Phytochemical Investigation of Traditional Bangladeshi Medicinal Plants

by Bishwajit Bokshi

Thesis submitted in fulfilment of the Doctor of Philosophy (**PhD)** requirements

under the supervision of **A/Prof. Alison Ung**
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Bishwajit Bokshi, declare that this thesis has been submitted to fulfill the requirements for obtaining PhD in the Faculty of Science, School of MAPS, UTS, Sydney.

This thesis paper is entirely my work unless specifically referenced or acknowledged. It also ensures that all sources and literature used are revealed in the thesis.

This work has not been submitted so far for qualification at any other educational institution.

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Impact of Covid-19 on research

The COVID-19 lockdown in two phases has created challenges regarding my project progress. With the lockdown, I experienced delays with the delivery of chemicals and reagents for biological assays. There were other obstacles to accessing the laboratories, university-provided software, and technical assistance required for my experiments. At first, the lockdown was received with optimism, to focus on writing and research. However, as the lockdown prolonged, the pressure and stress began to increase. The extended period at home provided obstacles and challenges that were deeply underestimated. I have lost approximately 3 months during a core period of my tenure, with which I would have delivered a better outcome. I am thankful for the encouragement and support I received from supervisors and team members during this time.

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List of Abbreviations

A. aspera	*Achyranthes aspera*
A. paniculata	*Andrographis paniculata*
ANOVA	Analysis of variance
APFA	*A. paniculata* acidic fraction
APFB	*A. paniculata* basic fraction
APFN	*A. paniculata* neutral fraction
BCS	Bovine calf serum
C. viscosum	*Clerodendrum viscosum*
COSY	^{1}H–^{1}H correlation spectroscopy
CVFA	*C. viscosum* acidic fraction
CVFB	*C. viscosum* basic fraction
CVFN	*C. viscosum* neutral fraction
DAG	Diacylglycerol
DPP-4	Dipeptidyl peptidase 4
EGCG	Epigallocatechin gallate
ER	Endoplasmic reticulum
FFA	Free fatty acid
GC-MS	Gas chromatography-mass spectrometry
GIP	Glucose-dependent insulinotropic
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter type 4
H. Indicum	*Heliotropium indicum*
HFD	High-fat diet
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum correlation
IKKβ	I_KB kinase-β
IPA	Isopropanol
IR	Insulin resistance
IRSs	Insulin receptor substrates
JNK1	JUN amino-terminal kinase 1
LDs	Lipid droplets
MSI	Metabolomics Standards Initiative

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide
2-NBDG	2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose
NCD	Noncommunicable diseases
NF-κB	Nuclear factor κB
NOESY	Nuclear overhauser effect spectroscopy
NPH	Neutral protamine Hagedorn
PERK	PRKR-like ER kinase
PFA	Paraformaldehyde
PI3K	Phosphatidylinositol 3-kinase
PKCs	Protein kinase C
*p*NPG	*p*-Nitrophenyl-*α*-D-glucopyranoside
PPAR	Peroxisome proliferator-activated receptors
ROS	Reactive oxygen species
SGLT2	Sodium-glucose co-transporter 2
SOCS	Cytokine signalling suppressors
STZ	Streptozotocin
T2D	Type 2 Diabetes
TH1	T helper 1
TLRs	Toll-like receptors
TZDs	Thiazolidinediones
UPR	Unfolded protein response
WHO	World Health Organisation

Abstract

Plants have provided novel compounds to develop drugs against various diseases, including Type 2 Diabetes (T2D). It is characterised by hyperglycaemia due to insulin resistance and insufficient insulin release, or both of them. This project aimed to identify novel antidiabetic compound/s or novel antidiabetic activities of known compounds isolated from two medicinal plants, *Andrographis paniculata* (*A. paniculata*) and *Clerodendrum viscosum* (*C. viscosum*).

A. paniculata crude extract was fractioned into acidic (APFA), basic (APFB), and neutral fractions (APFN). A total of three pure *ent*-labdane diterpenoids were isolated from APFN, and they are known andrographolide (A1) and 14-deoxyandrographolide (D1), and new *(3S),(4R),(5S),(9R),(10R)*-14-deoxy-11,12-didehydroandrographolide (D2). Other sub-fractions of APFN were subjected to further separation; however, no pure compounds were isolated from sub-fractions due to lack of purity and time constrain. APFA (0.131 g) and APFB (0.2057 g) were not feasible for purification to yield sufficient pure compounds for structural elucidation and biological assays.

All the three crude fractions and three pure compounds have been subjected to insulin-mediated glucose uptake assay in 3T3-L1 adipocytes. APFA significantly enhanced the glucose uptake by approximately 82% at 25 µg/ml; however, at 2.5 µg/ml, it failed to enhance glucose uptake significantly. At 10 and 100 µg/ml, APFB significantly enhanced the glucose uptake by 51% and 82%, respectively, whereas APFN significantly enhanced glucose uptake by 29% and 78%. Of the three pure compounds, A1 at 1 µM significantly enhanced the glucose uptake by approximately 38%; however, no significate uptake found at 10 µM. Both D1 and D2 significantly enhanced glucose uptake by approximately 45% and 65% at 1 µM and 10 µM, respectively.

The lipid accumulation study by Oil Red O staining reflected that APFA, AFFB, and APFN significantly increased lipids in 3T3-L1 adipocytes. APFA increased lipids by increasing the size of adipocytes (11% and 13% at 2.5 and 25 µg/ml, respectively), and APFB increased lipids by increasing the size of adipocytes by about 10% at both 10 and 100 µg/ml. APFN increased lipids by significantly increasing the size of adipocytes (9.17% at 10 µg/ml); however, it did not show any significant change at 100 µg/ml. A1 increased lipids by increasing the size of adipocytes by 9% at 10 µM without significant change at 1 µM. Increasing the number of new small adipocytes and adiponectin might increase lipid accumulation and increase glucose uptake. APFA, AFFB, and APFN, and A1 might increase glucose uptake following this mechanism. At both 1 and 10 µM, D1 decreased lipids by decreasing the size of adipocytes by

about 10% and the number of LDs by about 27%. D2 decreased lipids by decreasing the size of adipocytes by about 11% and the diameter of LDs by about 39% at both 1 and 10 µM. The decrease of lipid accumulation by D1 and D2 might increase glucose uptake by decreasing the level of free fatty acids, triglycerides, and adiponectin. None of the crude fractions of *A. paniculata* and pure compounds showed significant inhibition of the *α*-glucosidase enzyme. APFA, APFB, APFN, and A1 could control blood glucose levels by increasing glucose uptake and lipid accumulation. However, D1 and D2 may control blood glucose levels by increasing glucose uptake and decreasing lipid accumulation.

Three crude fractions of *C. viscosum* were fractioned into acidic (CVFA), basic (CVFB), and neutral fractions (CVFN) and subjected to insulin-mediated glucose uptake assay in 3T3-L1. CVFA was found to significantly increase glucose uptake by 30% and 46% at 10 µg/ml and 100 µg/ml, respectively, whereas CVFB significantly enhanced glucose uptake similar to CVFA, by 40% and 49% at 10 µg/ml and 100 µg/ml, respectively. The CVFN significantly improved glucose uptake by 56% and 52% at 10 µg/ml and 100 µg/ml, respectively.

The result of the lipid accumulation study showed that CVFB could not change the size of 3T3-L1 adipocytes, number, and dimeter of LDs/cell significantly. At 10 and 100 µg/ml, CVFA only significantly reduced LDs diameter by 38% and 49%, respectively, without significant change in the size of adipocytes and number of LDs/cell. CVFN significantly increased the number of LDs by 44.3% at 10 µg/ml. Overall, CVFA might increase glucose uptake by decreasing lipid accumulation, which might be due to the decrease of fatty acids or triglycerides. CVFN might increase glucose uptake by increasing lipid accumulation due to increased adiponectin and small new adipocytes.

No pure compounds were separated from either of the three fractions of *C. viscosum*. The phytochemicals in the crude extract of *C. viscosum* might undergo decomposition during the preparation of the crude extract or long duration of storage in refrigeration. Therefore, these results should be considered very preliminary, which requires further investigation.

In summary, two known compounds (A1 and D1) and a new compound (D2) have been separated from *A. paniculata*. This research demonstrated controlling blood glucose levels by APFN, which could justify the ethnopharmacological use of *A. paniculata*. Moreover, A1, D1, and D2 could serve as a novel treatment against T2D by increasing glucose uptake in 3T3-L1 cells at the low dose of 1 µM. Especially, D1 and D2 could be better therapy as they may control blood glucose levels by increasing glucose uptake and decreasing lipid accumulation.