

สารเคมีเพื่อการเกษตรจากดอกลำพอง *Datura metel* Linn.

นางสาวสิริจันทร์ พัฒนพงศ์สิริกุล

# ศูนย์วิทยทรัพยากร อุดมสตรีมหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตร์มหาบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2545

ISBN 974-17-9868-7

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

AGROCHEMICALS FROM THE FLOWERS OF *Datura metel* Linn.

Miss Sirichan Pattanapongsirikul

ศูนย์วิทยทรัพยากร  
อุบลราชธานีมหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2002

ISBN 974-17-9868-7

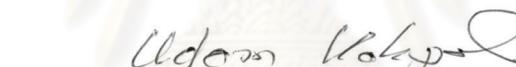
Thesis Title AGROCHEMICALS FROM THE FLOWERS OF *Datura metel*  
Linn.  
By Miss Sirichan Pattanapongsirikul  
Field of Study Chemistry  
Thesis Advisor Assistant Professor Warinthorn Chavasiri, Ph.D.

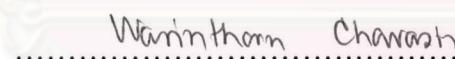
---

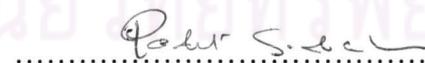
Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment  
of the Requirements for the Master's Degree

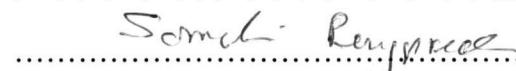
 ..... Dean of Faculty of Science  
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

THESIS COMMITTEE

 ..... Chairman  
(Professor Udom Kokpol, Ph.D.)

 ..... Thesis Advisor  
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

 ..... Member  
(Professor Padet Sidisunthorn, Ph.D.)

 ..... Member  
(Associate Professor Somchai Pengprecha, Ph.D.)

สิริจันทร์ พัฒนพงศ์สิริกุล : สารเคมีเพื่อการเกษตรจากดอกลำโพง *Datura metel* Linn. (AGROCHEMICALS FROM THE FLOWERS OF *Datura metel* Linn.) อ. ที่ปรึกษา: พศ. ดร. วринทร ชวนศิริ, 122 หน้า. ISBN 974-17-9868-7.

งานวิจัยนี้เป็นการรายงานการศึกษาองค์ประกอบทางเคมีของดอกลำโพงและฤทธิ์ทางชีวภาพทางการเกษตรเป็นครั้งแรก จากการทดสอบฤทธิ์ทางชีวภาพเบื้องต้นพบว่าสิ่งสกัดอัลคาลอยด์แสดงความเป็นพิษต่อหอยเชอร์ *Pomacea canaliculata* Lamark. นอกจากนี้พบว่า สิ่งสกัด เอทิลแอลกอฮอล์แสดงความเป็นพิษต่อไรสีน้ำตาล *Artemia salina* Linn. ในระดับปานกลางและมีฤทธิ์ยับยั้งการเจริญเติบโตของเมล็ดผักกาดหอม *Lactuca sativa* Linn. เมื่อแยกสารจากส่วนที่แสดงฤทธิ์ทั้งสองได้สาร 9 ตัว โดยอาศัยสมบัติทางเคมี ปฏิกิริยาเคมี และข้อมูลทางสเปกโตรสโคปีพบว่า โครงสร้างสารที่แยกได้แก่ ของผสมของเอสเทอโร่ โซ่ต่าง ของผสมสเตอรอยด์ เอสเทอโร่ ของผสม สเตอรอยด์ kaempferol scopolamine tropine aposcopolamine และอนุพันธ์ของ scopolamine 2 ตัว ผลการศึกษาฤทธิ์ทางชีวภาพชี้ว่า kaempferol แสดงฤทธิ์ความเป็นพิษต่อไรสีน้ำตาล ในระดับต่ำ scopolamine มีฤทธิ์ฆ่าหอยเชอร์และแสดงฤทธิ์ยับยั้งการออกของรากเมล็ดผักกาดหอมในระดับสูง และบังพบว่า tropine ออกฤทธิ์ฆ่าหนอนกระทุก *Spodoptera litura* ในระดับสูง.

# ศูนย์วิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา.....	เคมี.....	ลายมือชื่อนิสิต.....
สาขาวิชา.....	เคมี.....	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา.....	2545.....	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

# # 4372446523 : MAJOR CHEMISTRY

KEY WORD: *Datura metel* Linn/ Molluscicidal activity/ *Pomacea canaliculata* Lamark.

SIRICHAN PATTANAPONGSIRIKUL: AGROCHEMICALS FROM THE FLOWERS  
OF *Datura metel* LINN. THESIS ADVISOR: ASST. PROF. WARINTHORN  
CHAVASIRI, Ph.D., 122 pp. ISBN 974-17-9868-7.

This work was the first report on agricultural-based activity of the flowers of *Datura metel* Linn. From the preliminary biological screening test, the alkaloid crude extract revealed toxicity against *Pomacea canaliculata* Lamark. (golden apple snail). In addition, ethyl acetate crude extract displayed medium cytotoxicity against brine shrimp *Artemia salina* Linn. and plant growth inhibition against *Lactuca sativa*. The separation of two active fractions led to the isolation of nine substances. By means of physical properties, chemical reactions and spectroscopic evidences, the structures of four mixtures and four compounds could be deduced as a mixture of long chain esters, a mixture of steroid ester, a mixture of steroids, kaempferol, scopolamine, tropine, aposcopolamine, and two derivatives of scopolamine. The bioassay results indicated that kaempferol exhibited low cytotoxicity against brine shrimp. Moreover, scopolamine displayed the molluscicidal activity against golden apple snail and high root growth inhibition on *L. sativa*. In addition, tropine displayed insecticidal activity against *Spodoptera litura*.

# ศูนย์วิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

Department.....Chemistry.....Student's signature.....*Sirichan Pattanapongsirikul*  
 Field of study.....Chemistry.....Advisor's signature.....*Warin Mom Chavasiri*  
 Academic year.....2002.....Co-advisor's signature.....-

## **ACKNOWLEDGEMENT**

Firstly, the author would like to acknowledge all professors who taught her at the Department of Chemistry, Faculty of Science, Chulalongkorn University, especially to those in the Organic Chemistry division.

Secondary, the author would like to express her deepest gratitude to her advisor, Assistant Professor Dr. Warinthorn Chavasiri for his great advice, kind assistance, and encouragement throughout the course of this research. In addition, she is very grateful to Professor Dr. Udom Kokpol, Professor Dr. Padet Sidisunthorn, and Associate Professor Somchai Pengprecha, serving as the chairman and members of her thesis committee, respectively for their comments and suggestions. She would like to express her appreciation to Associate Professor Dr. Kingkaew Wattanasermkit Department of Biology, Faculty of Science, Chulalongkorn University for providing facilities and advice in brine shrimp lethality test. She would also like to express her special thanks to Assistant Professor Dr. Tirayut Vilaivan for performing 2D NMR spectra.

Thirdly, she wishes to express many thanks to the Graduate School for financial support as a part of this research work. Special thanks are acknowledged to Natural Products Research Unit for permission to use some equipment and instrument.

Finally, the author would also like to express her deepest gratitude to her parents and family members for their love, understanding, encouragement and advice throughout the course of her education. Moreover, she would like to thank her friends and members of Natural Products Research Unit for their friendship and help during her graduate study.

## CONTENTS

	<b>Pages</b>
Abstract in Thai.....	iv
Abstract in English.....	v
Acknowledgement.....	vi
List of Figures .....	x
List of Tables.....	xi
List of Schemes .....	xii
List of Abbreviations.....	xiii
<b>CHAPTER</b>	
1. INTRODUCTION.....	1
1.1 General characteristic of <i>D. metel</i> L. ....	3
1.2 Chemical constituents studies from <i>D. metel</i> L. ....	5
1.3 Biological activity study of <i>D. metel</i> L .....	11
1.4 The objective of this research .....	12
2. EXPERIMENTAL .....	13
2.1 Plant materials.....	13
2.2 Instruments and equipment .....	13
2.3 Chemical reagents .....	14
2.4 Chemical tests .....	14
2.5 Extraction procedure .....	14
2.6 Chemical reaction.....	15
2.7 Biological screening assay .....	16
Brine shrimp cytotoxic lethality test .....	16
Plant growth inhibition on <i>Lactuca sativa</i> L.....	16
Molluscicidal activity against <i>P. canaliculata</i> .....	17
Insecticidal activity by vial test contact toxicity .....	18
3. RESULTS AND DISCUSSION .....	19
3.1 The results of extraction.....	19
3.2 The results of biological activity screening test.....	20

## Contents (Continued)

	<b>Pages</b>
3.2.1 Brine shrimp cytotoxicity test .....	20
3.2.2 Plant growth inhibition on <i>Lactuca sativa</i> Linn.....	20
3.2.3 Molluscicidal activity.....	22
3.3 The results of the separation of crude extracts.....	23
3.3.1 Separation of ethyl acetate crude extract .....	23
3.3.2 Separation of alkaloid fraction.....	26
3.4 Purification, properties and structural elucidation of isolated substances .....	27
3.4.1 Purification, properties and structural elucidation of Mixture 1 .....	27
3.4.2 Purification, properties and structural elucidation of Mixture 2 .....	30
3.4.3 Purification, properties and structural elucidation of Mixture 3 .....	31
3.4.4 Purification, properties and structural elucidation of Compound 1.....	32
3.4.5 Purification, properties and structural elucidation of Compound 2.....	37
3.4.6 Purification, properties and structural elucidation of Compound 3.....	41
3.4.7 Properties and structural elucidation of Compound 4.....	45
3.4.8 Properties and structural elucidation of Compound 5.....	45
3.4.9 Properties and structural elucidation of Mixture 4.....	50
3.5 The results of biological activities test of isolated substances.....	57
3.5.1 The results of brine shrimp cytotoxicity test of isolated substances.....	57
3.5.2 The results of plant growth inhibition on <i>Lactuca sativa</i> L. of isolated substances.....	58

## Contents (Continued)

	Pages
3.5.3 The results of molluscicidal activity against <i>P. canaliculata</i> of isolated substances .....	60
3.5.4 The results of insecticidal activity on <i>Spodotera litura</i> of some isolated compounds.....	61
<b>4. CONCLUSION.....</b>	<b>64</b>
Proposal for future work .....	70
<b>REFERENCES.....</b>	<b>72</b>
<b>APPENDICES.....</b>	<b>77</b>
<b>VITA .....</b>	<b>122</b>


  
**ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย**

### List of Figures

Figures	Pages
1.1 Flower and stem (a) and Flower (b) of <i>D. metel</i> .....	5
1.2 Alkaloids found in <i>D. metel</i> .....	6
1.3 Steroids found in <i>D. metel</i> .....	7
1.4 Flavonoid found in <i>D. metel</i> .....	10
3.1 The effect of crude extracts of <i>D. metel</i> on A) the root growth of <i>Lactuca sativa</i> Linn. B) the shoot growth of <i>Lactuca sativa</i> Linn.....	21
3.2 The results of molluscicidal activity test.....	22
3.3 Chromatogram of Mixture 1a .....	28
3.4 HPLC chromatogram of Fraction AC1-3 .....	44
3.5 The result of isolated substances on A) root growth inhibition and B) shoot growth inhibition against <i>L. sativa</i> .....	59
3.6 Mortality of <i>P. canaliculata</i> at 72 hours .....	61
3.7 The mortality of neonate larvae of <i>S. litura</i> .....	62

ศูนย์วิทยบริการ  
จุฬาลงกรณ์มหาวิทยาลัย

## List of Tables

Tables	Pages
1.1 The different characteristics of three species in <i>Datura</i> spp.....	4
1.2 Alkaloids found in <i>D. metel</i> .....	5
3.1 The results of extraction.....	19
3.2 The results of brine shrimp cytotoxicity test.....	20
3.3 The effect of crude extracts of <i>D. metel</i> on the growth of <i>Lactuca sativa</i> L.	21
3.4 The results of molluscicidal activity test.....	22
3.5 The results of the separation of ethyl acetate crude extract (Fraction II)....	24
3.6 The results of the separation of Fraction EC1 .....	25
3.7 The results of the separation of the alkaloid fraction (Fraction III).....	26
3.8 The composition of steroids in Mixture 1a.....	29
3.9 The composition of steroids in Mixture 3.....	31
3.10 The comparison of $^1\text{H}$ and $^{13}\text{C}$ chemical shift assignments of Compound 1 with kaempferol .....	34
3.11 The $^1\text{H}$ and $^{13}\text{C}$ NMR chemical shift assignments of Compound 2 and reported scopolamine .....	39
3.12 The $^1\text{H}$ and $^{13}\text{C}$ NMR chemical shift assignments of Compound 3 and reported tropine .....	42
3.13 The $^1\text{H}$ and $^{13}\text{C}$ NMR chemical shift assignments of Compound 2 and 5 ....	48
3.14 The $^1\text{H}$ and $^{13}\text{C}$ NMR data of Component 1 of Mixture 4 .....	53
3.15 The $^1\text{H}$ and $^{13}\text{C}$ NMR data of Component 2 of Mixture 4 .....	55
3.16 The brine shrimp cytotoxicity test of isolated substances.....	57
3.17 Plant growth inhibition test of isolated substances .....	58
3.18 Mortality of <i>P. canaliculata</i> at 72 hours .....	60
3.19 Mortality of neonate larvae of <i>S. litura</i> .....	62
3.20 The LD <sub>50</sub> of selected compounds against <i>S. litura</i> by contact toxicity vial test.....	63
4.1 Isolated substances from the ethyl acetate extract of <i>D. metel</i> flowers .....	65
4.2 Isolated substances from alkaloid fraction of <i>D. metel</i> flowers .....	67

### List of Schemes

Schemes	Pages
2.1 The general extraction procedure.....	15
3.1 The summarized separation of ethyl acetate crude extract .....	25
3.2 The separation of alkaloid fraction .....	27
3.3 The proposed mass fragmentation pattern of Compound 1 .....	36
3.4 The possible mass fragmentation pattern of Compound 2.....	40
3.5 The mass fragmentation pattern of Compound 3.....	43
3.6 The possible mass fragmentation pattern of Compound 5.....	49
3.7 The proposed mass fragmentation pattern of Component 1 .....	54
3.8 The proposed mass fragmentation pattern of Component 2 .....	56


  
**ศูนย์วิทยทรัพยากร**  
**จุฬาลงกรณ์มหาวิทยาลัย**

### List of Abbreviations

b	broad (NMR)
°C	degree celsius
CDCl <sub>3</sub>	deuterated chloroform
CH <sub>2</sub> Cl <sub>2</sub>	dichloromethane, methylene chloride
CHCl <sub>3</sub>	chloroform
CIGAR	constant time inverse-detected gradient accordion rescaled long-rang heteronuclear multiple bond correlation
cm <sup>-1</sup>	unit of wavelength
d	doublet (NMR)
DEPT	the distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
DMSO-d <sub>6</sub>	deuterated dimethylsulfoxide
EtOAc	ethyl acetate
EtOH	ethanol
g	gram (s)
GC	gas chromatography
gCOSY	gradient correlation spectroscopy
gHSQC	gradient heteronuclear single quantum correlation
Hz	hertz
HPLC	high performance liquid chromatography
IR	infrared
J	coupling constant
kg	kilogram (s)
L	liter (s)
LC <sub>50</sub>	50% lethality concentration
LD <sub>50</sub>	50% lethality dose
m	multiplet (NMR)
M <sup>+</sup>	molecular ion
MeOH	methanol

### List of abbreviations (continued)

mg	milligram (s)
mL	milliliter (s)
m.p.	melting point
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ratio
nm	nanometer
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
ppm	part per million
R <sub>f</sub>	retardation factor
s	singlet (NMR)
t	triplet (NMR)
TLC	thin layer chromatography
UV	ultraviolet
Vol.	volume
wt	weight
δ	unit of chemical shift
μg	microgram (s)