



E46939

**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**

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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.


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ABSTRACT

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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 (H₂O₂). The protective effects of aTAQ on erythrocytes were by inhibiting the reactive oxygen species generation, methemoglobin formation, and decrease in glutathione level, which were caused by H₂O₂. aTAQ, also protected the plasmid DNA damage induced by both H₂O₂ and ferrous ion (Fe²⁺) 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 IC₅₀ 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 H₂O₂. 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.

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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	=	dichlorofluorescein
DTNB	=	5,5'-dithiobis (2-nitrobenzoic acid)
DPPH	=	1,1-diphenyl-2-picrylhydrazyl radicals
DMEM	=	Dulbecco's modified Eagle'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	=	Glutathione 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