A study of the chemistry of *Artocarpus heterophyllus* Lam. leaves and identification of active antioxidant and hypoglycemic fractions/ compounds

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M. Phil. **January 2016**

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of the Master of Philosophy in Chemistry on

January 2016

DECLARATION

"The work described in this thesis was carried out by me under the supervision of Prof. A. M. Abeysekera (Senior professor, Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda), Prof. (Mrs.) U.G. Chandrika (Professor, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda), Dr. (Mrs.) C. Padumadasa (Senior lecturer, Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda), Dr. A.K.E. Gunathilake (Senior lecturer, Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda) and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma."

14.06.2017 ..... Sranda .....

Date Signature

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the university for the purpose of evaluation.

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ABBREVIATIONS

^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
A. heterophyllus	*Artocarpus heterophyllus*
A_0	Absorbance of the control
A_1	Absorbance of fractions sample/standard
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
AGE	Advanced Glycation End product
ATP	Adenosine Triphosphate
BGL_{c0}	Blood glucose level of control 1st hour after administration of water immediately before the glucose load
BGL_{c1}	Blood glucose level of control 1 hour after administration of glucose load
BGL_{t0}	Blood glucose level of test group 1st hour after administration of sample immediately before the glucose load
BGL_{t1}	Blood glucose level of test group 1 hour after administration of glucose
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
COSY	Homonuclear correlation spectroscopy

COX-2	Cyclooxygenase 2
DKA	Diabetic keto acidosis
DMPD	*N*, *N*-dimethyl-*p*-phenylendiamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPP-IV	Dipeptidyl peptidase 4
DPPH	2,2-diphenyl-2-picrylhydrazyl
EA/W	Ethyl acetate fraction of the water extract
EI MS	Electron ionization mass spectrometry
FAG-MS	Fast atom bombardment mass spectra
FBG	Fasting Blood Glucose
FRAP	
GDM	Gestational Diabetes Mellitus
GIP	Glucose-dependent Insulinotropic Peptide
GLP-1	Glucagon-like Peptide-1
GLUT2	Glucose Transporter
HbA1C	Glycated haemoglobin (A1c), which identifies average plasma glucose concentration.
HBSS	Hanks Balanced Salt Solution, containing calcium chloride andmagnesium chloride
HDL	High Density Lipoprotein
HMBC	Heteronuclear Multiple Bond Correlation
HNC	Hyperosmolar non-ketonicoma
$HOCl^-$	Hydroxyl radicals, halogenated oxygen metabolites

HR-FAB MS	High Resolution Fast-Atom Bombardment mass spectrometry
HSQC	Heteronuclear Single-Quantum Correlation
IC_{50} value	Concentration of an inhibitor where the response (or binding) is reduced by half.
iNOS	inducible Nitric Oxide Synthase
IR Spectroscopy	Infrared Spectroscopy
J	Coupling constant
LA	Lactic acidosis
LDL	Low density lipoprotein
MGO	Methylglyoxal
MTT	tetrazolium
NADH	β-nicotanamide adenine dinucleotide (NADH)
NBT	Nitro blue tetrazolium
NCEs	New chemical entities
NOESY	Nuclear Overhauser Enhancement Spectroscopy
NPH	Isophane insulin (suspension of insulin with protamine)
PG	Propyl galate
PMS	Phenazine MethoSulphate
pNPG	p-nitrophenyl-α-D-glucopyranoside
PPAR-gamma	Peroxisome Proliferator-Activated Receptor-gamma
R_f	Retention factor
RNS	Reactive nitrogen species
ROS	Reactive Oxygen species

RPHPLC	Recycling Preparative High Performance Liquid Chromatography
SOZ	Serum Opsonized Zymosan
SRB	Sulforhodamine B
TBHQ	tertiary butyl hydroquinone
TLC	Thin Layer Chromatography
t_R	Retention time
UV-visible spectroscopy	Ultraviolet–visible spectroscopy
VLDL	Very low density lipoprotein

ACKNOWLEDGEMENTS

I take pride in acknowledging my supervisor, Prof. Ajit Abeysekera for his expert guidance, sincere advice and continuing support. His inspiring encouragements enabled me to carry out the research and awoke a scientific attitude in me. His kindness always gives me the new strength to face the problems of both research and life. To him, I am forever grateful.

The work embodied in this thesis would never been accomplished without the keen interest, precious attention and continuous encouragement of my supervisor Prof. Mrs. U.G. Chandrika. I am grateful to her for her guidance, thoughtful comments and suggestions and especially for her very kind support throughout the course of this research work and inspiring support and interest in my professional development.

I would like to express my sincere gratitude to my supervisor, Dr. Mrs. Chayanika Padumadasa for the invaluable time that I spent with her, for all of her advice, help and encouragement during the project. Without her attention and inspiration, I would not have been able to complete this project.

I wish to acknowledge my supervisor, Dr. A. K. E. Goonathilake for his support and encouraging especially at the commencement of this work.

I also wish to express my grateful appreciation of Prof. Iqbal Choudhary for giving me an opportunity to work in the HEJ Research Institute Research of Chemistry, Karachi. He generously offered his hand and his heart in support and guidance for me as a developing young researcher. I will be always grateful to him for his sincerity, kindness and encouragement throughout my research work.

I thank Dr. Vinitha Thadani for useful discussions and guidance on chromatography and for facilitating my visit to the HEJ Research Institute, Karachi.

Special thanks go to Mr. T. M. S. G. Thennakoon for valuable discussions on chromatography and for his guidance.

I also wish to express my heartiest gratitude to all academic and nonacademic staff of the department of Chemistry, University of Sri Jayewardenepura for their cooperation during my research studies. Special thanks go to Mr. Senadeera, Mr. Sisira and to Mr. Nilaj for facilitating my laboratory work.

I would also like to extend my appreciation to everyone at International center for chemical and biological Sciences, Karachi, who have contributed to make this work possible. Special thanks go to Dr. Muhammad Hafizur Rahman for guiding me through the animal studies which I have done at International center for chemical and biological Sciences, Karachi. I am so grateful to Dr. Mujeeb Ur Reheman for his help throughout my stay at HEJ Research Institute of Chemistry and Dr. *Achyut Adhikari* for helping me with the NMR analysis. Thanks are also due to Dr. Sammer Yousuf, Dr, Atia-tul-Wahab, Dr. Saima Rasheed and Ms. Sadia Siddiq and to all my friends at HEJ Research Institute specially to Dr.Thin Hling, Dr. Azeana Zahari, Dr. Shabbhir hussain, Dr. Hamdy Kashtoh and Dr. Yann Fongan for their care and help during my stay at the HEJ Research Institute of Chemistry.

I would like to acknowledge the assistance of Probationary lecturer, Department of Botany, University of Sri Jayewardenepura, Mr. Isuru Kariyawasam and Mrs. K. D. Degambada in the authentication of plant materials.

I feel extremely fortunate to have as friends my laboratory colleagues Ureshani, Durga, Kalpani and Tharangi with whom I shared not only the happiest times of my life but

also difficult moments of my life. I am especially grateful for them for their open ears and kind words which have sustained me along the way.

I thank my parents who are the beacon of my life for their abiding love and prayers and for their unfailing support. I have no words to acknowledge their contributions in my life. Without their help this thesis would not have been possible.

I would like to express my deepest love to my devoted loving husband Anura Wickramanayake who always supported in every aspect of my life including the completion of the arduous journey.

Last but not least, I am greatly indebted to my two sons Sanula and Danula who form the backbone and origin of my happiness.

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ABSTRACT

The aqueous extract of the senescent leaves of *Artocarpus heterophyllus* is traditionally used in Sri Lanka as a hypoglyceamic agent. It has been previously reported that the hypoglyceamic activity lies in the ethyl acetate fraction of the water extract (EA/W). The main purpose of this study was to identify hypoglyceamic fractions/ compounds from the EA/W fraction. The study also encompassed antioxidant, antiglycation, α-glucosidase inhibition and anti-inflammatory assays. The EA/W fraction was fractionated by chromatography on sephadex LH-20. Five fractions eluting with the following solvent systems were obtained. Fraction 1, dichloromethane/ hexane (4:1), Fraction 2, dichloromethane/acetone (3:2), Fraction 3, dichloromethane/acetone (1:4), Fraction 4, dichloromethane/ methanol (1:1) and Fraction 5, methanol. These fractions were screened for *in vivo* hypoglyceamic and antidiabetic activities and as well as *in vitro* antioxidant, antiglycation, α-glucosidase inhibitory and anti-inflammatory assays. Each fraction was tested for its effect on the blood glucose levels of fasted normal and diabetic rats. None of the fractions caused hypoglyceamia on normal rats. However there was significant ($p < 0.001$) reduction in the blood glucose level during the first three hours after giving the fractions 3, 4 and 5 to diabetic rats. Of the three fractions fraction 4 showed the highest hypoglyceamic activity. Fractions 3, 4 and 5 were also the

most active in the glucose tolerance test carried out on both normoglyceamic and diabetic rats. However fraction 3 was the most active in this assay. In both assays fractions 3, 4 and 5 at 50 mg/kg body weight showed activity of the same order as glibenclamide at 5 mg/ kg body weight. Fraction 4 was found to be the most active fraction in all the *in vitro* assays. In the DPPH radical quenching antioxidant assay it has a IC_{50} value of 21.69 ± 0.31 mg/ml compared with gallic acid which had IC_{50} value of 23.46 ± 0.43 mg/ml. In the superoxide anion radical scavenging assay it showed 98% percentage inhibition at 0.5 mg/ kg dose while quercetin showed 100% percentage inhibition. It has strong α - glucosidase inhibition activity with a IC_{50} value of 0.40 ± 0.01 mg/ml compared with acarbose which had a IC_{50} value of 0.54± 0.01 mg/mL. In the antiglycation assay it has a IC_{50} value 0.44 ± 0.01 mg/mL compared with rutin which had IC_{50} value of 0.18 ± 0.02 mg/mL. In the anti-inflammatory assay it has IC_{50} value of 16.9 ± 0.1 mg/mL compared with rutin which had IC_{50} value of 11.8 ± 1.91 mg/mL. As the thin layer chromatography analysis of fractions 3 and 4 indicated that they had several compounds in common, they were combined and subjected to chromatographic analysis. Five compounds were isolated using MCI gel, sephadex LH-20 and silica column chromatography, preparative thin layer chromatography and normal phase, reversed phase and size exclusion preparative high-performance liquid chromatography and their structures elucidated by using ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, COSY, NOESY, IR and UV-visible spectroscopy, EI, FAB and HR-FAB spectrometry. One of the five compounds, 3,4,6-trihydroxy-7-ene-megastigman-9-one **[104]**, was identified as the C5 epimer of lyratol E and is a new isomer. Of the four remaining compound 4-hydroxybenzoic acid **[93]** has been reported previously from the twigs of *A. heterophyllus.* The other remaining compounds 3,4-dihydroxy-7-ene-

megastigman-9-one **[103]**, 13-Hydroxy-3-oxo-α-ionol **[105]**, 3,4-dihydroxybenzoic acid **[106]** are known compounds but have not been reported previously from *A. heterophyllus*. This study indicates that the obtained hypoglycemic effect of the EA/W fraction is caused by the total effect of a number of compounds of similar structures and that it may not be possible to isolate a single highly active compound with hypoglyceamic activity from the senescent leaves of *A. heterophyllus.*

Key words: *Artocarpus heterophyllus*, hypoglycemic effect, antioxidant, antiglycation, alpha glucosidase inhibition, *epi*-lyratol E.