Electronic supplementary information (ESI)

Antihyperglycemic drug screening: 4-Nitrophenol intermittent pulse amperometry as a convenient α-glucosidase inhibitor assay

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Experimental sections

1. Chemicals and Materials

Ammonium sulfate suspension of type V rice α-glucosidase (α-GA), the α-GA substrate 4-nitrophenyl-α-D-glucopyranoside (4-NP-G, purity ≥ 99%), 4-nitrophenol (4-NP, purity ≥ 99%), lyophilized powder of type I *S. cerevisiae* α-glucosidase and analytical grade chemicals for acetate and phosphate buffer preparation were Sigma-Aldrich (St. Louis, USA) products and bought via local distributor S. M. Chemicals Supplies Co. Ltd. (Bangkok, Thailand. Please be aware that 4-NP, the released α-GL substrate redox label, is toxic due to its phenolic nature; During their application of 4-NP in the glucosidase inhibition assay users must follow strict safety protocols to protect laboratory personnel and the environment. Proper disposal procedures, use of personal protective equipment, and containment measures are essential to mitigate hazards and keep users and environment safe. 0.1 M acetate buffers of pH 4.0 or a phosphate buffer of 7.0 were used as the medium of the electrochemical glucosidase inhibition assay and were prepared with the buffer salts and deionized (DI) water. The three plant-based antidiabetic supplement samples were purchased from local drug stores.

2. Instrumentation

Cyclic voltammetry (CV) and intermittent pulse amperometry (IPA) were performed in a common beaker-type 3-electrode electrochemical (EC) cell with a Reference 600+ potentiostat from Gamry Instruments, Warminster, USA. The working-electrode (WE) was a 3-mm boron-doped diamond (BDD) electrode, the counter-electrode was a coiled Pt-wire, and the reference was a standard Ag/AgCl/3 M KCl electrode. Prior to use the BDD-WE surface was cleaned through a polishing treatment on a soft polishing pad that was soaked with an aqueous suspension of 0.4 µm alumina powder. Thorough rinsing with DI water and final drying with a cotton swab prepared the WE for application. OriginLab 2021b, Microsoft Excel, Microsoft PowerPoint and Inkscape were used as software for electrochemical data analysis and the preparation of the graphical displays of results.

3. Electrochemical detection of α-GA-catalysed 4-NP release from 4-NP-G

The IPA mode of amperometry used here followed the procedure design that was recently introduced as an optional electroanalytical scheme for 4-NP detection. However, the parameter set for IPA execution had to be adapted for the measurement of 4-NP release induced by the action of either rice or yeast α-GA on the model substrate 4-NP-G. Based on the supplier's information the rice α-GA was assayed in 0.1 M acetate buffer, pH 4.0 while for the yeast α-GA a sodium phosphate buffer with pH adjustment to 7.0 was used. Appropriate settings for the resting and detection potentials were extracted from CV data as shown in Fig. S1. The detection potentials were chosen as +1.065 and +0.95 V vs. Ag/AgCl/3 M KCl for the rice and yeast inhibition assays respectively, as these were the potentials with the most intense anodic 4-NP oxidation current in the assay buffers of pH 4.0 (rice) and 7.0 (yeast). The choice of the resting potential was, on the other hand, 0.0 V vs. Ag/AgCl/3M KCl, as here in either case virtually no anodic 4-NP current response was observed. To obtain optimal IPA recordings, the length of the IPA detection pulse and of the IPA resting period between individual pulses was set with the rice and yeast glucosidases to 0.5 s (rice/yeast) and 299.5 s (rice) and 99.5 s (yeast), respectively.

4. Supplementary Figures, schemes and tables

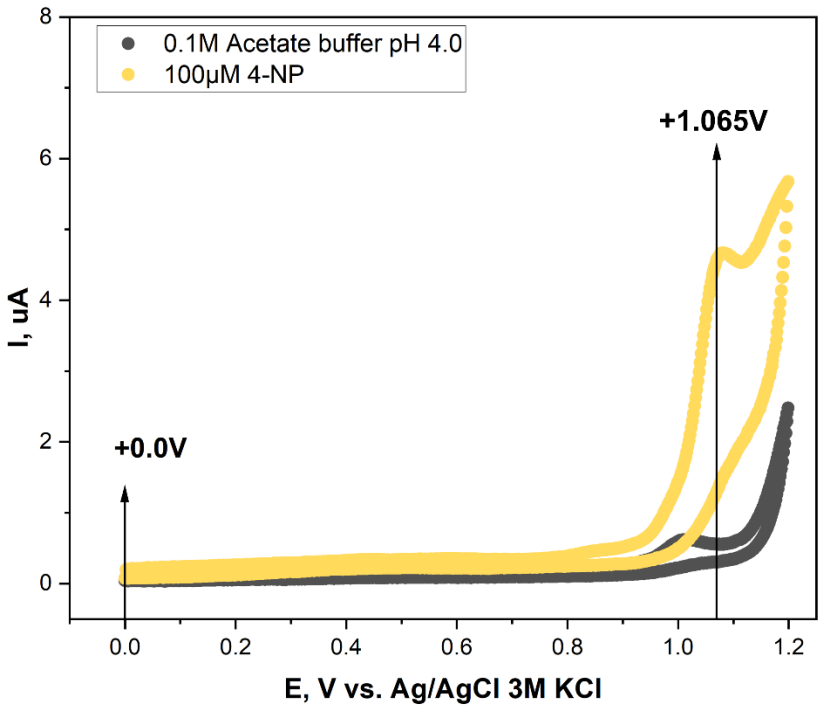

Fig. S1 Example of a cyclic voltammogram of 0.1 M acetate buffer pH 4.0 with (yellow) and without (black) 100 µM 4-NP. The scan speed was 100 mV s^{-1}. For the electrochemical glucosidase inhibition assay with IPA-based 4-NP readout the detection potential was the potential of the anodic 4-NP peak, namely + 1.065 V vs. Ag/AgCl/3 M KCl, while the IPA resting potential was 0 V vs. Ag/AgCl/3 M KCl.

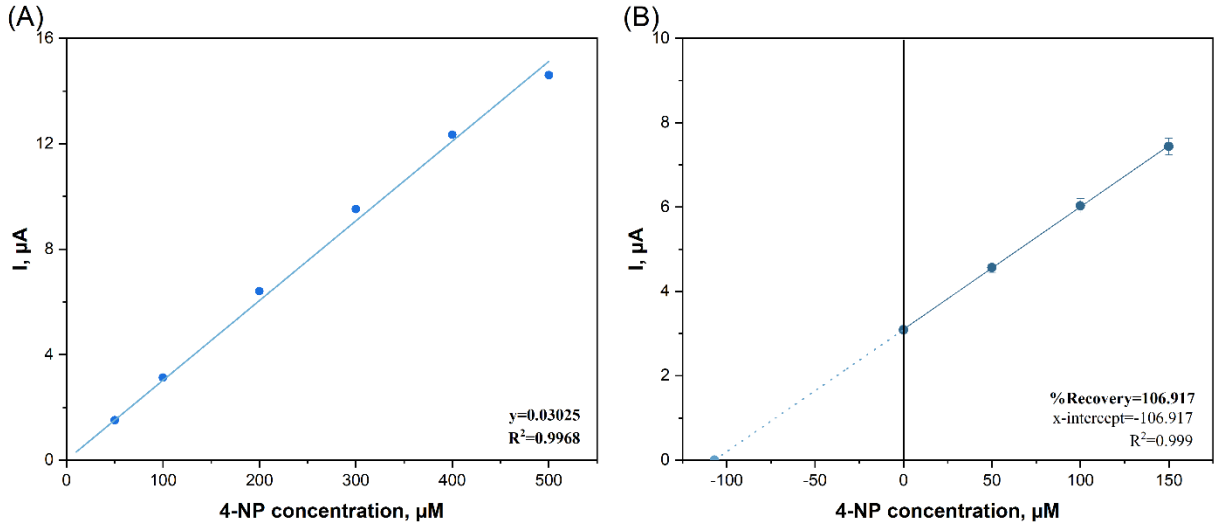

Fig. S2 (A) Calibration of the intermittent pulse amperometry (IPA) response for the electrochemical detection of 4-NP in 0.1 M acetate buffer pH 4.0, the assay buffer for the rice α-glucosidase inhibition assay. (B) The standard addition plot for the quantification of 100 µM 4-NP in 0.1 M sodium phosphate buffer, pH 7.0 by the intermittent pulse amperometry technique. A 3-mm boron-doped diamond (BDD) was the working electrode in the calibration and 4-NP quantification trials while a 3 Ag/AgCl/3M KCl probe and a coiled Pt wire served as the reference and counter-electrodes. For all required IPA-based current recordings the working electrode was held continually for 99.5 s at 0 V resting potential and 0.5 s at 0.95 V 4-NP detection potential.

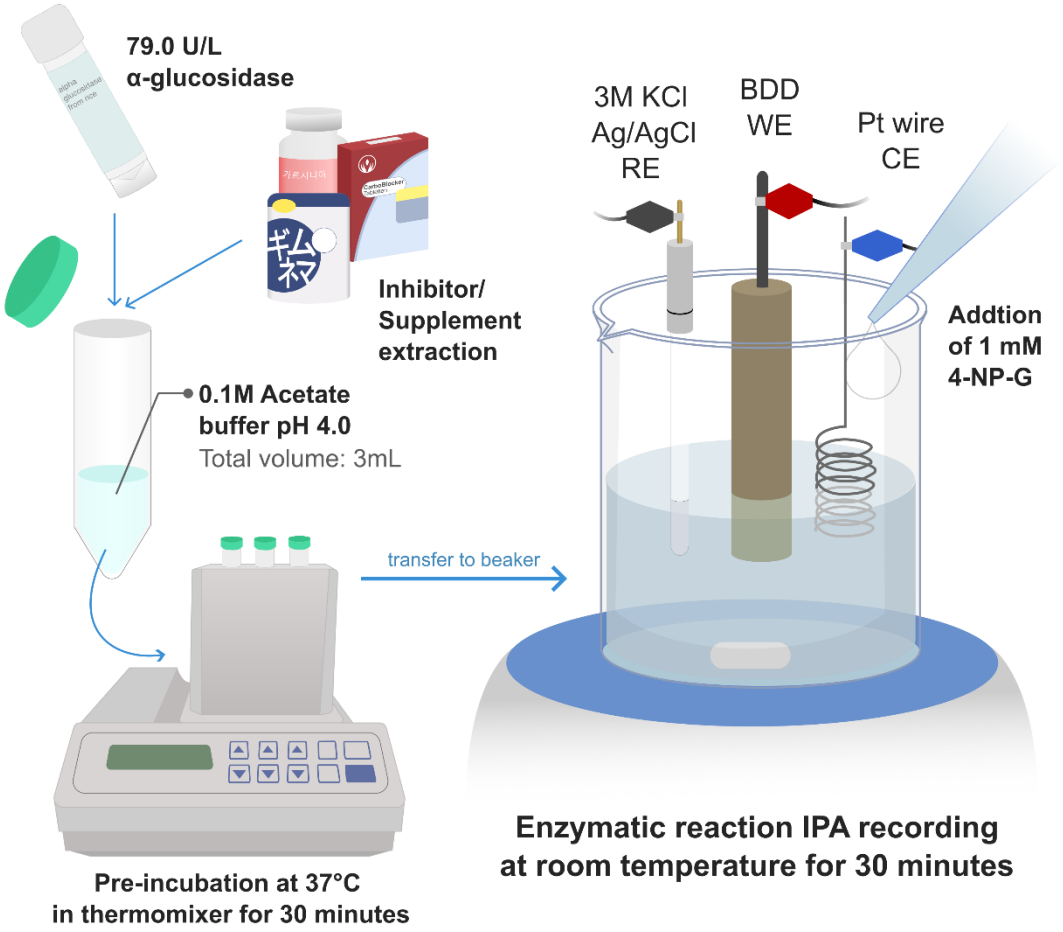

Fig. S3 Depiction of the procedure for the electrochemical α-glucosidase inhibition assay with intermittent pulse amperometry (IPA) used in the detection of enzymatically produced 4-NP. Rice or yeast α-glucosidase and an inhibitor (or supplement extract) were mixed in a plastic tube with 0.1 M acetate buffer, pH 4.0 (rice α-GA) or phosphate buffer 7.0 (yeast α-GA) and incubated at 37°C for 30 minutes. Then, the enzyme-inhibitor mixture was transferred gently to the beaker-type electrochemical cell with the boron-doped diamond working electrode, the Ag/AgCl/3 M KCl reference electrode and the coiled Pt wire CE. Enzymic activity was recorded with the IPA assay at room temperature. After 5 minutes of baseline recording in the gently stirred test solution, 4-NP-G was added to a final concentration of 1 mM and the recording was continued for another 30 minutes. IPA detection of 4-NP release from rice and yeast α-glucosidases action on 4-NP-G was carried out as described in Section 3.

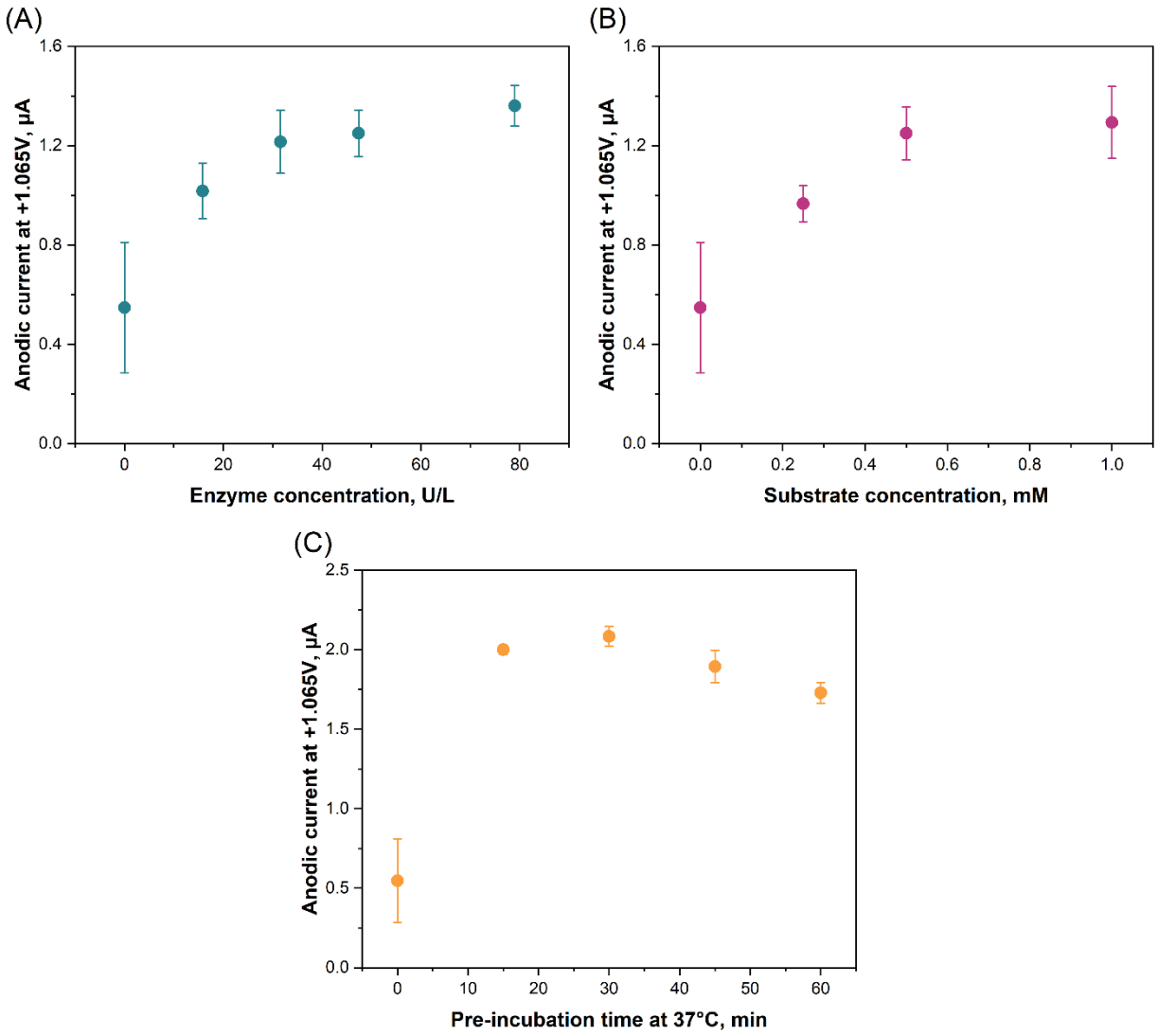

Fig. S4 Dependence of the anodic current in cyclic voltammograms (CVs) at detection potential (1.065 V vs. Ag/AgCl/3 M KCl) through the electrochemical cell with 0.1 M acetate buffer, pH 4.0 as functions of (A) the concentration of the rice α-glucosidase, (B) the concentration of the substrate 4-NP-G and (C) the time used for incubation of the glucosidase with the inhibitor acarbose prior to enzyme activity testing with α-GA inhibition assay as described in Section 3. Optimal conditions for the inhibition assay were 79.0 U/L of α-glucosidase, a substrate concentration of 1 mM and 30 min pre-incubation time.

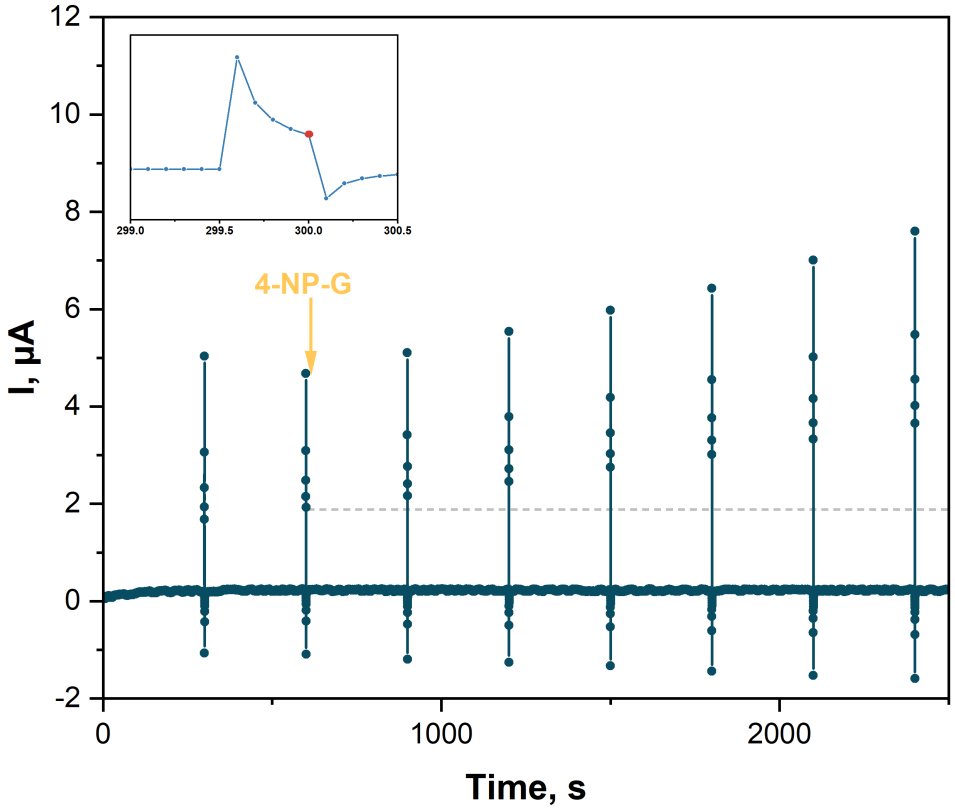

Fig. S5 An original IPA recording from rice α-glucosidase activity testing in 0.1M acetate buffer, pH 4.0 with 79.0 U/L enzyme, pre-incubation of the α-GA at 37°C for 30 minutes and 1 mM substrate (4-NP-G). 10 minutes after the start of IPA recording, 1 mM 4-NP-G was added to the assay medium in the electrochemical cell, while data acquisition continued for another 30 minutes (inset). The fifth current data point (red) within the 500 ms detection pulse was extracted and used for further data analysis.

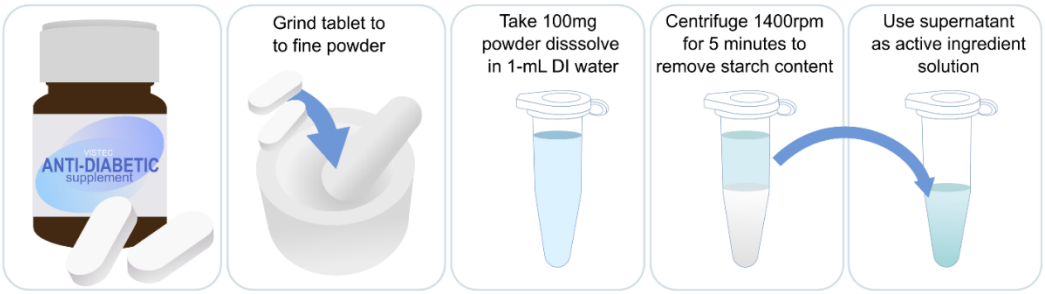

Fig. S6 Depiction of the procedure used for the preparation of aqueous solutions of the glucosaminidase inhibitor samples. Step 1: Crush and grind the supplement pill into fine powder. Step 2: Dissolve 100 mg of the freshly prepared supplement powder in 1 mL DI water and centrifuge the blend at 1400 rpm for 5 minutes to remove insoluble components. Step 3: Dilute the clean supplement extract with estimated active ingredients amount of 100 µg.

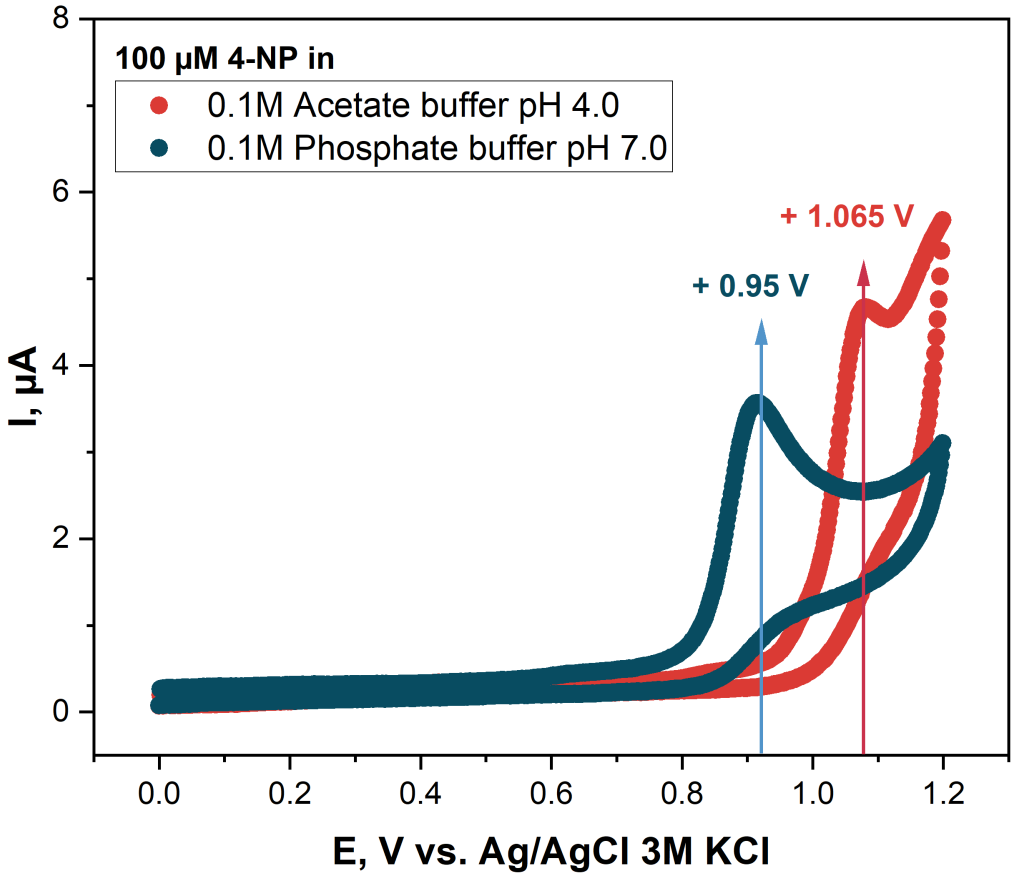

Fig. S7 Cyclic voltammograms of 4-NP 100 µM in 0.1M acetate buffer pH 4.0 (red) and 0.1 M phosphate buffer pH 7.0 (blue). The anodic oxidation peak responses that have been selected as a detecting potential for IPA assay are +1.065 V for acetate buffer and +0.95 V for phosphate buffer.

Table S1 List of commonly used glucosidase inhibitory assay methodologies.

Method	Target result	Ref.
In vivo screening		
Sucrose-induced diabetic-like fruit fly	Blood glucose	[1]
Diabetic mice with sample introduction via an oral uptake	Blood glucose Insulin tolerance test Oral glucose tolerance	[2]
Diabetic mice with sample injection	Blood glucose level Lipid profile Kidney function	[3]
In vitro screening		
Molecular docking	Molecule interaction on α-GL and inhibitor	[4]
Human colorectal adenocarcinoma cell (Caco-2) model	Glucose transportation	[5]
Personal glucose meter	Generated glucose product from α-GL hydrolyzation on maltose	[6]
Colorimetric assay	4-NP product from α-GL hydrolyzation on 4-NP-G	[7]
Electrochemical-based detection with thymol-lactic acid-MWCNTs/GCE	4-NP product from α-GL hydrolyzation on 4-NP-G	[8]
Electrochemical-based detection with magnetic nanoparticles/ pyrene boric acid/ graphite electrode	4-aminophenol from α-GL hydrolyzation on 4-aminophenyl-α-d-glucopyranoside	[9]
Electrochemical-based detection with α-GL/MWCNTs/SPCE	4-NP product from α-GL hydrolyzation on 4-NP-G	[10]
Magnetic ligand fishing	Chromatogram of binding magnetic/α-GL and inhibitor sample	[11]
Electrochemical-based IPA detection with bare BDD electrode	4-NP product from α-GL hydrolyzation on 4-NP-G	This work

[1] https://doi.org/10.3390/foods13040559

[2] https://doi.org/10.3390/nu14204413

[3] https://doi.org/10.1016/j.jep.2023.116241

[4] https://doi.org/10.1186/s12906-022-03706-x

[5] https://doi.org/10.1016/j.foodres.2023.113268

[6] **https://doi.org/10.3390/molecules26154638**

[7] 10.4103/0253-7613.161270

[8] https://doi.org/10.1016/j.talanta.2023.125313

[9] https://doi.org/10.1016/j.bios.2015.07.023

[10] 10.1186/s11671-016-1292-1

[11] https://doi.org/10.1016/j.talanta.2019.03.047