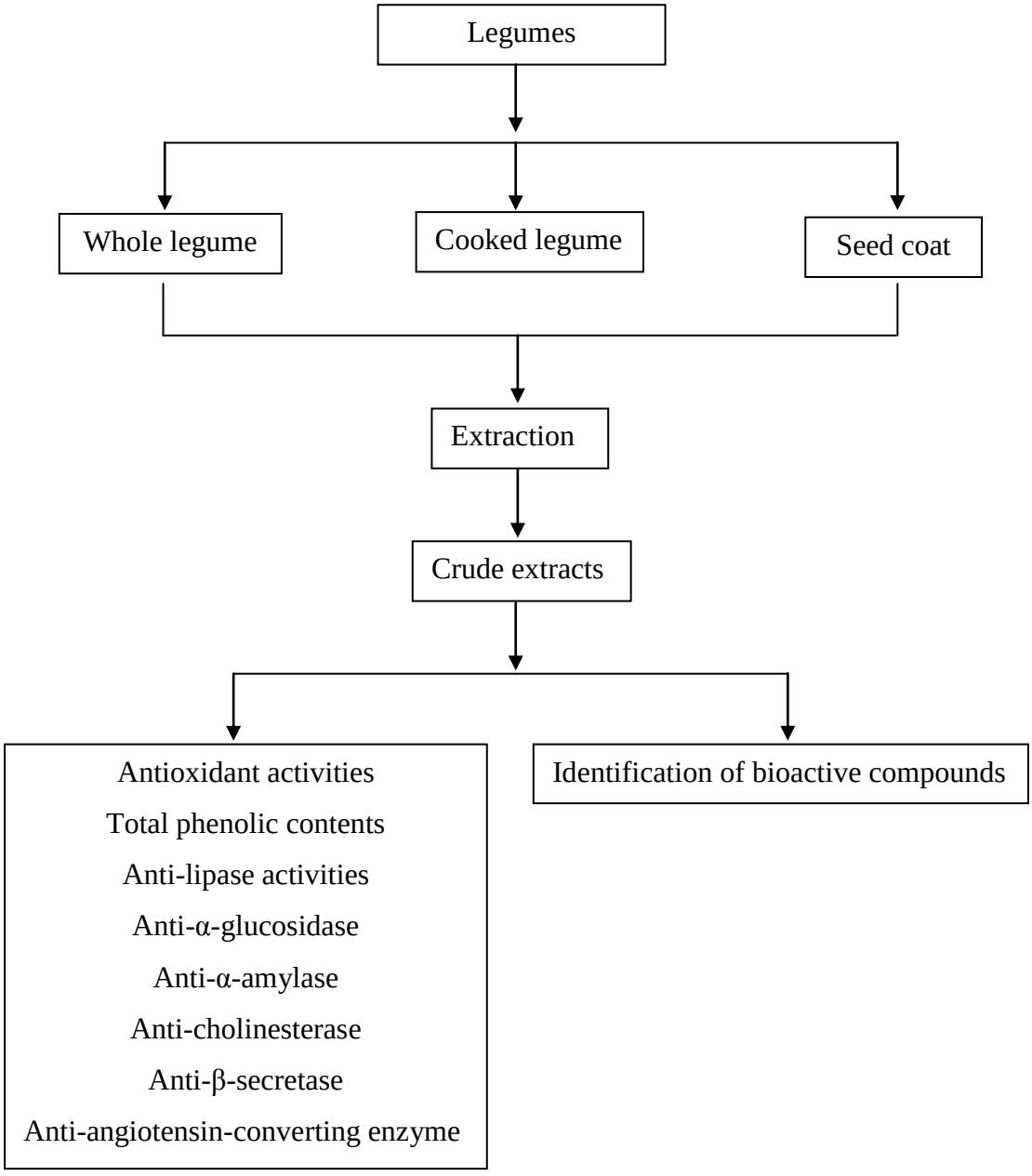
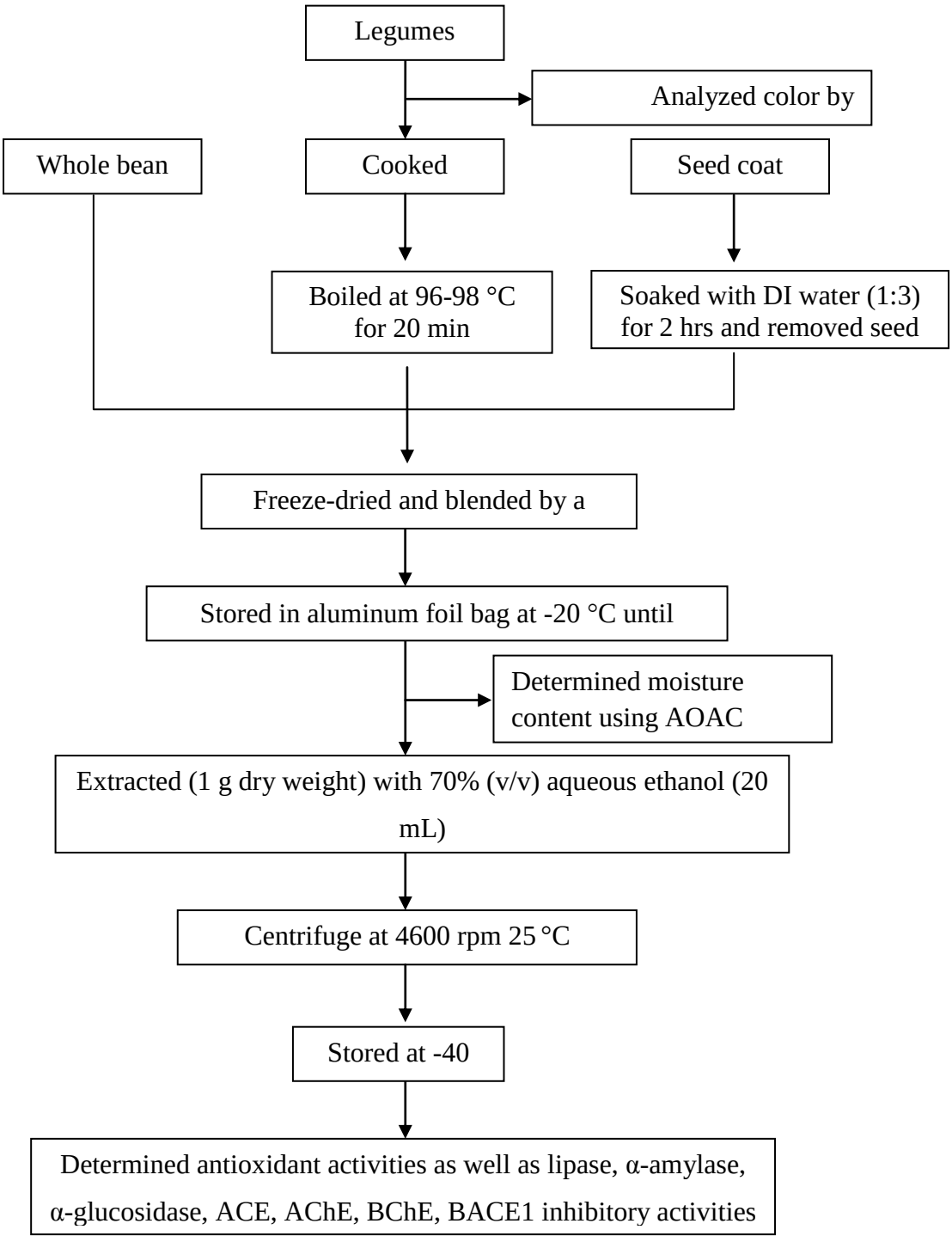
APPENDICES

APPENDIX A
OVERVIEW OF THIS STUDY



APPENDIX B
SAMPLE PREPARATION AND EXTRACTION



APPENDIX C
REAGENT PREPARATION

C1. Reagent preparations for flavonoid and phenolic acid analyses

Reagents

1. Methanol, pa
2. Hydrochloric acid (HCl), pa
3. Ascorbic acid, pa
4. Water, HPLC grade
5. Trifluoroacetic acid (TFA)
6. Acetronitrile, HPLC grade
7. Methanol, HPLC grade

Reagent preparation

1. 62.5% (v/v) methanol

Dilute 625 mL of methanol to 1000 mL with deionized water. Keep the solution at ambient temperature.

2. 6 M HCl

Add 200 mL of deionized water into a 500-mL volumetric flask and add 26 mL of HCl. Then, adjust the volume to 500 mL with deionized water and mix it. Keep the solution in ambient temperature.

3. 5% (w/w) TFA in methanol

Mix 210 μL of TFA and 1000 mL of methanol.

4. 0.5% (w/w) TFA

Mix 210 μL of TFA and 1000 mL of water.

5. 0.5% (w/w) TFA in acetronitrile

Mix 210 μL of TFA and 1000 mL of acetronitrile.

C2. Reagent preparations for Folin–Ciocalteu assay

Reagents

1. Folin-Ciocalteu reagent
2. Sodium bicarbonate (Na_2CO_3)
3. Gallic acid monohydrate

Reagent preparations

1. 10% (v/v) Folin-Ciocalteu reagent

Dilute 500 µL of Folin-Ciocalteu reagent (stock solution) to 5 mL with deionized water. Keep the reagent at ambient temperature.

2. 7.5% (w/v) Na_2CO_3 solution

Weight 7.5 g of Na_2CO_3 in a 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water and mix well. Keep the solution at ambient temperature.

3. 1000 µg/ml gallic acid solution (stock solution)

Weight 100 mg of gallic acid monohydrate in a 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water. Keep the solution in a freezer.

4. 200 µg/ml gallic acid solution (working solution for serial dilution)

Dilute 80 µL of 1000 µg/mL gallic acid solution (stock solution) to 400 µL with deionized water. After that, dilute 200 µg/mL gallic acid solution by serial dilution to 100, 80, 60, 40, 20 and 10 µg/mL, respectively. Keep the solution in an ice bath before using.

C3. Reagent preparations for antioxidant activity assays

DPPH radical scavenging assay

Reagents

1. Absolute ethanol
2. 2,2-diphenyl-1-picrylhydrazyl (DPPH)
3. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparation

1. 95% (v/v) aqueous ethanol

Dilute 950 mL of absolute ethanol to 1000 mL with deionized water. Keep the solution at ambient temperature.

2. 150 µM DPPH in 95% (v/v) aqueous ethanol

Weight 0.00148 g of DPPH into a 25-mL volumetric flask. Then, adjust the volume to 25 mL with 95% (v/v) aqueous ethanol and mix well. Keep the solution in an ice bath before using.

3. 8 mM trolox solution (stock solution)

Weigh 0.20 g of trolox into a 100-mL volumetric flask. Then, adjust the volume to 100 mL with 95% (v/v) aqueous ethanol and mix well. Keep the solution in a freezer.

4. 0.64 mM trolox solution (working solution for serial dilution)

Dilute 20 µL of 8 mM trolox solution (stock solution) to 250 µL with 95% (v/v) aqueous ethanol. After that, dilute 0.64 mM trolox solution by serial dilution to be 0.32, 0.16, 0.08, 0.04, 0.02 and 0.01 mM, respectively. Keep the solution in an ice bath before using.

Ferric reducing antioxidant power (FRAP) assay

Reagents

1. Glacial acetic acid
2. Hydrochloric acid (HCl)
3. 2,4,6-tripyridyl-s-triazine (TPTZ)
4. Sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$)
5. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)
6. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparation

1. 300 mM acetate buffer (pH 3.6)

Weight 3.1 g of sodium acetate trihydrate into a 1-L volumetric flask and add 16 mL of glacial acetic acid. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 10 mM TPTZ solution in 40 mM HCl

Weight 0.312 g of TPTZ into 100-mL volumetric flask. Then, adjust the volume to 100 mL with 40 mM HCl (0.4 mL of conc. HCl and 99.6 mL of deionized water) and mix well. Keep the solution in a refrigerator.

3. 20 mM $FeCl_3$•$6H_2O$ solution

Weight 0.5406 g of $FeCl_3$•$6H_2O$ into 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water and mix well. Keep the solution in a refrigerator.

4. FRAP reagent

Mixing the reagent of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM $FeCl_3$•$6H_2O$ solution in ratio of 10:1:1 respectively and warm in water bath at 37 °C before using.

5. 250 µM trolox solution (working solution for serial dilution)

Dilute 60 µL of 1000 µM trolox solution (stock solution, from ORAC assay) to 240 µL with deionized water. After that, dilute 250 µM trolox solution by serial dilution to be 125, 62.5 31.25, 15.625 and 7.8125 µM, respectively. Keep the solution in an ice bath before using.

Oxygen radical absorbance capacity (ORAC) assay

Reagents

1. Di-potassium hydrogen phosphate (K_2HPO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH)
4. Fluorescein sodium salt
5. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparation

1. ORAC buffer (stock solution)

- Prepare 0.75 M KH_2PO_4 solution (stock solution)

Weight 102.07 g of KH_2PO_4 into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

- Prepare 0.75 M K_2HPO_4 solution (stock solution)

Weight 130.64 g of K_2HPO_4 into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.
For ORAC buffer (stock solution) preparation, mix 351 mL of 0.75 M KH_2PO_4 solution (stock solution) and 603 mL of 0.75 M K_2HPO_4 solution (stock solution), this yields 954 mL of the ORAC buffer (stock solution). Keep the solution in a refrigerator.

2. ORAC buffer (working solution)

Take 100 mL of ORAC buffer (stock solution) into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well. Adjusted pH to 7.2 with a few drops of a NaOH concentrated solution. Keep the solution in a refrigerator.

3. 153 mM AAPH

Weigh 0.414 g of AAPH into a 10-mL volumetric flask. Then, adjust the volume to 10 mL with ORAC buffer (working solution) and mix well. Keep the solution in an ice bath before using.

4. 8.37 x 10–4 mM fluorescein solution (concentrated solution)

Weight 0.045 g of fluorescein sodium salt into a-100 mL volumetric flask. Then, adjust the volume to 100 mL with ORAC buffer (working solution). Keep the solution in a freezer.

5. 4.19 µM fluorescein solution (stock solution)

Dilute 100 µL of 8.37 x 10–4 mM fluorescein solution (concentrated solution) to 20 mL with ORAC buffer (working solution) and mix well. Keep the solution in a freezer.

6. Fluorescein solution (working solution)

Dilute 244 µL of 4.19 µM fluorescein solution (stock solution) to 12.5 mL with ORAC buffer (working solution) and mix well. Warm the solution in water bath at 37 °C before using.

7. 1000 µM trolox solution (stock solution)

Weigh 0.025 g of trolox into a 100-mL volumetric flask. Then, adjust the volume to 100 mL with ORAC buffer (working solution) and mix well. Keep the solution in a freezer.

C4. Reagent Preparations for Lipase Inhibitory Assays

Reagents

1. Absolute methanol
2. Tris(hydroxymethyl) aminomethane ($NH_2C(CH_2OH)_3$)
3. Di–potassium hydrogen phosphate (K_2HPO_4)
4. Potassium dihydrogen phosphate (KH_2PO_4)
5. Potassium Chloride (KCl)
6. Triton X–100
7. Ethylenediaminetetraacetic acid (EDTA)
8. 5,5'– dithiobis (2–nitro benzoic acid) (DTNB)
9. 2,3–dimercapto–1–propanol tributyrate (DMPTB)
10. Bovine serum albumin (BSA)
11. *Candida rugosa* lipase (Type 8, ≥700 unit/mg)

Reagent preparation

1. 50 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.2 (Assay buffer)

Weight 6.057 g of $NH_2C(CH_2OH)_3$, 0.7455 g of KCl and 0.3722 g of EDTA into 1 L beaker. Then, add deionized water about 500 mL, mix well and adjust pH to 7.2. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 10% (v/v) Triton X–100 in 50 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.2 (Substrate buffer)

Take 90 mL of assay buffer into 150 mL beaker. Then, add 10 mL of Triton X–100 and mix well. Keep the solution in a refrigerator.

3. 50 mM Tris, 10 mM KCl, 1 mM EDTA with 0.1% BSA, pH 8.0 (Enzyme buffer)

Take 250 mL of assay buffer into 250 mL beaker. Then, add 0.5 g of BSA and mix well. Adjust pH to 8.0 with a few drops of a NaOH concentrated solution. Keep the solution in a refrigerator.

4. 50 mM potassium phosphate buffer (KPB), pH 7.4

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water about 500 mL and mix well. Then, adjust pH to 7.4 with a few drops of a NaOH concentrated

solution. After that, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

5. 200 mM DTNB stock solution in Methanol

Weight 0.7927 g of DTNB into 10 mL volumetric flask and adjust the volume to 10 mL with absolute methanol.

6. 16 mM DTNB in 50 mM KPB

Take 920 µL of 50 mM KPB into 1.5 mL tube. Then, add 80 µL of 200 mM DTNB stock solution and mix well.

7. 10 mM DMPTB stock solution

Take 10 mL of substrate buffer into 15 mL tube and remove 33.45 µL. Then, add 33.45 µL of DMPTB solution and mix well. Keep the solution in a refrigerator.

8. 0.2 mM DMPTB

Take 10 mL of assay buffer into 15 mL tube and remove 200 µL. Then, add 200 µL of 10 mM DMPTB stock solution, mix well and store in an ice bath before used.

9. 1 mg/mL *Candida rugosa* lipase stock solution

Weight 1 mg of *Candida rugosa* lipase into 1.5 mL tube. Then, adjust the volume to 1 mL with enzyme buffer. Mix well and keep the solution in a refrigerator.

10. 0.002 mg/mL *Candida rugosa* lipase

Take 10 mL of enzyme buffer into 15 mL tube and remove 20 µL. Then, add 20 µL of 1 mg/mL *Candida rugosa* lipase stock solution, mix well and store in an ice bath before used.

C5. Reagent Preparations for α-amylase Inhibitory Assays

Reagents

1. Di–potassium hydrogen phosphate (K_2HPO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. Potassium Chloride (KCl)
4. *p*-Nitrophenyl-α-D-maltopentaoside (PNPG-5)
5. Porcine pancreatic α-amylase (Type 7, ≥10 unit/mg)

Reagent preparation

1. 50 mM potassium phosphate buffer KPB, pH 7

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water about 500 mL and mix well. Then, adjust pH to 7 with a few drops of a NaOH concentrated solution. After that, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 50 mM KPB, 200 mM KCl (Buffer)

Weight 1.491 g of KCl into 100 mL beaker. Then, add potassium phosphate buffer (KPB) about 100 mL, mix well. Keep the solution in a refrigerator.

3. 25 mM PNPG-5 stock solution

Take 1.053 mL of buffer into stock PNPG-5 bottle, mix well and keep the solution in a refrigerator.

4. 5 mM PNPG-5

Take 4 mL of buffer into 15 mL tube. Then, add 1 mL of PNPG-5 stock solution, mix well and store in an ice bath before used.

5. 40 mg/mL porcine pancreatic α-amylase

Weight 0.4 g of porcine pancreatic α-amylase into 50 mL tube and dissolve enzyme overnight. Then, centrifuge at 4,500 rpm for 10 minutes, filtrate and store in an ice bath before used.

C6. Reagent Preparations for α-glucosidase Inhibitory Assays

Reagents

1. Di–potassium hydrogen phosphate (K_2HPO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. *p*-nitrophenyl-α-D-glucopyranaide (PNPG)
4. *Saccharomyces cerevisiae* α-glucosidase (Type 1, ≥10 unit/mg)

Reagent preparation

1. 50 mM potassium phosphate buffer KPB, pH 7

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water about 500 mL and mix well. Then, adjust pH to 7 with a few drops of a NaOH concentrated

solution. After that, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 2 mM PNPG

Weight 0.006 g of PNPG into 15 mL tube. Then, add 10 mL of potassium phosphate buffer (KPB) mix well and store in an ice bath before used.

3. 100 U/mL *Saccharomyces cerevisiae* α-glucosidase stock solution

Take 1 mL of KPB, pH 7 into stock enzyme bottle mix well. Keep the solution in a refrigerator (-20 °C)

4. 0.1 U/mL *Saccharomyces cerevisiae* α-glucosidase

Take 9.99 mL of enzyme buffer into 15 mL tube. Then, add 0.01 mL of 0.01 U/mL *Saccharomyces cerevisiae* α-glucosidase stock solution, mix well and store in an ice bath before used.

C7. Reagent Preparations for ACE Inhibitory Assays

Reagents

1. Di–potassium hydrogen phosphate (K_2HPO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. Sodium hydroxide (NaOH)
4. Sodium Chloride (NaCl)
5. Hydrochloric acid (HCL)
6. *o*-Phthaldialdehyde
7. Hippuryl-histidyl-leucine (HHL)
8. Rabbit lung angiotensin-converting enzyme (≥2 unit/mg)

Reagent preparation

1. 100 mM potassium phosphate buffer KPB, pH 8.3

Take 47 mL of 1 M K_2HPO_4 stock solution and 3 mL of 1 M KH_2PO_4 stock solution into 500 mL beaker. Then, add deionized water (400 mL) and mix well. Adjust pH to 8.3 with a few drops of a NaOH concentrated solution. After that, adjust the volume to 500 mL with deionized water and mix well. Keep the solution in a refrigerator.

2. 0.025 M NaOH

Weight 0.1 g of NaOH into 100 mL beaker and adjust volume to 100 mL with deionized water.

3. 0.28 M NaOH

Weight 5.6 g of NaOH into 500 mL beaker and adjust volume to 500 mL with deionized water.

4. 3 M NaCl

Weight 17.53 g of NaCl into 100 mL beaker and adjust volume to 100 mL with deionized water.

5. 3 M HCl

Take 24.87 mL of 12.06 M HCl into 100 volumetric flask and adjust volume to 100 mL with deionized water.

6. 20 mg/mL *o*-phthaldialdehyde

Weight 40 mg of *o*-phthaldialdehyde into 2 mL tube. Then, add 2 mL of absolute methanol and mix well.

7. 3 mM Hippuryl-histidyl-leucine (HHL)

Weight 25.77 mg of HHL (429.27 g/mol) into 50 mL tube and add 4.165 mL of 0.025 NaOH. Then, 13.835 mL of 100 mM KPB, pH 8.3 and add 2 mL of 3 mM NaCl. Mix well and keep the solution in a refrigerator.

8. 1 U/mL ACE stock solution

Take 1 mL of KPB into stock enzyme bottle mix well. Keep the solution in a refrigerator (-20 °C)

9. 0.5 U/mL ACE

Take 0.5 mL of KPB, pH 8.3 into 1 mL tube. Then, add 0.5 mL of 1 U/mL ACE stock solution, mix well and store in an ice bath before used.

C8. Reagent preparations for cholinesterase inhibitory assays

AChE inhibitory assays

Reagents

1. Absolute methanol

2. Di-potassium hydrogen phosphate (K2HPO4)

3. Potassium dihydrogen phosphate (KH2PO4)

4. 5-5'-Dithiobis(2-nitrobenzoic acid) (DTNB)

5. Acetylthiocholine (ATCh)

6. *Electrophorus electricus* acetylcholinesterase (AChE)

Reagent preparation

1. 1 M K2HPO4 solution (stock solution)

Weight 228.23 g of K_2HPO_4 into 1-L beaker. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 1 M KH2PO4 solution (stock solution)

Weight 136.09 g of K_2HPO_4 into 1-L beaker. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

3. 50 mM potassium phosphate buffer (KPB), pH 7.0

Take 30.7 mL of 1 M K_2HPO_4 solution (stock solution) into 1 L volumetric flask and add 19.2 mL of 1 M KH_2PO_4 solution (stock solution) and then add deionized water (500 mL) and mix well. Then, adjust pH to 7.0 with a few drops of a NaOH concentrated solution. After that, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

4. 200 mM DTNB solution in methanol (stock solution)

Weight 0.7927 g of DTNB into 10-mL volumetric flask and adjust the volume to 10 mL with absolute methanol. Keep the solution at ambient temperature.

5. 16 mM DTNB solution in 50 mM KPB pH 7.0 (working solution)

Take 966 µL of 50 mM KPB pH7.0 into 1.5-mL tube. Then, add 84 µL of 200 mM DTNB solution (stock solution) and mix well.

6. 10 mM ACTh solution (stock solution)

Weight 28.918 mg of ACTh into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20 °C. (Molecular weight of ACTh = 289.18 g/mol)

7. 0.32 mM ACTh solution (working solution)

Dilute 184 µL of 10 mM ACTh solution (stock solution) to 5750 µL with 50 mM KPB pH 7.0. Keep the solution in an ice bath before using.

8. 0.01 mg/mL AChE solution (stock solution)

Weight 0.1 mg of AChE into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20 °C.

9. 0.0001 mg/mL AChE solution (working solution)

Dilute 82 µL of 0.01 mg/mL AChE solution (stock solution) to 8200 µL with 50 mM KPB pH 7.0 (working solution). Keep the solution in an ice bath before using.

BChE inhibitory assays

Reagents

1. Absolute methanol
2. Di-potassium hydrogen phosphate (K_2HPO_4)
3. Potassium dihydrogen phosphate (KH_2PO_4)
4. 5-5'-Dithiobis(2-nitrobenzoic acid) (DTNB)
5. Magnesium chloride ($MgCl_2$)
6. Butyrylthiocholine chloride (BTCh)
7. Equine serum butyrylcholinesterase (BChE)

Reagent preparation

1. 50 mM potassium phosphate buffer (KPB) pH 7.0 with 1mM $MgCl_2$

Weight 4.7605 mg of $MgCl_2$ and adjust the volume to 50 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution in a refrigerator. (Molecular weight of $MgCl_2$ = 95.21 g/mol)

2. 16 mM DTNB solution in 50 mM KPB pH 7.0 (working solution)

Take 966 µL of 50 mM KPB pH 7.0 into 1.5-mL tube. Then, add 84 µL of 200 mM DTNB solution (stock solution) and mix well.

3. 10 mM BCTh solution (stock solution)

Weight 22.578 mg of BCTh into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20 °C. (Molecular weight = 225.78 g/mol)

4. 0.4 mM BCTh solution (working solution)

Dilute 232 µL of 10 mM BCTh solution (stock solution) to 5800 µL with 50 mM KPB pH 7.0. Keep the solution in an ice bath before using.

5. 0.1 mg/mL BChE solution (stock solution)

Weight 1 mg of BChE into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 with 1mM $MgCl_2$. Mix well and keep the solution at -20 °C.

6. 0.0005 mg/mL BChE solution (working solution)

Dilute 41 µL of 0.1 mg/mL BChE solution (stock solution) to 8200 µL with 50 mM KPB pH 7.0 with 1mM $MgCl_2$. Keep the solution in an ice bath before using.

C9. Reagent preparations for BACE1 inhibitory assays

Reagents

Using β-Secretase (BACE1) Activity Detection Kit (Fluorescent)

1. 0.5 mg BACE1 substrate
2. Dimethyl sulfoxide (DMSO)
3. 3 units/µL BACE1 enzyme
4. Fluorescent assay buffer

Reagent preparation

1. 500 µM BACE1 substrate solution (stock solution)

Add 500 µL of DMSO into 0.5 mg BACE1 substrate. Keep the solution at -20 °C.

2. 50 µM BACE1 substrate solution (working solution)

Dilute 200 µL of 500 µM BACE1 substrate solution (stock solution) to 2000 µL with fluorescent assay buffer. Keep the solution in an ice bath before using.

3. 0.3 units/µL BACE1 enzyme

Dilute 20 µL of 3 units/µL BACE1 enzyme to 200 µL with fluorescent assay buffer. Keep the solution in an ice bath before using.

APPENDIX D
REACTION OF TPCS AND ANTIOXIDANT ACTIVITY ASSAYS

D1. TPCs by Folin-Ciocalteu assay

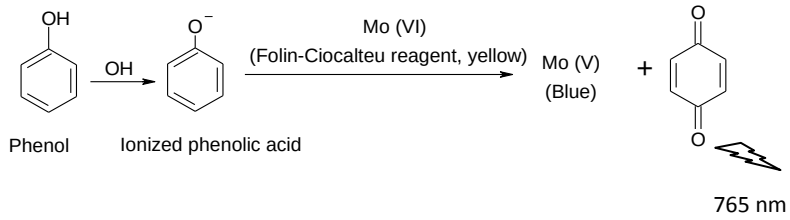


Figure D1.1 Reaction for determination of TPCs by Folin-Ciocalteu assay. The yellow Mo(VI) in phosphomolybdic–phosphotungstic acid reagent is reduced to blue Mo(V) complex by ionized phenolic group. The changed color is measured in a spectrophotometer at 765 nm.

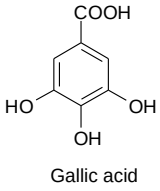


Figure D1.2 Chemical structure of gallic acid. It is a type of phenolic acid, which is a standard used in Folin-Ciocalteu Assay.

D2. Antioxidant activity

DPPH - radical scavenging assay

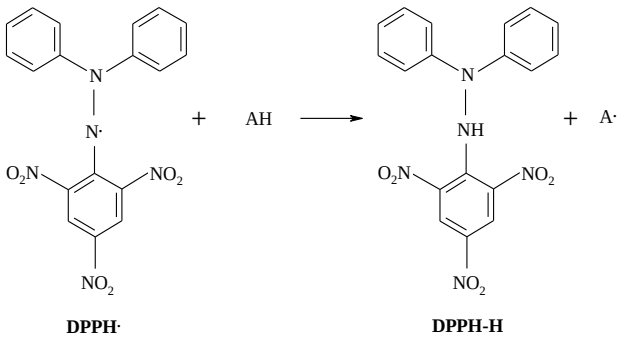


Figure D2.1 Reaction for determination of antioxidant activity by DPPH assay. The deep purple DPPH• radical reacts with antioxidants (AH) to produce a yellow DPPH–H compound. Antioxidant activity is measured with the loss of deep purple by absorbance at 520 nm.

Ferric reducing antioxidant power (FRAP) assay

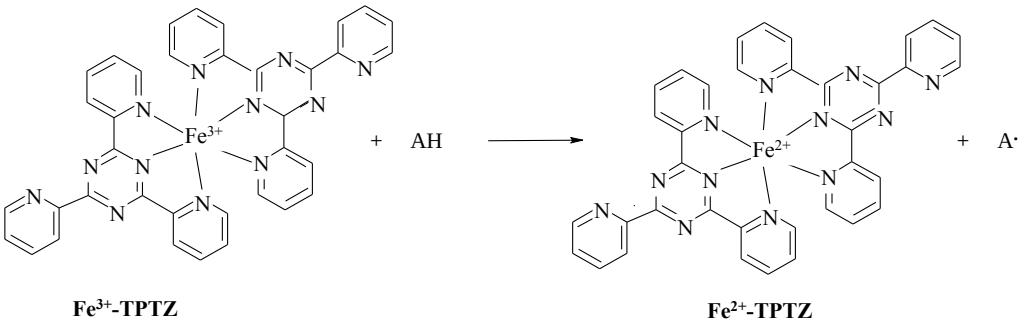


Figure D2.2 Reaction for determination of antioxidant activity by FRAP assay. The assay is measured by the reduction of brown Fe^{3+}–TPTZ to indigo Fe^{2+}–TPTZ by antioxidant (AH). The indigo color as indicator of product formation can be measured at a wavelength of 595 nm.

Oxygen radical absorbance capacity (ORAC) assay

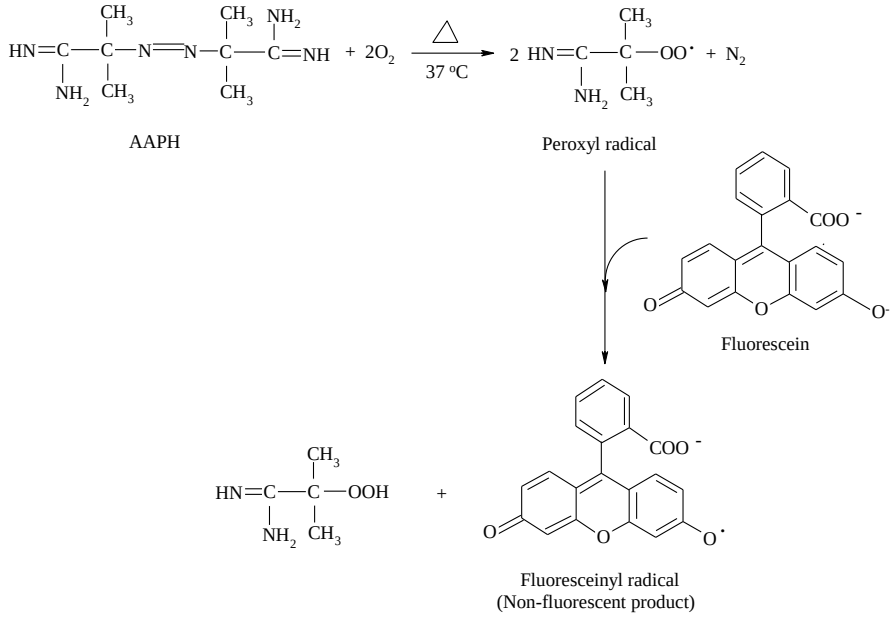


Figure D2.3 Reaction for determination of antioxidant activity by ORAC assay. AAPH is oxidized to generate nitrogen gas (N2) and two peroxyl radicals. The peroxyl radical then reacts with the fluorescein to form fluoresceinyl radical, a non–fluorescent product. The loss of fluorescein is measured with an excitation wavelength of 485 nm and emission wavelength of 528 nm.

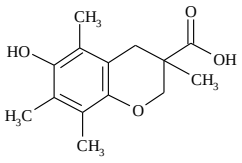


Figure D2.4 Chemical structures of trolox, a water soluble vitamin E analogue, which is a standard used in antioxidant assay.

APPENDIX E
ENZYME REACTION OF LIPASE ASSAY

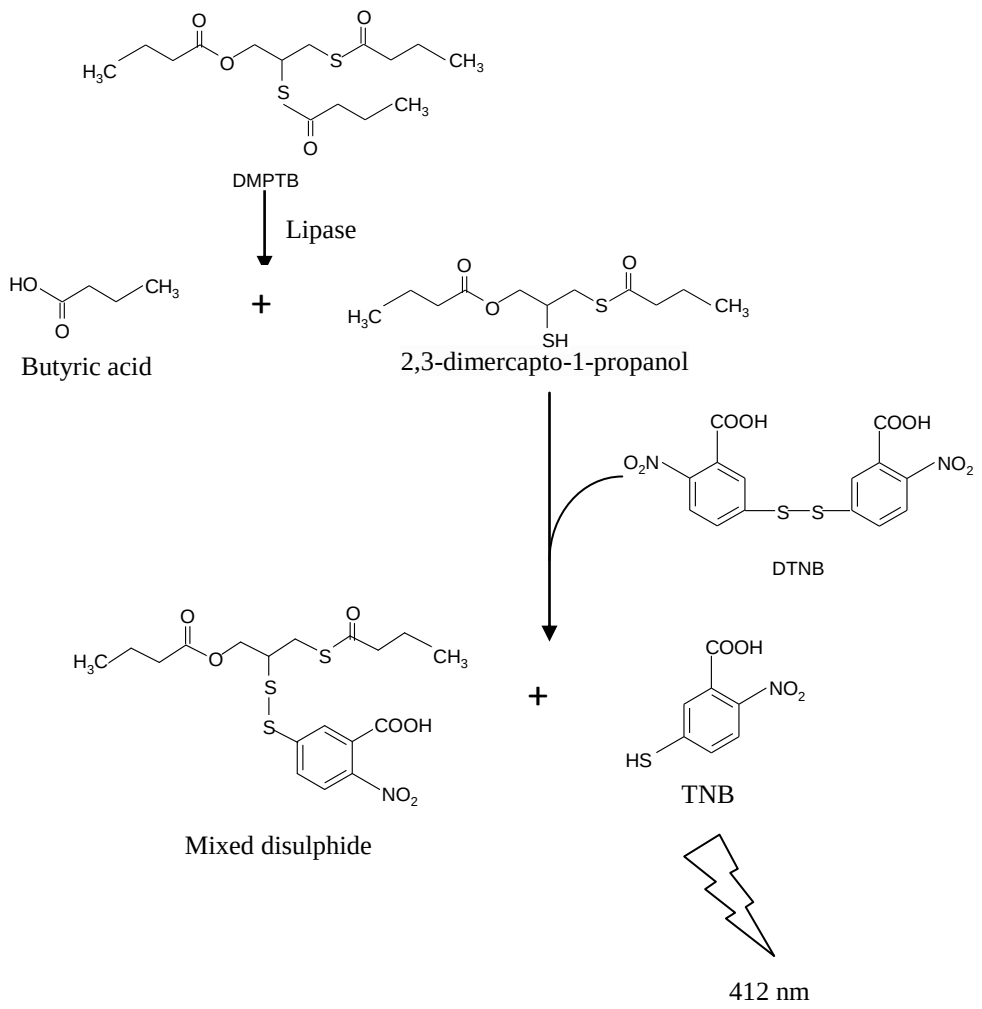


Figure E Lipase hydrolyzes 2,3–dimercapto–1–propanol tributyrate (DMPTB) into butyric acid and 2,3-dimercapto-1-propanol. Then, 2,3-dimercapto-1-propanol reacts with 5,5'– dithiobis (2–nitro benzoic acid) (DTNB), releasing mixed disulphide and 2-nitro-5-mercaptobenzoic acid (TNB). TNB has yellow color and can be absorbed at 412 nm.

APPENDIX F
ENZYME REACTION OF α-AMYLASE ASSAY

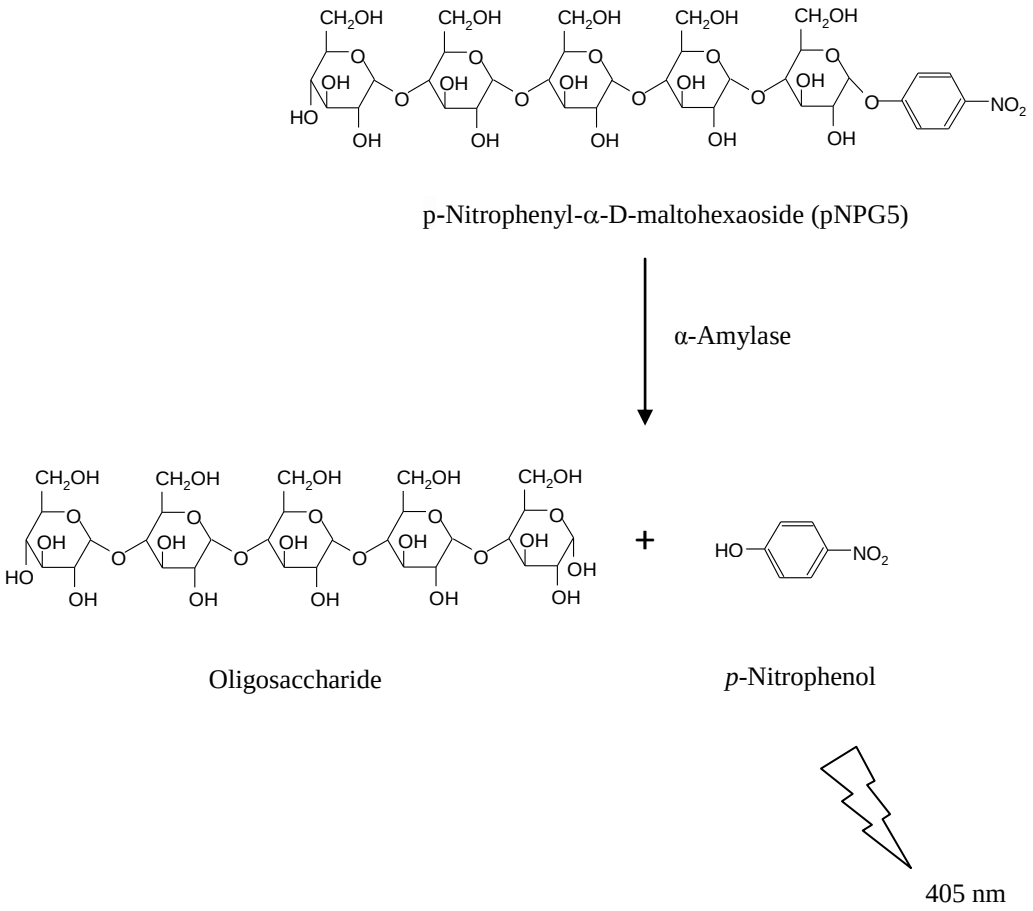


Figure F The α-amylase hydrolyzes *p*-nitrophenyl-α-D-maltohexaoside (pNPG5) into oligosaccharide and *p*-nitrophenol, which is absorbed at 405 nm.

APPENDIX G

ENZYME REACTION OF α-GLUCOSIDASE ASSAY

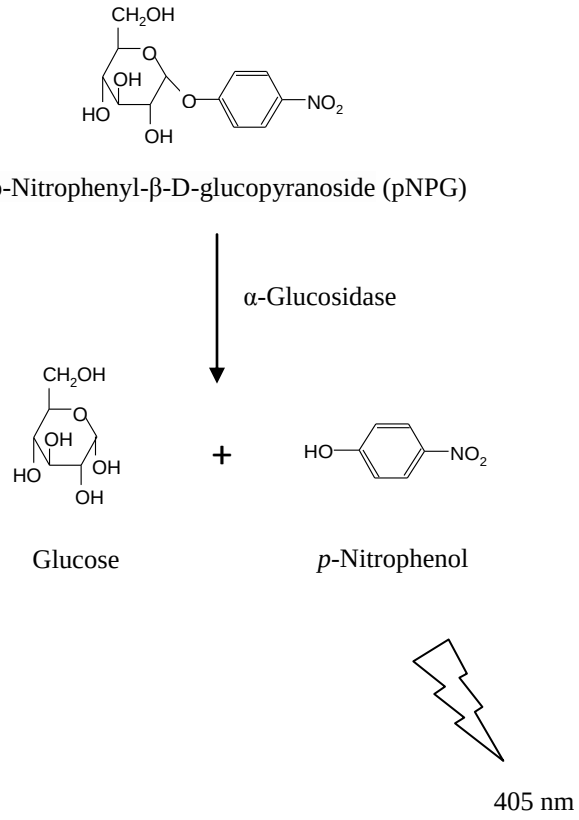


Figure G The α-glucosidase hydrolyzes *p*-nitrophenyl-β-D-glucopyranoside (pNPG) into glucose and *p*-nitrophenol, which can be absorbed at 405 nm.

APPENDIX H
CHOLINESTERASE INHIBITORY ASSAYS

AChE inhibitory assay

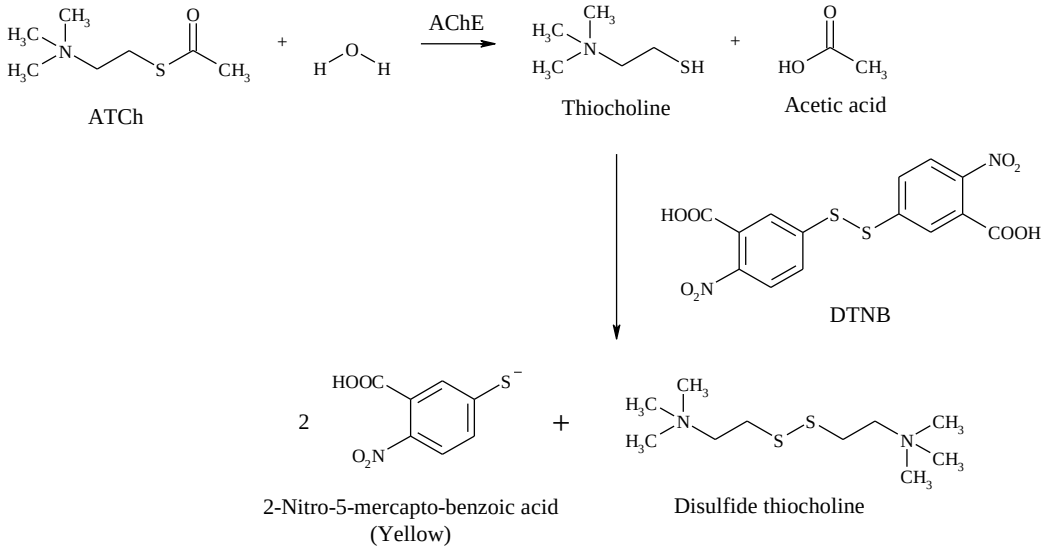


Figure H.1 Reaction of AChE inhibitory assays. ATCh is hydrolyzed by AChE to form thiocholine and acetic acid. Thiocholine is reacted with DTNB to generate color product, which can be measured at a wavelength of 412nm.

BChE inhibitory assay

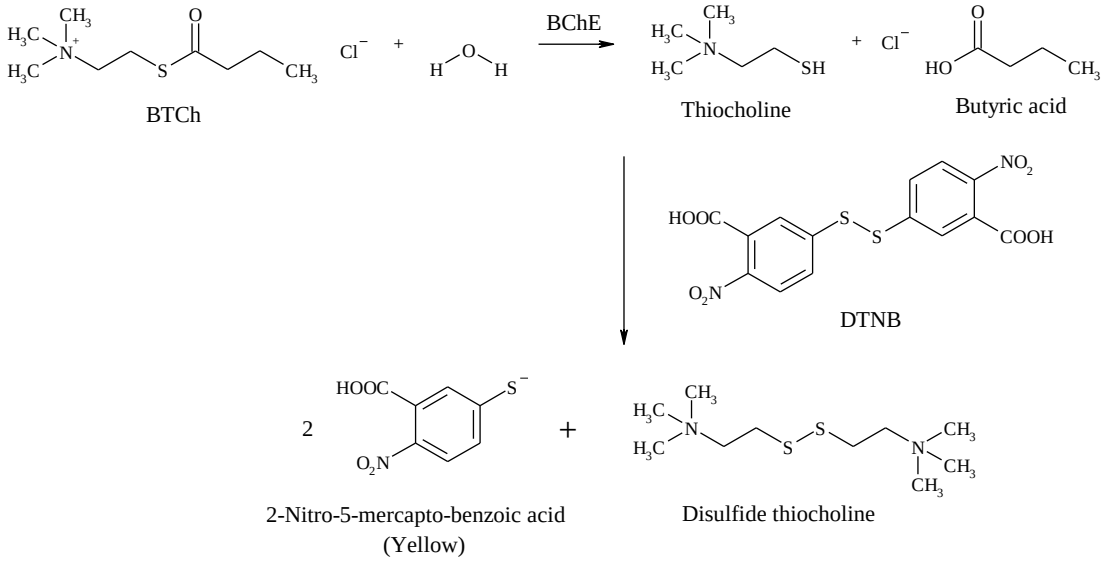


Figure H.2 Reaction of BChE inhibitory assays. The reaction is similar to AChE inhibitory activity assay. BChE hydrolyzes BTCh to thiocholine and butyric acid. The BChE inhibitory activity is measured from the color of the product between thiocholine and DTNB at 412 nm.

APPENDIX I
BACE1 INHIBITORY ASSAY

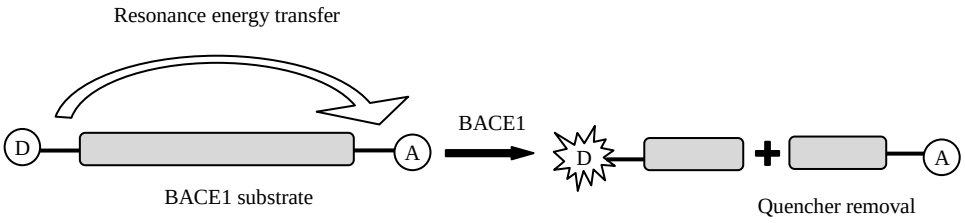


Figure I Reaction of BACE1 inhibitory assay. BACE1 substrate is cleaved from the quenching group (A) by BACE1 to increase fluorescence by fluorescent donor (D). The reaction can be measured at the excitation wavelength of 320 nm and the emission wavelength of 405 nm.

APPENDIX J
ENZYME REACTION OF ACE ASSAY

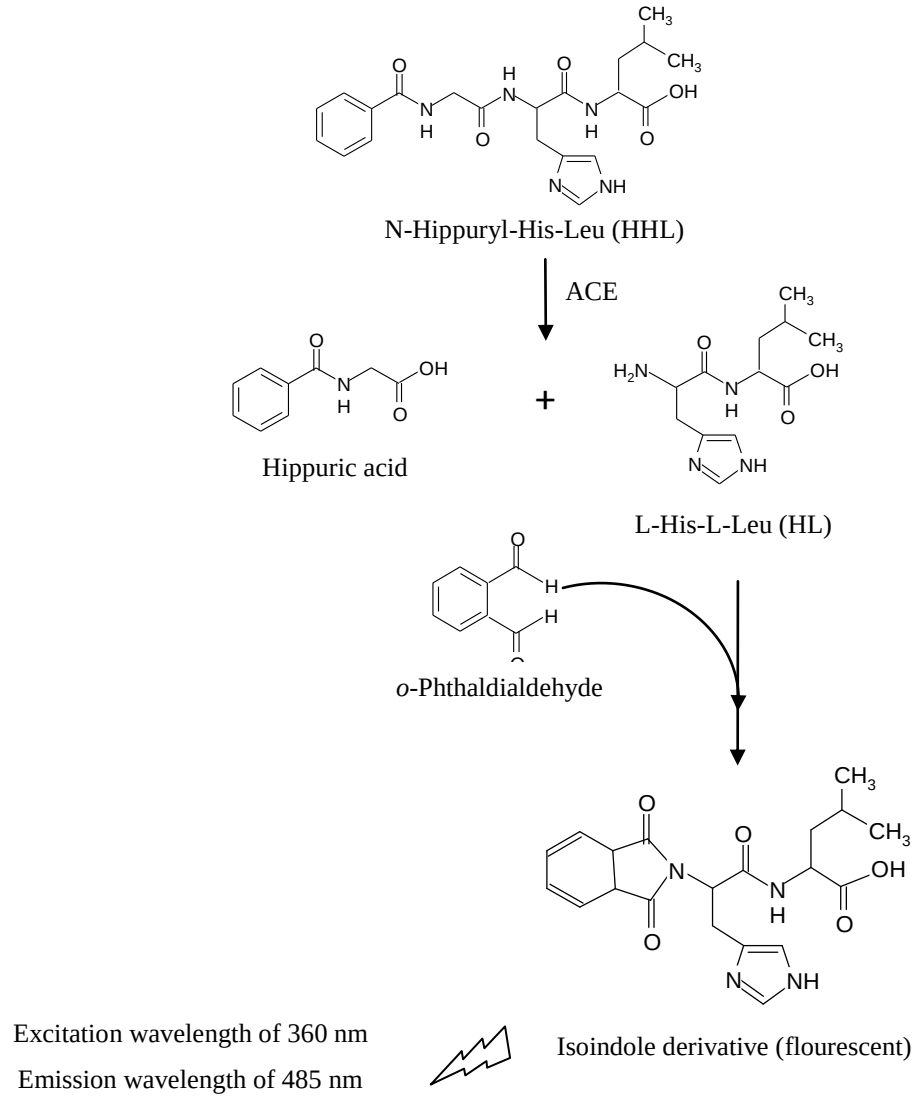
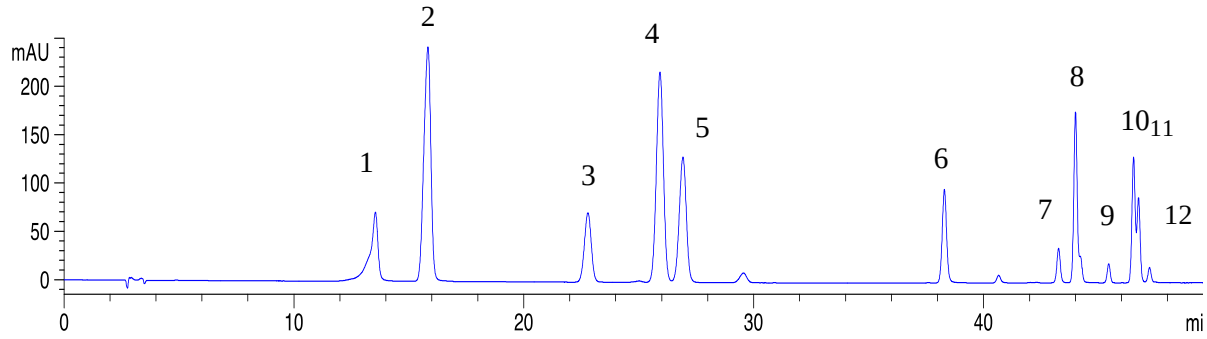


Figure J The ACE hydrolyzes *N*-Hippuryl-His-Leu (HHL) into hippuric acid and *L*-His-*L*-Leu (HL). Then, HL reacts with *o*-phthaldialdehyde, releasing isoindole derivative, a fluorescent product. The assay is monitored by measuring excitation wavelength of 360 nm and emission wavelength of 485 nm.

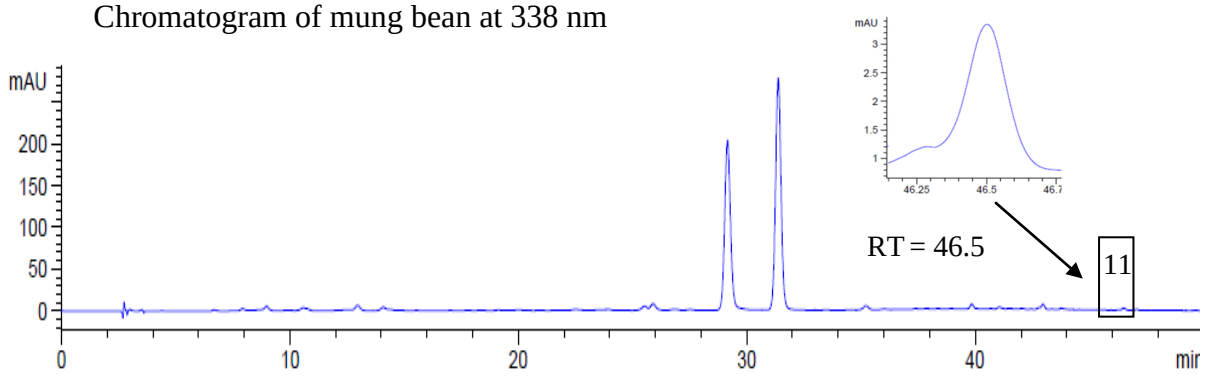
APPENDIX K

HPLC CHROMATOGRAM OF FLAVONOID AND PHENOLIC

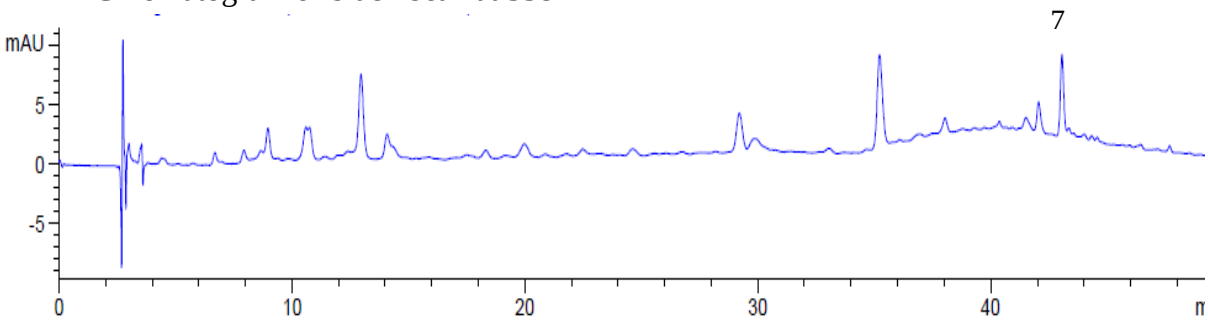
Chromatogram of standard at 338 nm



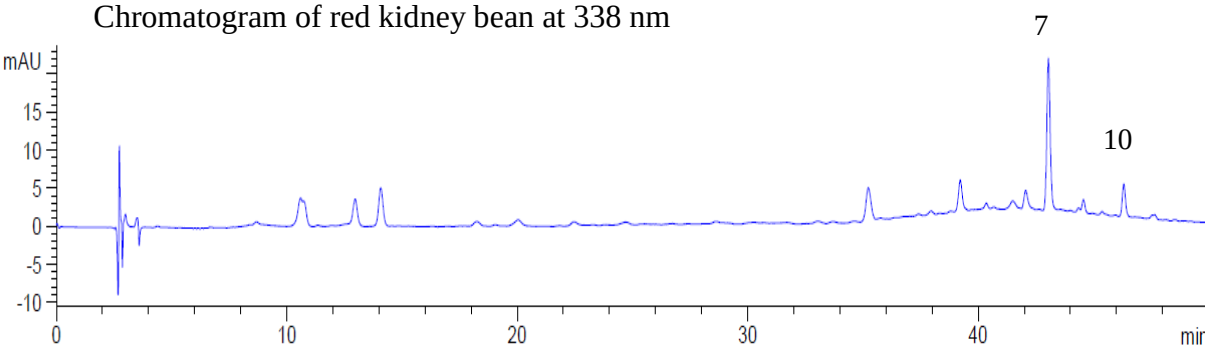
Chromatogram of mung bean at 338 nm



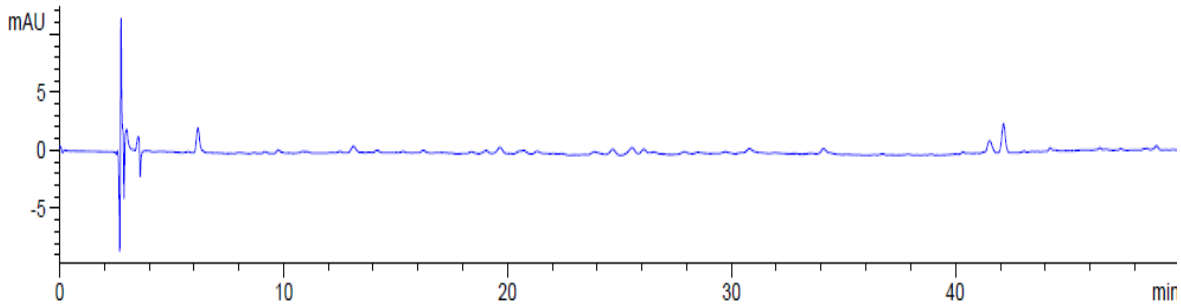
Chromatogram of black bean at 338 nm



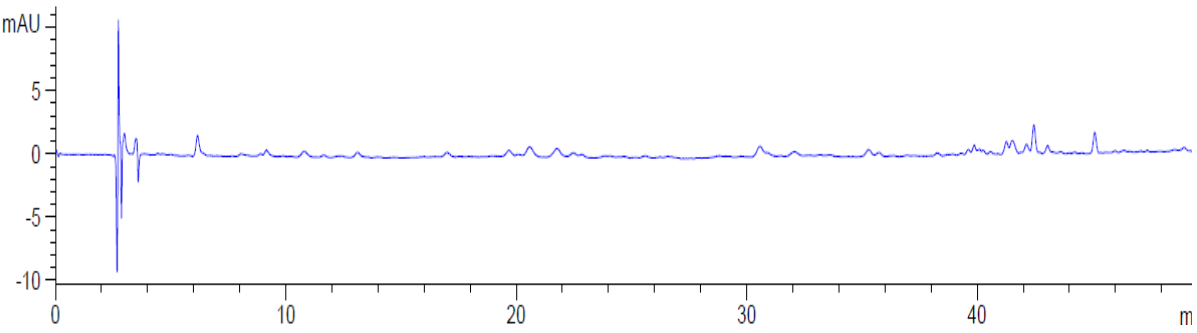
Chromatogram of red kidney bean at 338 nm



Chromatogram of white kidney bean at 338 nm



Chromatogram of soybean at 338 nm



Chromatogram of peanut at 338 nm

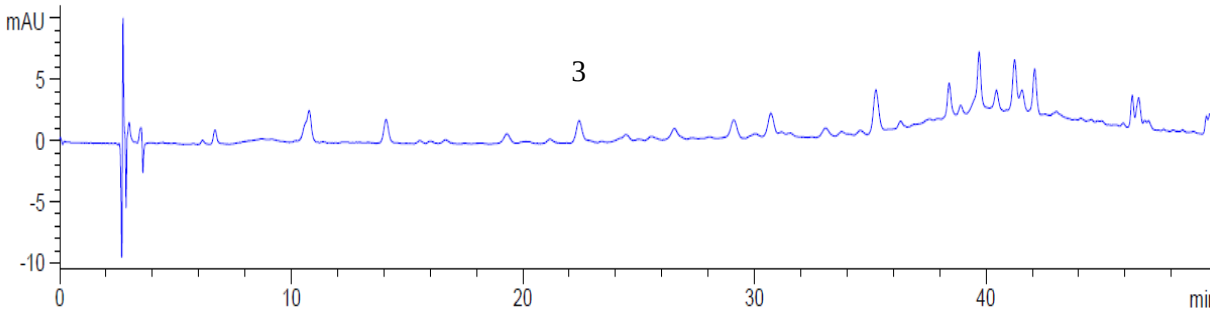


Figure K. The flavonoids and phenolic acids chromatogram of standards, mung bean, black bean, red kidney bean, white kidney bean, soybean and peanut (1: chlorogenic acid, 2: caffeic acid acid, 3: *p*-courmaric acid, 4: Ferulic acid, 5: sinapic acid, 6: myricetin, 7: quercetin, 8: luteolin, 9: hesperitin, 10: kaemferol, 11: apigenin and 12: isorhamnetin).