

ฤทธิ์ฆ่าหอยเชอรี่ของสารซาโปนินจากผลมะคำดีควาย



นางสาวทัศนีย์วรรณ ฝ่ายสุน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเกษตรศาสตรมหาบัณฑิต

สาขาวิชาเกษตรศาสตร์ ภาควิชาเกษตรศาสตร์

คณะเกษตรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

MOLLUSCICIDAL ACTIVITY OF SAPONINS FROM
SAPINDUS RARAK FRUITS ON *POMACEA CANALICULATA*

Miss Tatsaneewan Faysoon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy

Department of Pharmacognosy

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2006

Copyright of chulalongkorn University

491461

Thesis Title MOLLUSCICIDAL ACTIVITY OF SAPONINS FROM
 SAPINDUS RARAK FRUITS ON *POMACEA*
 CANALICULATA
By Miss Tatsaneewan Faysoon
Field of Study Pharmacognosy
Thesis Advisor Associate Professor Chaiyo Chaichantipyuth, Ph.D.

Accepted by the Faculty of Pharmaceutical Science, Chulalongkorn University
in Partial Fulfillment of the Requirements for the Master's Degree

.....*Pornpen Pramyothin*.....Dean of Faculty of Pharmaceutical Science
(Associate Professor Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

K. Likhit
.....Chairman
(Associate Professor Kittisak Likhitwitayawuid, Ph.D.)

Chaiyo Chaichantipyuth
.....Thesis Advisor
(Associate Professor Chaiyo Chaichantipyuth, Ph.D.)

Amorn Petsom
.....Member
(Associate Professor Amorn Petsom, Ph.D.)

S. Amnuoypol
.....Member
(Associate Professor Surattana Amnuoypol, Ph.D.)

ทัศนีย์วรรณ ฝ่ายสุน : ฤทธิ์ฆ่าหอยเชอรี่ของสารซาโปนินจากผลมะคำดีควาย
(MOLLUSCICIDAL ACTIVITY OF SAPONINS FROM *SAPINDUS RARAK*
FRUITS ON *POMACEA CANALICULATA*) อาจารย์ที่ปรึกษา : รศ. ดร. ชัยโย
ชัยชาญทิพยุทธ, 136 หน้า

จากการศึกษาองค์ประกอบทางเคมีของผลมะคำดีควาย (*Sapindus rarak* DC.) สามารถสกัดแยกสารบริสุทธิ์จากสิ่งสกัดเมทานอลได้สารซาโปนินสองชนิด คือสาร Sapindoside B และ Mukurozi-saponin E₁ การพิสูจน์เอกลักษณ์และโครงสร้างทางเคมีของสารทั้งสองกระทำโดยการวิเคราะห์ข้อมูลทางสเปกโตรสโคปีจาก IR, MS, 1-D NMR และ 2-D NMR เมื่อนำสารประกอบที่แยกได้ทั้งสองมาทดสอบฤทธิ์ฆ่าหอยเชอรี่ (*Pomacea canaliculata*) พบว่าที่ระดับความเข้มข้น 7 มิลลิกรัมต่อลิตร ของสารทั้งสอง มีผลทำให้หอยเชอรี่ตาย 100 เปอร์เซ็นต์ ภายใน 72 ชั่วโมง โดยสาร Sapindoside B แสดงความเป็นพิษต่อหอยเชอรี่ที่ระดับความเข้มข้น LC₅₀ ที่ 24 ชั่วโมง มีค่าเท่ากับ 4.34 มิลลิกรัมต่อลิตร และสาร Mukurozi-saponin E₁ แสดงความเป็นพิษต่อหอยเชอรี่ที่ระดับความเข้มข้น LC₅₀ ที่ 24 ชั่วโมง มีค่าเท่ากับ 4.28 มิลลิกรัมต่อลิตร และเมื่อนำสารประกอบที่แยกได้ทั้งสองมาทดสอบการยับยั้งเซลล์มะเร็งพบว่าสารทั้งสองมีฤทธิ์ปานกลางในการยับยั้งเซลล์มะเร็งกระเพาะอาหาร (KATO-3) โดยมีค่า IC₅₀ ที่ 5.55 และ 6.17 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ

ภาควิชา.....เกษตรศาสตร์..... ลายมือชื่อนิสิต.....ทัศนีย์วรรณ ฝ่ายสุน.....
สาขาวิชา.....เกษตรศาสตร์..... ลายมือชื่ออาจารย์ที่ปรึกษา.....ชัยโย ชัยชาญทิพยุทธ.....
ปีการศึกษา.....2549.....

467 65632 33 : MAJOR PHARMACOGNOSY

KEYWORDS : MOLLUSCICIDAL/ SAPONIN/ *SAPINDUS RARAK*/
POMACEA CANALICULATA

TATSANEEWAN FAYSOON : MOLLUSCICIDAL ACTIVITY OF
SAPONINS FROM *SAPINDUS RARAK* FRUITS ON *POMACEA
CANALICULATA*. THESIS ADVISOR: ASSOC. PROF. CHAIYO
CHAICHANTIPYUTH, Ph.D., 136 pp.

The chemical and biological study of a methanolic extract prepared from the fruits of *Sapindus rarak* DC. led to the isolation of sapindoside B and mukurozi-saponin E₁. Their structures were determined by interpretation of spectroscopic data (IR, MS, 1-D NMR and 2-D NMR). Sapindoside B and mukurozi-saponin E₁ showed the molluscicidal activity with 100 % mortality at 7 ppm against *Pomacea canaliculata* after treating 72 h. and showed the LC₅₀ values at 4.34 and 4.28 ppm, respectively after treating 24 h. The MTT colorimetric assay for cytotoxic activity of Sapindoside B and mukurozi-saponin E₁ showed moderate cytotoxicity against human gastric carcinoma (KATO-3) activity level with IC₅₀ values of 5.55 and 6.17 µg/ml, respectively.

Department of..Pharmacognosy.. Student's signature...*นิตานันท์ อายสุ*.....
Field of study...Pharmacognosy.. Advisor's signature...*ชโย ไชยคุณ*.....
Academic year.....2006.....

ACKNOWLEDGEMENTS

The author wishes to express her deepest gratitude to her thesis advisor, Associate Professor Dr. Chaiyo Chaichantipyuth, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his guidance, suggestions, encouragement and support throughout the course of this study.

I would like to thank Associate Professor Dr. Kittisak Likhitwitayawuid, Associate Professor Dr. Amorn Petsom, Associate Professor Dr. Surattana Amnuoyopol for their valuable suggestions and comments as committee members.

In addition, I wish to thank the Institute of Biotechnology and Genetic Engineering for all facilities and grant. Appreciation is also extended to the Graduate School of Chulalongkorn University for granting partial support to conduct this research.

A large debt of gratitude is owed to the author's teachers, friends, and all the staff members of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, who kindly offered their assistance, encouragement, and helpful comments throughout this research. Although she has received the generosity of too many of them to list individually in this page, her appreciation is beyond words.

CONTENTS

	PAGE
ABSTRACT (Thai).....	iv
ABSTRACT (English).....	v
ACKNOWLEDGEMENTS	vi
CONTENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xv
LIST OF SCHEME.....	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER	
I INTRODUCTION	
1. <i>Sapindus rarak</i> DC.	
1.1 General characterization of <i>Sapindus rarak</i> DC.....	1
1.2 The classification of <i>Sapindus rarak</i> DC.....	2
2. Saponins	
2.1 General introduction.....	3
2.2 Definitions.....	3
3. <i>Pumacea canaliculata</i> Lamarck.	
3.1 Taxonomy of the golden apple snails.....	5
3.2 General characteristics of the golden apple snails.....	6
3.3 The life cycle of golden apple	10
4. State of problem	12
5. Objectives of study.....	14
6. Scope of study.....	14

	PAGE
CHAPTER	
II. HISTORICAL	
1. Chemical constituents of genus <i>Sapindus</i>	15
2. Literature Review	
2.1 Literature review on molluscicidal activity of saponins.....	32
III EXPERIMENTAL	
1. Source of Plant Material.....	34
2. General Techniques	
2.1 Analytical Thin Layer Chromatography (TLC).....	34
2.2 Column Chromatography (CC)	
2.2.1 Quick Column Chromatography.....	35
2.2.2 Conventional Column Chromatography.....	35
2.2.3 Column Chromatography on Diaion HP-20.....	36
2.3 Spectroscopic Techniques	
2.3.1 Infrared (IR) Absorption Spectra.....	36
2.3.2 Mass Spectra (MS).....	37
2.3.3 Nuclear Magnetic Resonance Spectrometer.....	37
2.4 Physical Property Measurement Apparatus	
2.4.1 Melting Points.....	37
2.4.2 Optical Rotations.....	37
2.5 Solvents.....	38
2.6 Chemical test for detection.....	38

	PAGE
CHAPTER	
3. Molluscicidal activity testing	
3.1 Materials	
3.1.1 Tested animals.....	38
3.1.2 Chemicals.....	38
3.2 Methods	
3.2.1 Preparation of tested snails.....	39
3.2.2 Molluscicidal test.....	39
3.2.3 Statistical Procedure.....	40
4. Extraction and Isolation	
4.1 Extraction.....	41
4.2 Isolation.....	42
4.3 Physical and spectral data of the isolated Compounds Sp1 and Sp2	
4.3.1 Compound Sp1.....	57
4.3.2 Compound Sp1.....	57
5. Biological activity test	
5.1 Cytotoxicity test.....	58
IV RESULTS AND DISCUSSION	
1. Structure determination of the isolated compounds	
1.1 Structure determination of compound Sp1.....	60
1.2 Structure determination of compound Sp2.....	69
2. Results of Molluscicidal Activity.....	83
3. Results of Cytotoxicity.....	85

	PAGE
CHAPTER	
V CONCLUSION.....	87
REFERENCES	88
APPENDICES.....	94
VITA.....	136

LIST OF TABLES

TABLE		PAGE
1	The chemical constituents of <i>Sapindus mukorossi</i>	15
2	The chemical constituents of <i>Sapindus emarginatus</i>	16
3	The chemical constituents of <i>Sapindus trifoliatus</i>	17
4	The chemical constituents of <i>Sapindus saponaria</i>	17
5	The chemical constituents of <i>Sapindus delavayi</i>	18
6	The results of molluscicidal activity testing against <i>P. canaliculata</i> at 24 hours intervals of methanol extract and chemical controls.....	41
7	The partition of methanol extract.....	42
8	The results of molluscicidal activity testing of chloroform layer and aqueous layer against <i>P. canaliculata</i> at 24 hours intervals.....	43
9	Combination of fractions from aqueous layer.....	44
10	The results of molluscicidal activity testing of fraction A-1-A-4 against <i>P. canaliculata</i> at 24 hours intervals.....	45
11	Combination of fractions from A-1.....	46
12	The results of molluscicidal activity testing of fraction B-1-B-6 against <i>P. canaliculata</i> at 24 hours intervals.....	47
13	Combination of fractions from B-6.....	48
14	The results of molluscicidal activity testing of fraction C-1-C-4 against <i>P. canaliculata</i> at 24 hours intervals.....	49
15	Combination of fractions from C-1.....	50

TABLE	PAGE
16	The results of molluscicidal activity testing of fraction D-1-D-4 against <i>P. canaliculata</i> at 24 hours intervals.....51
17	Combination of fractions from D-4.....52
18	The results of Molluscicidal activity testing of compound Sp1 and compound Sp2 against <i>P. canaliculata</i> at 24 hours intervals.....52
19	The FT-IR absorption band assignments of Compound Sp1.....61
20	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp1 and Sapindoside B.....64
21	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp1 and Sapindoside B (Cont.).....65
22	¹³ C- NMR Spectral Data of the Sugar part of Compound Sp1 and Sapindoside B.....66
23	¹³ C and ¹ H- NMR spectral data of the Compound Sp1.....67
24	¹³ C and ¹ H- NMR spectral data of the Compound Sp1 (Cont.).....68
25	The FT-IR absorption band assignments of Compound Sp2.....69
26	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp2 and Mukurozi-saponin E ₁73
27	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp2 and Mukurozi-saponin E ₁ (Cont.).....74

TABLE	PAGE
28	¹³ C- NMR Spectral Data of the Sugar part of Compound Sp2 and Mukurozi-saponin E ₁75
29	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp1 and Compound Sp2.....76
30	¹³ C- NMR Spectral Data of the Aglycone part of Compound Sp1 and Compound Sp2 (Cont.)..... 77
31	¹³ C- NMR Spectral Data of the Sugar part of Compound Sp1 and Compound Sp2 (Cont.).....78
32	¹³ C and ¹ H-NMR spectral data of the Compound Sp2.....79
33	¹³ C and ¹ H-NMR spectral data of the Compound Sp2 (Cont.).....80
34	¹ H- NMR spectral data of the Compound Sp1 and Compound Sp2.....81
35	¹ H- NMR spectral data of the Compound Sp1 and Compound Sp2 (Cont.).....82
36	The results of molluscicidal activity testing against <i>P. canaliculata</i> at 24 hours intervals of methanol extract, compound Sp1, compound Sp2 and chemical controls.....83
37	Cytotoxicity data of the saponins from <i>Sapindus rarak</i>85
38	The results of molluscicidal activity testing of methanol extract against <i>P. canaliculata</i> at 24 hours intervals.....122
39	The results of molluscicidal activity testing of aqueous layer against <i>P. canaliculata</i> at 24 hours intervals.....123
40	The results of molluscicidal activity testing of fraction A-1 against <i>P. canaliculata</i> at 24 hours intervals.....124

LIST OF FIGURES

FIGURES	PAGE
1	Skeletal types of genin found in the three principal classes of saponin..... 4
2	Monodesmosidic and bidesmosidic saponins.....5
3	The structure of the shell of <i>P.canaliculata</i> (Lamarck.).....7
4	The structure of <i>P. canaliculata</i> shell and operculum.....7
5	The structure of the mentle of <i>Pomacea canaliculata</i>8
6	The difference of shell of male and female <i>P.canaliculata</i>9
7	The life cycle of golden apple snails.....10
8	Structure of compounds previously isolated from genus <i>Sapindus</i>19
9	Structure of compound Sp1(Sapindoside B).....63
10	Structure of compound Sp2(Mukurozi- saponin E ₁).....72
11	The IR spectrum of Compound Sp1.....96
12	The EI-MS spectrum of Compound Sp1.....97
13	The 400 MHz ¹ H - NMR spectrum of Compound Sp1.....98
14	The expansion of ¹ H - NMR spectrum of Compound Sp1.....99
15	The expansion of ¹ H - NMR spectrum of Compound Sp1.....100
16	The expansion of ¹ H - NMR spectrum of Compound Sp1.....101
17	The 100 MHz ¹³ C - NMR spectrum of Compound Sp1.....102
18	The 400 MHz HMBC spectrum of Compound Sp1.....103
19	The expansion of HMBC spectrum of Compound Sp1.....104
20	The expansion of HMBC spectrum of Compound Sp1.....105
21	The 400 MHz HMQC spectrum of Compound Sp1.....106
22	The IR spectrum of Compound Sp2.....107

FIGURES	PAGE
23 The EI-MS spectrum of Compound Sp2.....	108
24 The 400 MHz ^1H -NMR spectrum of Compound Sp2.....	109
25 The expansion of ^1H -NMR spectrum of Compound Sp2.....	110
26 The expansion of ^1H -NMR spectrum of Compound Sp2.....	111
27 The expansion of ^1H -NMR spectrum of Compound Sp2.....	112
28 The expansion of ^1H -NMR spectrum of Compound Sp2.....	113
29 The 100 MHz ^{13}C -NMR spectrum of Compound Sp2.....	114
30 The 400 MHz HMBC spectrum of Compound Sp1.....	115
31 The expansion of HMBC spectrum of Compound Sp2.....	116
32 The expansion of HMBC spectrum of Compound Sp2.....	117
33 The expansion of HMBC spectrum of Compound Sp2.....	118
34 The 400 MHz HMQC spectrum of Compound Sp2.....	119
35 The 400 MHz ^1H - ^1H COSY spectrum of Compound Sp2.....	120

LIST OF SCHEME

SCHEME	PAGE
1 Extraction and isolation of the fruits from <i>Sapindus rarak</i>	54
2 Extraction and isolation of the fruits from <i>Sapindus rarak</i> (Cont.).....	55
3 Extraction and isolation of the fruits from <i>Sapindus rarak</i> (Cont.).....	56

LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	Acetic acid
br	Broad (for NMR spectral data)
c	Concentration
°C	Degree Celcius
CDCl ₃	Deuterated chloroform
CH ₃	Methyl
CHCl ₃	Chloroform
CH ₃ OH	Methanol
cm	Centimeter
cm ⁻¹	Reciprocal centimeter (unit of wave number)
¹³ C- NMR	Carbon-13-Nuclear Magnetic Resonance
CU1	CU-one (Tea seed powder contained 12 % Saponins)
d	Doublet (for NMR spectral data)
dd	Doublet of doublets (for NMR spectral data)
DDI	Double de-ionized water
2D	Two Dimensional
DEPT	Distortionless Enhancement by polarization Transfer
EIMS	Electron Impact Mass Spectroscopy
EtOAc	Ethyl Acetate
FT-IR	Fourier Transform Infrared spectrophotometer
g	Gram
¹ H- ¹ H COSY	Homonuclear (Proton-Proton) Correlation Spectroscopy

HMBC	¹ H-detected Heteronuclear Multiple Bond Coherence
KBr	Potassium bromide
¹ H-NMR	Proton Nuclear Magnetic Resonance
Hz	Hertz
<i>J</i>	Coupling constant
KBr	Potassium bromide
Kg	Kilogram
L	Litre
LC ₀	0 % lethality concentration
LC ₁₀₀	100 % lethality concentration
LC ₅₀	500 % lethality concentration
LC ₉₀	900 % lethality concentration
M	Multiplet (for NMR spectral data)
MeOH	Methanol
mg	Milligram
MHz	Mega-hertz
ml	Millilitre
mm	Millimetre
MS	Mass spectrometry
m/z	Mass to charge ratio
n-BuOH	Normal butanol
nm	Nanometer
NMR	Nuclear Magnetic Resonance Spectrometer
NOESY	Nuclear Overhauser Enhancement Spectroscopy

ODS	Octadodecyl
ppm	Part per million
q	Quartet (for NMR spectral data)
R _f	Retardation factor
RSD	Relative standard deviation
S	Singlet (for NMR spectral data)
t ½	Time half life
t	Triplet (for NMR spectral data)
t	Tonnes
Tig	Tigloyl
TLC	Thin layer chromatography
UV	Ultraviolet
w/w	Weight by weight
WHO	World health organization
δ	Chemical Shift
ε	Molar absorption
[α] ²⁹ _D	Specific Rotation at 29 °C on Sodium D line (nm)