

APPENDIX A

List of chemicals and materials used in this study

Chemicals/Materials	Source
100% mrthanol	BDH, England
1-Methoxy-5-methylphenazine methosulfate	AppliChem, Germany
2 [N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]	Invitrogen, USA
-2-deoxy-D-glucose 2-NBD glucose	
2,2-diphenyl-1-picrylhydrazyl radical	Sigma-Aldrich, USA
3-Isobutyl-1-methylxanthine	Sigma-Aldrich, USA
95% Ethanol	BHD, England
Acetic acid	BHD, England
Aluminium chloride	BHD, England
Anisaldehyde	BHD, England
Antibiotic antimycotic solution	Invitrogen, USA
Anti-cytokine capture antibody	BioLegend,CA
Bovine serum albumin	PAA Laboratories GmbH, Austria
Calf serum	GIBCO, USA
Cell culture 25 and 75 cm ³ flask and well plates	Corning, USA

Cell culture 35 mm and 100 mm dish and well plate	Corning, USA
Chloroform	BHD, England
Dexamethasone	Sigma-Aldrich, China
Diethylpyrocarbonate (DEPC)	AppliChem, Germany
Dimethylsulfoxide (analytical grade)	RCI Labscan Limited, Thailand
Disodium hydrogen phosphate	BDH, England
Dulbeco's Modified Eagle's Medium (DMEM)	GIBCO, USA
Ferulic acid	Sigma-Aldrich, USA
Fetal bovine serum (FBS)	HyClone, USA
Folin-ciocalteu reagent	BDH, England
Free Glycerol Reagent	Sigma-Aldrich, USA
Gallic acid	Sigma-Aldrich, USA
Griess reagent	Sigma-Aldrich, USA
HEPES	Calbiochem, Taiwan
Insulin, Human Recombinant	Sigma-Aldrich, USA
Isopropyl alcohol	BDH, England
Lipopolysaccharides	Sigma-Aldrich, USA
Low-glucose DMEM medium	GIBCO, USA
Oligo-(dT)-18 primers	Bio Basic Inc.,
Penicillin-Streptomycin	GIBCO, USA

Potassium acetate	BHD, England
Potassium chloride	BHD, England
Potassium dihydrogen phosphate	BHD, England
Potassium phosphate	BHD, England
Primers	WARD MADIC LTD, Thailand
Quercetin	Sigma-Aldrich, USA
Recovery™ Cell Culture Freezing Medium	GIBCO, USA
Reverse transcriptase	Invitrogen, USA
RevertAid™ First Stand cDNA Synthesis Kit	Fermentas, Canada
Ribonuclease inhibitor	Fermentas, Canada
RiboZol reagent	Amresco, USA
RNase Away	Molecular Bio Products, USA
Sodium chloride	Merck, Germany
Sodium dihydrogen orthophosphate 1-hydrate	BDH, England
Sodium hydrogen carbonate	BDH, England
Sodium nitrite	QReC, New Zeland
Sulfuric acid	BHD, England
THUNDERBIRD™ SYBG® qPCR Mix	Toyobo, USA
Tris	Research Organic, USA
Trizol reagent	Invitrogen, USA

Trypan Blue	Sigma-Aldrich, USA
Trypsin	Invitrogen, USA
Tumor necrosis factor (TNF- α)	PEPROTECH, USA
Tween-20	USB Corporation, USA
Vacuum filter/Storage bottle	Corning, USA
WST-1 reagent	AppliChem, Germany



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APPENDIX B

List of instrument used in this study

Instrument	Company
Abi 7500 Fast Real-Time PCR system	Applied Biosystems
Autoclave	Tomy autoclave SS-240
Automatic pipette	Thermoscientific
Carbon dioxide incubator	Thermoscientific
Centrifuge	Kubota Corporation
Freezer (-80°C)	Forma Scientific
Freezer (-20°C)	TOSHIBA
Glassware	Pyrex and Scott duran
Hot air oven	Heraeus
Hot plate stirrer	Torrey Pines Scientific, USA
Inverted microscope	NIKON

Laminar flow biological cabinet Class II	NU AIRE
Liquid nitrogen tank	International Cryogenics, Inc.
Magnetic stirrer	Thermolyne
Micro-plate spectrophotometer	Bio-Tek Instrument
Nano Drop spectrophotometer	Thermoscientific
pH meter	LG, Korea
Refrigerator	SIEMENS
Thermocycler	BIO-RAD, England
Vortex	Scientific industries
Water bath	Science/Electronic, USA

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APPENDIX C

Reagents and buffers preparation

1. Reagents for cell culture

1.1 Incomplete DMEM serum free medium

DMEM powder	13.5	g
HEPES	2.603	g
NaHCO ₃	3.7	g

Add DW to 1,000 ml, adjust pH 7.4 and sterile by Millipore filter membrane (0.22 µm) and stored at 4°C

1.2 Complete DMEM medium

1.2.1 Fetal Bovine serum medium

Fetal Bovine Serum	10	mL
DMEM	88	mL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.2 Fetal Calf serum medium

Fetal Calf Serum	10	mL
DMEM	88	mL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.2 MDI medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
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DMEM	86.8	mL
Insulin	100	μL
IBMX	200	μL
Dexamethasone	100	μL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.3 Insulin medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
DMEM	87.9	mL
Insulin	100	μL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.4 FBS insulin medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
DMEM	87.5	mL
Insulin	50	μL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.3 Phosphate buffer saline (PBS pH 7.4)

NaCl	8	g
KCl	0.2	g
Na ₂ HPO ₄	1.15	g
KH ₂ PO ₄	0.2	g
DW	800	mL

Adjust pH 7.4 using 1M HCl, then adjust volume to 1,000 mL

2. DEPC treated water

Deionized water	1000	mL
DEPC	0.1	mL

3. IBMX Solution (make fresh)

IBMX	0.0575 g	
0.35N KOH	0.5	mL
DW	0.5	mL

Dissolve IBMX in a solution made of 0.5N KOH to a final concentration of 0.0115g/mL and then filter sterilize through a 0.22 mm syringe filter.

4. Insulin Stock Solution

Insulin	0.01	g
0.02M HCl	10	mL

167 uM (1mg/mL) in 0.02M HCl and then filter sterilized through 0.22 mm filter. Can store at -20°C for long term, 4°C short term.

5. Dexamethasone Stock Solution

Dexamethasone	0.0197	g
100% ethanol	5	mL

Freezer Stock: 10mM of Dex in 100% ethanol (store at -20C)

Dexamethasone	100	μL
PBS (filter)	900	μL

Working Stock: Dilute Freezer stock to 1mM in PBS. Filter sterilize and store at 4 °C.

6. Reagent for determination of phenolic content

6.1 7% Na_2CO_3

Na_2CO_3	7	g
DI	100	mL

6.2 Folin-Ciocalteu's phenol reagent

Dilute before use with DI 1:1

7. Reagent for determination of flavonoid content

7.1 5% NaNO_2 solution

NaNO_2	5	g
DI	100	mL

7.2 10% AlCl_3 solution

AlCl_3	10	g
DI	100	mL

7.3 1M NaOH

NaOH	4	g
DI	100	mL

8. Reagent for DPPH assay

8.1 0.2 mM DPPH

DPPH	0.8	mg
Absolute MeOH	10	mL

9. 0.05% Tween-20 wash buffer for ELISA

Tween-20	500	μL
PBS pH 7.4	1,000	μL
10 Mobile phase for HPLC		
TFA	0.1	g
Methanol	100	mL
10 Protein Electrophoresis and Western Blot Analysis		
10.1 RIPA contains proteinase inhibitor (prepared before used)		
Protease inhibitor cocktail kit	1	tablet
RIPA buffer	10	mL
10.2 TRIS Buffered Saline (TBS)		
Tris-base	2.42	g
NaCl	8	g
DI	800	mL
Adjust pH to 7.4 then adjust volume with DI to 1,000 mL		
10.3 0.15% Tween-20-Tris-buffer saline (T-TBS)		
TBS	1,000	μL
50% Tween 20	3	mL
10.4 10% SDS		
SDS	10	g
DI	100	mL
10.5 10% APS		

APS	1	g
DI	10	mL

10.6 Separating gel 10% SDS-PAGE

DI	4.6	mL
30%Acrylamide	2.67	mL
Tris-buffer pH 8.8	2.5	mL
10%SDS	100	μL
10%APS	100	μL
TEMED	10	μL

10.7 Stacking gel 4%

DI	2.3	mL
30%Acrylamide	0.67	mL
Tris-buffer pH 6.8	1	mL
10%SDS	40	μL
10%APS	40	μL
TEMED	4	μL

10.8 Running buffer

Tris-base	3.04	g
Glycine	14.4	g
10%SDS	10	mL
DI	1,000	mL

10.9 Blotting buffer

Tris-base	3.04	g
Glycine	14.4	g
Methanol	200	mL
DI	800	mL

10.10 Blocking reagent (3%BSA)

BSA	0.3	g
TBS-T	10	mL

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APPENDIX D

The chromatogram of total phenolics of ABAE and ABEE were determined by high performance liquid chromatography (HPLC) using the following condition:

Column: Phenomenex Luna C18 (2)(size 250 x 4.6 mm, 5 μ m)

Mobile phase: A; 0.1% TFA in H₂O

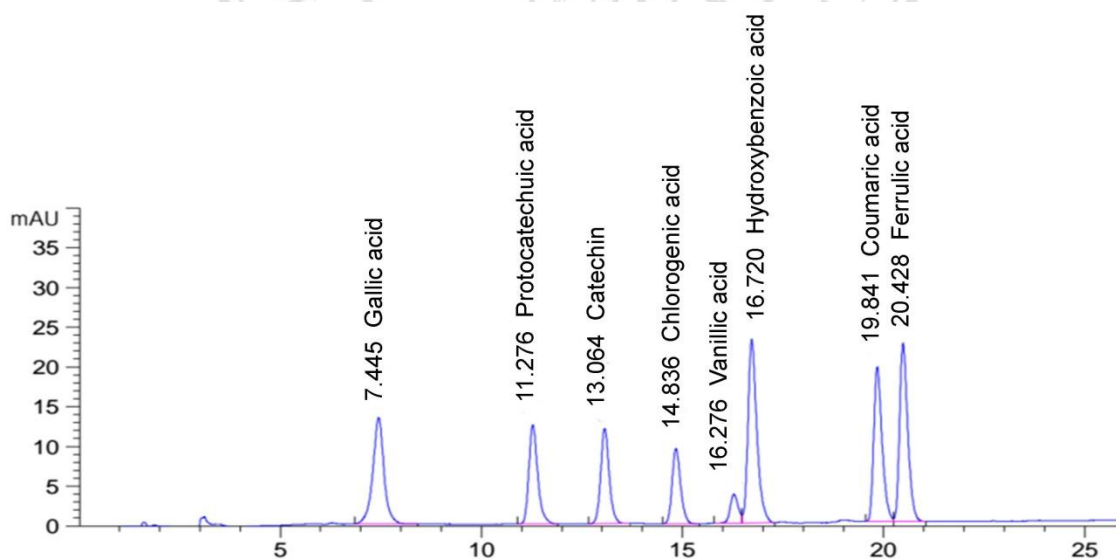
B; Methanol 100%

Injection: 10 mg/ml (10 μ l)

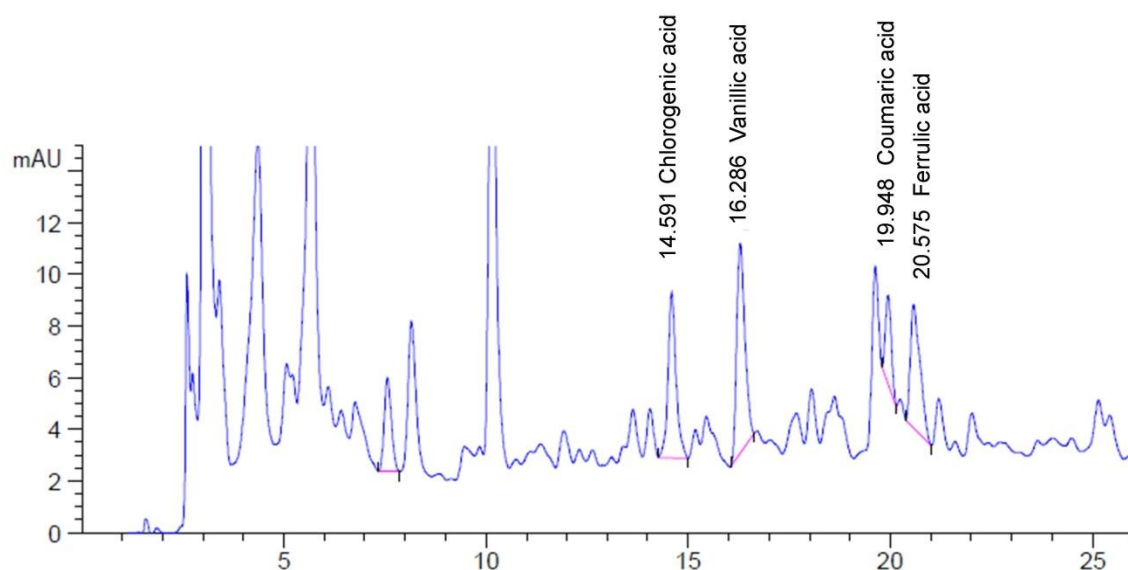
Flow rate: 1.0 ml/min

Detection: UV 280 nm

(A) Standard phenolic compound mixed



(B) Aqueous extract of *Anoectochilus burmannicus* (ABAE)



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(C) Ethanolic extract of *Anoectochilus burmannicus* (ABEE)

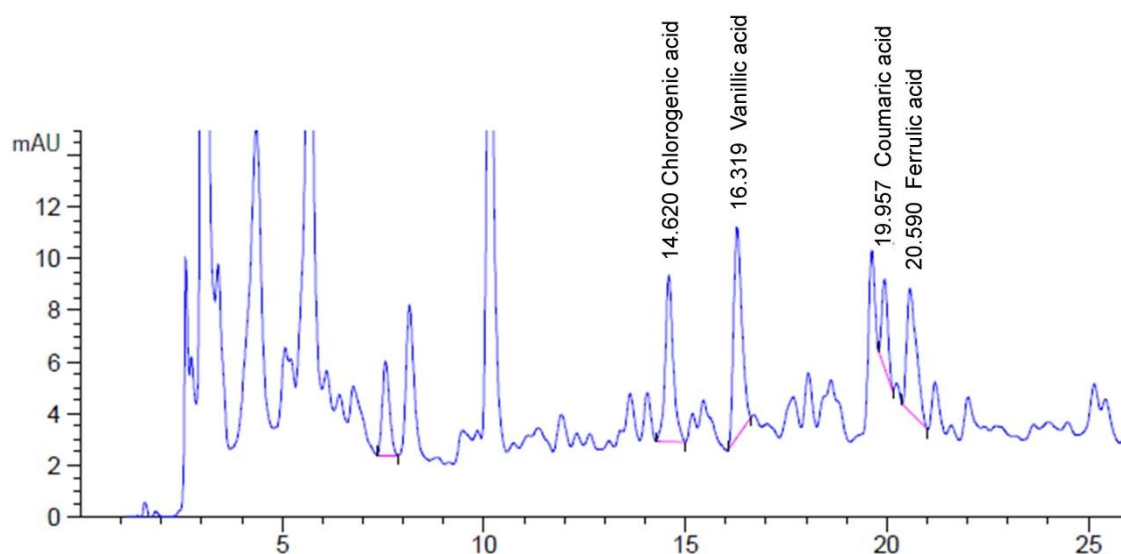


Figure 3.12 HPLC chromatogram of total phenolics in ABAE and ABEE. (A) Standard phenolic compound mixed, (B) Aqueous extract of *Anoectochilus burmannicus* (ABAE), (C) Ethanolic extract of *Anoectochilus burmannicus* (ABEE)

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Publication	1. Phatcharaporn Budluang, Arisa Bonness, Ariyaphong Wongnoppavich. and Pornsiri Pitchakarn. Anti-inflammatory activity of aqueous extract of <i>Anoectochilus burmannicus</i> in RAW 264.7 macrophage. The first International Conference on Pharmacy Education and Research Network of ASEAN. Bangkok, Thailand, December 2-4, 2015. (Proceeding and oral presentation)



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