

องค์ประกอบทางเคมีจากใบพลู *Piper betle* Linn. และฤทธิ์ทางชีวภาพ



นางสาวน้ำฝน กูเจริญไพศาล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมี ภาคเคมี

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2540

ISBN 974-638-420-1

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

28 S.A. 2548

114076427

**CHEMICAL CONSTITUENTS FROM THE LEAVES OF
Piper betle Linn. AND THEIR BIOLOGICAL ACTIVITY**

Miss Numphon Koocharoenpisa

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

Department of Chemistry

Graduate School

Chulalongkorn University

Academic year 1997


ISBN 974-638-420-1

Thesis Title Chemical Constituents from the Leaves of *Piper betle* Linn.
 and their Biological Activity
By Miss Numphon Koocharoenpibal
Department Chemistry
Thesis Advisor Associate Professor Udom Kokpol, Ph.D.


Accepted by the Graduate School, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Master's Degree.



..... Dean of Graduate School
(Professor Supawat Chutivonges, M.D.)

Thesis Committee:


..... Chairman
(Associate Professor Siri Varothai, Ph.D.)


..... Thesis Advisor
(Associate Professor Udom Kokpol, Ph.D.)


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


..... Member
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

พิมพ์ครั้งที่ ๑ ฉบับนี้จัดทำขึ้นเพื่อจำหน่ายแก่สมาชิกของชมรมพฤกษศาสตร์แห่งประเทศไทย

นำเสน **ฤทธิ์ทางชีวภาพ : องค์ประกอบทางเคมีจากใบพลู *Piper betle* Linn. และฤทธิ์ทางชีวภาพ (CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Piper betle* Linn. AND THEIR BIOLOGICAL ACTIVITY) อ. ที่ปรึกษา : รศ. ดร. อุดม ก๊กผล; 211 หน้า. ISBN 974-638-420-1.**

ผลการทดสอบฤทธิ์ทางชีวภาพเบื้องต้นของใบพลู *Piper betle* Linn. พบว่าถึงสกัดในเอทานอล แสดงความเป็นพิษต่อ brine shrimp (*Artemia salina* Linnaeus) ทำการแยกสิ่งสกัดเป็นส่วนๆพบว่า ส่วนที่ละลายในคลอโรฟอร์มแสดงฤทธิ์ทางชีวภาพดีที่สุด เมื่อทำการแยกสิ่งสกัดด้วยวิธีทางโครมาโทกราฟี สามารถแยกสารได้ 10 ชนิด โดยอาศัยสมบัติทางกายภาพ และ ข้อมูลทางสเปกโทรสโกปี สามารถพิสูจน์สูตรโครงสร้างของสารได้ คือ 1) สารผสมระหว่าง 2,6-dimethyl cyclohexanol และ 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone, 2) สารผสมแอลกอฮอล์โซ่ตรง ($C_{17} - C_{33}$), 3) สารผสมของเอสเตอร์โซ่ตรง, 4) lupeol, 5) สารผสมแอลกอฮอล์โซ่ตรง (C_{21}, C_{23}), 6) 2-propenyl hexanoate, 7) friedelan-3 β -ol, 8) chavibetol 9) สารผสมสเตอรอยด์ 3 ชนิด คือ campesterol, stigmasterol และ β -sitosterol และ 10) allylpyrocatechol สารทั้งหมดที่แยกได้ พบว่า chavibetol และ allylpyrocatechol แสดงความเป็นพิษต่อ brine shrimp (*Artemia salina* Linnaeus) ได้ดีที่สุดด้วยค่า LC_{50} 2.55 และ 16.36 $\mu\text{g/ml}$ ในเวลา 24 ชั่วโมง ตามลำดับ นอกจากนี้ในการแยกสิ่งสกัดบิวทานอล ยังพบสารอีก 6 กลุ่ม

ภาควิชา ๒๕๕
สาขาวิชา ๒๕๕
ปีการศึกษา ๒๕๕๐

ลายมือชื่อนิสิต นำเสน อุดม ก๊กผล
ลายมือชื่ออาจารย์ที่ปรึกษา อุดม ก๊กผล
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม —

C825232 : MAJOR CHEMISTRY

KEY WORD: *Piper betle* Linn. / CHEMICAL CONSTITUENTS / BIOLOGICAL ACTIVITY

NUMPHON KOOCHAROENPISAL : CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Piper betle* Linn. AND THEIR BIOLOGICAL ACTIVITY.

THESIS ADVISOR : ASSOC. PROF. UDOM KOKPOL ,Ph.D. 211 pp.

ISBN 974-638-420-1.

The preliminary bioassay results of the leaves of *Piper betle* Linn. indicated that the ethanol crude extract exhibited cytotoxicity activity against brine shrimp (*Artemia salina* Linnaeus). Ethanol crude extract was separated to eight fractions and its chloroform fraction revealed the most biological activity. Fractionation of crude chloroform extract using column chromatography led to the isolation of ten substances. By means of physical properties, and spectroscopic data, the structures of compounds were elucidated. They were found to be 1) a mixture of 2,6-dimethyl cyclohexanol and 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone, 2) a mixture of straight long chain alcohols (C₁₇-C₃₃), 3) a mixture of straight long chain esters, 4) lupeol, 5) a mixture of straight long chain alcohol (C₂₁, C₂₃), 6) 2-propenyl hexanoate, 7) friedelan-3β-ol, 8) chavibetol, 9) a mixture of steroids (campesterol, stigmasterol and β-sitosterol), and 10) allylpyrocatechol.

The bioassay results indicated that chavibetol and allylpyrocatechol exhibited cytotoxicity activity against brine shrimp (*Artemia salina* Linnaeus) with LC₅₀ values of 2.55 and 16.36 µg/ml at 24 hours, respectively. In addition, fractionation of crude butanol extract led to the isolation of six groups of substances.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา.....
สาขาวิชา.....
ปีการศึกษา.....

ลายมือชื่อผู้คิด.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

ACKNOWLEDGMENT

The author wishes to express her deep gratitude to her advisor Associate Professor Dr. Udom Kokpol, for his very kind assistance, generous guidance and encouragement throughout the course of this research. She is grateful to Dr. Kingkaew Wattanasermkit, Department of Biology, Chulalongkorn University, for brine shrimp bioassay experiments. Thanks are also extended to Assistant Professor Dr. Warinthorn Chavasiri, Associate Professor Gaysorn Veerachato and Dr. Santi Tip-pyang of the Natural Products Research Unit, Department of Chemistry, Chulalongkorn University for their helpful discussion and support.

Appreciations are also expressed to the Graduate School of Chulalongkorn University and the Natural Products Research Unit of Department of Chemistry of Chulalongkorn University for their financial support of this research work. In addition, she wishes to thank, Professor Dr. Padet Sidisunthorn, Associate Professor Dr. Siri Varothai, Associate Professor Dr. Warinthorn Chavasiri and Dr. Roderick Bates for their comments and suggestion.

Special thanks is given to collaborative partners at Beijing Medical School who kindly performed and provided a preliminary results of cytotoxicity tests against various cell lines.

A deep affectionate gratitude is acknowledged to her parents and friends for their love, understanding, encouragement and support throughout the entire study. Without them, the author would have never been able to achieve this goal.

จุฬาลงกรณ์มหาวิทยาลัย

CONTENTS

	page
Abstract in Thai.....	iv
Abstract in English.....	v
Acknowledgment	vi
List of Figures.....	x
List of Schemes.....	xiv
List of Tables.....	xv
List of Abbreviations.....	xviii
 CHAPTER	
I INTRODUCTION.....	1
1.1 Chemical constituents studies of <i>Piper</i> genus.....	3
1.2 Research in phamacology study and chemical constituents of <i>Piper betle</i> Linn.....	52
 II EXPERIMENTAL.....	 58
2.1 Plant materials.....	58
2.2 Instruments and equipment	58
2.3 Chemical reagents	59
2.4 Physical separation techniques.....	60
2.5 Extraction for peliminary sreening test.....	60
2.6 Bioassay experiments	62
- The inhibition effect for tumor cell line	62
- Brine shrimp (<i>Artemia salina</i> Linnaeus) cytotoxic lethality test.....	63

III RESULTS AND DISCUSSION	64
3.1 The result of extraction	64
The result of biological activity screening tests of the ethanol crude extract	
3.1.1 - Anticell line cytotoxicity test.....	64
3.1.2 - Brine Shrimp Cytotoxicity Test	66
3.2 Chemical constituents of the leaves of <i>Piper betle</i> Linn.	70
3.3 Separation of chloroform crude extract (Fraction I).....	72
3.4 Purification and properties of substances from <i>Piper betle</i> Linn.	79
3.4.1 Purification and properties of Mixture 1	79
3.4.2 Purification and properties of Mixture 2.....	80
3.4.3 Purification and properties of Mixture 3	81
3.4.4 Purification and properties of Compound 4	81
3.4.5 Purification and properties of Mixture 5	82
3.4.6 Purification and properties of Compound 6	83
3.4.7 Purification and properties of Compound 7	83
3.4.8 Purification and properties of Compound 8	84
3.4.9 Purification and properties of Compound 9	85
3.4.10 Purification and properties of Mixture 10	86
3.4.11 Purification and properties of Compound 11	87
3.5 Structural elucidation of the isolated substances from the leaves of <i>Piper betle</i> Linn.	88
3.5.1 Structural elucidation of Mixture 1	88
3.5.2 Structural elucidation of Mixture 2	90
3.5.3 Structural elucidation of Mixture 3	94
3.5.4 Structural elucidation of Compound 4	94
3.5.5 Structural elucidation of Mixture 5	100
3.5.6 Structural elucidation of Compound 6	102
3.5.7 Structural elucidation of Compound 7	103
3.5.8 Structural elucidation of Compound 8	107
3.5.9 Structural elucidation of Mixture 9	110

3.5.10 Structural elucidation of Mixture 10	117
3.5.11 Structural elucidation of Compound 11	118
3.6 Separation of <i>n</i> -butanol crude extract (Fraction II)	122
3.7 Purification, properties and structural elucidation for fractions of <i>n</i> -butanol crude extract	129
3.7.1 Purification, properties and structural elucidation of PBBU 1.....	129
3.7.2 Purification, properties and structural elucidation of PBBU 2	131
3.7.3 Purification, properties and structural elucidation of PBBU 3.....	132
3.7.4 Purification, properties and structural elucidation of PBBU 4.....	132
3.7.5 Purification, properties and structural elucidation of PBBU 5.....	133
3.7.6 Purification, properties and structural elucidation of PBBU 6.....	134
3.8 Study on biological activity of isolated compounds	135
3.9 Anticell line cytotoxicity test of isolated compounds.....	136
3.10 The results of brine shrimp (<i>Artemia salina</i> Linnaeus) cytotoxic lethality test of isolated compounds.....	139
IV CONCLUSION AND SUGGESTION FOR FUTURE WORKS.....	140
References	146
Appendices	157
Vita	211

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

Figures	Page
1. Leaves and stems of <i>Piper betle</i> Linn.....	2
2. The chemical constituents of some plants in <i>Piper</i> Genus.....	30
3. Some chemical constituents from <i>Piper betle</i> Linn.....	56
4. The IR spectrum of Mixture 1	158
5. The ¹ H NMR spectrum of Mixture 1	158
6. The ¹³ C NMR spectrum of Mixture 1	159
7. The GLC analysis results of Mixture 1	160
8. The mass spectrum of Mixture 1 at retention time 12.29 min	161
9. The mass spectrum of Mixture 1 at retention time 26.62 min	162
10. The mass spectrum of Mixture 1 at retention time 26.62 min	163
11. The IR spectrum of Mixture 2	164
12. The ¹ H NMR spectrum of Mixture 2	164
13. The ¹³ C NMR spectrum of Mixture 2	165
14. The GLC analysis results of Mixture 2	166
15. The GLC analysis results of standard long chain aliphatic alcohol (C= 14, 16, 18, 20 and 22).....	166
16. The calibration curve of standard long chain aliphatic alcohol.....	167
17. The IR spectrum of Mixture 3	168
18. The IR spectrum of Compound 4	168
19. The mass spectrum of Compound 4	169
20. The ¹ H NMR spectrum of Compound 4	170
21. The ¹³ C NMR spectrum of Compound 4	170
22. The DEPT 90 ¹³ C NMR spectrum of Compound 4	171
23. The DEPT 135 ¹³ C NMR spectrum of Compound 4	171
24. The GLC analysis results of Mixture 5 and standard long chain alcohol	172
25. The calibration curve of standard long chain aliphatic alcohol.....	173
26. The IR spectrum of Mixture 5	174

27. The IR spectrum of Compound 6	174
28. The ^1H NMR spectrum of Compound 6	175
29. The GLC analysis results of Compound 7	176
30. The mass spectrum of Compound 7	177
31. The mass spectrum of Compound 7	178
32. The IR spectrum of Compound 7	179
33. The mass spectrum of Compound 7	179
34. The ^1H NMR spectrum of Compound 7	180
35. The ^{13}C NMR spectrum of Compound 7	180
36. The DEPT 90 and DEPT 135 ^{13}C MNR spectrum of Compound 7.....	181
37. The IR spectrum of Compound 8	181
38. The mass spectrum of Compound 8	182
39. The ^1H NMR spectrum of Compound 8	184
40. The ^{13}C NMR spectrum of Compound 8	185
41. The DEPT 90 and DEPT 135 ^{13}C MNR spectrum of Compound 8.....	186
42. The IR spectrum of Mixture 9	187
43. The mass spectrum of Mixture 9	187
44. The ^1H NMR spectrum of Mixture 9	187
45. The ^{13}C NMR spectrum of Mixture 9	187
46. The DEPT 90 and DEPT 135 ^{13}C MNR spectrum of Mixture 9	189
47. The GLC analysis results of Mixture 9 and standard steroid (campesterol, β -sitosterol and stigmasterol).....	190
48. The ^1H NMR spectrum of Mixture 10	191
49. The ^{13}C NMR spectrum of Mixture 10	191
50. The GLC analysis results of Mixture 9 and standard steroid (campesterol, β -sitosterol and stigmasterol).....	192
51. The IR spectrum of Compound 11	193
52. The mass spectrum of Compound 11	194
53. The ^1H NMR spectrum of Compound 11	195
54. The ^{13}C NMR spectrum of Compound 11	195

55. The DEPT 90 and DEPT 135 ¹³ C MNR spectrum of Compound 11.....	196
56. The IR spectrum of PBBu 1.....	197
57. The ¹ H NMR spectrum of PBBu 1	198
58. The ¹³ C NMR spectrum of PBBu 1	198
59. The DEPT 90 and DEPT 135 ¹³ C MNR spectrum of PBBu 1.....	199
60. The mass spectrum of PBBu 1	200
61. The IR spectrum of PBBu 2	200
62. The GC-MS analysis results of PBBu 2	201
63. The mass spectrum of PBBu 2	201
64. The mass spectrum of PBBu 2	202
65. The IR spectrum of PBBu 3	202
66. The GC-MS analysis results of PBBu 3	203
67. The mass spectrum of a component in PBBu 3.....	204
68. The IR spectrum of PBBu 4	204
69. The GC-MS analysis results of PBBu 4	202
70. The mass spectrum of a component in PBBu 4 at Rt 23.43 min.....	205
71. The IR spectrum of PBBu 5	205
72. The GC-MS analysis results of PBBu 5	206
73. The mass spectrum of a component in PBBu 5 at Rt 13.00 min	206
74. The mass spectrum of a component in PBBu 5 at Rt 13.00 min and diethoxydimethyl silane.....	207
75. The mass spectrum of a component in PBBu 5 at Rt 23.67min.....	207
76. The mass spectrum of a component in PBBu 5 at Rt 26.97 min.....	208
77. The mass spectrum of a component in PBBu 5 at Rt 26.97 min and 2(3H)-benzothiazolone.....	209
78. The IR spectrum of PBBu 6	209
79. The GC-MS analysis results of PBBu 6	210
80. The mass spectrum of a component in PBBu 6 at Rt 35.15 min.....	210

LIST OF SCHEMES

Schemes	Page
1. Procedure for preparation of crude extract samples for screening of inhibitory activity of tumors cell in vitro and for the brine shrimp toxicity test	61
2. The procedure of brine shrimp cytotoxic lethality test.....	63
3. The procedure of extraction.....	71
4. The possible mass fragmentation pattern of Compound 4.....	97
5. Fragmentation of Compound 7.....	106
6. The possible mass fragmentation pattern of Mixture 9 for β -sitosterol (R= C ₂ H ₅), Campesterol (R= CH ₃) and stigmasterol.....	113

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Tables	Page
1. The chemical constituents of some plants in <i>Piper</i> genus	4
2. The chemical constituents of <i>Piper betle</i> Linn.....	55
3. The results of the preliminary screening of the ethanol crude extract, results of cytotoxicity againts cell line.....	65
4. The results of the preliminary screening of each crude extract of <i>Piper betle</i> Linn.(Anticell line Cytotoxicity Test).....	67
5. The results of brine shrimp cytotoxicity test.....	70
6. The results of separation of the crude chloroform extract by column chromatography	73
7. The results of the separation of the eluted fractions No. 8-14 by silica gel column chromatography	74
8. The results of the separation of fractions No. 21-22.....	75
9. The results of the separation of fractions No. 5-7.....	75
10. The results of the separation of fractions No. 26-30.....	76
11. The results of the separation of fractions No. 37-57.....	77
12. The results of the separation of fractions No. 58-101.....	77
13. The results of the separation of fractions of eluted fractions No. 89-101 by silica gel.....	78
14. The results of the separation of fractions No. 105-110.....	79
15. The IR absorption band assignments of Compound 1.....	88
16. The retention time of Compound 1	89
17. The IR absorption band assignments of Mixture 2	90
18. Retention time of standard long chain aliphatic primary alcohol acid Mixture 2	92
19. Various type of saturated long chain aliphatic primary alcohol found in Mixture 2	93
20. The IR absorpion band assignments of Mixture 3	94

21. The IR absorption band assignments of Compound 4.....	95
22. The ¹ H-NMR chemical shift assignments of Compound 4.....	96
23. The ¹³ C-NMR chemical shift assignment of lupeol and Compound 4.....	98
24. The IR absorption band assignments of Mixture 5	100
25. Retention time of standard long chain aliphatic primary alcohol and Mixture 5.....	101
26. Various type of saturated long chain aliphatic primary alcohol found in Mixture 5	101
27. The IR absorption band assignments of Compound 6	102
28. The IR absorption band assignments of Compound 7.....	103
29. The assignment of the ¹³ C-NMR spectrum of Friedelan-3β-ol and Compound 7	104
30. The IR absorption band assignments of Compound 8.....	107
31. The comparison of ¹³ C-NMR spectrum of chavibetol and Compound 8..	109
32. The IR absorption band assignments of Mixture 9	110
33. The ¹³ C-NMR chemical shift assignments of Mixture 9.....	112
34. Retention time of the mixture of three standard steroids.....	115
35. The structure of campesterol, stigmasterol and β-sitosterol.....	116
36. The retention time from the gas chromatogram of Mixture 10 compared with standard steroids	117
37. The IR absorption band assignments of Compound 11	118
38. The comparison of ¹³ C-NMR spectrum of allylpyrocatechol and Compound 11.....	119
39. The results of cardiac glycoside test of crude <i>n</i> -butanol extract.....	120
40. The results of saponin test of crude <i>n</i> -butanol extract.	121
41. The results of separation of the crude <i>n</i> -butanol extract by column chromatography	122
42. The results of the separation of the eluted fractions No. 3-7 by silica gel column chromatography.	123

43. The results of the separation of fractions No. 2-5 by silica gel column chromatography	124
44. The results of the separations of fractions No. 8-19 (Bu 3)	124
45. The results of the separations of fractions No. 8-17	125
46. The results of the separations of fractions No. 18-22.....	126
47. The results of the separations of fractions No. 20-28.....	126
48. The results of the separations of fractions No. 3-8.....	127
49. The results of the separation of fractions No. 3.....	127
50. The results of brine shrimp cytotoxic lethality test of various fractions derived from the separation of <i>n</i> -butanol crude extract.....	128
51. The comparison of the ¹³ C-NMR signals of D-Fructose and PBBu 1....	130
52. The results of antitumor activity against <i>Human Bladder Carcinoma</i> (BIU).....	136
53. The results of antitumor activity against <i>Human Erythroleukemia</i> <i>Carcinoma</i> (K562).....	137
54. The results of antitumor activity against <i>Human Gastric Carcinoma</i> (BGC-823).....	137
55. The results of antitumor activity against <i>Human Nasopharyngeal</i> <i>Carcinoma</i> (KB).....	138
56. The results of antitumor activity against <i>Human Leukemia Carcinoma</i> (HL-60).....	138
57. The results of brine shrimp cytotoxic lethality test of isolated compounds.....	139
58. All substances isolated from chloroform crude extract of the leaves of <i>Piper betle</i> Linn.	141

LIST OF ABBREVIATIONS

b	broad
cm ⁻¹	unit of wavelength
°C	degree celsius
cont.	continue
¹³ C NMR	carbon 13 nuclear magnetic resonance
d	doublet (NMR)
DEPT	distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
FID	Flame ionization detector
Fig.	Figure
g	gram
GC-MS	gas chromatography-mass spectroscopy
GLC	gas liquid chromatography
¹ H NMR	proton nuclear magnetic resonance
IR	infrared
J	coupling constant (NMR)
kg	kilogram
m	multiplet (NMR)
m/e	mass to charge ratio
MS	mass spectrometry
M ⁺	molecular ion
ml	milliliter
mg	milligram
MW	molecular weight
m.p.	melting point
No.	number
ppm	part per million
R _f	rate of flow in chromatography

Rt	retention time
LC ₅₀	concentration that caused 50 % lethality
wt. by wt.	weight by weight
δ	chemical shift
s	singlet (NMR)
t	triplet (NMR)
TLC	thin layer chromatography



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย