 254 nm for the CQA derivatives and kudinoside D, respectively. Figure 1 shows the chromatograms obtained from IL-ext and each standard for the CQA derivatives and kudinoside D.

2.5 | Effects of IL-Ext on Blood Glucose Levels in α -Starch-Or Glucose-Loaded Mice

Male ICR mice (5–7 weeks old, weighing 28.8 ± 2.4 g) were used in this study. All mice were allowed free access to food and water under a standard 12h light/12h dark cycle in a temperature-controlled room (23°C ± 2°C). The animals were fasted for 16–20 h before the beginning of the experiment but were allowed free access to water during fasting. The IL-ext (0, 100, or 500 mg/kg body weight, BW) was suspended in an α -starch (4.0 g/kg BW) solution, which was then administered orally to the fasted mice. Blood glucose levels in the tail vein were monitored using a LAB Gluco instrument at the indicated time points (after loading α -starch, 15, 30, 45, 60, and 120 min). Blood glucose levels in the tail vein before loading α -starch were plotted as 0 min in Figure 1. All animal experiments were performed in accordance with the Institutional Animal Care and Use requirements of Kindai University (permit number: KAPS-2020-024).

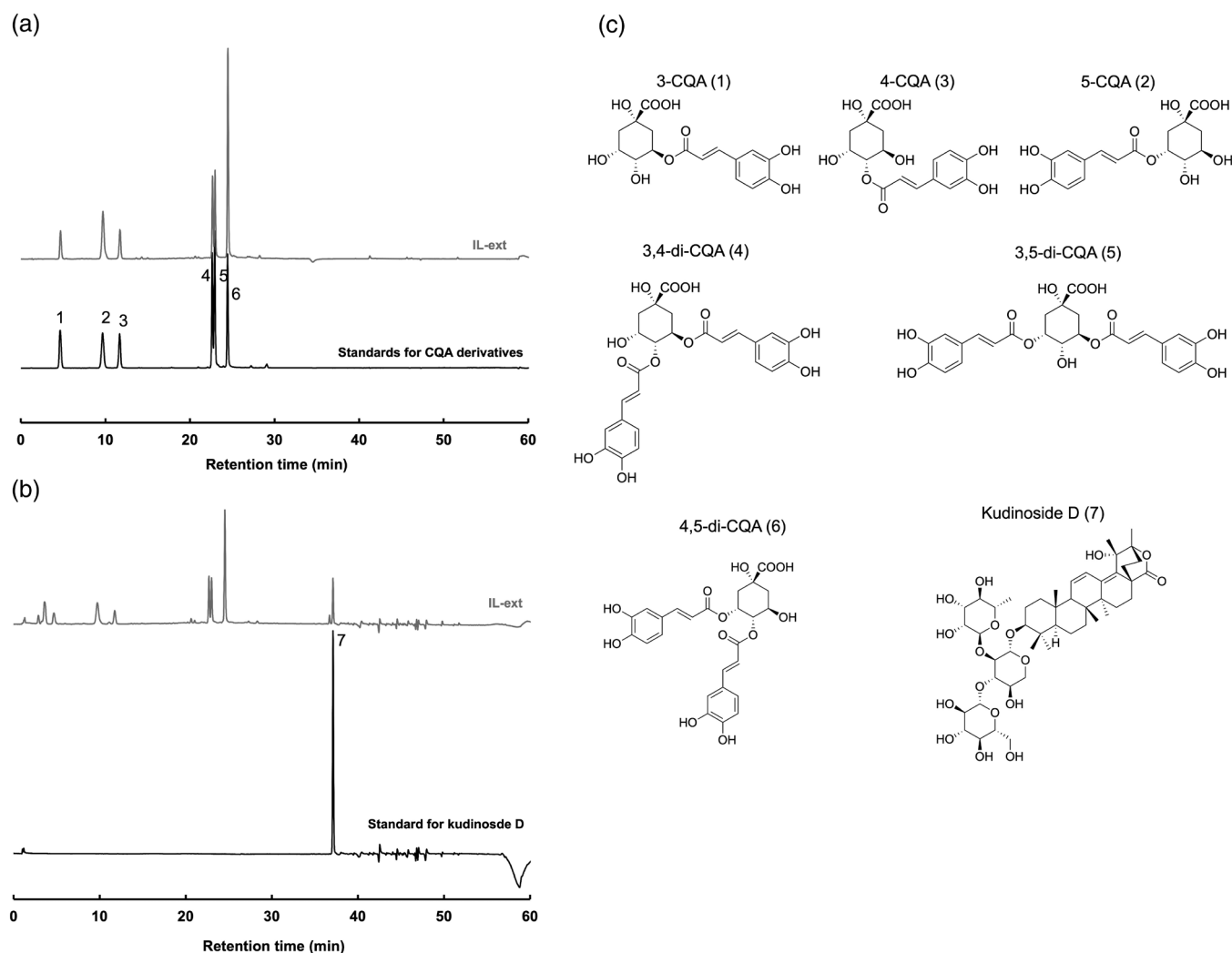


FIGURE 1 | Chromatograms obtained in the HPLC-UV analysis of IL-ext and the CQA derivative and kudinoside D standards. Seven peaks were detected in 0.5 mg/mL IL-ext diluted in 50% acetonitrile at 325 nm (a) and 254 nm (b). Retention times of these seven peaks matched those of 100 μ g/mL of the standards for 3-CQA (1), 5-CQA (2), 4-CQA (3), 3,4-di-CQA (4), 3,5-di-CQA (5), 4,5-di-CQA (6), and kudinoside D (7) diluted in 50% acetonitrile (c).

2.6 | Maltase Activity Assay

The maltase activity assay was performed using an α -Glucosidase Inhibitory Activity Assay Kit (Wako). Briefly, the maltose solution was preincubated for 3 min at 37°C in the presence or absence of IL-ext or its fractions (0.005–1 mg/mL). The IL-ext and its fractions were dissolved in DMSO, and the assay was performed in the presence of 1% DMSO (final concentration) for all experiments, including the untreated control. The reaction was initiated by adding maltase solution for 30 min at 37°C. The reaction was terminated by boiling the mixture for 3 min and placing it on ice. The amount of glucose produced was measured using a LabAssay Glucose kit (Wako).

2.7 | Data Analyses

IC₅₀ values were determined using GraphPad Prism 9 (GraphPad Software, La Jolla, CA).

2.8 | Statistical Analyses

All in vivo and in vitro data are presented as mean $\pm$ SD and SEM values, respectively. Statistical analyses were performed using Student's *t*-test or analysis of variance (ANOVA), followed by Dunnett's test, with the significance threshold set at $p < 0.05$. GraphPad Prism 9 (GraphPad Software, La Jolla, CA) was used to perform Dunnett's test.

3 | Results

3.1 | Inhibitory Effect of IL-Ext on Increases in Blood Glucose After α -Starch Loading in Mice

To evaluate the inhibitory effect of IL-ext on increases in post-prandial blood glucose levels, α -starch with IL-ext (100 or 500 mg/kg BW) was orally administered to mice. IL-ext suppressed the increase in blood glucose levels at 15, 30, and 45 min after oral

loading with α -starch (Figure 2). The area under the curve (AUC) of the delta blood glucose concentration-time at 0–120 min after α -starch loading was calculated as listed in Table 1. Acarbose, an α -glucosidase inhibitor, was used as a positive control.

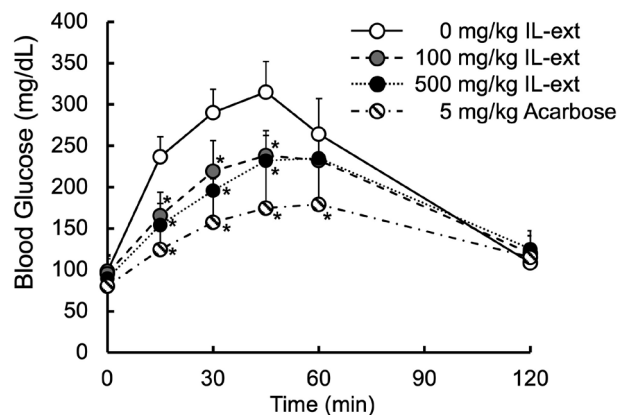


FIGURE 2 | Effect of IL-ext on the increase in blood glucose level after α -starch loading in mice. Time course of blood glucose levels after oral administration of 4 g/kg BW of α -starch with 0 (control, white circles with solid line), 100 (gray circles with dashed line), or 500 (black circles with dotted line) mg/kg BW of IL-ext. As a positive control (striped circles with dot-dashed line), 5 mg/kg BW of acarbose was administered with 4 g/kg BW of α -starch. Blood glucose levels in tail vein were measured 15, 30, 45, 60, and 120 min after loading α -starch. Each point represents the mean $\pm$ SD ($n=12-15$). *: Significant difference from the control based on Dunnett's test results after ANOVA ($p<0.05$).

TABLE 1 | AUC of blood glucose after administrating α -starch.

	AUC (mg-min/dL)
Control	14,738 $\pm$ 1949
100 mg/kg BW of IL-ext	10,949 $\pm$ 1904*
500 mg/kg BW of IL-ext	11,205 $\pm$ 1860*
5 mg/kg BW of Acarbose	8009 $\pm$ 3085*

Abbreviation: BW, body weight.

*A significant difference from the control, based on the results of Dunnett's test after ANOVA ($p<0.05$).

TABLE 2 | Contents of the CQAs and kudinoside D in fractions prepared from IL-ext.

Fractions	Content (mg/g)						Kudinoside D
	3-CQA	4-CQA	5-CQA	3,4-di-CQA	3,5-di-CQA	4,5-di-CQA	
IL-ext	21.7	27.4	44.6	34.0	28.6	76.3	17.6
H ₂ O-eluted fraction	64.3	65.9	95.0	N.D.	N.D.	N.D.	N.D.
MeOH-eluted fraction	N.D.	9.8	6.7	57.8	37.4	105.9	40.8
EtOAc-soluble fraction	N.D.	N.D.	N.D.	25.4	97.1	149.7	14.6
Fr.1	3.9	24.1	16.8	166.0	98.1	156.2	N.D.
Fr.2	N.D.	N.D.	N.D.	5.6	10.8	186.8	49.8
Fr.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	131.1

Abbreviation: N.D., Not detected.

3.2 | Components in IL-Ext and Its Fractions

The levels of CQAs and kudinoside D in IL-ext and its fractions were quantified via HPLC (Table 2). Other components were detected through liquid chromatography–tandem mass spectrometry (LC–MS/MS), and their levels in the fractions relative to those in the IL-ext were calculated (Tables S1–S3). Mono-CQAs were more concentrated in the H₂O-eluted fraction than in IL-ext, whereas mono-CQAs were not detected in the EtOAc-soluble fractions (Table 2). Di-CQAs and kudinoside D were found in the MeOH-eluted and EtOAc-soluble fractions but not in the H₂O-eluted fraction. Di-CQAs were concentrated in the MeOH-eluted and EtOAc-soluble fractions, and kudinoside D was concentrated in the MeOH-eluted fraction and to the same extent in the EtOAc-soluble fraction compared to the levels in IL-ext. We further partitioned the MeOH-eluted fraction into Fr.1–3. Almost all CQAs were eluted in Fr.1, whereas kudinoside D was not detected in Fr.1. Although Fr.2 contained 4,5-di-CQA to the same degree as Fr.1, the amounts of 3,4- and 3,5-di-CQAs were markedly lower than those of Fr.1, and mono-CQAs were not detected in Fr.2. Additionally, the kudinoside D content in Fr.2 was comparable to that in the MeOH-eluted fraction. In Fr.3, CQAs were not detected, but kudinoside D was concentrated compared to that in the MeOH-eluted fraction. Ultimately, we regarded the H₂O-eluted fraction, Fr.1, and Fr.3 as mono-CQAs, di-CQAs, and kudinoside D-enriched fractions, respectively.

3.3 | Effect of IL-Ext on Carbohydrate Digestive Enzymes

We examined the inhibitory potential of IL-ext and its fractions against maltase. Maltase activity was inhibited by IL-ext in a concentration-dependent manner (Figure 3a). Although the inhibitory effects of MeOH-eluted and EtOAc-soluble fractions on maltase tended to be stronger than that of IL-ext, the H₂O-eluted fraction had a weaker effect than IL-ext (Table 3). In further fractions from the MeOH-eluted fraction, Fr.1 and Fr.2 inhibited maltase more strongly than Fr.3 (Figure 3b and Table 3). These results indicate that mono-CQAs and terpenoid saponins were not the primary components involved in the inhibitory effect of IL-ext on maltase. We further evaluated the inhibitory potencies of commercially available standards for

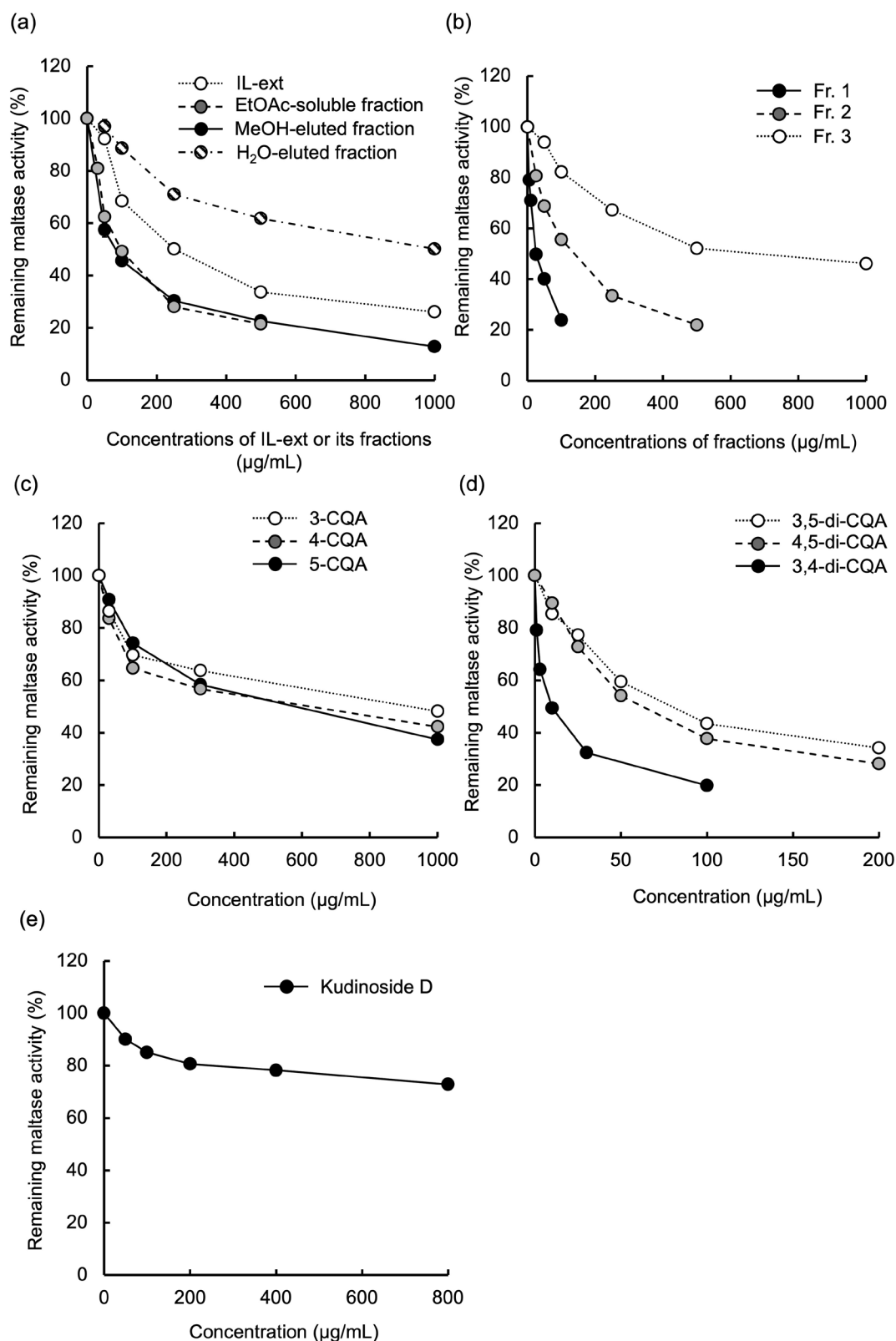


FIGURE 3 | Inhibitory effect of IL-ext, its fractions, and standards for CQA derivatives and kudinoside D on maltase. (a) Maltase inhibition by IL-ext (white circles with dotted line) or its fractions, H₂O-eluted (striped circles with dot-dashed line), MeOH-eluted (black circles with solid line), and EtOAc-soluble fractions (gray circles with dashed line). (b) Maltase inhibition by further fractions from MeOH-eluted fraction, Fr.1 (black circles with solid lines), Fr.2 (gray circles with dashed lines), and Fr.3 (white circles with dotted lines). (c-e) Maltase inhibition by standards of mono-CQAs (c), di-CQAs (d), and kudinoside D (e). 3-CQA and 3,5-di-CQA are indicated by white circles with dotted lines; 4-CQA and 4,5-di-CQA are indicated by gray circles with dashed lines; and 5-CQA, 3,4-di-CQA, and kudinoside D are represented by black circles with solid lines in the respective graphs. Each bar represents mean $\pm$ SEM ($n=4$).

TABLE 3 | IC₅₀ values of fractions from IL-ext against human-derived maltase.

Fraction	IC ₅₀ (μg/mL)	95% CI (μg/mL)
IL-ext	272.5	242.7–306.0
H ₂ O-eluted fraction	834.7	742.3–941.5
MeOH-eluted fraction	90.3	77.8–104.5
EtOAc-soluble fraction	104.2	94.4–115.0
Fr.1	27.0	24.6–29.6
Fr.2	121.6	114.7–128.9
Fr.3	614.8	549.9–688.5

Abbreviation: CI, Confidence interval.

TABLE 4 | IC₅₀ values of compounds contained in IL-ext against human-derived maltase.

Compound	IC ₅₀ (μg/mL)	95% CI (μg/mL)
3-CQA	682.4	485.1–958.7
4-CQA	428.0	304.8–600.5
5-CQA	469.7	390.1–565.8
3,4-di-CQA	9.0	6.7–12.0
3,5-di-CQA	81.1	74.7–88.0
4,5-di-CQA	65.4	61.3–69.7
Kudinoside D	> 800	—
Acarbose	0.214	0.157–0.294

CQAs and kudinoside D. Although the inhibitory potencies of 3-, 4-, and 5-CQA on maltase were comparable (Figure 3c), 3,4-di-CQA inhibited maltase more strongly than 3,5-di- or 4,5-di-CQA (Figure 3d). Kudinoside D showed a much weaker inhibition of maltase than the CQAs (Figure 3e). The IC₅₀ values for maltase are listed in Table 4. The IC₅₀ of kudinoside D for maltase could not be estimated because of its weak inhibitory potential and poor water solubility.

Furthermore, we calculated the concentrations of CQAs and kudinoside D in each fraction at the IC₅₀ for maltase and compared them with the IC₅₀ value of the individual standard components for maltase, as presented in Table 5. The concentrations of CQAs and kudinoside D in each fraction at IC₅₀ were calculated using the levels of the components (Table 2) and the IC₅₀ of each fraction for maltase (Table 3). When the component concentration ratio for the IC₅₀ of each fraction for maltase to the IC₅₀ of the respective components for maltase ([C]/IC₅₀) was greater than 1, the concentration of the respective components in the IC₅₀ of each fraction reached the IC₅₀ of the components that were estimated using their standards. Because only the [C]/IC₅₀ values of 3,4-di-CQA in IL-ext, MeOH-eluted fraction, and Fr.1 exceed 1 (Table 5), our results indicate that 3,4-di-CQA is the primary component regarding maltase inhibition in IL-ext.

TABLE 5 | Inhibitory effects of CQAs and kudinoside D in IL-ext on maltase.

Components	IL-ext		H ₂ O-eluted fraction		MeOH-eluted fraction		EtOAc-soluble fraction		Fr.1		Fr.2		Fr.3	
	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀	[C] (μg/mL)	[C]/IC ₅₀
3-CQA	6.4	0.01	26.1	0.04	N.D.	—	N.D.	—	0.5	0.00	N.D.	—	N.D.	—
4-CQA	8.1	0.02	26.7	0.06	2.0	0.00	N.D.	—	3.4	0.01	N.D.	—	N.D.	—
5-CQA	13.2	0.03	38.5	0.08	1.4	0.00	N.D.	—	2.4	0.01	N.D.	—	N.D.	—
3,4-di-CQA	10.0	1.12	N.D.	—	11.7	1.30	2.6	0.29	23.2	2.58	1.57	0.17	N.D.	—
3,5-di-CQA	8.5	0.10	N.D.	—	7.6	0.09	9.9	0.12	13.7	0.17	3.03	0.04	N.D.	—
4,5-di-CQA	22.5	0.34	N.D.	—	21.5	0.33	15.3	0.23	21.9	0.33	52.40	0.80	N.D.	—
Kudinoside D	5.2	N.D.	N.D.	—	8.3	N.D.	1.5	N.D.	N.D.	—	13.97	N.D.	364.7	N.D.

Note: The IC₅₀ values and contents of the components in each fraction are from Tables 2, 3, and 4. Abbreviations: [C], calculated concentration of components in IC₅₀ of each fraction; [C]/IC₅₀, ratio of concentration-IC₅₀ of each component; N.D., not determined.

4 | Discussion

Simultaneous administration of IL-ext decreased glucose absorption into blood circulation after oral loading with α -starch; however, the dose-dependent effect of IL-ext was not observed (Table 1). We suspended IL-ext in α -starch solution to administer it to mice because of its poor solubility in water. Since the water content in the gastrointestinal tract of a fasted mouse is reported to be 0.81 ± 1.3 mL [18], the concentration of IL-ext in the intestinal tract after administration (100 or 500 mg/10 mL/kg) was estimated to be over 2 mg/mL, the maximum soluble concentration of IL-ext in water. Therefore, the concentration of IL-ext would have been saturated in the intestinal fluid. Assuming that 100 mg/kg BW of IL-ext is necessary to exert an inhibitory effect on dietary glucose absorption, 6 g of IL-ext has to be taken by humans (60 kg BW). As the single dose of acarbose is maximally 0.1 g, the effect of IL-ext on dietary glucose absorption seems to be weaker than that of acarbose.

Continuous drinking of 0.05% ethanol extract of kuding tea, including IL leaves, prevents an increase in blood glucose levels after loading glucose by improving glucose tolerance in the high-fat-diet-fed mice [19]. Unlike this, we show that IL-ext can prevent the increase of blood glucose after loading α -starch in normal-diet-fed mice that have normal glucose tolerance. The inhibition of dietary glucose absorption by acarbose monotherapy is useful for reducing BW and decreasing blood triglyceride level in nondiabetic obese people [20]. Moreover, acarbose can extend lifespan in mice [21]. Thus, tea brewed from IL leaves is potentially useful as “beauty-slimming tea” and “longevity tea” by preventing dietary glucose absorption.

The profiles of the IL-ext contents were close to those reported in the methanol extract of dried IL leaves [17]. However, the IL-ext prepared in this study tended to contain more aqueous constituents than the reported methanol extracts of IL leaves. This appears to depend on the solvent used. Since we used 60% aqueous ethanol as the solvent for extraction from dried IL leaves, aqueous components were well extracted into IL-ext.

Consistent with a previous study [15], di-CQAs inhibited maltase more strongly than mono-CQAs (Table 4). Although inhibitory potencies on yeast-derived α -glucosidase are the same among di-CQAs [15], our results indicate that 3,4-di-CQA shows the strongest inhibition on maltase among di-CQAs (Table 4). There is no difference in inhibitory potencies on yeast-derived α -glucosidase among each di-CQA (data not shown), reflecting a difference in the recognition of di-CQAs as inhibitors between human-derived maltase and yeast-derived α -glucosidase.

The present study has limitations. We only investigated the inhibitory effect of IL-ext on the increase in blood glucose levels after α -starch loading. To verify 3,4-di-CQA as a key component in IL-ext for exhibiting this effect, it is necessary to perform an in vivo study using a di-CQA-enriched fraction and/or 3,4-di-CQA standard. However, any constituent contained in IL-ext possibly affects other factors rather than maltase, including other carbohydrate digestive enzymes, glucose transporters,

and incretin, to inhibit increases in blood glucose levels after α -starch loading. We confirm that IL-ext and its fractions inhibit α -amylase activity (Table S4). Moreover, IL-ext suppressed the increase in blood glucose levels after glucose loading (data not shown). Thus, the inhibitory effect of IL-ext on postprandial increases in blood glucose levels must involve complex mechanisms, and 3,4-di-CQA-mediated maltase inhibition seems to be one of them. In addition, we investigated the inhibitory potentials of IL-ext, its fractions, and compounds on maltase activity using the Mutarotase-Glucose oxidase method for glucose quantification. As reductive substances, such as phenolic compounds including CQAs, can interfere with the colorimetric reaction in this method, the IC_{50} for maltase activities may not be estimated accurately in this study. Although we demonstrated that IL-ext can suppress postprandial increases in blood glucose levels in vivo for the first time, further investigation is necessary to understand its mechanisms.

5 | Conclusion

We demonstrate for the first time that IL-ext exerts an inhibitory effect on postprandial increases in blood glucose levels. Our results indicate that maltase inhibition by di-CQAs, especially 3,4-di-CQA, is one of the mechanisms underlying the effects of IL-ext. Thus, we successfully provide empirical evidence of the utility of ingesting IL leaves as “beauty-slimming tea” and “longevity tea.”

Acknowledgments

We thank Kindai University Joint Research Center for the use of its facilities.

Disclosure

All animal experiments were performed in accordance with the Institutional Animal Care and Use requirements of Kindai University (KAPS-2020-024).

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. L. Li, L. J. Xu, G. Z. Ma, Y. M. Dong, Y. Peng, and P. G. Xiao, “The Large-Leaved Kudingcha (*Ilex Latifolia* Thunb and *Ilex Kudingcha* C.J. Tseng): A Traditional Chinese Tea With Plentiful Secondary Metabolites and Potential Biological Activities,” *Journal of Natural Medicines* 67, no. 3 (2013): 425–437, <https://doi.org/10.1007/s11418-013-0758-z>.
2. A. Y. Woo, J. M. Jiang, C. F. Chau, et al., “Inotropic and Chronotropic Actions of *Ilex Latifolia* Inhibition of Adenosine-5'-Triphosphatases as a Possible Mechanism,” *Life Sciences* 68, no. 11 (2001): 1259–1270, [https://doi.org/10.1016/S0024-3205\(00\)01024-9](https://doi.org/10.1016/S0024-3205(00)01024-9).
3. H. Wu, Y. L. Chen, Y. Yu, J. Zang, Y. Wu, and Z. He, “*Ilex Latifolia* Thunb Protects Mice From HFD-Induced Body Weight Gain,” *Scientific Reports* 7, no. 1 (2017): 14660, <https://doi.org/10.1038/s41598-017-15292-x>.

4. T. T. Zhang, C. Y. Zheng, T. Hu, J. G. Jiang, J. W. Zhao, and W. Zhu, "Polyphenols From *Ilex Latifolia* Thunb. (a Chinese Bitter Tea) Exert Anti-Atherosclerotic Activity Through Suppressing NF- κ B Activation and Phosphorylation of ERK1/2 in Macrophages," *MedChemComm* 9, no. 2 (2018): 254–263, <https://doi.org/10.1039/C7MD00477J>.
5. R. Y. Gan, D. Zhang, M. Wang, and H. Corke, "Health Benefits of Bioactive Compounds From the Genus *Ilex*, a Source of Traditional Caffeinated Beverages," *Nutrients* 10, no. 11 (2018): 1682, <https://doi.org/10.3390/nu10111682>.
6. M. A. Ouyang, Y. Q. Liu, H. Q. Wang, and C. R. Yang, "Triterpenoid Saponins From *Ilex Latifolia*," *Phytochemistry* 49, no. 8 (1998): 2483–2486, [https://doi.org/10.1016/S0031-9422\(98\)00164-2](https://doi.org/10.1016/S0031-9422(98)00164-2).
7. Z. Wang, M. N. Clifford, and P. Sharp, "Analysis of Chlorogenic Acids in Beverages Prepared From Chinese Health Foods and Investigation, In Vitro, of Effects on Glucose Absorption in Cultured Caco-2 Cells," *Food Chemistry* 108, no. 1 (2008): 272–369, <https://doi.org/10.1016/j.foodchem.2007.10.083>.
8. K. Nishimura, T. Miyase, and H. Noguchi, "Triterpenoid Saponins From *Ilex Kudinchia*," *Journal of Natural Products* 62, no. 8 (1999): 1128–1133, <https://doi.org/10.1021/np990128y>.
9. Y. Che, Q. Wang, R. Xiao, et al., "Kudinoside-D, a Triterpenoid Saponin Derived From *Ilex Kudingcha* Suppresses Adipogenesis Through Modulation of the AMPK Pathway in 3T3-L1 Adipocytes," *Fitoterapia* 125 (2018): 208–216, <https://doi.org/10.1016/j.fitote.2017.11.018>.
10. W. Zhang, J. Guo, H. Qiu, C. Wang, Q. Q. Chen, and B. Liu, "Quantitation of Kudinoside A, Kudinoside D and Kudinoside F in Human Plasma Using a High Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometric Method," *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 972 (2014): 1–5, <https://doi.org/10.1016/j.jchromb.2014.09.023>.
11. X. W. Jiang, J. P. Bai, Q. Zhang, et al., "Caffeoylquinic Acid Derivatives Protect SH-SY5Y Neuroblastoma Cells From Hydrogen Peroxide-Induced Injury Through Modulating Oxidative Status," *Cellular and Molecular Neurobiology* 37, no. 3 (2017): 499–509, <https://doi.org/10.1007/s10571-016-0387-7>.
12. J. K. In, J. K. Kim, J. S. Oh, and D. W. Seo, "5-Caffeoylquinic Acid Inhibits Invasion of Non-Small Cell Lung Cancer Cells Through the Inactivation of p70s6K and Akt Activity: Involvement of p53 in Differential Regulation of Signaling Pathways," *International Journal of Oncology* 48, no. 5 (2016): 1907–1912, <https://doi.org/10.3892/ijo.2016.3436>.
13. K. Azuma, K. Ippoushi, M. Nakayama, H. Ito, H. Higashio, and J. Terao, "Absorption of Chlorogenic Acid and Caffeic Acid in Rats After Oral Administration," *Journal of Agricultural and Food Chemistry* 48, no. 11 (2000): 5496–5500, <https://doi.org/10.1021/jf000483q>.
14. O. Muraoka, S. Ying, K. Yoshikai, et al., "Synthesis of a Nitrogen Analogue of Salacinol and Its Alpha-Glucosidase Inhibitory Activity," *Chemical and Pharmaceutical Bulletin* 49, no. 11 (2001): 1503–1505, <https://doi.org/10.1248/cpb.49.1503>.
15. D. Xu, Q. Wang, W. Zhang, et al., "Inhibitory Activities of Caffeoylquinic Acid Derivatives From *Ilex Kudingcha* C. J. Tseng on α -Glucosidase From *Saccharomyces Cerevisiae*," *Journal of Agricultural and Food Chemistry* 63, no. 14 (2015): 3694–3703, <https://doi.org/10.1021/acs.jafc.5b00420>.
16. Y. Sakamoto, N. Inoue, Y. Nakanishi, et al., "Hepatoprotective Principles From the Rhizomes of *Picrorhiza Kurroa*," *Biological and Pharmaceutical Bulletin* 46, no. 6 (2023): 848–855, <https://doi.org/10.1248/bpb.b23-00167>.
17. C. Fan, J. Deng, Y. Yang, et al., "Multi-Ingredients Determination and Fingerprint Analysis of Leaves From *Ilex Latifolia* Using Ultra-Performance Liquid Chromatography Coupled With Quadrupole Time-Of-Flight Mass Spectrometry," *Journal of Pharmaceutical and Biomedical Analysis* 84 (2013): 20–29, <https://doi.org/10.1016/j.jpba.2013.05.039>.
18. E. L. McConnell, A. W. Basit, and S. Murdan, "Measurement of Rat and Mouse Gastrointestinal pH, Fluid and Lymphoid Tissue, and Implications for In Vivo Experiments," *Journal of Pharmacy and Pharmacology* 60, no. 1 (2008): 63–70, <https://doi.org/10.1211/jpp.60.1.0008>.
19. S. Fan, Y. Zhang, N. Hu, et al., "Extract of Kuding Tea Prevents High-Fat Diet-Induced Metabolic Disorders in C57BL/6 Mice via Liver X Receptor (LXR) β Antagonism," *PLoS One* 7, no. 12 (2012): e51007, <https://doi.org/10.1371/journal.pone.0051007>.
20. A. Q. Yu, J. Le, W. T. Huang, et al., "The Effects of Acarbose on Non-Diabetic Overweight and Obese Patients: A Meta-Analysis," *Advances in Therapy* 38, no. 2 (2021): 1275–1289, <https://doi.org/10.1007/s12325-020-01602-9>.
21. D. E. Harrison, R. Strong, S. Alavez, et al., "Acarbose Improves Health and Lifespan in Aging HET3 Mice," *Aging Cell* 18, no. 2 (2019): e12898, <https://doi.org/10.1111/accel.12898>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.