ANTIOXIDANT AND RELATING BIOLOGICAL ACTIVITIES OF FLAVONOIDS EXTRACTED FROM SEED HUSK OF *Tamarindus indica* Linn.

PUKSIRI SINCHAIYAKIT

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree in Biological Science
May 2011

ANTIOXIDANT AND RELATING BIOLOGICAL ACTIVITIES OF FLAVONOIDS EXTRACTED FROM SEED HUSK OF Tamarindus indica Linn.

PUKSIRI SINCHAIYAKIT

A Thesis Submitted to the Graduate School of Naresuan University

in Partial Fulfillment of the Requirements

for the Doctor of Philosophy Degree in Biological Science

May 2011

This thesis entitled "Antioxidant and relating biological activities of flavonoids extracted from seed husk of *Tamarindus indica* Linn." submitted by Puksiri Sinchaiyakit in partial fulfillment of the requirements for the Doctor of Philosophy Degree in Biological Science is hereby approved.

..... Chair
(Assistant Professor Surisak Prasarnpun, Ph.D.)

..... Committee
(Professor Maitree Suttajit, Ph.D.)

..... Committee
(Associate Professor Supakorn Pongbangpho, Ph.D.)

..... Committee
(Assistant Professor Sarin Sriprang, Ph.D.)

..... Committee
(Assistant Professor Wandee Wattanachaiyingcharoen, Ph.D.)

..... Committee
(Associate Professor Somdet Srichairatanakool, Ph.D.)

..... Committee
(Professor Malyn Chulasiri, Ph.D.)

Approved

.....
(Assistant Professor Kanungnit Pupatwibul, Ph.D.)
Dean of the Graduate School

May 2011

ACKNOWLEDGEMENTS

I am grateful to thank my thesis advisor, Professor Maitree Suttajit, for his invaluable guidance, supervision and constant inspiration throughout the study. Also, I am greatly thankful to my co-advisors, Associate Professor Supakorn Pongbangpho, Assistant Professor Sarin Sriprang, and Assistant Professor Wandee Wattanachaiyingcharoen.

My appreciation is conveyed to Naresuan University (NU), Chiangmai University (CMU), Thailand and University of North Texas and Health Science Center (UNTHSC), USA for their available scientific facilities. I wish to express my grateful thank to the Royal Golden Jubilee Scholarship under the Thailand Research Fund for their financial support throughout my Ph.D. Program study.

I deeply express my gratitude to Professor Robert Gracy, University of Texas at San Antonio, Texas, USA for his kind assistance, valuable discussion and encouragement. I owe my deeply gratitude to Dr.Yoji Ezure, CMU, for sacrificing his time and supporting me to develop an understanding of the research and manuscript writing. Professor Neeraj Agarwal, UNTHSC, Texas, USA is acknowledged for guiding me in cell culture.

I am sincerely grateful to Associate Professor Somdet Srichairatanakool, Dr. Udompan Kansuwan and other staffs in the Faculty of Medicine, CMU for their kind assistance and support my study. Furthermore, I would like to thank Assistant Professor Surisak Prasanpan, NU; Associate Professor Samlee Mankhertkorn, CMU; Associate Professor Dumrong Santiarworn, CMU; Associate Professor Chaiwat Chaiyasut, CMU; Associate Professor Surapote Wongyai, Rangsit University; Assistant Professor Nantarit Chokethaworn, Maejo University and Mr. Prasit Chanarat, CMU, for their kind supports and suggestions in a number of ways.

Unforgettable assistance and kind support by friends and persons including Dr. Nasapon Povichit, Dr. Prasert Rattana, Dr. Gannika Pumthong, Dr. Pakritsadang Keawsuriya, Dr. Maneesh Kumar, and many others are very much appreciated.

The last but not the least, I would like to thank my family for supporting me spiritually throughout my life.

Puksiri Sinchaiyakit

Title	ANTIOXIDANT AND RELATING BIOLOGICAL ACTIVITIES OF FLAVONOIDS EXTRACTED FROM SEED HUSK OF Tamarindus indica Linn.
Author	Puksiri Sinchaiyakit
Advisor	Professor Maitree Suttajit, Ph.D.
Co - Advisor	Associate Professor Supakorn Pongbangpho, Ph.D. Assistant Professor Sarin Sriprang, Ph.D. Assistant Professor Wandee Wattanachaiyingcharoen, Ph.D.
Academic Paper	Thesis Ph.D. in Biological Science, Naresuan University, 2010
Keywords	Antioxidant, tamarind seed husk, tannins, polymeric proanthocyanidins, flavonoids

ABSTRACT

Free radicals from endogenous and exogenous sources have demonstrated to be implicated in many diseases. Flavonoids, which have high antioxidant activity, are attractive and increasingly receiving interest as nutritional compounds suitable for promoting human health. In the present study, it was aimed to investigate the chemistry of antioxidant flavonoids in tamarind (*Tamarind indica* Linn.) seed husk (TaSH) and their antioxidant activities and biological functions.

The TaSH extract with acetone/water (7:3, v/v), called TA, has potent antioxidant activity by scavenging free radicals, inhibiting free radical formation, enhancing autoxidation of ferrous ion and chelating ferric ion. TA was characterized by qualitative chemical tests, TLC, HPLC, UV and IR spectrophotometry.

The small molecular weight phenolic compounds (aTAQ) including flavonoids in TA prepared by fractionating with acetone using silica gel quick column chromatography could prevent hemolysis induced by hydrogen peroxide (H_2O_2). The protective effects of aTAQ on erythrocytes were by inhibiting the reactive oxygen species generation, methemoglogin formation, and decrease in glutathione level, which were caused by H_2O_2. aTAQ, also protected the plasmid DNA damage induced by both H_2O_2 and ferrous ion (Fe^{2+}) in both single-strand and double-strand breakage.

Very high content (more than 39%) of polymeric proanthocyanidins in TaSH was confirmed. And the crude ethanolic extract of TaSH (TE) with high content of polymeric proanthocyanidins, about 94%, was prepared from TaSH by one pot extraction using ethanol/water (3:2, v/v) at 60 °C. This extraction method will be a very useful method for manufacturing the polymeric proanthocyanidins products.

The crude TE was further purified with Sephadex LH20 to give the fraction called aTES. From the structural analysis of aTES, the main compounds in TaSH were found to belong to condensed tannins (polymeric proanthocyanidins). The condensed tannins in TaSH did not contain a galloyl group. The ratio of procyanidins to prodelphinidins was 2:3, and the average degree of polymerization of aTES was 7. The antioxidant activities using DPPH and ABTS assays were investigated. The IC_{50} values of aTES were 4.2±0.2 (DPPH assay) and 6.2±0.3 μg/mL (ABTS assay).

It is concluded that the antioxidative flavonoids highly contained in tamarind seed husk were chemically proved to be polymeric proanthocyanidins. Their antioxidant activities and biological functions are closely comparable with those of grape seed extract especially in the prevention of hemolysis of red blood cells and the protection of DNA oxidative breakage induced by H_2O_2. Therefore, TaSH should be used as an alternative source for proanthocyanidins and could be further developed for a diet supplement as the same as grape seed extract.

LIST OF CONTENT

LIST OF CONTENT (CONT.)

LIST OF CONTENT (CONT.)

Chapter **Page**

LIST OF TABLES

LIST OF TABLES (CONT.)

LIST OF FIGURES

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

ABBREVIATION

AAPH	=	2,2'-azobis (2-amidinopropane) hydrochloride
a.u.	=	Arbitrary units
Abs	=	Absorbance
ABTS	=	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
BHT	=	Butylated hydroxyl toluene
°C	=	Degree of Celsius
DCFDA	=	2',7'-dichlorofluorescein diacetate
DCFH	=	dichlorofluorescin
DTNB	=	5.5'-dithiobis (2-nitrobenzoic acid)
DPPH	=	1,1-diphenyl-2-picrylhydrazyl radicals
DMEM	=	Dulbecco's modified Eaggle's medium
DMSO	=	Dimethyl sulphoxide
DNA	=	Deoxyribonucleic acid
ETC	=	Electron transport chain
Fe	=	Iron
g	=	Gram
G-6-PD	=	Glucose-6-phosphate dehydrogenase
GS	=	Lung cancer cells line
GSE	=	Grape seed extract from Akopharma
GE	=	Crude extract from grape seed by ethanol/water (3:2, v/v)
aGES	=	Acetone fraction of GE by Sephadex LH20 column chromatography.
GO	=	Glucose oxidase
GSH	=	Reduced glutathione
GSHPX	=	Glutathione peroxidase
GSSG	=	Glutatione disulfide (oxidized form)
Hb	=	Hemoglobin

ABBREVIATION (CONT.)

HEPES	=	(N-[2-hydroxyethyl]pirerazine-N′-[2-ethanesulfonic acid])
HPLC	=	High performance liquid chromatography
HT-22	=	The mouse hippocampal ganglion cells
IC_{50}	=	The half maximal inhibitory concentration
IR	=	Infrared
KS	=	Leukemia cells line
LDH	=	Lactate dehydrogenase
μg	=	Microgram
M	=	Molar
MDA	=	Malondialdehyde
metMb	=	Metmyoglobin
metHb	=	Methemoglobin
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mM	=	Millimolar
MTT	=	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide
NADH	=	Nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	=	Nicotinamide adenine dinucleotide phosphate
NADPH	=	Nicotinamide adenine dinucleotide phosphate (reduced form)
nd	=	Not detected
ND	=	Not determined
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NO	=	Nitric oxide
OD	=	Optical density
ON	=	Oxidation number of oxygen

ABBREVIATION (CONT.)

OPC	=	Oligomeric proanthocyanidins
PBS	=	Phosphate buffer saline
PA	=	Proanthocyanidins
PC	=	Procyanidins
PD	=	Prodelphinidins
PRPs	=	Salivary proline-rich protein
RBCs	=	Red blood cells
R_f	=	Retention factor
RGC-5	=	Cultures of retinal ganglion cells
RCS	=	Reactive chlorine species
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
rpm	=	Revolution per minute
S.E.	=	Standard error
sec	=	Second
S.D.	=	Standard deviation
SDS-PAGE	=	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOD	=	Superoxide dismutase
SPSS	=	Statistical package for the social sciences
SSA	=	5-sulfosalicylic acid solution
TBB	=	Tris borate buffer
TS	=	Tamarind seed
TaSH	=	Tamarind seed husk
TA	=	Crude extract from TaSH by acetone/water (7:3, v/v)
aTAS	=	Acetone fraction of TA by Sephadex LH20 column chromatography.

ABBREVIATION (CONT.)

eTAS	=	Ethanol fraction of TA by Sephadex LH20 column chromatography.
mTAS	=	Methanol fraction of TA by Sephadex LH20 column chromatography.
aTAQ	=	Acetone fraction of TA by quick silica gel column chromatography.
mTAQ	=	Methanol fraction of TA by quick silica gel column chromatography.
TE	=	Crude extract from tamarind seed husk by ethanol/water (3:2, v/v)
aTES	=	Acetone fraction of TE by Sephadex LH20 column chromatography.
mTES	=	Methanol fraction of TE by Sephadex LH20 column chromatography.
TBA	=	Thiobarbituric acid
TBARS	=	Thiobarbituric acid reactive substances
TBB	=	Tris borate buffer
TEAC	=	Trolox equivalent antioxidant capacity
TIFF	=	Tagged image file format
TLC	=	Thin layer chromatography
TNB	=	5-thio-2-nitrobenzoic acid
UV	=	Ultraviolet
VIS	=	Visible
v/v	=	Volume by volume
w/v	=	Weight by volume