# ALLELOPATHIC POTENTIAL OF *SPIRULINA PLATENSIS*

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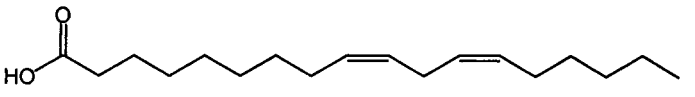
# บทคัดย่อ


งานวิจัยนี้เป็นการศึกษาศักยภาพทางอัลลีโลพาทีของสารสกัดจากสาหร่ายสไปรูลินา พลาเทนซิส ต่อพืชทดสอบสองชนิดได้แก่ผักโขมจีน (*Amaranthus tricolor* L.) และหญ้าข้าวนก (*Echinochloa crus-galli* (L) Beauv.) พบว่า สารสกัดชั้นน้ำสามารถยับยั้งการงอกของผักโขมจีนได้สมบูรณ์ที่ระดับความเข้มข้น 1.25 เปอร์เซ็นต์โดยน้ำหนัก และยับยั้งการงอกของหญ้าข้าวนกได้สมบูรณ์ที่ระดับความเข้มข้น 5 เปอร์เซ็นต์โดยน้ำหนัก สารออกฤทธิ์ทางชีวภาพในชั้นน้ำคือ C-phycocyanin (C-PC **29**) สามารถยับยั้งความยาวรากของผักโขมจีนได้มากกว่าความยาวต้นแต่ไม่มีผลต่อการงอกของเมล็ด ส่วนผลต่อหญ้าข้าวนกพบว่า C-PC **29** สามารถยับยั้งรากได้เพียงเล็กน้อยที่ระดับความเข้มข้นสูงสุด และยังพบว่า สารสกัดด้วยน้ำร้อนและ C-PC **29** ที่สลายตัวสามารถยับยั้งการงอกและการเจริญเติบโตของพืชทดสอบเช่นเดียวกับสารสกัดชั้นน้ำและ C-PC **29**

นอกจากนั้นผลทางอัลลีโลพาทีของสารสกัดหยาบชั้นเฮกเซน เอทิลแอซิเตต และเมทานอลต่อพืชทดสอบพบว่า สารสกัดชั้นเอทิลแอซิเตตแสดงผลในการยับยั้งการงอกและการเจริญเติบโตของผักโขมจีนได้ดีที่สุด สารสกัดส่วนย่อยจากชั้นเอทิลแอซิเตตถูกจำแนกองค์ประกอบด้วยเทคนิค GC-MS โครมาโทกราฟี สเปกโทรสโกปีและสเปกโทรเมทรีพบว่า ประกอบด้วยสารกลุ่มกรดไขมันที่เป็นองค์ประกอบหลักคือ palmitic acid **10** และ linoleic acid **12** และองค์ประกอบย่อยเช่น stearic acid **13** palmitoleic acid **15** oleic acid **14** $\gamma$-linolenic acid **11** และ 5,8,11-eicosatrienoic acid **53** รวมทั้งสารองค์ประกอบย่อยอื่นๆเช่น 2-ethyl-3-methylmaleimide **50** dihydroactinidiolide **51** และ 4-oxo-$\beta$-ionone **52** เมื่อทำการแยกสารองค์ประกอบหลักในสารสกัดส่วนย่อยที่ยับยั้งการงอกและการเจริญเติบโตของผักโขมจีนได้ดีที่สุดพบว่าประกอบด้วยสารกลุ่ม norisoprenoids 4 ชนิดคือ 3-hydroxy-$\beta$-ionone **59** 3-hydroxy-5$\alpha$,6$\alpha$-epoxy-$\beta$-ionone **56** 3-hydroxy-5$\beta$,6$\beta$-epoxy-$\beta$-ionone **57** และ loliolide **58** โดยที่สาร **51-52** และ **56-59** สามารถแยกได้เป็นครั้งแรกจากสาหร่ายสไปรูลินา พลาเทนซิส และจากการศึกษาปรากฏการณ์การเสริมฤทธิ์กัน (synergistic phenomenon) ของ linoleic acid **12**

และ dihydroactinidiolide **51** พบว่าสารผสม **12**:**51** ในอัตราส่วน 5:5 และ 3:7 สามารถยับยั้งการงอกและการเจริญเติบโตของผักโขมจีนได้ดีกว่าสารบริสุทธิ์ทั้งสองชนิด



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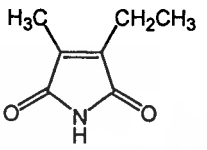

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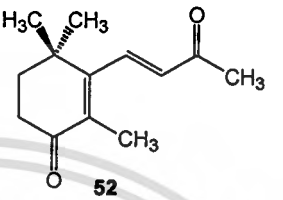

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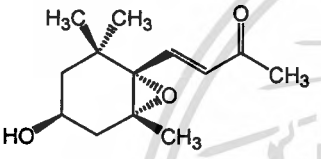

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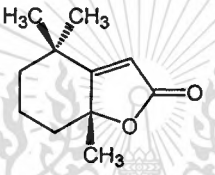

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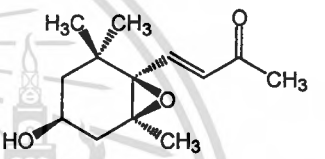

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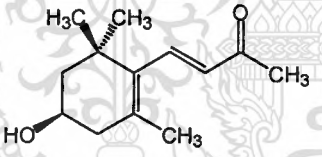

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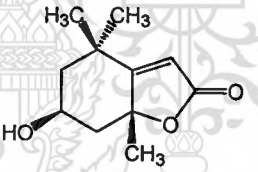

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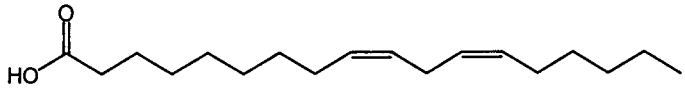
## ABSTRACT

The allelopathic potential of *Spirulina platensis* extracts on 2 tested plants, Chinese amaranth (*Amaranthus tricolor* L.) and Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) was investigated. The aqueous extract completely inhibited seed germination of Chinese amaranth and Barnyardgrass at concentrations of 1.25 and 5% by weight, respectively. The highest allelopatic effects of C-phycocyanin (C-PC **29**), reduced root length of Chinese amaranth more than the shoot length but had no effect on seed germination. As for Barnyardgrass, C-phycocyanin (C-PC **29**) slightly inhibited on shoot and root length at the highest applied concentration. In addition, the hot aqueous extract and denatured C-PC showed the allelopathic effects on tested plants similar to those of aqueous extract and C-PC **29**. The allelopathic effects of 3 crude organic extracts obtained from sequential solvent extraction were also studied on the tested plants. The results found that crude ethyl acetate extract had the highest inhibitory activity on Chinese amaranth.

The constituents of the high active fractions and subfractions obtained from crude ethyl acetate extract were determined by GC-MS, chromatography, spectroscopy and spectrometry techniques. The results showed that these fractions and subfractions consisted of major fatty acids, palmitic acid **10** and linoleic acid **12**, minor fatty acids, for examples, stearic acid **13**, palmitoleic acid **15**, oleic acid **14**, γ-linolenic acid **11** and 5,8,11-eicosatrienoic acid **53**, including other minor compounds, 2-ethyl-3-methylmaleimide **50**, dihydroactinidiolide **51**, and 4-oxo-β-ionone **52**. Isolation of the highest activity subfractions on Chinese amaranth found that these subfractions consisted of 4 norisoprenoids, 3-hydroxy-β-ionone **59**, 3-hydroxy-5α,6α-epoxy-β-ionone **56**, 3-hydroxy-5β,6β-epoxy-β-ionone **57** and loliolide **58**. The compounds **51-52** and **56-59** are isolated here for the first time from *S. platensis*. Furthermore, the synergistic phenomenon between linoleic

acid **12** and dihydroactinidiolide **51** was investigated. The observation found that the mixed ratios of **12**:**51** in 5:5 and 3:7 had a higher inhibitory activity on Chinese amaranth than the pure compounds.



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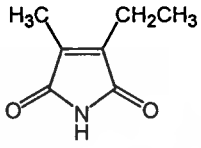

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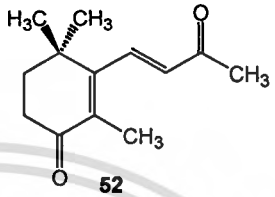

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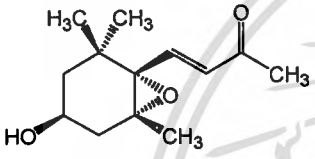

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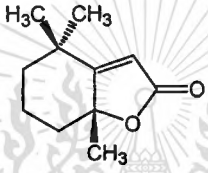

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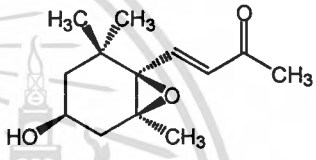

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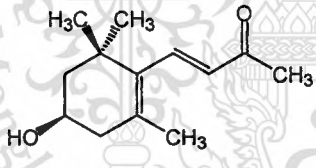

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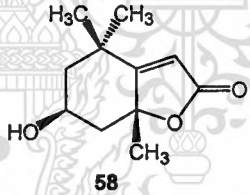

**58**

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# CONTENTS

Page

ABSTRACT (in Thai).....I

ABSTRACT (in English).....III

ACKNOWLEDGMENTS.....V

CONTENTS.....VI

LISTS OF TABLES.....IX

LISTS OF FIGURES.....XII

LISTS OF SCHEMES AND FLOW CHARTS.....XV

LIST OF ABBREVIATIONS.....XVI

CHAPTER 1 INTRODUCTION.....1

1.1 Statement and significance of the problems.....1

1.2 Objectives.....3

1.3 Scope of the study.....3

1.4 Process of the study.....3

1.4.1 Extraction, isolation and purification.....3

1.4.2 Analyse and characterise the structure of allelochemical compounds by spectroscopic methods.....3

1.5 Expected Results.....4

CHAPTER 2 THEORY AND LITERATURE REVIEWS.....5

2.1 Allelopathy.....5

2.2 Allelochemicals.....5

2.2.1 Mode of release of allelochemicals.....6

2.2.2 Mechanisms of action of allelopathic agents.....8

2.3 *Spirulina platensis*.....10

2.3.1 Nutrients and other chemicals.....10

2.3.2 Biological activities of *Spirulina*.....14

# CONTENTS (Continued)

Page

2.4. Literature reviews.....15

CHAPTER 3 RESEARCH METHODOLOGY.....21

3.1 Chemicals.....21

3.2 Apparatus.....21

3.3 Instruments.....22

3.4 General directions.....22

3.5 Allelopathic effects of aqueous extract on seed germination and seedling growth.....24

3.6 Allelopathic effects of C-phycocyanin **29** on seed germination and seedling growth.....25

3.7 Allelopathic effects of fractions from crude aqueous extract on seed germination and seedling growth.....25

3.8 Allelopathic effects of denatured C-phycocyanin bioassay.....26

3.9 Allelopathic effects of hot aqueous extract on seed germination and seedling growth.....27

3.10 Allelopathic effects of crude organic extracts on seed germination and seedling growth.....27

3.11 Allelopathic effects of fractions from the highest activity crude organic extract on seed germination and seedling growth.....28

3.12 Allelopathic effects of subfractions from the highest activity fractions on seed germination and seedling growth.....28

3.13 Allelopathic effects of fatty acids on seed germination and seedling growth.....29

3.14 Allelopathic effects of isolated allelochemicals on seed germination and seedling growth.....31

3.15 Synergistic phenomenon of linoleic acid **12** and dihydroactinidiolide **51**.....31

CHAPTER 4 RESULTS AND DISCUSSION.....33

4.1 Allelopathic effects of aqueous extract on seed germination and seedling growth..... 33

4.2 Allelopathic effects of C-phycocyanin **29** on seed germination and seedling growth.....34

4.3 Allelopathic effects of fractions from the aqueous extract on seed germination and seedling growth.....38

# CONTENTS (Continued)

Page

4.4 Allelopathic effects of denatured C-phycocyanin on seed germination and seedling growth....43
4.5 Allelopathic effects of hot aqueous extract on seed germination and seedling growth....45
4.6 Allelopathic effects of crude organic extracts on seed germination and seedling growth....47
4.7 Allelopathic effects of fractions from the highest activity crude organic extract on seed germination and seedling growth....50
4.8 Allelopathic effects of 7 subfractions from E6 on seed germination and seedling growth....58
4.9 Isolation and identification of active compounds from fractions E5-E6 and subfractions E6F3-E6F5....63
4.10 Allelopathic effects of 6 subfractions from E13 on seed germination and seedling growth....75
4.11 Isolation and identification of active compounds from subfractions E13F2-E13F4....78
4.12 Allelopathic effects of pure compounds from subfractions E6F3-E6F5 and E13F2-E13F4....96
4.12.1 Fatty acids....96
4.12.2 $C_{11}$ and $C_{13}$ norisoprenoids....108
4.13 Synergistic phenomenon of linoleic acid **12** and dihydroactinidiolide **51**....118

CHAPTER 5 CONCLUSIONS....124
5.1 Suggestions....125

REFERENCES....126
APPENDICES....144
Appendix A: Calibration curve of standard fatty acids....145
Appendix B: IR, ESMS and NMR spectra of alleochemical compounds....147
BIOGRAPHY....173

# LISTS OF TABLES

Table Page

3.1 GC-MS conditions .....30

4.1 Allelopathic effects of aqueous extract of *S. platensis* on seed germination and seedling growth of Chinese amaranth and Barnyardgrass.....33

4.2 Allelopathic effects of C-PC **29** on (a) Chinese amaranth (b) Barnyardgrass.....36

4.3 Absorbance, purification and concentrations of C-PC **29**.....38

4.4 Allelopathic effects of fractions F1-F3 from crude aqueous extract on seed germination and seedling growth of Chinese amaranth and Barnyardgrass.....40

4.5 Allelopathic effects of denatured C-PC on seed germination and seedling growth of Chinese amaranth and Barnyardgrass.....44

4.6 Allelopathic effects of hot aqueous extract on seed germination and seedling growth of Chinese amaranth and Barnyardgrass.....45

4.7 Allelopathic effects of crude organic extracts on seed germination and seedling growth of Chinese amaranth and Barnyardgrass.....49

4.8 Allelopathic effects of 23 fractions from crude EtOAc extract on seed germination of Chinese amaranth.....52

4.9 Allelopathic effects of 23 fractions from crude EtOAc extract on seedling growth of Chinese amaranth.....53

4.10 Allelopathic effects of 23 fractions from crude EtOAc extract on seed germination of Barnyardgrass.....56

4.11 Allelopathic effects of 23 fractions from crude EtOAc extract on seedling growth of Barnyardgrass.....57

4.12 Allelopathic effects of 7 subfractions from E6 on seed germination of Chinese amaranth.....60

4.13 Allelopathic effects of 7 subfractions from E6 on seedling growth of Chinese amaranth.....60

4.14 Allelopathic effects of 7 subfractions from E6 on seed germination of Barnyardgrass.....61

4.15 Allelopathic effects of 7 subfractions from E6 on seedling growth of Barnyardgrass.....62

4.16 Chemical compositions of fractions E5-E6 and subfractions E6F3-E6F5.....65-66

4.17 $^{13}C$ NMR and DEPT spectroscopic data of dihydroactinidiolide **51** in $CDCl_3$.....70

4.18 $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of dihydroactinidiolide **51** in $CDCl_3$.....70

# LISTS OF TABLES (Continued)

Table Page


4.19 $^{1}H$ and $^{13}C$ NMR of dihydroactinidiolide **51** in $CDCl_3$ compared to literature data.....71

4.20 $^{13}C$ NMR and DEPT spectroscopic data of 4-oxo-β-ionone **52** in $CDCl_3$.....72

4.21 $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of 4-oxo-β-ionone **52** in $CDCl_3$.....73

4.22 $^{1}H$ and $^{13}C$ NMR of 4-oxo-β-ionone **52** in $CDCl_3$ compared to literature data.....73

4.23 Allelopathic effects of 6 subfractions from E13 on seed germination of Chinese amaranth.....76

4.24 Allelopathic effects of 6 subfractions from E13 on seedling growth of Chinese amaranth.....76

4.25 Allelopathic effects of 6 subfractions from E13 on seed germination of Barnyardgrass.....78

4.26 Allelopathic effects of 6 subfractions from E13 on seedling growth of Barnyardgrass.....78

4.27 Pure compounds from E13F2-E13F4.....79

4.28 $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-β-ionone **59** in $CDCl_3$.....80

4.29 $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of 3-hydroxy-β-ionone **59** in $CDCl_3$.....80

4.30 $^{1}H$ and $^{13}C$ NMR of 3-hydroxy-β-ionone **59** in $CDCl_3$ compared to literature data.....81

4.31 $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$....83

4.32 $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$.....84

4.33 $^{1}H$ and $^{13}C$ NMR of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$ compared to literature data.....85

4.34 $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$.....87

4.35 $^{1}H$-$^{13}C$ HETCOR correlations in $CDCl_3$ and $^{1}H$-$^{1}H$ COSY correlations in $CD_3COCD_3$ of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**.....88

4.36 $^{1}H$-$^{13}C$ NMR of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$ compared to literature data....89

4.37 $^{13}C$ NMR and DEPT spectroscopic data of loliolide **58** in $CDCl_3$.....91

4.38 $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of loliolide **58** in $CDCl_3$.....91

4.39 $^{1}H$ and $^{13}C$ NMR of loliolide **58** in $CDCl_3$ compared to literature data.....92

4.40 Allelopathic effects of commercial fatty acids from E6F3-E6F5 on seed germination and seedling growth of Chinese amaranth.....97

4.41 Allelopathic effects of commercial fatty acids from E6F3-E6F5 on seed germination and seedling growth of Barnyardgrass.....99

# LISTS OF TABLES (Continued)

Table Page

4.42 Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on seed germination and seedling growth of Chinese amaranth.....109

4.43 Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on seed germination and seedling growth of Barnyardgrass.....111

4.44 Allelopathic effects of a mixture between linoleic acid **12** and DAD **51** on seed germination and seedling growth of Chinese amaranth.....121

# LISTS OF FIGURES

Figure Page

2.1 Mode of release of allelochemicals....6

2.2 *Spirulina platensis*....10

4.1 Allelopathic effects of aqueous extract of *S. platensis* at concentrations of 0.625-5% on (a) Chinese amaranth and (b) Barnyardgrass....34

4.2 Chemical structure of phycocyaninbilin chromophore (open chain tetrapyrrole) of C-PC **29**....35

4.3 UV-VIS absorption spectra of (a) aqueous extract and (b) commercial C-PC **29**....35

4.4 Allelopathic effects of C-PC **29** at concentrations of 62.5-1,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass....37

4.5 UV-VIS absorption spectra of fractions F1-F3....39

4.6 Allelopathic effects of fractions F1-F3 from crude aqueous extract of *S. platensis* at concentrations of 250-2,000 ppm on Chinese amaranth....41

4.7 Allelopathic effects of fractions F1-F3 from crude aqueous extract of *S. platensis* at concentrations of 250-2,000 ppm on Barnyardgrass....42

4.8 UV-VIS absorption spectrum of denatured C-PC....43

4.9 Allelopathic effects of denatured C-PC at concentrations of 62.5-1,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass....44

4.10 Allelopathic effects of hot aqueous extracts of *S. platensis* at concentrations of 0.625-5% on (a) Chinese amaranth and (b) Barnyardgrass....45

4.11 UV-VIS absorption spectrum of hot aqueous extract....46

4.12 Allelopathic effects of crude organic extracts of *S. platensis* at concentrations of 250-4,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass....48

4.13 Allelopathic effects of fractions, E5-E7 and E11-E14, from crude EtOAc extract at concentrations of 250-2,000 ppm on Chinese amaranth....54-55

4.14 Allelopathic effects of fractions E5 and E6 from crude EtOAc extract at concentrations of 250-2,000 ppm on Barnyardgrass....58

4.15 Allelopathic effects of subfraction E6F3-E6F5 at concentrations of 125-1,000 ppm on Chinese amaranth....59

4.16 Allelopathic effects of subfractions E6F3-E6F5 at concentrations of 125-1,000 ppm on Barnyardgrass....62

# LISTS OF FIGURES (Continued)

Figure Page

4.17 Allelopathic effects of subfractions E13F2-E13F4 at concentrations of 125-1,000 ppm on Barnyardgrass....77

4.18 Allelopathic effects of commercial fatty acids at concentrations of 62.50-1,000 ppm on Chinese amaranth....98

4.19 Allelopathic effects of commercial fatty acids at concentrations of 62.50-1,000 ppm on Barnyardgrass....100

4.20 Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F3-E6F5 and E13F2-E13F4 on Chinese amaranth....110

4.21 Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on Barnyardgrass....112

4.22 The allelopathic effects of 5 mixtures of linoleic acid **12** : DAD **46** in ratios 9:1 (b), 7:3 (c), 5:5 (d), 3:7 (f) and 1:9 (g), and both of pure compounds, (a) and (h), at concentrations of 62.5-500 ppm on Chinese amaranth....119-120

4.23 The allelopathic effects of 5 mixtures of linoleic acid **12** : DAD **46** and both of pure compounds at concentrations 62.5-500 ppm on Chinese amaranth (a) inhibition on seed germination, (b) inhibition on shoot length and (c) inhibition on root length....122

A1 Internal standard calibration curve for (a) methyl palmitate, (b) methyl stearate, (c) methyl oleate, (d) methyl linoleate and (e) methyl-γ-linolenate....145-146

B1 (a) IR and (b) ESMS spectra of dihydroactinidiolide **51**....147

B2 (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of dihydroactinidiolide **51** in $CDCl_3$....148

B3 (a) DEPT 135 and (b) COSY spectra of dihydroactinidiolide **51** in $CDCl_3$....149

B4 HETCOR spectrum of dihydroactinidiolide **51** in $CDCl_3$....150

B5 (a) IR and (b) ESMS spectra of 4-oxo-β-ionone **52**....151

B6 (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 4-oxo-β-ionone **52** in $CDCl_3$....152

B7 (a) DEPT 135 and (b) COSY spectra of 4-oxo-β-ionone **52** in $CDCl_3$....153

B8 HETCOR spectrum of 4-oxo-β-ionone **52** in $CDCl_3$....154

B9 (a) IR and (b) ESMS spectra of 3-hydroxy-β-ionone **59**....155

B10 (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 3-hydroxy-β-ionone **59** in $CDCl_3$....156

B11 (a) DEPT 135 and (b) COSY spectra of 3-hydroxy-β-ionone **59** in $CDCl_3$....157

# LISTS OF FIGURES (Continued)

Figure Page

B12 HETCOR spectrum of 3-hydroxy-β-ionone **59** in $CDCl_3$.....158

B13 (a) IR and (b) ESMS spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56**.....159

B14 (a) $^1H$ NMR and (b) $^{13}C$ NMR spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$.....160

B15 (a) DEPT 135 and (b) COSY spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$.....161

B16 HETCOR spectrum of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$.....162

B17 (a) IR and (b) ESMS spectra of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**.....163

B18 (a) $^1H$ NMR and (b) $^{13}C$ NMR spectra of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$.....164

B19 (a) DEPT 135 spectrum in $CDCl_3$ and (b) COSY spectrum in $CD_3COCD_3$ of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**.....165

B20 HETCOR spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$.....166

B21 HMBC spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CD_3COCD_3$.....167

B22 NOESY spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CD_3COCD_3$.....168

B23 (a) IR and (b) ESMS spectra of loliolide **58**.....169

B24 (a) $^1H$ NMR and (b) $^{13}C$ NMR spectra of loliolide **58** in $CDCl_3$.....170

B25 (a) DEPT 135 and (b) COSY spectra of loliolide **58** in $CDCl_3$.....171

B26 HETCOR spectrum of loliolide **58** in $CDCl_3$.....172

# LISTS OF SCHEMES AND FLOW CHARTS

Scheme Page

4.1 Degradation process of allelochemical DIBOA **39**.....47

4.2 Degradation products of β-carotene **26**.....68

Flow chart Page

3.1 The extraction procedures and allelopathic bioassay of aqueous extract.....26

3.2 The extraction procedures and allelopathic bioassay of crude organic extracts.....32

4.1 The extract of allelochemical compounds from E5-E6, E6F3-E6FF5 and E13F2-E13F4.....95

# LIST OF ABBREVIATIONS

| | |
|---|---|
| a.m.u. | Atomic Mass Unit |
| A | Absorbance |
| *br* | broad |
| *brm* | broad multiplete |
| *c* | concentration (g/100 mL) |
| °C | degree Celsius |
| $CD_3COCD_3$ | deuterated acetone |
| $CDCl_3$ | deuterated chloroform |
| cm | centimetre |
| $cm^{-1}$ | wave number unit |
| COSY | Homonuclear COrrelation SpectroscopY |
| C-PC | C-phycocyanin |
| *d* | doublet |
| 2D NMR | two dimension nuclear magnetic resonance spectroscopy |
| *dd* | doublet of doublets |
| *ddd* | doublet of doublets of doublets |
| *dddd* | doublet of doublets of doublets of doublets |
| DEPT | Distortionless Enhancement by Polarization Transfer |
| DMRT | Duncan's Multiple Range Test |
| DNA | DeoxyriboNucleic Acid |
| *dq* | doublet of quartet |
| *dt* | doublet of triplet |
| $EC_{50}$ | Effective Concentration 50 |
| ESIMS | ElectroSpray Ionization Mass Spectrometry |
| FAME | Fatty Acid Methyl Ester |
| FID | Flame Ionization Detector |
| FT-IR | Fourier Transform-InfraRed |
| FT-NMR | Fourier Transform-Nuclear Magnetic Resonance |
| G+ | gram positive |

# LIST OF ABBREVIATIONS (Continued)

| | |
|---|---|
| G- | gram negative |
| µg | microgram |
| GC | Gas Chromatography |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| HETCOR | HETeronuclear chemical shift CORrelation |
| HIV | Human Immunodeficiency Virus |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HPLC | High Performance Liquid Chromatography |
| hr | hour |
| Hz | Hertz |
| $IC_{50}$ | 50% Inhibitory Concentration |
| *J* | coupling constants (Hz) |
| Kg | Kilogram |
| L | Litre |
| $LC_{50}$ | Lethal Concentration |
| LC-Q | Liquid Chromatography-Quadrupole |
| $LD_{50}$ | Lethal Dose, 50% |
| lit. | literature |
| µL | microlitre |
| *m* | multiplet |
| M | Molar |
| µM | micromolar |
| MeOH | Methanol |
| mg | milligram |
| MHz | Megahertz |
| MIC | Minimum Inhibitory Concentration |
| min | minute |
| mL | millilitre |
| mm | millimetre |
| mp | melting point |

# LIST OF ABBREVIATIONS (Continued)

| | |
|---|---|
| MS | Mass Spectrometry |
| MW | Molecular Weight |
| m/z | a value of mass divided by charge |
| NMR | Nuclear Magnetic Resonance Spectroscopy |
| NOESY | Nuclear Overhauser Effect SpectroscopY |
| *P* | Probability |
| PBMC | Peripheral Blood Mononuclear Cells |
| ppm | part per million |
| rel. intensity | relative intensity |
| $R_f$ | rate of flow |
| RNA | RiboNucleic Acid |
| rpm | roll per minute |
| RT | Retention Time |
| *s* | singlet |
| sp. | Species |
| *t* | triplet |
| TLC | Thin Layer Chromatography |
| UV | Ultra-Violet radiation |
| UV-VIS | Ultra-Violet-VISible spectroscopy |
| v | volume |
| $\nu_{max}$ | maximum adsorption frequency |
| $\alpha$ | axial |
| $\beta$ | equatorial |
| $\lambda_{max}$ | maximum absorption wavelength |
| $\delta_H$ | Proton chemical shift (ppm) |
| $\delta_C$ | Carbon chemical shift (ppm) |
| $[\alpha]_D$ | Specific rotation |

# CHAPTER 1

# INTRODUCTION

## 1.1 Statement and significance of the problems

The major problem of the world's agricultural efficiency depends largely on controlling a variety of diseases and pests especially weeds. Weeds, simply defined as plants growing in an undesired location, compete with crops for resources, lower crop yields, and can contaminate the crop with their seeds thereby perpetuating the problem into subsequent growing seasons [1-2]. Furthermore, due to mankind's ever increasing mobility, exotic plant species have been extensively introduced to new locations around the world. Left uncontrolled weeds will spoil the appearance of a garden and deprive cultivated plants of water, space, light and nutrients. Sometimes weeds can grow so vigorously that they choke out carefully natured plants. They also can harbour pasts and diseases and can multiply very quickly either vegetativly by roots and shoots or through the productions of seeds, often in large numbers. A number of different methods can be used to control environmental weeds for examples, by preventive, chemical, biological, mechanical and cultural control. Different situations require different approaches, depending on such factors as, the species of weed being managed, levels of weed infestation and resources available such as time, labours, equipment and finances. Integrated weed management that include a combination of control methods is likely to be most effective in controlling weeds without allowing the weeds to become resistant to herbicides [3].

Weeds are major problem in crop productions in Thailand that the main weed control method is chemical control. Herbicides have several different modes of action, some will only effect broadleaf plants are called selective herbicide and some are non-selective. Widespread use of synthetic herbicides has resulted in herbicide-resistant weeds, and public concerns over the impact synthetic herbicides have on human health and the environments are increasing. These concerns are shifting attention to alternative weed control technologies based on natural products [4-5].

Allelopathy is most commonly defined as direct or indirect detrimental effect of one plant including microorganisms on the germination, growth, or development of either plant

through the production of chemical that escape into the environment [6-7]. Allelopathic interactions between plants have been implicated in the patterning of vegetation and weed growth in agricultural system and in inhibition of growth of several crops [8-10]. In recent years, allelopathy in agriculture has received considerable attention for two reasons; the first, allelochemicals decrease the agricultural and sivicultural yield and the second, allelochemicals can be beneficial as natural pesticides. Thus the science of allelopathy is gaining momentum. The indiscriminate application of synthetic pesticides has resulted in increasing resistance in organisms, causing severe environmental pollution and health hazard. Use of allelochemicals from plants for this purpose would be environmentally friendly, due to the fact that natural chemicals are renewable and easily degradable [11].

Although these allelopathic phenomena are well recognized in the terrestrial plant kingdom, very little is known about those for marine plants [12]. The earlier studies on cyanobacterial allelopathy were conducted in the beginning of the 20$^{th}$ century, and in the 1930s it was suggested that algal toxins played a role in phytoplankton succession by affecting some species more than other [6]. Some cyanobacteria have been reported to be responsible for the allelopatic properties on some plants by affecting the germination and growth of other species. For example, *Nostoc* 31, showed allelopathic activity against cyanobacteria *Anabaena* 7120 [13]. To date, some cyanobacterial allelochemicals have been isolated and characterized [14].

*Spirulina platensis* is a photosynthetic cyanobacterium that possesses biological activity and it widely cultivated to produce biological active food additives and to treat several diseases [15]. New effective medicinal preparations are based on it [16]. Water soluble proteins and phycocyanin pigment in addition to the rich assortment of biologically active compounds (BAC) from *Spirulina* biomass hold theoretical and practical interest [17-19]. Several studies show that *S. platensis* or its extracts can prevent or inhibit cancer in humans and animals. In vitro studies suggest that polysaccharides of *Spirulina* enhance cell nucleus enzyme activity and DNA repair synthesis [20-21]. Furthermore, the author found that the preliminary study of aqueous and crude organic extracts of *S. platensis* revealed the inhibitory effect on seed germination and seedling growth of Chinese mustard [22]. This research aim is alternatively investigated the new activity, allelopathic potential, of *S. platensis* on seed germination and seedling growth of tested plants.

## 1.2 Objectives

1. To investigate the allelopathic potential of aqueous extract, crude organic extracts, fractions, and subfractions from *S. platensis* on tested plants: Chinese amaranth (*Amaranthus tricolor* L.) and Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.).
2. To isolate and study the activity of the allelochemicals from crude extracts of *S. platensis*.
3. To determine the structure of allelochemical compounds by spectroscopic methods.

## 1.3 Scope of the study

1. Investigation the allelopathic potential of aqueous extract and crude organic extracts of *S. platensis* by petri dish test.
2. Extraction and isolation allelochemicals from crude organic extracts of *S. platensis*.
3. Investigation the activity of allelochemicals by vial test.
4. Determination of the structure of allelochemicals by spectroscopic methods.

## 1.4 Process of the study

### 1.4.1 Extraction, isolation and purification

1. Extract dried *S. platensis* by distilled water and hot distilled water and test on their allelopathic potential.
2. Extract dried *S. platensis* by Sequential Solvent Extraction (hexane, ethyl acetate and methanol) and test on their allelopathic potential.
3. Isolate allelochemicals from the highest inhibitory fractions or subfractions and study their allelopathic potential.

### 1.4.2 Analyse and characterise the structure of allelochemical compounds by spectroscopic methods

## 1.5 Expected Results

1. Explanation the new activity, allelopathic potential, of *S. platensis*.
2. Identification the allelochemicals from crude aqueous and organic extracts of *S. platensis*.
3. The effectiveness of allelochemicals from *S. platensis* on inhibition of growth may be the basic result for weed control.

# CHAPTER 2

# THEORY AND LITERATURE REVIEWS

## 2.1 Allelopathy

The term allelopathy, was introduced by Molisch in 1937 [23], and was derived from the Greek words allelon "of each other" and pathos "to suffer", the term generally refer to biochemical interactions between all types of plants including microorganisms. His discussion indicated that he meant the term to cover both inhibitory and stimulatory reciprocal biochemical interactions. In 1984, Rice [6] defined the term allelopathy as any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment. Some use the term in a wider sense, for instance entomologists, who include the effects of secondary compounds on plant-insect interactions.

In 1996 the International Allelopathy Society defined allelopathy as follow: "Any process involving secondary metabolites produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agricultural and biological system (excluding animals), including positive and negative effect" [24]. In the following, the term is used in accordance with Rice [6], but effects of the chemical compounds involved in plant-plant interactions and the effects of allelopathic plants are discussed in a broader perspective than strictly related to the plant-plant interactions.

## 2.2 Allelochemicals

Chemicals released from plants and imposing allelopathic influences are termed allelochemicals or allelochemics. Most allelochemicals are classified as secondary metabolites and are produced as offshoots of the primary metabolic pathways of the plant. Often, there functioning in the plant is unknown, but some allelochemicals are known also to have structural functions (e.g. as intermediates of lignification) or to play a role in the general defense against herbivores and plant pathogens [25a-b, 26].

Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flower [6]. The mode of release of allelochemicals from donor plant into the environment comprises an important part of allelopathy. A variety of allelochemicals are synthesized and stored in different plant cells, either freely or bound form

[27] and are released into the environment in response to various abiotic and biotic stresses. The mode of release of a particular allelochemical depends on its chemical nature [6, 28-29].

### 2.2.1 Mode of release of allelochemicals

Four major routes of entry of allelochemicals into the environment have been recognized, based on their chemistry: volatilization, leaching, root exudation, and decomposition of plant residue [6, 29] as shown in Figure 2.1.

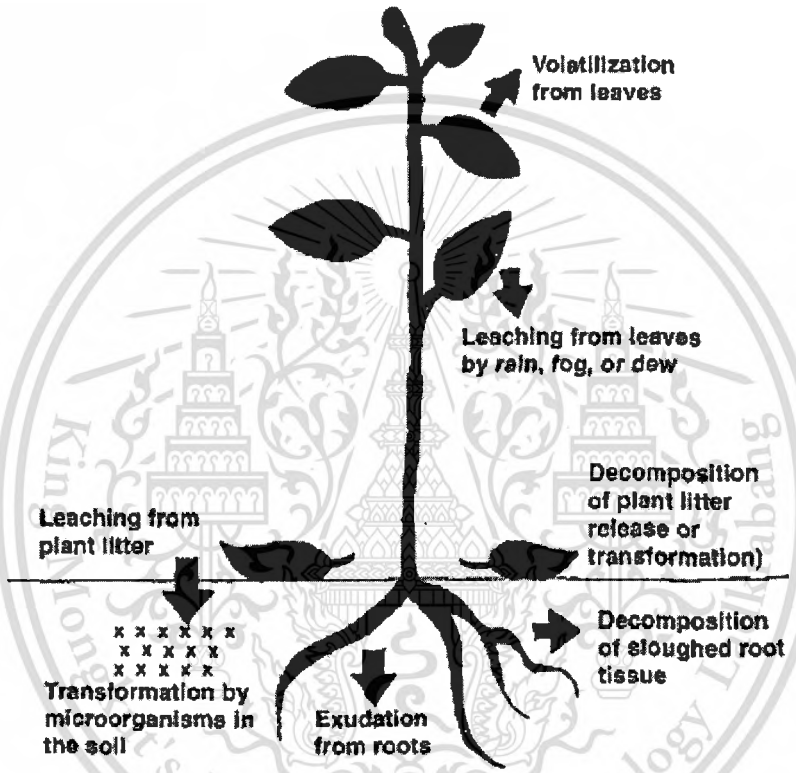
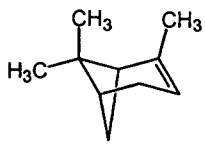


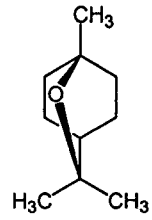

**Figure 2.1** Mode of release of allelochemicals [30 ]

#### 1 Volatilization

The release of allelochemicals by volatilization is often confined to plants producing terpenoids and monoterpenes [31]. The allelopathic potential of volatiles is well documented. The common general that release volatiles include *Artemisia*, *Salvia*, *Parthenium*, and *Eucalyptus* [6]. *Eucalyptus* has been known to contain a variety of terpeniods that are toxic to germination and seedling growth of numerous crop plants, for examples, α-pinene **1** and 1, 8-cineole **2** [32].




α-Pinene **1**




1, 8-Cineole **2**

**2 Leaching**

Leaching is the removal of substances from plants by the action of rain, snow, fog, dew, or mist. It offers a greater portion of allelochemicals to the environment [33]. The degree of leachability, however, depends on the type of plant tissue, the age of the plant, and the amount and nature of precipitation [34]. The major allelochemicals released via leaching include a variety of organic and inorganic substances such as phenolic compounds, terpenoids, and alkaloids [6], and most of these chemicals have been well characterized for their toxic effects on the surrounding plants and microorganisms under field and laboratory conditions [35].

**3 Root exudation**

In several instances, the suppression in crop yields has been attributed to toxins released by roots of various crop and weed species adjacent to crop plants [36]. Several of the compounds released via roots are known to reduce seed germination, root and shoot length, nutrient uptake, and nodulation [37-38]. Through root exudates comprise only 2-12% of the total photosynthates of the plant, they contribute a great deal toward allelopathy, as most of the well-known allelochemicals are root exudates. It has been observed that factors such as plant age, nutrition, light, and moisture would influence root exudation both quantitatively and qualitatively [39].

**4 Decomposition of plant residue**

The decomposition of plant residues adds a large quantity of allelochemicals to the rhizosphere [40]. The inhibitory potential of several compounds released during decomposition have been listed. Factors that influence this process include the nature of plant residue, soil type, and the conditions of the decomposition [41]. The decomposing plant materials are never equally distributed throughout the soil, and therefore as the roots grow through the soil, at some points they may come in contact with decomposing residue and be affected by allelochemicals. The compounds released into the soil are subjected to transformation by soil microflora and produce even more biologically active products than the present compounds [42]. Investigations using aqueous plant extracts have shown that water soluble inhibitors present in crop plants can be quickly released during decomposition.

The toxicity arising from plant residues provides some of the challenging problems and opportunities for agronomists and weed scientists. Toxins from the decomposing crop residues can affect young crop plants sown between mature plants, that is, in relay cropping. Likewise the stubble of the preceding crop in multiple sequential cropping can affect the next crop. In areas where stubble mulch farming is practiced for soil and water conservation, toxins from the stubbles have proved toxic to certain crops in rotation. These residues influence not only the crop emergence, growth, and productivity, but also influence similar aspects of weed growth.

**2.2.2 Mechanisms of action of allelopathic agents** [6]

Though much evidence has been documented on growth inhibition due to allelochemicals, the physiological and molecular mechanisms leading to such inhibition have not been critically investigated. The most probable reason for the lack of understanding of the mechanism of action is that most of the allelopathic effects under natural conditions are either synergistic or additive, and it is difficult to study the mode of action of a particular allelochemical separately. Though few attempts have been made to elucidate the mode of action, no definite mechanism of action for any particular chemical has been proposed. The threshold concentration of the compound required to affect plant processes varied with the type of the compounds, plant species, and the concentration. Rice explained the mechanisms of action of allelopathic agents on plants processes that the detail as showed below.

1 Effects on division, elongation, and ultrastructure of the cell.

2 Effects on hormone-induced growth.

3 Effects on membrane permeability.

4 Effects on mineral uptake.

5 Effects on easily available phosphorus and potassium in soils.

6 Effects on stomatal opening and photosynthesis.

7 Effects on respiration.

8 Inhibition of protein synthesis and changes in lipid and organic acid metabolism.

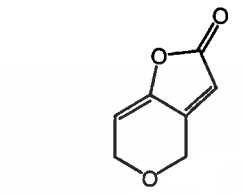
9 Possible inhibition of porphyrin synthesis.

10 Inhibition or stimulation of specific enzymes.

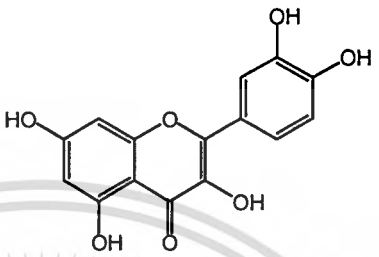
11 Effects on corking and clogging of xylem elements, stem conductance of water, and internal water relation.

12 Miscellaneous mechanisms.

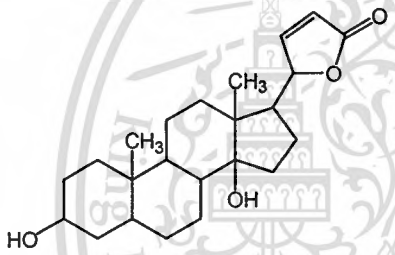
There are many thousands of chemical compounds produced by microorganisms or higher plants, but only a limited number of them have been identified as toxins involved in allelopathy. The types of allelochemical compounds can be identified, for examples, simple unsaturated lactones, long-chain fatty acids, flavonoids, terpenoids, steroid, phenols, benzoic acid, cinnamic acid **9** and derivatives, as shown below [6].



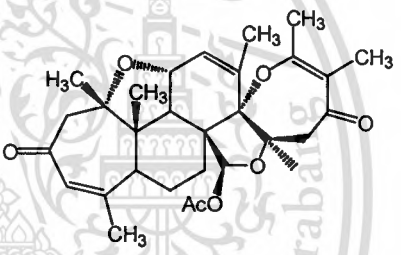
Patulin **3**



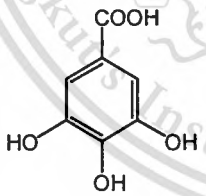
Quercetin **4**



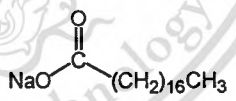
Digitoxigenin **5**



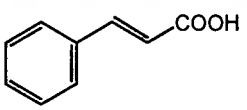
Brevione E **6**



Gallic acid **7**



Sodiumstearate **8**



Cinnamic acid **9**

These compounds showed the different activity to the target organisms. For example, Brevione E **6** is a diterpenoid derivative that was isolated from *Penicillium brevicompactumm* Dierckx. It completely inhibited shoot length of wheat at $10^{-4}$ M [43].

## 2.3 *Spirulina platensis* [44]

Blue green alga, *S. platensis*, is classified as part of the Monera Kingdom, Subkingdom-Eubacteria, Cyanobacteria Division, Cyanophyceae Class, Nostacles Order, Oscillatoriaceae Family, *Spirulina* Genus, and *platensis* Species [45]. It is photoautotrophic microorganisms widely distributed in nature. The morphology of its cell (Figure 2.2) is followed: thallus blue-green; trichomes slightly constructed at the cross-walls, 6-8 mm broad, not attenuated at the ends or only a little attenuated, more or less regularly spirally coiled; spirals 26-36 mm broad, distances between the spirals 43-57 mm; cells nearly as long broad, or shorter than broad, 2-6 mm long, cross-walls granulated; end-cells broadly rounded.

*Spirulina* has been used as human food for centuries, because it contains many nutrients, such as proteins, vitamins, minerals, carbohydrates, and essential fatty acids. The chemical compositions in *S. platensis* as discussed below.


**Figure 2.2** *Spirulina platensis*

### 2.3.1 Nutrients and other chemicals

#### 1 Protein

*Spirulina* contains unusually high amounts of protein, between 55 and 77% by dry weight, depending upon the source. It is a complete protein, containing all essential amino acid, though with reduced amounts of methionine, cysteine, and lysine, as compared to standard proteins such as that from meat, eggs, or milk. However, it is superior to all standard plant protein, such as that from legumes [46-47].

#### 2 Nucleic acids

One of the main concerns about the consumption of microorganisms is their high content of nucleic acids that may cause disease such as gout. *Spirulina* contains 2.2-3.5% of RNA and 0.6-1% of DNA, which represents less than 5% of these acids, based on dry weight [46].

### 3 Carbohydrates

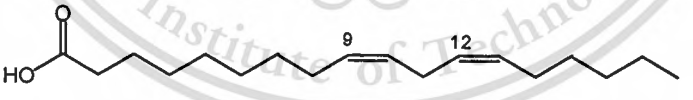
*S. platensis* contains about 13.6% carbohydrates; some of these are glucose, mannose, galactose, xylose, and rhamnose [48]. *Spirulina* does not have cellulose in its cell wall, a feature that makes it an appropriate and important foodstuff for people with problems of poor intestinal absorption, and geriatric patients [49]. A new high molecular weight polysaccharide, with immunostimulatory activity has been isolated from *Spirulina* and is called "Immulina". This highly water-soluble polysaccharide represents between 0.5 and 2.0% (w/w) of the dry microalgae [50].

### 4 Essential Fatty Acids

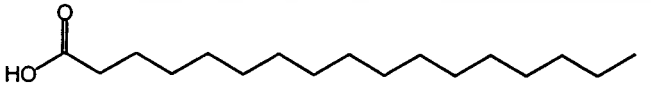
*Spirulina* is rich in palmitic acid **10**, γ-linolenic acid (GLA) **11**, linoleic acid (LA) **12**, and also provides stearic acid **13**, oleic acid **14**, palmitoleic acid **15**, α-linolenic acid (ALA) **16**, stearidoic acid (SDA) **17**, docosahexaenoic acid (DHA) **18**, and arachidonic acid (AA) **19** [47, 51].


Palmitic acid **10**

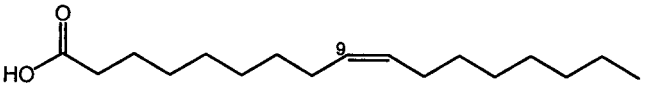

γ-Linolenic acid **11**



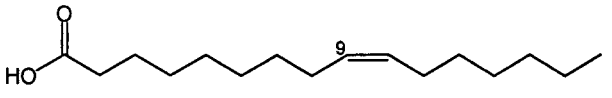

Linoleic acid (LA) **12**



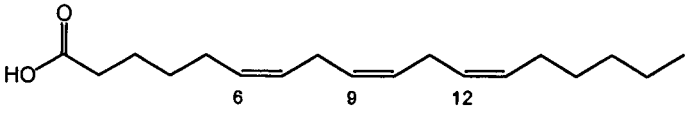

Stearic acid **13**



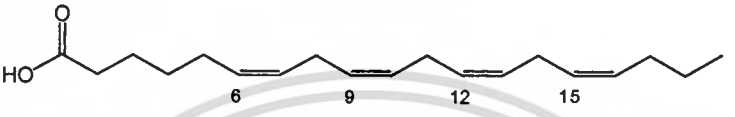

Oleic acid **14**



Palmitoleic acid **15**

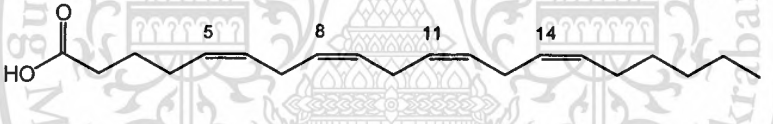


α -Linolenic acid (ALA) **16**



Stearidonic acid (SDA) **17**

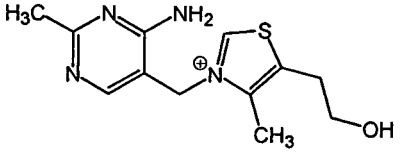
Docosahexaenoic acid (DHA) **18**



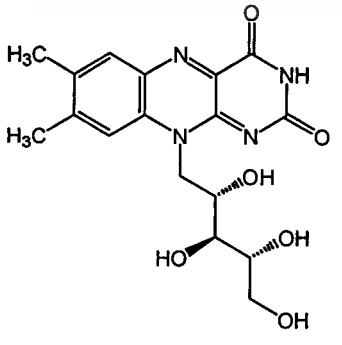
Arachidonic acid (AA) **19**

## 5 Vitamins

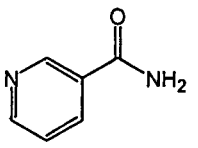
*Spirulina* contains vitamin $B_1$ (thiamine) **20**, $B_2$ (riboflavin) **21**, $B_3$ (nicotinamide) **22**, $B_6$ (pyridoxine), $B_9$ (folic acid), $B_{12}$ (cyanocobalamin), vitamin C **23**, vitamin D, and vitamin E **24** [47, 51].



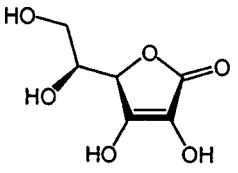
Thiamine ($B_1$) **20**



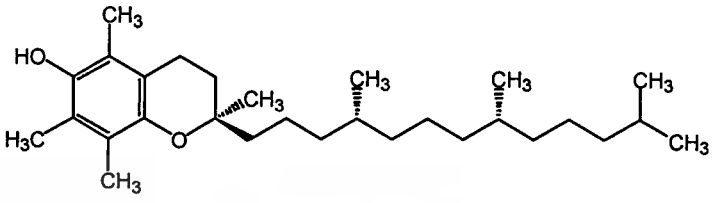
Riboflavin ($B_2$) **21**



Nicotinamide ($B_3$) **22**



L-Ascorbate (C) **23**



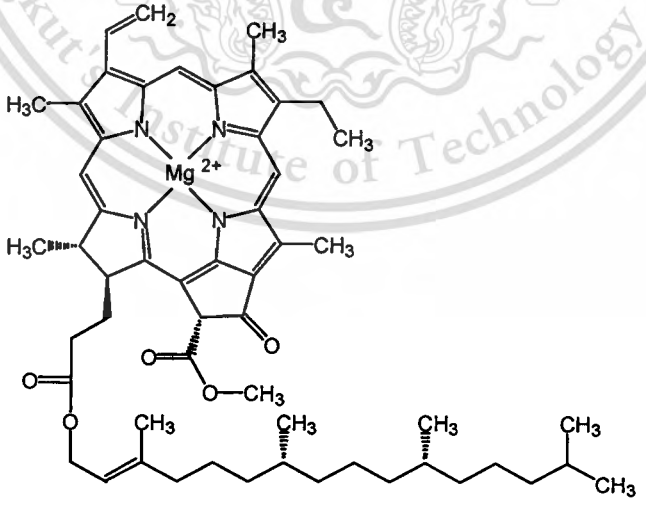
Tocopherol (E) **24**

**6 Minerals**

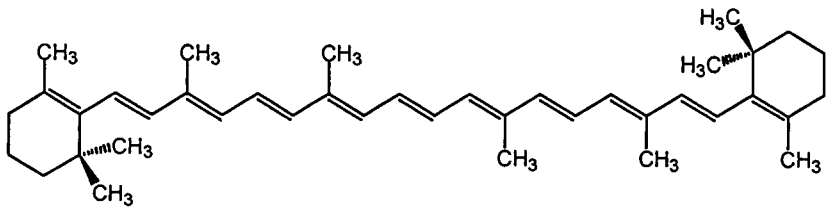
*Spirulina* is a rich source of potassium, and also contains calcium, chromium, cupper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc [47, 51].

**7 Photosynthetic Pigments**

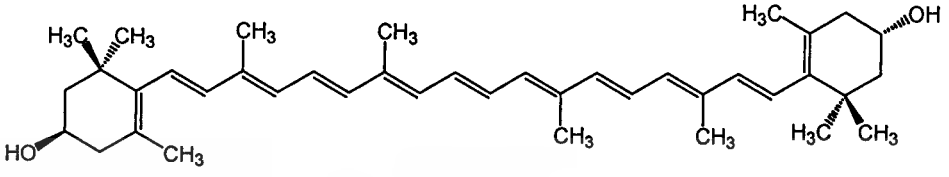
*Spirulina* contains many pigments including chlorophyll-a **25**, xanthrophyll, β-carotene **26**, echinenone, myxoxanthophyll, zeaxanthin **27**, canthaxanthin **28**, diatoxanthin, 3'-hydroxyechinenone, β-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins, C-phycocyanin **29** and allophycocyanin [44].



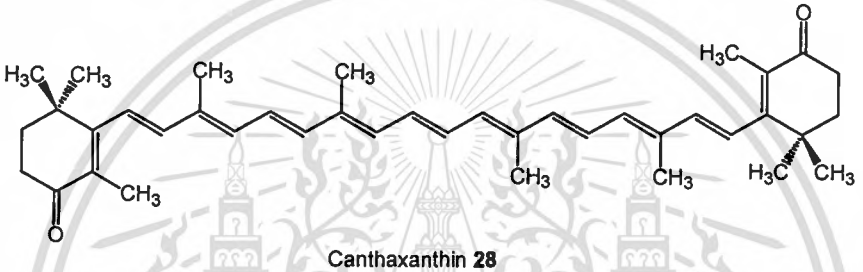
Chlorophyll-a **25**



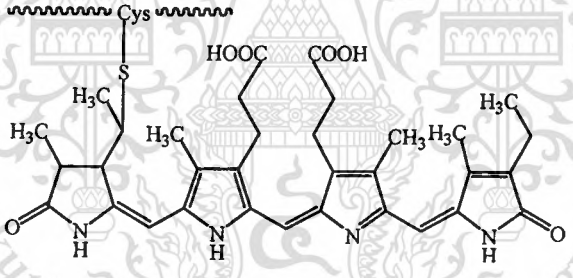
β-carotene **26**



Zeaxanthin **27**



Canthaxanthin **28**



C-Phycocyanin **29**

### 2.3.2 Biological activities of *Spirulina*

The cyanobacterium, *S. platensis*, is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and fatty acids. It has gained more and more attention, not only for foods aspects but also on the development of potential pharmaceuticals. In vitro research found that *S. platensis* extract inhibits HIV replication in human T-cells, peripheral blood mononuclear cells (PBMC), and Langerhans cells [52]. Many animal researches showed that *Spirulina* helps prevent heart damage caused by chemotherapy using Doxorubicin, without interfering with its anti-tumor activity [53]. Moreover, *Spirulina* reduces the severity of strokes and improves recovery of movement after a stroke; reverses age-related declines in memory and learning; and prevents and treats hay fever [54-56]. For clinical trials, *Spirulina* is effective for the clinical

improvement of melanosis and keratosis due to chronic arsenic poisoning improves weight-gain and corrects anemia in both HIV-infected and HIV-negative undernourished children; and protects against hay fever [57-59].

## 2.4 Literature reviews

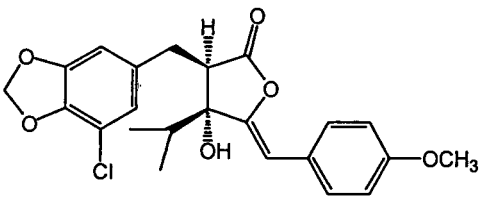
Algal allelopathy was apparently first not by Harder [60] who recorded autoinhibition in old cultures of *Nostoc punctiforme*. Biochemical interactions as a cause for phytoplankton succession were first proposed by Akehurst [61]. Several researches and information about allelopathic interaction between cyanobacteria, cyanobacteria-plant, cyanobacteria-algae, and cyanobacteria-microorganism have been reported, as in the following:

Lefevre and Nisbet [62] reported that a culture of *Phormidium uncinatum* increased the concentration of dissolved organic matter in the medium by almost eightfold in 40 days. The experiment was repeated using a medium in which *P. uncinatum* had grown for only 12 days, and the results were identical. Their evidence indicated that the required ingredients in the medium were sufficient for good growth of *Scenedesmus*. When the medium was considerably diluted with distilled water, *Scenedesmus* started dividing immediately.

Fogg and Westlake [63] reported that in pure cultures of *Anabaena cylindrical*, 5 to over 50% of the total nitrogen assimilated dissolves in the medium as peptide nitrogen. They demonstrated that species of algae from the other phyla liberate extracellular peptides also and that peptides occur commonly in natural water. They suggested that peptides may exert important effects on the growth of organisms in water by forming complexes with other dissolved substances.

Using multiple diffusion chamber, Martin et al. [64] found that the blue-green algae, *Gomphosphaeria aponina*, was allelopathic to the red tide algae, *Gymnodinium breve*, but *G. breve* had no effect on *G. aponina*.

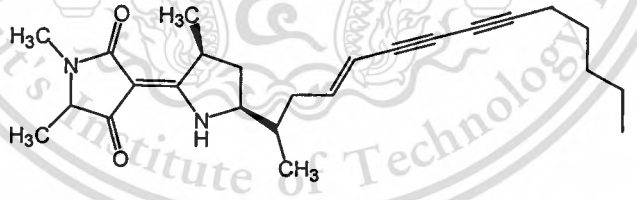
In 1982, Pignatello et al. [65] reported the isolation of a chlorine-containing allelochemical from *Scytonema hofmanni*. The allelochemical was named cyanobacterin **30** which the strucutre is a diaryl substitution γ-lactone. This compound inhibited algae and other cyanobacteria but not other non-photosynthetic bacteria or protozoa [66-67]. It had a minimum effect dose of 2 mg/mL (4.6 μM) against *Synechococcus* cells, but no effect on purple photosynthetic bacteria which lack photosystem II.




Cyanobacterin **30**

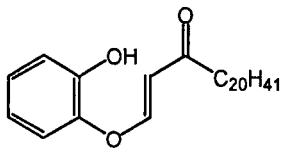
Murakami et al. [68] discovered seven autolytic substances, monogalactosyl diacylglycerols (MGDGs), produced by the cyanobacterium, *Phormidium tenue*. These compounds showed the autolytic activity on *P. tenue* growth. Moreover, if MGDGs contain unsaturated fatty acids, such as linoleic acid **12** and linolenic acids (**11** or **16**), lysed the alga in lower concentrations, while those saturated fatty acids caused no lysis even at 100 ppm.

In 1991, Gross et al. [69] isolated lipophilic allelopathic compound against other cyanobacteria, cholophytes, and diatom, fischerellin, from *Fischerella muscicola* and *Fisherella ambigue*. This compound is a potent inhibitor of oxygenic photosynthetic organisms, with a minimum inhibitory concentration (MIC) of 14 nM against *Synechococcus* PCC 6911 in 54 hrs growth assay. In 1996, the structure of this allelochemical was renamed more precisely fischerellin A **31** by Hangmann and Jüttner [70].



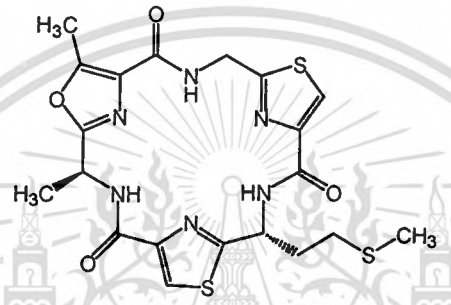

Fisherellin A **31**

Bagchi et al. [71-72] described algicidal activity in a bloom-forming cyanobacteria, *Oscillatoria laetevirens*, against cyanobacteria, *Synechococcus* sp.. They found that the $IC_{50}$ was approximately 1 μM with *Synechococcus* thylakoids. The algicidal metabolite was later characterized to have a molecular $C_{29}H_{48}O_3$, with the most probable postulated structure as compound **32**.



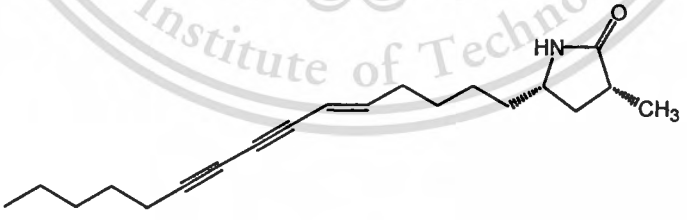

**32**

Jüttner et al. [13] isolated cyclic peptide containing thiazole and oxazole moieties, nostocyclamide M **33**, from *Nostoc* 31. This compound shows allelopathic activity against cyanobacteria, *Anabena* 7120.




Nostocyclamide M **33**

In 1997, Papke et al. [73] isolated and identified a new 2-pyrrolidinone with a polyunsaturated side chain from the cyanobacterium *F. muscicola.* The new compound, which was named fischerellin B **34**, was exhibited algicidal properties.



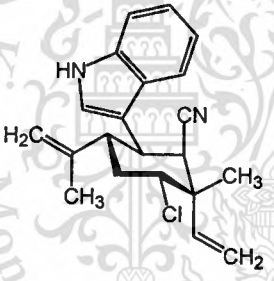

Fisherellin B **34**

In 1999, Schlegel et al. isolated mainly one hundred and ninety-eight cyanobacterial strains, that samples were collected from diverse habitats in Australia, Indonesia, Nepal, Thailand and Vietnam. These cyanobacterial stains were screened for their antibiotic activity against green algal species of the genera *Coelastrum*, *Scenedesmus* and *Monoraphidium* [74]. They reported

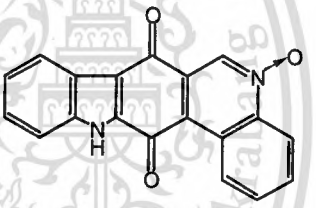
the discovery of ten strains of *Fischerella*, seven of *Nostoc* and three of *Calothrix* that produce antialgal compounds with a broad activity spectrum.

Pushparaj et al. [75] studied the allelopathic activity of organic extracts from *Nodularia harveyana*, a dinitrogen-fixing cyanobacterial. The active substances were lypophilic and exhibited strong allelopathic activity against other axenic cyanobacteria, antibiotic activity against G+ pathogenic bacteria and antifungal activity against two plant pathogens. The extracts were toxic ($LC_{50}$ at 30 μL) for grazers such as a rotifer (*Brachionus calyciflorus*) and a crustacean (*Thamnocephalus platyurus*).

Doan et al. [76] studied the allelopathic actions of the alkaloid 12-*epi*-hapalindole E isonitrile **35,** that isolated from cyanobacterium *Fischerella* sp., and the indolophenanthridine calothrixin A **36** from a *Calothrix* sp., on organisms including G+ and G- bacteria, cyanobacteria, fungi, eukaryotic green algae, protozoa and mammalian cells. They found that compounds **35** and **36** showed $IC_{50}$ values varied considerably to be 3-45 μM.

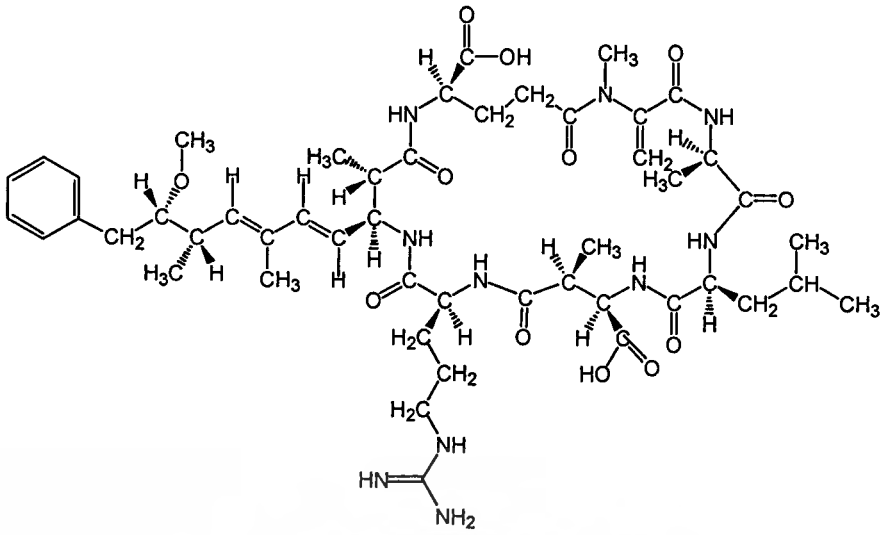



12-*epi*-Hapalidole E isonitrile **35**



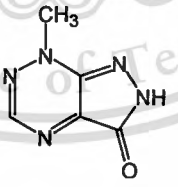

Calothrixin A **36**

Singh et al. [77] purified micocystin-LR **37** from cyanobacterium *Microcystis aeruginosa*. At a concentration of 50 μg/mL, this toxin caused complete inhibition of growth followed by cell lysis in *Nostoc muscorum* and *Anabaena* BT1 after 6 days of toxin addition. The addition of toxin (25 μg/ mL) to the culture suspensions of the *Nostoc* and *Anabaena* strains caused instant and drastic loss of $O_2$ evolution.



Micocystin-LR **37**

In 2003, Hirata et al. [78] investigated the effect of nostocine A **38**, the violet pigment releases from *Nostoc spongiaeforme*, on the growth of six green algae and seven cyanobacterium. They found that nostocine A **38** exhibited growth inhibitory activity comparable to paraquat, and the activity tended to be stronger to green algae than to cyanobacteria. Furthermore, nostocine A **38** showed the allelopathic activity on the growth of Barnyardgrass. Results found that nostocine A **38** exhibited strong inhibitory activity to the root elongation of Barnyardgrass. From this experiment, they suggested that nostocine A **38** may act as a toxin or an allelochemical to breeding organisms in nature.



Nostocine A **38**

Suikkanen et al. [79] investigated the allelopathic effects of three cyanobacterial species (*N. spumigena, Aphanizomenon flos-aquae* and *Anabaena lemmermannii*) that frequently form mass-occurrences in the Baltic Sea. They exposed monocultures of three phytoplankton species (*Thalassiosira weissflogii*, *Rhodomonas* sp. and *Prymnesium parvum*) to cell-free filtrates of the three cyanobacteria, and quantified allelopathic effects with cell counts. They also investigated

the role of the growth phase of cyanobacteria in their allelopathy, by comparing the effects of an exponential and a stationary phase culture of *N. spumigena*. All tested cyanobacteria inhibited the growth of *Rhodomonas* sp., but none of them affected *P. parvum*.

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Chemicals

1. Hexane, commercial grade (ZEN POINT)
2. Ethyl acetate, commercial grade (ZEN POINT) and analytical grade (CARLO ERBA)
3. Methanol, commercial grade (ZEN POINT)
4. Distilled water
5. Anhydrous magnesium sulfate (UNILAB)
6. Silica gel, 0.02-0.06 mm (Scharlau GE0048)
7. MERCK silica gel 60 (< 0.063 mm)
8. C-Phycocyanin **29** (C-PC) (Sigma-Aldrich, USA)
9. Sodium bicarbonate, analytical grade (LAB-SCAN)
10. Sulfuric acid, analytical grade (Mallinkrodt Chemicals)
11. Anisaldehyde (PANREAC QUIMICA SA)
12. Palmitic acid **10**, γ-linolenic acid **11**, linoleic acid **12**, stearic acid **13** and oleic acid **14**, analytical grade (Fluka and Riedel-deHaën, Switzerland)

## 3.2 Apparatus

1. Petri dish (95 mm in diameter)
2. Vial (2 cm diameter X 4.5 cm height)
3. Beaker
4. Graduated cylinder
5. Erlenmeyer flask
6. Volumetric flask
7. Test tube
8. Spatula
9. Pasteur pipette
10. Stirring rod
11. Glass column
12. Glass funnel

13. Graduated pipette
14. Thermometer
15. TLC aluminium sheet (silica gel $F_{254}$, MERCK)
16. Germination paper
17. Filter paper Whatman No. 1
18. Parafilm

## 3.3 Instruments

1. Oven
2. Magnetic stirrer (Fisher scientific)
3. Rotary evaporator (Rotavapor R-114, BÜCHI)
4. Melting point apparatus (GALLENKAMP SANYO)
5. Analytical balance (Denver Instrument Company TC-254)
6. UV-VIS Double Beam Spectrometer (Thermo Electron Corporation, model He λ ios α)
7. Fourier Transform Infrared Spectrometer, FT-IR (Spectrum GX 60237, Perkin Elmer)
8. Fourier Transform Nuclear Magnetic Resonance, FT-NMR 300 MHz (Avance DPX 300, BRUKER)
9. Freez Dryer (Virtis advantage data center wizard 2.0, Virtis)
10. Gas Chromatography-Mass Spectrometer (GC: Agilent Technologies Model 6890N, MS: Agilent Technologies Model 7683)
11. Finnigan LC-Q MS Detector Electrospray
12. Polarimeter (JASCO-1020)

## 3.4 General directions

All organic solvents were distillated by simple distillation under their boiling point temperature.

Melting points were determined on electrothermal melting apparatus GALLEKAMP SANYO.

Analytical thin layer chromatography (TLC) was performed on MERCK silica gel 60 $F_{254}$ aluminium sheet. Spots were visualized by UV light (254 and 366 nm) or by dipping with anisaldehyde reagent. The plates were then heated for 5 min at 110°C. Flash column

chromatography was performed on MERCK silica gel 60 (< 0.063 mm). Column chromatography was performed on Scharlau GE 0048 silica gel 60 : 0.02-0.06 mm.

**Spectroscopy**

Infrared (IR) spectra were recorded on FT-IR Perkin Elmer: Spectrum GX 60237 as neat films or dissolve in dichloromethane using potassium chloride plates.

$^{1}H$ and $^{13}C$ NMR were recorded on FT-NMR BRUKER: Avance DPX 300 (300 MHz) spectrometer at the Department of Chemistry, Faculty of Science, KMITL. Samples for NMR spectroscopy were recorded as solution in $CDCl_3$ or in $CD_3COCD_3$. Tetramethylsilane (TMS) was used as an internal standard. Coupling constants (*J*) are quoted in Hertz (Hz). $^{1}H$ NMR spectra were reported in the form $\delta_H$ (integration, multiplicity and coupling constants assignments). The proton assignments were based on coupling constants and related literature data. The $^{13}C$ NMR spectra were recorded in the form $\delta_C$ (assignments).

**Statistical analysis**

All experiments were performed in four replications. The data were analyzed at the seventh day and transformed into percentages of control. Germination percentages, root and shoot lengths were subjected to one-way analysis of variance and the Duncan's Multiple Range Test (DMRT) to determine significant among mean values at the probability level of 0.05.

Inhibition (% of control) was calculated as follows:

$$\text{Inhibition (\% of control)} = (100 - (\text{sample extracts/control}) \times 100))$$

**Bioassay seeds**

Two tested plants, including Chinese amaranth (*Amaranthus tricolor* L.) and Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) were used. Commercial Chinese amaranth seeds were purchased from Thai Seed & Agriculture Co., Ltd., Bangkok. The seeds of Barnyardgrass were collected in August 2006 from paddy fields of Minburi district, Bangkok. The germination randomly selected seeds was always > 80%.

**Material**

Dried *S. platensis* was obtained from the Department of Fisheries Science, Faculty of Agricultural Technology, KMITL. Algae were cultured photoautotrophically in Zarouk's medium [80]. The cultures were gassed with 0.03% $CO_2$ in air and algae were cultivated at 25°C with pH 10.5. The cultivate flasks were illuminated by continuous cool white fluorescent lamps

at 3,000 lux. The cells were harvested at the late exponential phase by centrifugation and dried in an oven at 40°C.

**Spectroscopic measurement**

The UV-VIS absorption spectra of aqueous extracts and C-PC **29** were measured on a UV-VIS double beam spectrometer with a 1 cm path length from 200-1,000 nm. The ratio of $A_{620}/A_{280}$ gives the purity of C-PC **29**, wherein $A_{620}$ is the maximum absorbance of C-PC **29** and $A_{280}$ is the absorbance of total protein [81, 82]. C-PC **29** concentration (mg/mL) was calculated as follow:

Concentration of C-PC **29** (mg/mL) $= [A_{620}-(0.474xA_{652})]/5.34$

## 3.5 Allelopathic effects of aqueous extract on seed germination and seedling growth

**General procedure for the preparation of aqueous extract**

Dried *S. platensis* was fronzen at -20°C for 24 hrs to break the cells, subsequently 5 grams of dried broken cells were placed in a 125 mL Erlenmeyer flask and 95 mL of distilled water was added. The mixture was stirred for 10 min at room temperature. The flask was sealed with Parafilm and kept in a refrigerator at 4°C for 24 hrs. After that the mixture was centrifuged at 5,000 rpm for 20 min, and the blue supernatant (5% by weight of stock solution) was diluted appropriately with distilled water to obtain the final concentrations of 5, 2.5, 1.25 and 0.625%, respectively. Cell debris was discarded. Afterwards, the fiftyfold diluted stock solution was determined by UV-VIS absorption spectra.

**General bioassay**

The aqueous extract of *S. platensis* was treated in completely randomized design with 4 replications. Twenty seeds of each tested plant were placed in a 9 cm petri dish, lined with germination paper and 5 mL of the extract were added. Controls received only distilled water (5 mL/petri dish). All treatments were covered and placed at room temperature (average 32 °C daytime and 28 °C night time) and natural light conditions (6.00 am – 6.00 pm). Germinated seed were counted for 7 days period and percentages of germination were calculated. After 7 days, shoot and root length of germinated seeds in all treatment were recorded. Differences in the percentages of seed germination and root and shoot length were assessed by the analysis of variance and the Duncan's Multiple Range Test (DMRT).

## 3.6 Alllelopathic effects of C-phycocyanin 29 on seed germination and seedling growth

Commercial C-PC **29** was purchased from Sigma-Aldrich, U.S.A. Ten milligrams of C-PC **29** were dissolved in distilled water using 10 mL volumetric flask to obtain 1,000 ppm of stock solution and the UV-VIS absorption spectrum was measured. The stock solution was diluted to 500, 250, 125, and 62.5 ppm, respectively. Five hundred microlitres of each concentration were pipetted in a vial lined with germination paper. Ten seeds of each tested plants were placed on the germination paper. The vials were sealed with Parafilm and grown for 7 days. Germination tests were conducted in the same manner as described in the previous experiment.

## 3.7 Allelopathic effects of fractions from crude aqueous extract on seed germination and seedling growth

### Preparation of fractions of *S. platensis* aqueous extract

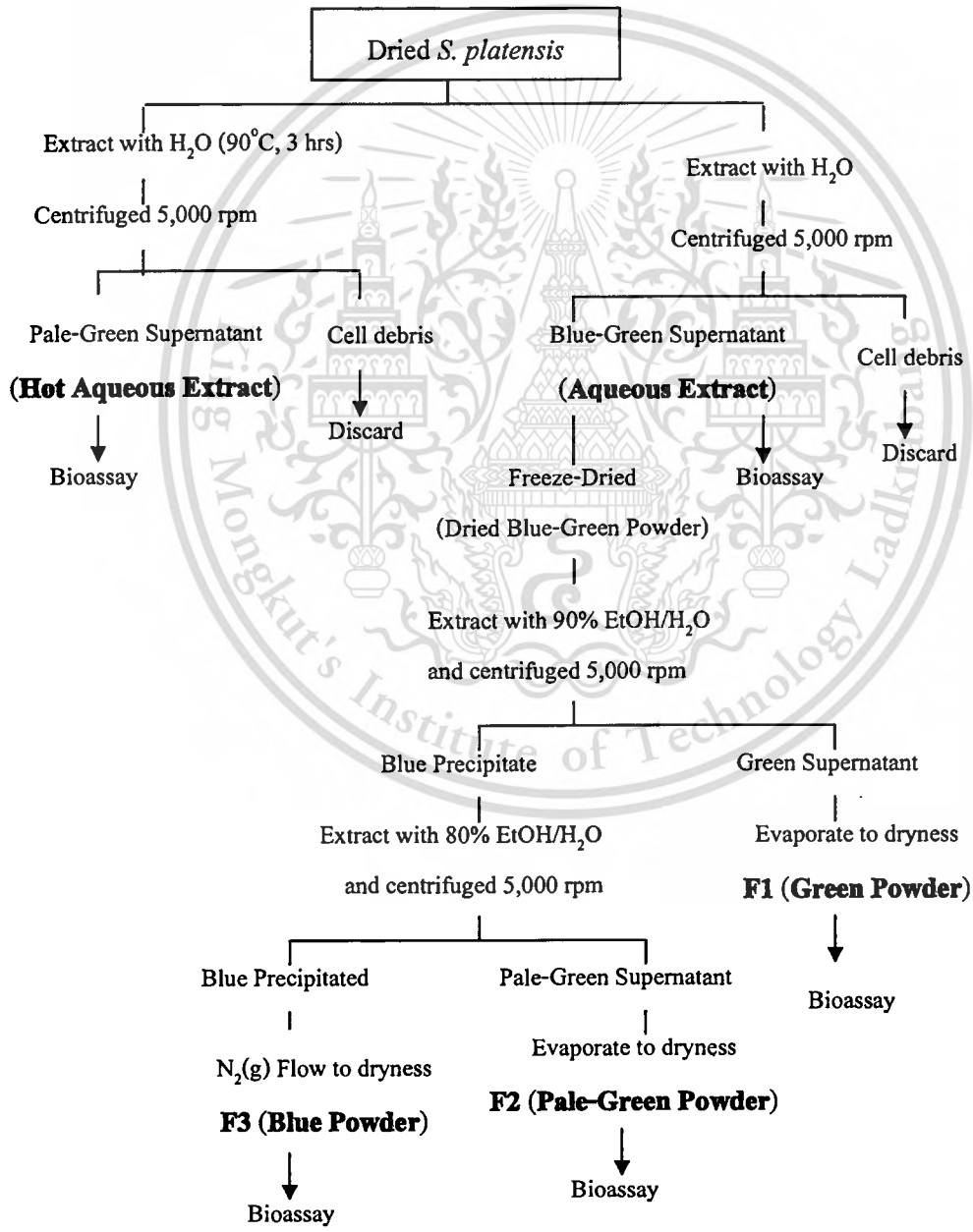
As shown in Flow chart 3.1, fifty millitres of 5% aqueous solution were dehydrated under a vacuum (Freez Dryer, Virtis advantage data center wizard 2.0, Virtis) to receive a blue-green powder. After that the powder was extracted with 90% (v/v) aqueous ethanol and centrifuged at 5,000 rpm for 20 min, a green supernatant and a blue precipitated were obtained. The precipitated was extracted with 80% (v/v) aqueous ethanol and centrifuged as described in the previous experiment to obtain a blue precipitated and a pale-green supernatant. The green and the pale-green supernatant were then evaporated to dryness on a rotary vacuum evaporator at 40°C to give a green powder (F1), a pale-green powder (F2) and a blue precipitate, respectively [83]. The latter was dried under $N_2$ (g) flowed to become a blue powder (F3).

### Allelopathic effects of fractions of *S. platensis* aqueous extract bioassay

Twenty milligrams of each fraction were dissolved with distilled water in 10 mL volumetric flask (2,000 ppm stock solution), then the stock solution was measured UV-VIS absorption spectra and calculated C-PC **29** (mg/mL) as described in section spectroscopic measurement. After that the stock solutions were diluted to give final concentrations at 2,000, 1,000, 500, and 250 ppm, respectively. Each fraction was studied the allelopathic activity as described in section 3.6.

### 3.8 Allelopathic effects of denatured C-phycocyanin bioassay

A flask of 1,000 ppm stock solution of commercial C-PC **29** was sealed with Parafilm, then the stock solution was heated on a hot bath at 90°C for 3 hrs. The stock solution was cooled down at room temperature. The UV-VIS absorption spectra of the stock solution was measured, then the concentration of C-PC **29** (mg/mL) was calculated as described in section 3.4. After that the denatured C-PC stock solution was diluted with distilled water to yield the final concentrations at 1,000, 500, 250, 125 and 62.5 ppm, respectively. The denatured C-PC was studied the allelopathic activity as described in section 3.6.




**Flow chart 3.1** The extraction procedures and allelopathic bioassay of aqueous extract

## 3.9 Allelopathic effects of hot aqueous extract on seed germination and seedling growth

The method for preparing the hot aqueous extract was performed using fifty millitres of 5% aqueous solution of *S. platensis* which was stirred at 90°C for 3 hrs. The extracted mixture was cooled down to room temperature and kept in refrigerator. After that the extracted mixture was centrifuged at 5,000 rpm for 20 min, and the 5% hot aqueous was diluted appropriately with distilled water to obtain the final concentrations at 5, 2.5, 1.25 and 0.625%, respectively. The allelopathic activity of hot aqueous extract was studied as described in section 3.6. To determine the composition of C-PC **29** of hot aqueous extract, the 5% by weight stock solution was again diluted fiftyfold to give a final concentration 0.1% by weight. Sample was determined C-PC **29** and the concentration of C-PC **29** (mg/mL) calculated as described in section 3.4.

## 3.10 Allelopathic effects of crude organic extracts on seed germination and seedling growth

### General procedure for the preparation of crude organic extracts

Four kilograms of dried *S. platensis* were frozen at -20°C for 24 hrs and extracted with hexane (2 x 6 L) for 7 days period at room temperature. The extract was then filtrated through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness by a rotary vacuum evaporator at 40°C to give crude hexane extract. The residue was then similarly extracted with ethyl acetate (EtOAc, 2 x 6 L) and methanol (MeOH, 2 x 6 L) to obtain crude ethyl acetate and methanol, respectively.

### General bioassay

The three dried samples concentrated from hexane, ethyl acetate and methanol were again dissolved in each solvent to compare their allelopathic effects. Five hundred microlitres of each of these crude extract solutions at concentrations of 40,000, 20,000, 10,000, 5,000 and 2,500 ppm were pipetted in a 9.5 cm petri dish lined with germination paper and evaporated to dryness for 3 hrs at room temperature. For the distilled water control, 5 mL of distilled water was applied to petri dish. After evaporation, 5 mL of distilled water was added onto the germination paper to give the final concentrations at 4,000, 2,000, 1,000, 500 and 250 ppm and then 20 seeds of tested plant were placed on germination paper and grown for 7 days. Germination tests were conducted in the same manner as in the previous experiment.

## 3.11 Allelopathic effects of fractions from the highest activity crude organic extract on seed germination and seedling growth

**General procedure for the isolation of the highest activity crude organic extract** [84]

The highest activity crude organic extract was removed the solvent in vacuo, then mixed with silica gel. The mixture was subjected to column chromatography (Silica gel), eluted with hexane and hexane with increasing amounts of ethyl acetate and methanol. Each compound was collected as it elutes from the column in a test tube and the mixture was tested by TLC, using anisaldehyde reagent as an indicator. The process was discontinued when the compound(s) desired was (were) off the column. The solvent was evaporated from the test tubes and weighed the crude to determine the collected fractions.

**General bioassay**

All fractions were again dissolved in ethyl acetate to compare their inhibitory effect. Fifty microlitres of each fraction at concentrations of 20,000, 10,000, 5,000 and 2,500 ppm were pipetted in a vial (4.5 X 2 cm) lined with germination paper and evaporated to dryness for 3 hrs at room temperature. After evaporation, 0.5 mL of distilled water was added onto the germination paper to yield the final concentrations at 2,000, 1,000, 500 and 250 ppm and then 10 seeds of tested plants were placed on germination paper and each vial was sealed with Parafilm and grown for 7 days. Germination tests were conducted in the same manner as in the previous experiment.

## 3.12 Allelopathic effects of subfractions from the highest activity fractions on seed germination and seedling growth

**General procedure for the isolation of fractions**

The method for isolation of the fractions by column chromatography was performed as described in section 3.11.

**General bioassay**

All subfractions were again dissolved in ethyl acetate to compare their inhibitory effect. Fifty microlitres of each of these subfractions at concentrations of 10,000, 5,000, 2,500 and 1,250 ppm were pipetted in a vial lined with germination paper and evaporated to dryness for 3 hrs at room temperature. After evaporation, 0.5 mL of distilled water was added onto the germination paper to yield the final concentrations at 1,000, 500, 250 and 125 ppm, and then 10 seeds of each

tested plant were placed on germination paper and each vial was sealed with Parafilm and grown for 7 days. Germination tests were conducted in the same manner as in the previous experiment.

## 3.13 Allelopathic effects of fatty acids on seed germination and seedling growth

### General procedure for the identification of fatty acids

The results from $^{1}H$ and $^{13}C$ NMR showed that the high activity fractions and subfractions contained fatty acid. In order to determine the fatty acid composition, the fractions and subfractions were converted to fatty acid methyl ester (FAME) and analysed by the standard GC-MS method.

**The preparation of fatty acid methyl esters**: The samples (~30 mg) were methylated with 2% $H_2SO_4$ in MeOH (20 mL) and the resulting solution stirred at room temperature for 30 min. After this period, the reaction was heated until reflux at $80^0$C for 2 hrs whereupon the reaction was completed by thin layer chromatography. The mixture was cooled to room temperature and the excess methanol was removed under reduce pressure. The crude product was dissolved with ethyl acetate (20 mL) and washed with 0.5 M $NaHCO_3$ (2 X 40 mL), brine (10 mL), dried ($Na_2SO_4$), filtered through Whatman no. 1 and evaporated to dryness under reduce pressure to yield the fatty acid methyl ester products. These products were prepared to concentration of 1,500 ppm in ethyl acetate before analysed by GC-MS.

The GC-MS analysis was performed on an Agilent 6890 GC equipped with a flame ionization detector (FID). Automated split injection was executed using an Agilent 7683 autosampler. The instrumental configuration and analytical conditions are summarized in Table 3.1.

The total run time of the active fractions was 30 min. The GC oven temperature programme was 50°C held for 1 min, at a rate 25°C/min to 230, and held for 13 min. Fatty acid methyl ester and other compounds were identified by comparing the retention time (RT) of the integrated peak of the individual FAME with those of authentic standard FAMEs and databank of the Wiley7n. 1 library. Quantitative determinations were compared by using 500 ppm heptadecanoic acid methyl ester as internal standard (Appendix A). The peak area that less than 1% not qualified and the mass range was scanned from 50 to 500 a.m.u. A standard curve was plotted for each FAME using the weight and area ratios with respect to the internal standard.

Standard curves of FAMEs were prepared at concentrations of 250, 500, 1,000 and 2,000 ppm using ethyl acetate as solvent that contained the concentration of internal standard at 500 ppm (Appendix A).

**Table 3.1** GC-MS conditions [85]

| Instrumentation | |
|---|---|
| Chromatographic system : | Agilent 6890 GC |
| Inlet | Split |
| Detector | FID or Agilent 5973 MSD |
| Automatic sampler | Agilent 7683 |
| Liner | Split iner (p/n 5183-4647) |
| Column | 30 x 0.25 mm ID, 0.25μm DB-Wax (J& W 122-7032) |
| Experimental Conditions GC-FID | |
| Inlet temperature | 240°C |
| Injection volume | 1 μL |
| Split ratio | 1/100 |
| Carrier gas | Helium, flow rate 1 mL/min |
| Head pressure | - |
| Oven temperature | 50°C, 1 min, 25°C/min to 200°C, 3°C/min to 230°C, 13 min. |
| Detector temperature | 230°C |
| Detector gases | Helium |

**General bioassay**

The pure fatty acids were dissolved with ethyl acetate to yield concentrations of 10,000, 5,000, 2,500, 1,250 and 625 ppm, respectively. Then fifty microlitres of each of these concentrations were pipetted in a vial lined with germination paper and evaporated to dryness for 3 hrs at room temperature. After evaporation, 0.5 mL of distilled water was added onto the germination paper to give the final concentrations at 1,000, 500, 250, 125 and 62.5 ppm and then 10 seeds of tested plant were placed on germination paper and each vial was sealed by Parafilm and grown for 7 days. Statistical analysis, bioassay procedures and conditions, were similar to the previous work.

## 3.14 Allelopathic effects of isolated allelochemicals on seed germination and seedling growth

### General procedure for the isolation of allelochemicals

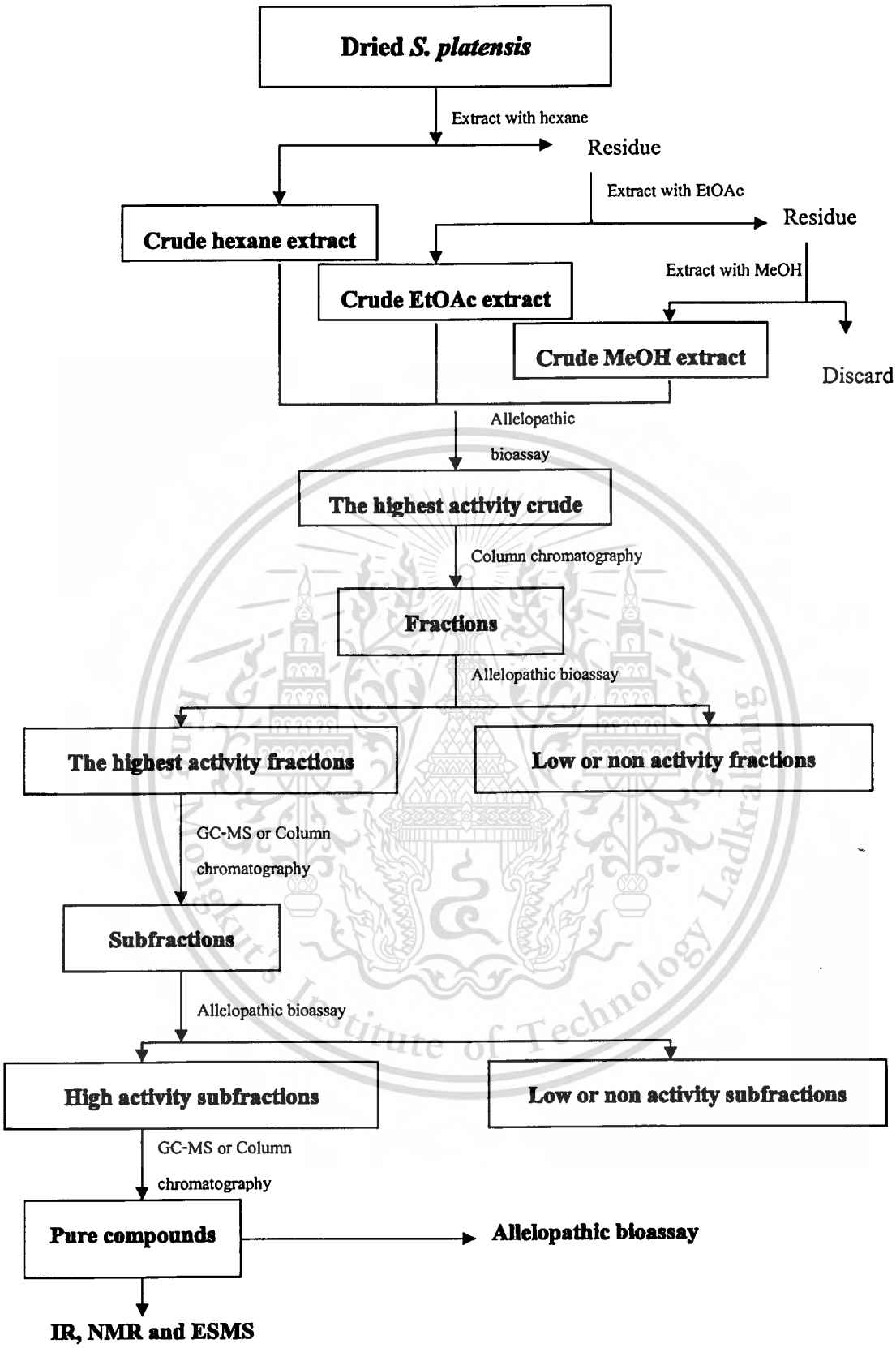
The method use for isolation of allelochemical by flash column chromatography was performed as described in section 3.11. All compounds were characterized and identified by FT-IR, $^{1}$H NMR, $^{13}$C NMR spectroscopy and MS spectrometry.

### General bioassay

All pure compounds were compared the allelopathatic effects on tested plants at the concentrations of 62.5-1,000 ppm. The procedure was described in section 3.13. A summary for the research procedures is illustrated in Flow chart 3.2.

## 3.15 Synergistic phenomenon of linoleic acid 12 and dihydroactinidiolide 51

Linoleic acid **12** and dihydroactinidiolide **51** were mixed in the weight ratio (**12**:**51**) of 9:1, 7:3, 5:5, 3:7 and 1:9. Each ratio was diluted with ethyl acetate to produce concentrations at 5,000, 2,500, 1,250, and 625 ppm, respectively. Then fifty microlitres of each of these concentrations were pipetted in a vial lined with germination paper and evaporated to dryness for 3 hrs at room temperature. After evaporation, 0.5 mL of distilled water was added onto the germination paper to give the final concentrations at 500, 250, 125 and 62.5 ppm and then 10 seeds of tested plant were placed on germination paper and each vial was sealed with Parafilm and grown for 7 days. The statistical analysis, bioassay procedures and concentrations, were similar to the previous work.




**Flow chart 3.2** The extraction procedures and allelopathic bioassay of crude organic extracts.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Allelopathic effects of aqueous extract on seed germination and seedling growth

Aqueous extract of *S. platensis* showed strong inhibitory effect on seed germination and seedling growth of tested plants, as discussed in the results below.

**Chinese amaranth**

Results in Table 4.1 indicated that all concentrations of the aqueous extract of *S. platensis* had an inhibitory effect on seed germination and seedling growth of Chinese amaranth. The aqueous extract of *S. platensis* at a concentration of 0.625% inhibited seed germination by 20% and at concentrations of 1.25-5% showed complete inhibition. As for seedling growth, it was found that the aqueous extract at a concentration of 0.625% inhibited shoot and root length by 45.38 and 68.35% respectively, as shown in Figure 4.1 (a).

**Table 4.1** Allelopathic effects of aqueous extract of *S. platensis* on seed germination and seedling growth of Chinese amaranth and Barnyardgrass

| | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| Concentrations (% by weight) | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0c | 0c | 0c | 0c | 0d | 0d |
| 0.625 | 20b | 45.38b | 68.35b | -2.90c | 0.14d | 16.54c |
| 1.25 | 100a | 100a | 100a | 24.64b | 34.00c | 44.88b |
| 2.5 | 100a | 100a | 100a | 95.65a | 93.22b | 100a |
| 5 | 100a | 100a | 100a | 100a | 100a | 100a |

Values with same letters in each column there are not significantly different at $P$=0.05

**Barnyardgrass**

The allelopathic effects of aqueous extract on seed germination and seedling growth of Barnyardgrass were summarized in Table 4.1. Significant reduction of germination and shoot length were observed at a concentration of 1.25%, while root length was affected at a

concentration of 0.625%. At concentrations of 0.625 and 1.25% inhibited root length by 16.54 and 44.88% whereas at a concentration of 2.5% root length was completely inhibited. At concentrations of 1.25 and 2.5% inhibited seed germination by 24.64 and 95.65%, and reduced shoot length by 34 and 93.22%. The extract completely inhibited seed germination and shoot length at a concentration of 5% (Figure 4.1 (b)).

(a)

(b)

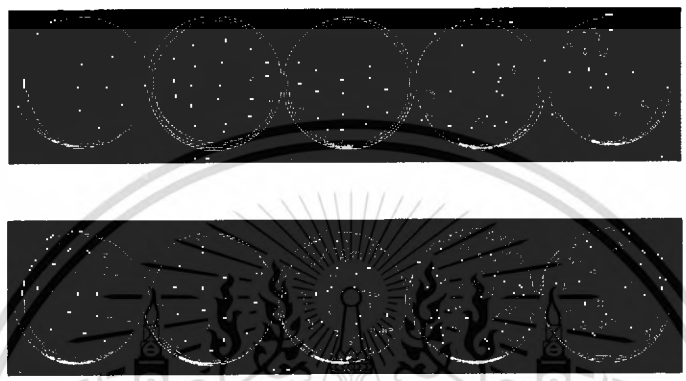



**Figure 4.1** Allelopathic effects of aqueous extract of *S. platensis* at concentrations of 0.625-5% on (a) Chinese amaranth and (b) Barnyardgrass

## 4.2 Allelopathic effects of C-phycocyanin 29 on seed germination and seedling growth

The results obtained from this study showed that *S. platensis* aqueous extract significantly inhibited all the tested plants. In previous studies [44, 86-89] reported that billiproteins was one of the major metabolites of *S. platensis* aqueous extract. Biliproteins are brilliantly coloured, water-soluble proteins with linear tetrapyrole prosthetic groups (bilin) chromophore. In their function state, biliproteins are covalently linked to specific systeine residues of the proteins (Fig. 4.2). They also form light-harvesting antenna complexes of cyanobacteria, called phycobilisomes (PBS). The biliproteins are broadly classified into three groups based on UV-VIS spectroscopic properties: phycoerythrin (PE) $\lambda_{max}$ 540-570 nm, phycoerythrobilin; phycocyanin (PC) $\lambda_{max}$ 610-620 nm, and allophycocyanin (APC) $\lambda_{max}$ 650-655. Antenna systems of *S. platensis* are composed of C-PC **29** and APC at approximate ratio of 10:1 ratio and the protein fraction may contain up to 20% of C-PC **29** [44, 90-92]. C-PC **29** is not only used as nutrient ingredients and natural dyes for food and cosmetics, but also has therapeutic

values such as cyclooxygenase-2 inhibition, antioxidant and anti-inflammatory effects, anticancer effect and prevention of renal injury [93-98].

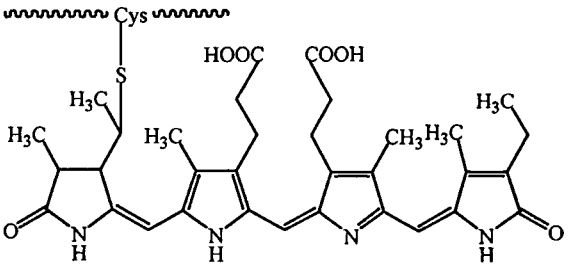



**Figure 4.2** Chemical structure of phycocyaninbilin chromophore (open chain tetrapyrrole) of C-PC **29**

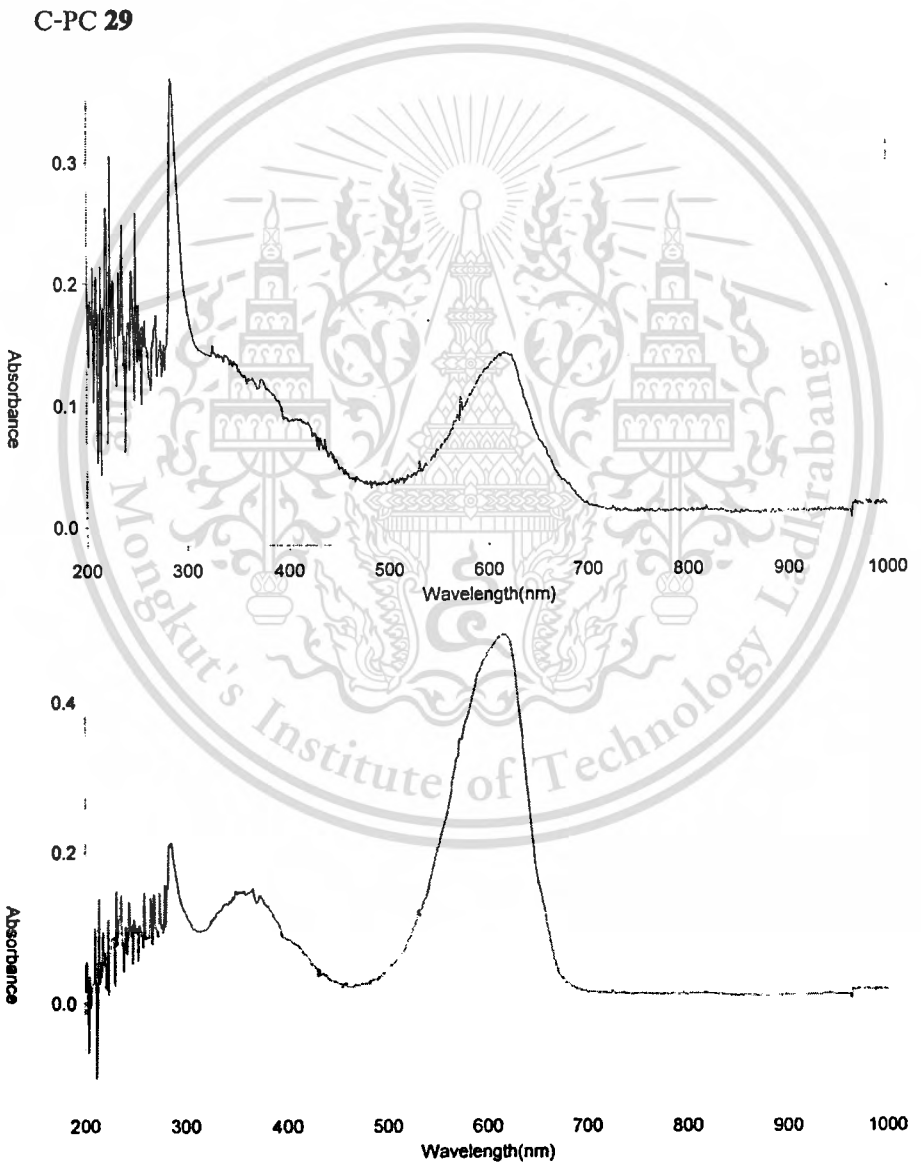



**Figure 4.3** UV-VIS absorption spectra of (a) aqueous extract and (b) commercial C-PC **29**

The purity of the aqueous extract and commercial C-PC **29** was evaluated according to the purity ratio, $A_{620}/A_{280}$. The fractions that exhibited the purity ratio >4.0 were considered as high purity C-PC **29** [81]. As shown in Figure 4.3 and Table 4.3, the UV-VIS absorption spectrum of aqueous extract showed that *S. platensis* contained C-PC **29** (peak at 620 nm) and the $A_{620}/A_{280}$ ratio of the blue supernatant was 1.17, while the purity ratio of compared with commercial C-PC **29** was 4.25. Since C-PC **29** is the major component in aqueous extract that may exhibit allelopathic effects on tested plants. In order to support the research's hypothesis, the allelopathic effects of C-PC **29** on Chinese amaranth and Barnyardgrass was also investigated.

**Table 4.2** Allelopathic effects of C-PC **29** on (a) Chinese amaranth (b) Barnyardgrass

| Concentrations (ppm) | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0a | 0cd | 0e | 0a | 0b | 0bc |
| 62.5 | 0a | -9.09d | -0.27e | 2.86a | -1.98c | -2.51c |
| 125 | 5a | -1.77cd | 10.37d | 0a | -0.06b | 1.72b |
| 250 | 2.5a | 4.29bc | 47.75c | 0a | -0.42b | -0.13bc |
| 500 | 5a | 10.35b | 70.80b | 5.71a | -0.30b | -0.26bc |
| 1,000 | 7.5a | 53.03a | 82.81a | 2.86a | 13.42a | 20.58a |

Values with same letters in each column there are not significantly different at $P$=0.05

The allelopathic assay of C-PC **29** did not exhibit inhibitory effect on seed germination of both tested plants, however, C-PC **29** did exhibit significant shoot length inhibition on Chinese amaranth at concentrations of 500 and 1,000 ppm by 10.35 and 53.03%, respectively. At concentrations of 125-1,000 ppm root length was inhibited by 10.37-82.81%. As for Barnyardgrass, it was found that only the highest concentration of 1,000 ppm exhibited an effect on shoot and root length. In contrast, at a concentration of 62.5 ppm shoot length was promoted by 1.98% as shown in Table 4.2 and Figure 4.4.

(a)

(b)

1,000 500 250 125 62.5 control

**Figure 4.4** Allelopathic effects of C-PC **29** at concentrations of 62.5-1,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass

The results from this experiment showed that C-PC **29** inhibited the growth of both tested plants. It has been reported that the allelopathic of protein such as, extracellular peptides from *A. cylindrical* [63], nostocyclamide M **33** from *Nostoc* 31 [13] and micocystin-LR **37** from *M. aeruginosa* [77] exhibited the allelopathic effects on algae and plants. In 1962 Jørgensen [99] isolated chlorophyllide pigments, with structure closely related to C-PC **29**, these were isolated from ether and ethanol extracts of *Chlorella vulgaris*, *Chlamydomonas reinhardii*, or *Scenedesmus quadricauda*. These compounds inhibited the growth of *Bacillus subtilis* after the chlorophyllides were transformed by light. Hirata et al. [78] investigated the effect of nostocine A **38**. The violet pigment is released from blue-green algae, *N. spongiaeforme*. It affected the growth of green algae, seven cyanobacterium, and Barnyardgrass. They found that the nostocine A **38** exhibited strong inhibitory activity on the root elongation of Barnyardgrass. These findings were in accordance with the experimental results indicating that C-PC **29** had an inhibitory effect on root more than shoot length. Several researches have reported the effect of protein on plants, for example, elicitins, one group of protein elicitors, are secreted from many plant pathogen fungal genus *Phytopthora* and some *Pythium*. These compounds can cause a hypersensitive response, including leaf necrosis and cell death, and induce systemic acquired resistance in some plant species [100-101]. The results indicated that the necrotic proteins had a toxic effect to plant hosts, similar to the experimental results showing that the protein C-PC **29** can inhibit the growth

of tested plants. In the natural environment some algal toxins are generally contained within the algal cell, but are released into the water when the cell is damaged or died [102]. In the same manner, *S. platensis* may act with an allelopathic phenomenon when cell damaged or died but may not affect when it is still alive. The experiment results suggested that the active C-PC **29** pigment may act as a toxin or has an allelochemical effects on breeding organisms in nature.

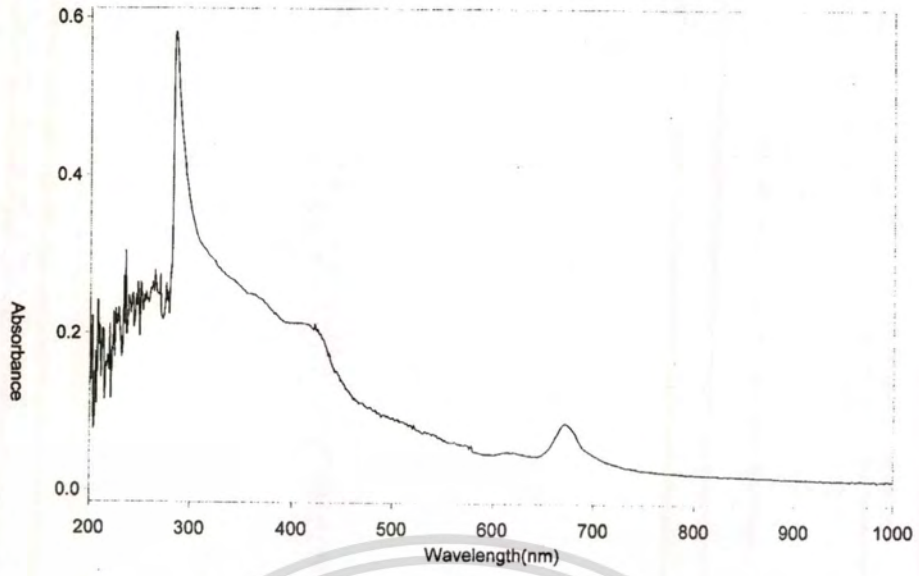
## 4.3 Allelopathic effects of fractions from the aqueous extract on seed germination and seedling growth

The aqueous extract was dehydrated using a freeze-dryer to obtain a blue-green powder. One gram of the blue-green powder was fractionated into 3 fractions, 445.9 mg of F1 (44.59%), 105 mg of F2 (10.50%), and 416.5 mg of F3 (41.65%). Based on the UV-VIS spectra the purity of C-PC **29** of these fractions was determined at $A_{620}/A_{280}$. The result from Table 4.3 and Figure 4.5 indicated that all fractions had a lower ratio than aqueous extract. The blue precipitate fraction (F3) showed an absorption band at 620 nm which indicated the C-PC **29** component. A high absorption of around 280 nm indicated that F3 had a higher protein content than F2 and F1 [81, 103-104]. The fraction F1 consisted of chlorophyll that had an absorbance band at 678 nm [105].

**Table 4.3** Absorbance, purification and concentrations of C-PC **29**

| Samples | $A_{280}$ | $A_{620}$ | Purity ($A_{620}/A_{280}$) | C-PC **29** (mg/mL) |
|---|---|---|---|---|
| Aqueous extract (5%) fiftyfold dilution | 0.118 | 0.138 | 1.169 | 0.020 |
| Hot aqueous extract (5%) fiftyfold dilution | 0.165 | 0.063 | 0.381 | 0.007 |
| F1 (2,000 ppm) | 0.249 | 0.052 | 0.209 | 0.005 |
| F2 (2,000 ppm) | 0.266 | 0.086 | 0.323 | 0.009 |
| F3 (2,000 ppm) | 0.552 | 0.357 | 0.647 | 0.040 |
| C-PC **29** (1,000 ppm) | 0.110 | 0.468 | 4.250 | 0.076 |
| Denatured C-PC (1,000 ppm) | 0.127 | 0.059 | 0.492 | 0.007 |

F1




F2


F3

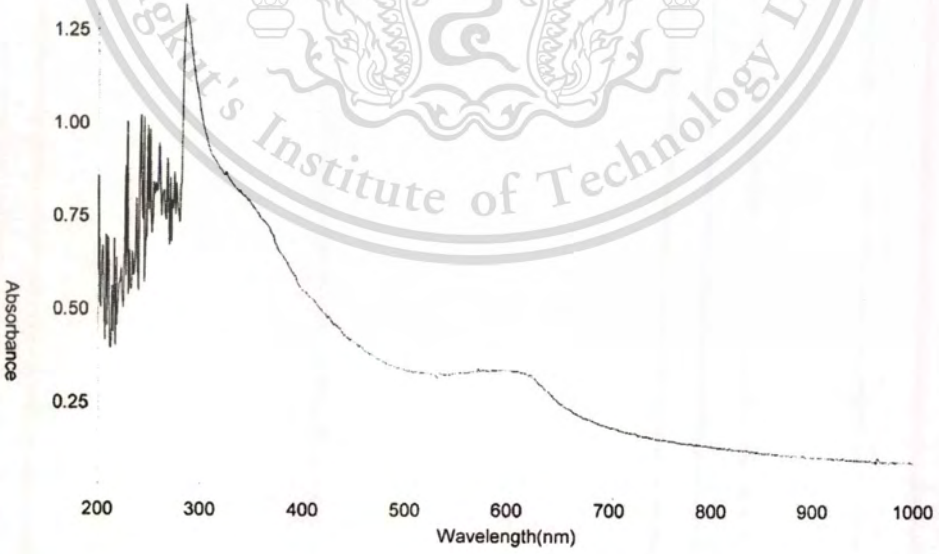



**Figure 4.5** UV-VIS absorption spectra of fractions F1-F3

**Table 4.4** Allelopathic effects of fractions F1-F3 from crude aqueous extract on seed germination and seedling growth of Chinese amaranth and Barnyardgrass

| Fractions-concentrations (ppm) | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0c | 0d-f | 0f-h | 0a | 0h | 0c |
| F1-250 | -2.63c | 1.58d | 5.26ef | 0a | -3.74i | -16.25ef |
| F1-500 | 2.63bc | -6.98fg | -12.54j | 0a | -0.29h | -18.08fg |
| F1-1,000 | 2.63bc | 2.03d | 16.56d | 0a | -0.69h | -9.95de |
| F1-2,000 | 7.90bc | 11.934c | 39.32c | 2.70a | 18.53e | 18.41b |
| F2-250 | 0c | -21.40h | -6.50i | 0a | -1.38hi | -21.06fg |
| F2-500 | 5.26bc | -8.33g | 4.18e-g | 0a | 16.86e | -24.71g |
| F2-1,000 | 2.63bc | 0.45de | 6.50e | 0a | 25.95d | -2.65cd |
| F2-2,000 | 13.16b | 34.91b | 55.73b | 0a | 40.16b | 25.54b |
| F3-250 | 0c | -4.95d-g | -3.41hi | 2.70a | 4.60g | -5.97cd |
| F3-500 | 0c | -6.98fg | -0.93gh | 0a | 8.17f | 0c |
| F3-1,000 | 2.63bc | -5.86e-g | 18.42d | 2.70a | 31.82c | -4.48cd |
| F3-2,000 | 50a | 56.08a | 77.24a | 8.11a | 52.82a | 69.65a |

Values with same letters in each column are not significantly different at $P$=0.05

**Chinese amaranth**

As shown in Table 4.4 and Figure 4.6, the results from the allelopathic assay indicated that fractions F2 and F3 at a concentration of 2,000 ppm inhibited the germination of Chinese amaranth whereas fraction F1 had no effect. In case of seedling growth, all fractions at a concentration of 2,000 ppm had an inhibitory effect on shoot length and at concentrations of 1,000-2,000 ppm inhibited root length. Especially at a concentration of 2,000 ppm fraction F3 had the highest inhibitory effect on shoot and root length of Chinese amaranth by 56.08 and 77.24%, respectively. By contrast, fraction F2 at concentrations of 250-500 ppm promoted shoot length, while at a concentration of 500 ppm fraction F1 promoted root length.

F1

F2

F3

2,000 1,000 500 250 control

**Figure 4.6** Allelopathic effects of fractions F1-F3 from crude aqueous extract of *S. platensis* at concentrations of 250-2,000 ppm on Chinese amaranth

**Barnyardgrass**

The effect of fractions F1-F3 on Barnyardgrass showed that none of these fractions had any effect on seed germination. Fractions F1 at a concentration of 2,000 ppm, F2 at concentrations of 500-2,000 ppm, and F3 at concentrations of 250-2,000 ppm had inhibitory effect on shoot length, especially fraction F3 at a concentration of 2,000 ppm which had the highest inhibitory effect on shoot length by 52.82%. Regarding the effect on root length, it was found that at a concentration of 2,000 ppm all fractions inhibited root length. The highest effect was fraction F3 which inhibited root length by 69.65%. Fractions F1 at concentrations of 250-1,000 ppm and F2 at concentrations of 250-500 ppm promoted root length but other concentrations had no effect (Table 4.4 and Figure 4.7).

F1

F2

F3

2,000 1,000 500 250 control

**Figure 4.7** Allelopathic effects of fractions F1-F3 from crude aqueous extract of *S. platensis* at concentrations of 250-2,000 ppm on Barnyardgrass

The results from Table 4.3 demonstrated that the blue precipitate fraction (F3) which had the highest constituent of C-PC **29** exhibited the strongest allelopathic effect on the tested plants. From the observation of the experiment, it was found that the colour of 3 fractions was paleness with compare to an aqueous extract. All fractions of C-PC **29** had purity ratio lower than an aqueous extract. It should be indicated that C-PC **29** may denature during extraction processes. This result was in agreement with Pasco et al. [106] who described the de-colouration of C-PC **29** when it was dissolved with organic solvents such as aqueous ethanol. Aqueous extraction at beyond 40°C would cause phycocyanin to become unstable [105]. Furthermore, if the phycocyanin becomes completely denatured, it will precipitate out of the solution. The results from this experiment, the author believes that the denatured compositions such as de-colourized C-PC can play an allelopathic role.

## 4.4 Allelopathic effects of denatured C-phycocyanin on seed germination and seedling growth

The purity of the C-PC **29** aqueous solution (pH = 7) was evaluated according to the purity ratio, $A_{620}/A_{280}$. The result found that after heated C-PC **29** solution at 90°C for 3 hrs the absorbance ratio was reduced from 4.25 to 0.49. The absorbance peak at 620 nm was lower and at 280 nm was higher (Figure 4.8), and the colour changed from a deep blue solution to colourless. These results indicated that C-PC **29** was denatured in these conditions. Similarly, several reports referred to the effect of pH and temperature on the degradation or denaturation of aqueous C-PC **29** extract [104-105, 107-109]. The results found that when the temperature was high, denaturation increased while the extract was more stable at the lower pH. These results showed that the denaturation and pH were inversely proportional with respect to the denaturation of phycobiliprotein. The denaturation of phycoprotein is accompanied by a large loss of visible absorption caused by a change in the chromophores from a linear to cyclic conformation. The extract is denatured its deep-blue colour changing to light-blue and finally to a loss of colour depending on the heating temperature and time length. Moreover, the absorption peak at 620 nm decreased while at 280 nm and 370 nm it increased [103-105, 106, 109-111].

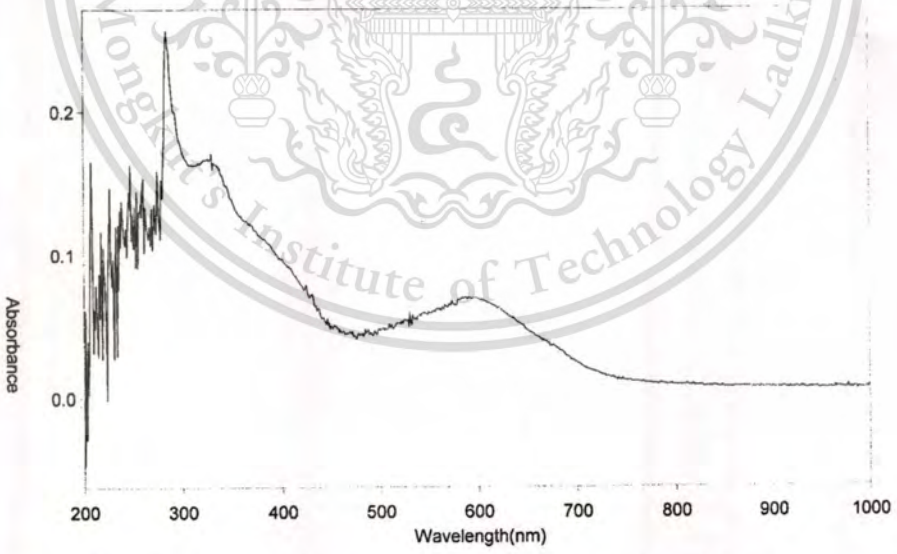



**Figure 4.8** UV-VIS absorption spectrum of denatured C-PC

The alleopathic assay results from Table 4.5 indicated that denatured C-PC had no effect on seed germination of tested plants but showed a high inhibitory activity on the growth of seedling of Chinese amaranth. It also showed a small inhibitory effect on Branyardgrass growth.

When the effect of denatured C-PC and C-PC **29** solutions was compared, it was found that both solutions had an inhibitory effect on seedling growth of Chinese amaranth and Barnyardgrass. The denatured C-PC had an inhibitory effect on shoot of Chinese amaranth lower than C-PC **29** solution. Except at a concentration of 62.5 ppm, all the applied concentrations of the denatured C-PC inhibited the root length of Chinese amaranth that similar to C-PC **29** showed this effect at concentrations of 125-1,000 ppm (Figure 4.9). These results indicated that although C-PC **29** was denatured but its allelopathic effect was slightly changed. In order to confirm this result, the allelopathic effects of hot aqueous extract of *S. platensis* were investigated on tested plants.

**Table 4.5** Allelopathic effects of denatured C-PC on seed germination and seedling growth of Chinese amaranth and Barnyardgrass

| Concentrations (ppm) | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0a | 0c | 0e | 0a | 0b | 0c |
| 62.5 | 0a | 0.75c | 2.29e | 0a | -0.59b | -2.33c |
| 125 | 0a | 1.50c | 32.80d | -2.78a | -1.18b | -3.26c |
| 250 | 2.63a | 2.81c | 38.42c | 0a | -1.45b | -2.64c |
| 500 | 0a | 10.86b | 43.23b | 2.78a | 0.054b | 6.37b |
| 1,000 | 2.63a | 26.40a | 75.69a | 2.78a | 10.75a | 27.02a |

Values with same letters in each column are not significantly different at $P$=0.05

(a)

(b)

1,000 500 250 125 62.5 control

**Figure 4.9** Allelopathic effects of denatured C-PC at concentrations of 62.5-1,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass.

## 4.5 Allelopathic effects of hot aqueous extract on seed germination and seedling growth

Results from Table 4.6 showed that all the applied concentrations of hot aqueous extract of *S. platensis* had allelopathic effects on seed germination and seedling growth of Chinese amaranth, and the extract completely inhibited at a concentration of 1.25% (Figure 4.10). Similarly to the result of denatured C-PC and C-PC **29**, the hot aqueous extract exhibited lower inhibitory effect on shoot of Chinese amaranth than an aqueous extract.

**Table 4.6** Allelopathic effects of hot aqueous extract on seed germination and seedling growth of Chinese amaranth and Barnyardgrass

| Concentrations (% by weight) | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0c | 0c | 0c | 0c | 0c | 0d |
| 0.625 | 13.75b | 17.37b | 46.11b | 0c | -6.83d | 10.79c |
| 1.25 | 100a | 100a | 100a | 23.19b | 20.89b | 53.31b |
| 2.5 | 100a | 100a | 100a | 97.10a | 96.61a | 100a |
| 5 | 100a | 100a | 100a | 100a | 100a | 100a |

Values with same letters in each column are not significantly different at $P$=0.05

(a)

(b)

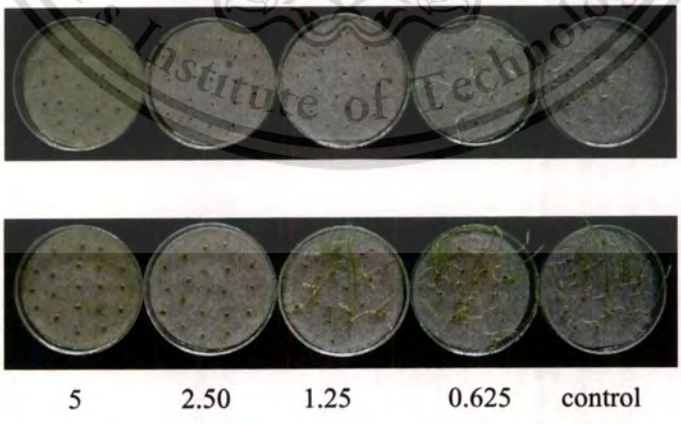



**Figure 4.10** Allelopathic effects of hot aqueous extracts of *S. platensis* at concentrations of 0.625-5% on (a) Chinese amaranth and (b) Barnyardgrass

As for Barnyardgrass the hot aqueous extract at a concentration of 0.625% did not show any inhibitory effect on seed germination whereas the extract promoted shoot length and inhibited root length. The hot extract at a concentration of 1.25% inhibited seed germination and the growth of seedling and at a concentration of 5% completely inhibited seed germination. The root length of tested plants was the most affected and completely inhibited at a concentration of 2.5%.

After determined a purity ratio of C-PC **29** in the hot aqueous extract was determined, the result found that the hot aqueous extract had $A_{620}/A_{280}$ 0.38 and exhibited a very small absorbance band at 620 nm (Figure 4.11). This extract calculated the concentration of C-PC **29** and showed that the concentration was reduced from 0.020 to 0.007 mg/mL as shown in Table 4.3. It should be indicated that a denatured of C-PC **29** was in this solution. The result was in agreement with the previous experiment that denatured C-PC played an allelopathic role. These results are still doubtful because the allelopathic effects of denatured C-PC may depend on the concentration of each extract. It is possible that the hot aqueous extraction may yield crude extract more than aqueous extraction at room temperature. Then the aqueous extract and the hot aqueous extract were dehydrated to dryness and the amount of crude extract in each solution was calculated. The results clearly showed that both solutions had very closely concentrations (107.20 mg/ 3 mL of 5% stock aqueous extract ~ 35,733.33 ppm and 106 mg/3 mL of 5% stock hot aqueous extract ~ 35,333.33 ppm).

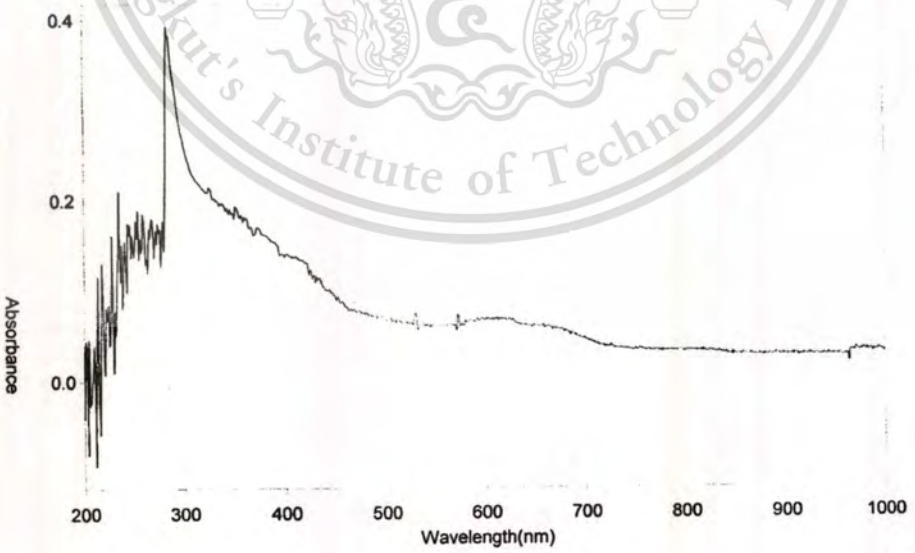
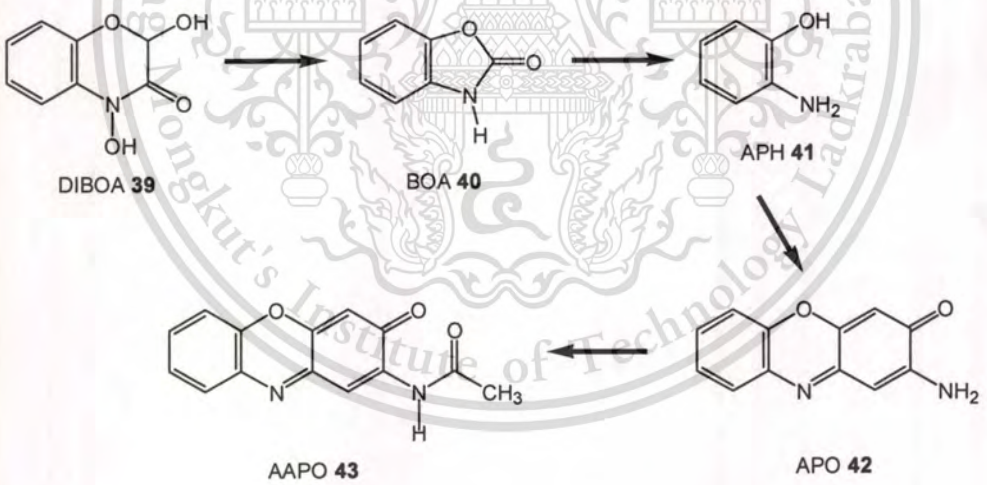



**Figure 4.11** UV-VIS absorption spectrum of hot aqueous extract

The finding in this experiment indicated that the denatured C-PC showed allelopathic activity on the tested plants. These results were in agreement with several researches on allelopathic activity of degraded compounds. A good example was DIBOA **39** (2,4-dihydroxy-1,4-benzoxazin-3-one), an allelochemical produced from many important commercial cultivates including wheat, maize and rice, with phototoxic activity. The degradation of this compound in soil by fungi or bacteria yields a wide variety of compounds such as 2-aminobezoxazolinone (BOA) **40**, 2-aminophenol (APH) **41**, 3-aminophenoxazin-2-one (APO) **42**, and 3-acetamidophenoxazin-2-one (AAPO) **43** (Scheme 4.1). These degradation derivatives, APO **42**, showed excellent allelopathic effect on tested plant [112-117]. Zhou et al. [109] investigated the antioxidant activity of C-PC **29**. The result found that the ability of scavenging hydroxyl radicals greatly increased when this compound denatured, then they concluded that phycobilin moiety is the main part of PC **29** involved in scavenging hydroxyl radicals. From these reasons and experimental results may suggest that although C-PC **29** denatured but it can show allelopathic role, that may depending on the allelopathic mechanism of denatured compound on tested plants but it is still unknown.




**Scheme 4.1** Degradation process of allelochemical DIBOA **39**

## 4.6 Allelopathic effects of crude organic extracts on seed germination and seedling growth

Dried *S. platensis* 4 Kg was extracted by Sequential Solvent Extraction to obtain three crude organic extracts. The weight and percentages of crude extract were showed below.

| | |
|---|---|
| Dried *S. platensis* | 4.00 Kg (100%) |
| Crude hexane extract | 25.90 g (0.65%) |
| Crude ethyl acetate extract (EtOAc) | 125.90 g (3.15%) |
| Crude methanol extract (MeOH) | 795.67 g (19.89%) |


**Figure 4.12** Allelopathic effects of crude organic extracts of *S. platensis* at concentrations of 250-4,000 ppm on (a) Chinese amaranth and (b) Barnyardgrass

**Table 4.7** Allelopathic effects of crude organic extracts on seed germination and seedling growth of Chinese amaranth and Barnyardgrass

| Concentrations (ppm) | % Inhibition on Chinese amaranth | | | % Inhibition on Barnyardgrass | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0ef | 0c | 0de | 0a | 0a-c | 0a |
| Hexane 250 | 0ef | -1.81cd | -10.58fg | 1.32a | -2.32c-e | 2.53a |
| Hexane 500 | -1.45ef | -0.21c | -5.57ef | 0a | -1.61b-d | 1.19a |
| Hexane 1,000 | 0ef | 1.39c | -0.37de | 1.32a | 0.40a-c | -0.14a |
| Hexane 2,000 | 2.90d-f | 12.38b | 0.98de | 2.63a | -8.31gh | 1.40a |
| Hexane 4,000 | 36.23c | 21.34b | 10.95bc | 2.63a | -2.87c-f | 0.28a |
| EtOAc 250 | 1.35d-f | -5.59c-e | -48.89j | 1.32a | 1.51ab | -14.74c |
| EtOAc 500 | 1.35d-f | -6.26c-e | -45.52j | 1.32a | 2.57a | -9.19b |
| EtOAc 1,000 | 5.41de | -0.80c | -18.08gh | 2.63a | -1.36b-d | -12.77c |
| EtOAc 2,000 | 50b | 13.98b | 6.41cd | -1.32a | -1.41b-d | -14.25c |
| EtOAc 4,000 | 95.95a | 76.70a | 80.69a | 3.95a | -2.06c-e | -24.91d |
| MeOH 250 | -2.90f | -12.96d-f | -14.25fg | 0a | -4.18d-f | 2.74a |
| MeOH 500 | -1.45ef | -12.81d-f | -23.83h | 2.63a | -5.34e-g | -13.47c |
| MeOH 1,000 | -2.90f | -16.20ef | -36.36i | 0a | -10.37h | -25.68d |
| MeOH 2,000 | 4.35d-f | -20.32f | -12.46fg | 2.63a | -17.12i | -35.37e |
| MeOH 4,000 | 8.70d | -17.08ef | 16.20b | 2.63a | -5.94fg | -40.35f |

Values with same letters in each column there are not significantly different at $P$=0.05

Results, as shown in Table 4.7 and Figure 4.12, indicated that the effect of crude organic extracts of *S. platensis* on Chinese amaranth at high concentrations applied. Crude hexane and EtOAc extracts had an inhibitory effect on Chinese amaranth while crude MeOH reduced germination and root length, but no inhibitory effect on shoot length. In contrast, crude EtOAc at concentrations of 250-1,000 ppm and crude MeOH at concentrations of 250-2,000 ppm promoted root length, and all applied concentrations of crude MeOH promoted shoot length of Chinese amaranth. Crude EtOAc at a concentration of 4,000 ppm had the highest inhibitory effect on seed germination, shoot and root length of Chinese amaranth 95.95, 76.70, and 80.69%, respectively. As for Barnyardgrass, all the applied concentrations of crude organic extracts had no inhibitory activity on the germination and growth of the tested plant, but showed a moderate promotion

effect. Crude MeOH, especially, showed a moderate promotion effect on the root length of Barnyardgrass.

The results from crude organic extracts showed that the high polarity extract, MeOH, promoted seed germination and seedling growth of tested plants. It has been expected that the MeOH extract consisted of high polarity compounds, for examples, sugars, proteins, vitamins, pigments, minerals and plant growth regulator compounds. Especially, sugars and minerals are the basic nutrients that plants require for growth [118].

### 4.7 Allelopathic effects of fractions from the highest activity crude organic extract on seed germination and seedling growth

Allelopathic assayed of crude ethyl acetate extract indicated the highest inhibitory activity, 65 g (100%) of the crude was isolated by column chromatography to obtain 23 fractions as shown below.

Fraction E1 (1,682.50 mg, 2.59%) eluted by hexane, was obtained as a mixture of yellow oil and orange solid.

Fraction E2 (950.30 mg, 1.46%) eluted by hexane, was obtained as a mixture of brown oil and brown solid.

Fraction E3 (1,558.90 mg, 2.40%) eluted by hexane, was obtained as a mixture brown oil and brown solid.

Fraction E4 (682.80 mg, 1.05%) eluted by gradient solvent range 0-1% EtOAc in hexanes, was obtained as a brown oil.

Fraction E5 (1,516 mg, 2.33%) eluted by gradient solvent range 1-5% EtOAc in hexanes, was obtained as a brown wax.

Fraction E6 (1,194.80 mg, 1.84%) eluted by 5% EtOAc in hexane, was obtained as a dark green oil containing a dark green wax.

Fraction E7 (1,947.60 mg, 3.00%) eluted by gradient solvent range 5-8% EtOAc in hexane, was obtained as a dark green wax.

Fraction E8 (2,329 mg, 3.58%) eluted by gradient solvent range 8-9% EtOAc in hexane, was obtained as a dark green wax.

Fraction E9 (1,663 mg, 2.56%) eluted by gradient solvent range 9-16% EtOAc in hexane, was obtained as a dark green wax.

Fraction E10 (1,647.10 mg, 2.53%) eluted by gradient solvent range 17-22% EtOAc in hexane, was obtained as a dark green wax.

Fraction E11 (700 mg, 1.08%) eluted by gradient solvent range 23-24% EtOAc in hexane, was obtained as a dark green wax.

Fraction E12 (827 mg, 1.27%) eluted by 25% EtOAc in hexane, was obtained as a dark green wax.

Fraction E13 (762 mg, 1.17%) eluted by gradient solvent range 25-27% EtOAc in hexane, was obtained as a dark green wax.

Fraction E14 (2,430.50 mg, 3.74%) eluted by gradient solvent range 28-33 EtOAc in hexane, was obtained as a dark green wax.

Fraction E15 (929.10 mg, 1.43%) eluted by gradient solvent range 34-44% EtOAc in hexane, was obtained as a dark green wax.

Fraction E16 (1,628.60 mg, 22.51%) eluted by gradient solvent range 48-62% EtOAc in hexane, was obtained as a dark brown wax.

Fraction E17 (499.80 mg, 0.77%) eluted by gradient solvent range 64-66% EtOAc in hexane, was obtained as a dark brown wax.

Fraction E18 (10,410.50 mg, 16.02%) eluted by gradient solvent range 66-88% EtOAc in hexane, was obtained as a dark brown wax.

Fraction E19 (2,910.80 mg, 4.48%) eluted by gradient solvent range (90% EtOAc in hexane-pure EtOAc, and 3-5% MeOH in EtOAc), was obtained as a dark brown wax.

Fraction E20 (4,789.6 mg, 7.37%) eluted by gradient solvent range 5-10% MeOH in EtOAc, was obtained as a dark brown wax.

Fraction E21 (8,857 mg, 13.63%) eluted by 10% MeOH in EtOAc, was obtained as a dark brown wax.

Fraction E22 (2,687.50 mg, 4.13%) eluted by 10% MeOH in EtOAc, was obtained as a dark brown wax.

Fraction E23 (2,311.50 mg, 3.56%) eluted by 10% MeOH in EtOAc, was obtained as a dark brown wax.

Total 54,917.20 mg, 84.49%.

**Table 4.8** Allelopathic effects of 23 fractions from crude EtOAc extract on seed germination of Chinese amaranth

| Fractions | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm |
| Control | 0a | 0a | 0de | 0j |
| E1 | -3.03a | 6.06a | 3.03c-e | 36.36e |
| E2 | 3.03a | -3.03a | 15.15bc | 69.70c |
| E3 | 0a | 0a | 9.09b-d | 21.21f-h |
| E4 | 0a | 3.03a | 6.06b-e | 30.30ef |
| E5 | -3.03a | 3.03a | 15.15bc | 72.73c |
| E6 | -3.03a | 0a | 36.36a | 84.85b |
| E7 | 3.03a | 3.03a | 15.15bc | 66.67c |
| E8 | 3.03a | 0a | 3.03c-e | 0j |
| E9 | -3.03a | 0a | 0de | 0j |
| E10 | 0a | 0a | 0de | 15.15h |
| E11 | 0a | -3.03a | 18.18b | 72.73c |
| E12 | 0a | 0a | 3.03c-e | 90.91ab |
| E13 | 0a | 3.03a | 42.42a | 100a |
| E14 | -3.03a | -3.03a | 0de | 51.52d |
| E15 | 0a | 3.03 | 6.06b-e | 12.12hi |
| E16 | -3.03a | -3.03 | 0de | 3.03ij |
| E17 | -3.03a | 0a | 0de | 0j |
| E18 | -6.06a | 0a | 3.03c-e | 3.03ij |
| E19 | -6.06a | 0a | 0de | 18.18gh |
| E20 | -3.03a | -6.06a | -6.06e | -6.06j |
| E21 | 0a | 0a | 3.03c-e | 15.15h |
| E22 | -6.06a | -6.06a | 3.03c-e | 27.27e-g |
| E23 | -3.03a | 0a | 15.15bc | 21.21f-h |

Values with same letters in each column there are not significantly different at $P$=0.05

**Chinese amaranth**

The allelopathic effects of 23 fractions on the germination of Chinese amaranth found that fractions E5-E7, E11, E13 and E23 at concentrations of 1,000-2,000 ppm and fractions E1-E4, E10, E14-E15, E19 and E21-E22 at a concentration of 2,000 ppm significantly inhibited seed

germination, particularly E13 showed complete inhibition at a concentration of 2,000 ppm, however other fractions had no effect on seed germination (Table 4.8).

**Table 4.9** Allelopathic effects of 23 fractions from crude EtOAc extract on seedling growth of Chinese amaranth

| Fractions | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm |
| Control | 0a | 0b-e | 0e-g | 0h-i | 0a-d | 0a | 0b | 0g-i |
| E1 | -2.11ab | -4.38c-g | -14.91i-k | 16.21ef | -11.06e-g | -29.05g | -34.67hi | -3.06hi |
| E2 | -13.45cd | -4.38c-g | -11.51h-j | 20.10e | -25.53ij | -2.81a-c | 0b | 43.02d |
| E3 | -16.69d | -14.59i-k | -29.66l | -12.32j | -26.83ij | -27.54fg | -43.22i | -15.88jk |
| E4 | -4.86a-c | -3.73c-f | -18.31jk | 7.62f-h | -22.81hi | -28.34g | -23.32fh | 7.84fg |
| E5 | -6.00a-c | -14.10h-k | -1.62e-h | 87.03b | -1.51a-e | -18.59d-f | -16.68e-g | 83.92bc |
| E6 | -0.65ab | 10.86a | 68.40a | 90.28ab | -3.22a-f | 0.10a | 69.95a | 89.95b |
| E7 | -14.42cd | -1.30b-e | 10.86b-d | 34.04d | -33.97jk | -11.16cd | -3.52b-d | 48.74d |
| E8 | -17.67d | -11.18f-i | -11.67h-j | -3.08ij | -18.39g-i | -11.76cd | -15.78d-g | -8.54ij |
| E9 | -14.26cd | -27.55l | -23.01kl | -29.17k | 1.61a-c | -18.79d-f | -23.02fh | -26.63l |
| E10 | -21.72d | -15.07i-k | -9.24g-j | 0.65g-i | -35.48jk | -21.61fg | -6.63b-e | 8.84fg |
| E11 | -34.85e | -21.88kl | -11.83h-j | 74.88c | -41.91k | -11.46cd | -8.94b-e | 78.89cc |
| E12 | -15.07cd | -12.80g-j | -6.00f-i | 85.41b | -13.57f-h | -5.93a-c | -14.67c-g | 75.88c |
| E13 | -19.29d | -20.75j-l | -15.88i-k | 100a | -9.95d-g | -8.44a-c | -23.52fh | 100a |
| E14 | -15.40cd | -9.08e-i | 1.13d-g | 91.09ab | -18.59g-i | -22.11fg | 1.31b | 79.90c |
| E15 | -11.18b-d | -11.02f-i | -12.97i-k | 11.02eg | -19.50g-i | -10.85cd | -24.02fh | -6.93i |
| E16 | -5.51a-c | -13.13g-j | -13.00i-k | -12.16j | -7.34c-f | -12.06c-e | -26.23gh | -28.04l |
| E17 | -5.51a-c | -5.51d-h | -12.97i-k | -11.99j | -6.33c-f | -21.06e-g | -37.69i | -20.10kl |
| E18 | 0.81a | -1.62c-e | -5.19e-i | -3.24ij | -2.71a-f | -5.53a-c | -18.69e-g | 4.22gh |
| E19 | -5.83a-c | -3.73c-f | -0.97e-h | 39.22d | -5.53b-f | -10.05b-d | -12.66c-f | 48.44d |
| E20 | 0.49a | -2.43c-f | 4.38c-f | 8.75f-h | 4.92ab | -11.16cd | 3.42b | 2.21gh |
| E21 | 3.57a | 3.08a-d | 5.35c-e | 8.27f-h | 5.73a | -2.31a-c | -7.54b-e | 14.57f |
| E22 | 2.76a | 7.13ab | 18.48b | 35.17d | 2.91a-c | -1.01ab | -2.41bc | 42.71d |
| E23 | 2.43a | 3.73a-c | 11.67bc | 36.30d | -1.51a-e | -10.15b-d | -7.04b-e | 32.66e |

Values with same letters in each column there are not significantly different at $P$=0.05

The inhibitory effect on shoot length showed that fractions E6 at concentrations of 500-2,000 ppm, E7, E22 and E23 at concentrations of 1,000-2,000 ppm and E1-E2, E5, E11-E15 and E19 at a concentration of 2,000 ppm significantly reduced shoot length. In case of root lengths, fraction E6 at concentrations of 1,000-2,000 ppm, E2, E5, E7, E11-E14, E19 and E21-E23 at a concentration of 2,000 ppm significantly suppressed the growth of root length. In contrast, other fractions and other concentrations promoted root length or did not affect on root length (Table 4.9 and Figure 4.13).

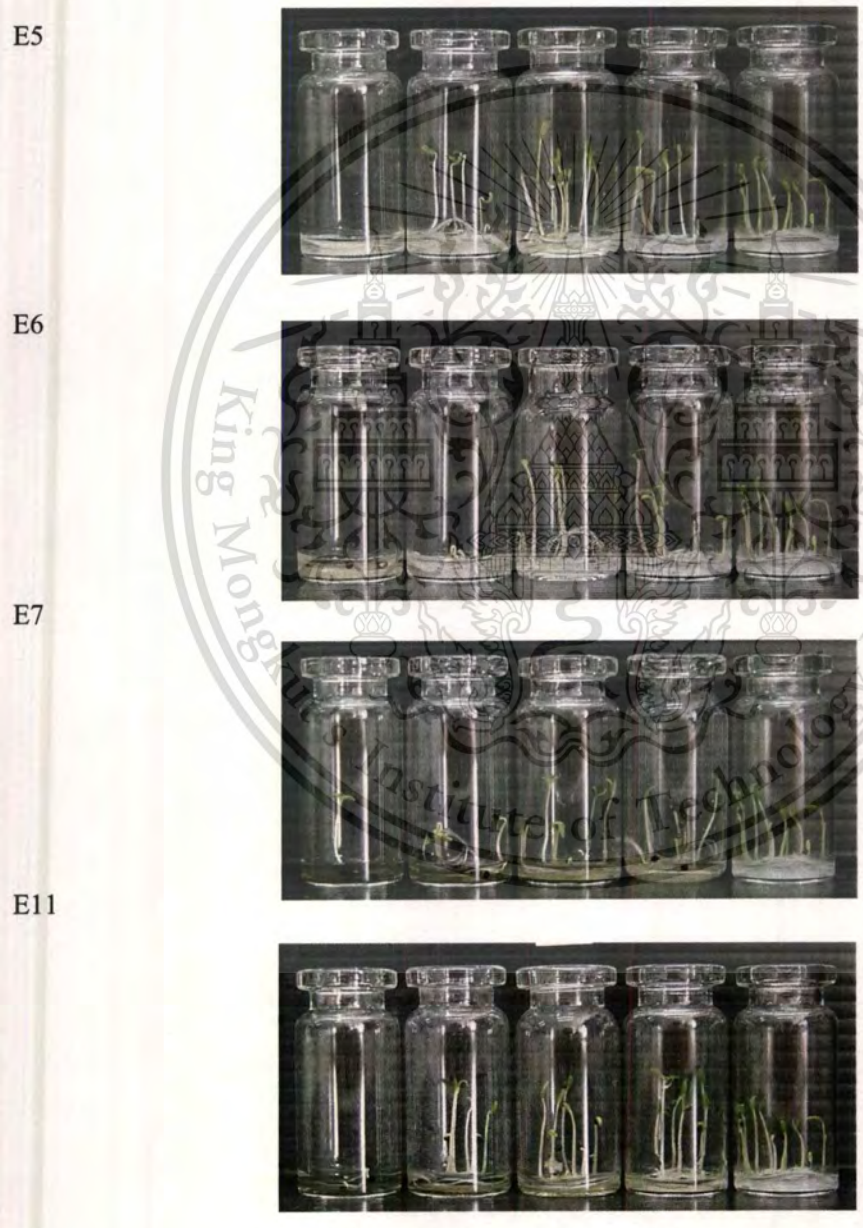



**Figure 4.13** Allelopathic effects of fractions, E5-E7 and E11-E14 from crude EtOAc extract at concentrations of 250-2,000 ppm on Chinese amaranth

E12

E13

E14

2,000 1,000 500 250 control

**Figure 4.13** Allelopathic effects of fractions, E5-E7 and E11-E14 from crude EtOAc extract at concentrations of 250-2,000 ppm on Chinese amaranth (Continued)

**Barnyardgrass**

The result for Barnyardgrass showed that fraction E5 at a concentration of 2,000 ppm and E6 at concentrations of 500-2,000 ppm exhibited an inhibitory effect on seed germination. In case of shoot length, fractions E5 at concentrations of 1,000-2,000 ppm, E6 at concentrations of 500-2,000 ppm, and E22 at a concentration of 2,000 ppm inhibited shoot length. For root length, it was found that E2 at concentrations of 1,000-2,000 ppm, E3 at a concentration of 500 ppm and E6 at a concentration of 250 ppm showed promotion root length. In contrast, fractions E5, E6 and E21 at concentrations of 1,000-2,000 ppm and fractions E7, E8, E19, E20, E22 and E23 at a concentration of 2,000 ppm significantly reduced root length. The E6 fraction at a concentration of 2,000 ppm caused the highest inhibitory effect on seed germination, the growth of shoot and root length 91.11, 49.47 and 72.12%, respectively (Table 4.10-4.11 and Figure 4.14).

**Table 4.10** Allelopathic effects of 23 fractions from crude EtOAc extract on seed germination of Barnyardgrass

| Fractions | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm |
| Control | 0a | 0b | 0b | 0c |
| E1 | -5.26a | 0b | 5.26b | 5.26c |
| E2 | 0a | 2.63ab | 2.63b | 0c |
| E3 | 5.26a | 5.26ab | 5.26b | 2.63c |
| E4 | 0a | 2.63ab | 0b | 0c |
| E5 | -2.63a | 2.63ab | 7.89b | 18.42b |
| E6 | 0a | 18.42a | 71.05a | 92.11a |
| E7 | 0a | 2.63ab | 2.63b | 0c |
| E8 | 2.63a | -2.63b | 2.63b | 0c |
| E9 | 0a | 0b | 2.63b | 5.26c |
| E10 | 2.63a | -2.63b | 2.63b | 7.89bc |
| E11 | 0a | 0b | 5.26b | 7.89bc |
| E12 | 2.63a | 5.26ab | 0b | -2.63c |
| E13 | 0a | 5.26ab | 0b | 2.63c |
| E14 | 5.26a | -2.63b | 5.26b | 7.89bc |
| E15 | 2.63a | 5.26ab | 5.26b | 0c |
| E16 | -5.26a | 0b | 0b | 0c |
| E17 | 2.63a | 0b | 2.63b | -2.63c |
| E18 | 5.26a | -2.63b | -2.63b | 2.63c |
| E19 | -2.63a | 5.26ab | -2.63b | 5.26c |
| E20 | 0a | 5.26ab | 2.63b | 2.63c |
| E21 | 2.63a | 2.63ab | 0b | 0c |
| E22 | 5.26a | 2.63ab | 5.26b | 2.63c |
| E23 | 0a | 5.26ab | 5.26b | 5.26c |

Values with same letters in each column there are not significantly different at $P$=0.05

**Table 4.11** Allelopathic effects of 23 fractions from crude EtOAc extract on seedling growth of Barnyardgrass

| Fractions | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm |
| Control | 0ab | 0bc | 0c | 0d | 0a | 0ab | 0c-f | 0g |
| E1 | -7.85b | 4.78bc | -0.18c | 6.84cd | -5.18ab | 2.59ab | -5.53fg | 0.82g |
| E2 | -3.12ab | -2.43bc | -3.12c | -3.45d | -0.59a | -3.41bc | -10.12g | -17.76h |
| E3 | 3.45ab | 0.69bc | 5.74bc | 2.62cd | 0.59a | -11.53c | 6.12c-e | 7.06e-g |
| E4 | 3.63ab | 6.20bc | 6.16bc | 6.56cd | 7.41a | 4.35ab | 2.94c-f | 6.82e-g |
| E5 | 4.73a | 6.34bc | 12.10b | 30.11b | 5.18a | 1.18ab | 25.88b | 46.71b |
| E6 | 5.24a | 16.77a | 44.88a | 49.47a | -16.47b | -4.71bc | 52.94a | 72.12a |
| E7 | 4.23a | 0.64bc | 0.41bc | 6.29cd | 7.06a | -4.24bc | 1.18c-f | 20d |
| E8 | 3.26ab | -1.06bc | -2.02c | 5.28cd | 3.29a | 7.29a | 0c-f | 15.41d-f |
| E9 | -4.09ab | -3.90c | 2.94bc | 4.36cd | -3.88ab | 2.35ab | 0.82c-f | 7.18e-g |
| E10 | -3.63ab | 3.49bc | 5.47bc | 1.52cd | -0.24a | 2.71ab | 6.12c-e | 0.71g |
| E11 | 3.72ab | 5.10bc | 3.49bc | 4.78cd | 3.88a | -3.53bc | 6.24c-e | 2.47g |
| E12 | 2.16ab | 3.90bc | 7.49bc | 5.37cd | 5.53a | 8.35a | 6.24c-e | 2.35g |
| E13 | 3.17ab | -2.53bc | 5.93bc | 3.12cd | 3.06a | -3.53bc | 5.53c-e | -1.18g |
| E14 | 5.14a | 7.44b | 8.22bc | 1.33d | 8.24a | 5.06ab | 5.88c-e | -3.65g |
| E15 | 0.87ab | 1.65bc | 4.23bc | 6.02cd | 3.29a | 2.24ab | 7.06cd | 6fg |
| E16 | 3.22ab | 5.51bc | 5.05bc | 6.20cd | 5.29a | 3.76ab | -1.53d-f | 7.53e-g |
| E17 | 4.04a | 3.40bc | 5.93bc | 2.66cd | 1.06a | 2.35ab | 5.88c-e | 5.76fg |
| E18 | -1.93ab | 4.13bc | 3.03bc | 3.26cd | -1.65a | 5.18ab | 6c-e | 2.71g |
| E19 | 2.94ab | 5.10bc | 2.89bc | 8.22cd | 8.12a | 3.65ab | 6.71cd | 16.12d-f |
| E20 | 4.00a | 4.00bc | 4.32bc | 4.09cd | 6a | 7.06a | 8.24c | 17.53de |
| E21 | 3.77ab | -1.42bc | 2.89bc | 10.51cd | 7.18a | -0.82ab | 26b | 31.18c |
| E22 | 2.25ab | 3.17bc | 4.59bc | 16.35c | 1.88a | -0.12ab | 5.18c-e | 36.24c |
| E23 | 2.89ab | 3.26bc | -0.28c | 10.28cd | -0.71a | 7.88a | -2.82e-g | 16.47d-f |

Values with same letters in each column there are not significantly different at $P$=0.05

E5

E6

2,000 1,000 500 250 control

**Figure 4.14** Allelopathic effects of fractions E5 and E6 from crude EtOAc extract at concentrations of 250-2,000 ppm on Barnyardgrass

## 4.8 Allelopathic effects of 7 subfractions from E6 on seed germination and seedling growth

As mentioned in the previous experiment, fraction E6 exhibited high allelopatic effects on seed germination and seedling growth of both tested plants. In order to investigate the active compounds, fraction E6 was fractionated into seven subfractions and the allelopathic activity studied at concentrations of 125-1,000 ppm.

The weight and percent by weight of seven subfractions with compared to E6 954 mg (100%) as shown below.

Subfraction E6F1 (19.20 mg, 2.01%) eluted by gradient solvent range (0-2% EtOAc in hexanes), was obtained as a light yellow wax.

Subfraction E6F2 (141.30 mg, 14.81%) eluted by 3% EtOAc in hexane, was obtained as a white solid.

Subfraction E6F3 (255.20 mg, 26.75%) eluted by 4% EtOAc in hexane, was obtained as a mixture of white solid and yellow wax.

Subfraction E6F4 (105.20 mg, 11.03%) eluted by gradient solvent range (4-5% EtOAc in hexanes), was obtained as a dark green oil.

Subfraction E6F5 (186.50 mg, 19.55%) eluted by 5% EtOAc in hexane, was obtained as a dark brown oil.

Subfraction E6F6 (121.10 mg, 12.69%) eluted by gradient solvent range (6-9% EtOAc in hexanes), was obtained as a dark brown oil.

Subfraction E6F7 (113.80 mg, 11.93%) eluted by gradient solvent range (10% EtOAc in hexane-pure EtOAc), was obtained as a dark brown oil.

Total 942.30 mg, 98.77%.

**Chinese amaranth**

As shown in Table 4.12, the allelopathic effects of seven subfractions from E6 on Chinese amaranth found that subfractions E6F3 at a concentration of 1,000 ppm, E6F4 at concentrations of 250-1,000 ppm and E6F5-E6F7 at concentrations of 500-1,000 ppm inhibited seed germination. In particular, E6F4 at a concentration of 500 ppm had a complete inhibitory effect on the germination of Chinese amaranth seeds.


**Figure 4.15** Allelopathic effects of subfraction E6F3-E6F5 at concentrations of 125-1,000 ppm on Chinese amaranth

**Table 4.12** Allelopathic effects of 7 subfractions from E6 on seed germination of Chinese amaranth

| Subfrations | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 125ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0b | 0c | 0c |
| E6F1 | 0a | 3.33b | 0c | 6.67c |
| E6F2 | 0a | 6.67b | 0c | -3.33c |
| E6F3 | -3.33a | 6.67b | 6.67c | 100a |
| E6F4 | 0a | 66.67a | 100a | 100a |
| E6F5 | 0a | 6.67b | 93.33a | 100a |
| E6F6 | -3.33a | 3.33b | 33.33b | 43.33b |
| E6F7 | 3.33a | 3.33b | 26.67b | 50b |

Values with same letters in each column there are not significantly different at $P$=0.05

**Table 4.13** Allelopathic effects of 7 subfractions from E6 on seedling growth of Chinese amaranth

| Subfrations | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0bc | 0bc | 0d | 0ab | 0b | 0d | 0d |
| E6F1 | -2.96a | -16.67e | -24.63d | -18.15e | -1.58b | -29.57e | -38.48f | -18.10e |
| E6F2 | 1.67a | -3.89b-d | -17.59d | -17.59e | 2.57a | -0.79b | 0.40d | -1.29d |
| E6F3 | 0.74a | -0.37bc | 7.41b | 100a | 0.20ab | -6.73c | 20.57c | 100a |
| E6F4 | -0.37a | 64.81a | 100a | 100a | 2.08a | 58.46a | 100a | 100a |
| E6F5 | -0.56a | -6.48cd | 94.44a | 100a | -14.54c | -3.66bc | 95.05b | 100a |
| E6F6 | -2.59a | -8.89d | -1.85c | 19.81c | 0ab | -12.76d | -4.45d | 24.73c |
| E6F7 | 0.37a | 2.78b | 6.30b | 46.67b | -14.05c | -13.16d | -9.20e | 41.64b |

Values with same letters in each column there are not significantly different at $P$=0.05

The effect on seedling growth of Chinese amaranth found that subfractions E6F4 at concentrations of 250-1,000 ppm, E6F5 at concentrations of 500-1,000 ppm and E6F3, E6F6-E6F7 at a concentration of 1,000 ppm showed an inhibitory effect on the growth of seedling. The strongest effect was found in subfraction E6F4 at a concentration of 500 ppm that exhibited

completely inhibition on seedling growth, while subfractions E6F1 at concentrations of 250-1,000 ppm and E6F2 at concentrations of 500-1,000 ppm promoted shoot length. The promotion of root length was found in subfractions E6F1 at concentrations of 250-1,000 ppm, E6F3 and E6F6 at a concentration of 250 ppm, E6F5 at concentrations of 125-250 ppm and E6F7 at concentrations of 125-500 ppm (Table 4.13 and Figure 4.15).

**Barnyardgrass**

The results from Table 4.14 indicated that subfraction E6F7 at a concentration of 1,000 ppm, E6F3 and E6F5 at concentrations of 500-1,000 ppm and E6F4 at concentrations of 250-1,000 ppm exhibited allelopathic potential on seed germination of Barnyardgrass. Subfractions E6F3 and E6F7 at a concentration of 1,000 ppm, E6F5 at concentrations of 500-1,000 ppm, and E6F4 at concentrations of 250-1,000 ppm reduced shoot length. As for root length, subfractions E6F3 and E6F7 at a concentration of 1,000 ppm and E6F4-E6F5 at concentrations of 500-1,000 ppm exhibited an inhibitory effect. However, subfractions E6F5 at a concentration of 125 ppm and E6F6 at concentrations of 125-250 ppm promoted root length. The subfraction E6F4 at a concentration of 1,000 ppm exhibited the highest inhibitory activity on the germination of seed and shoot length by 94.59 and 95.69%, respectively and completely inhibited root length (Figure 4.16).

**Table 4.14** Allelopathic effects of 7 subfractions from E6 on seed germination of Barnyardgrass

| Subfrations | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 125ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0b | 0c | 0e |
| E6F1 | -2.70a | 2.70b | 0c | 2.70de |
| E6F2 | -2.70a | 2.70b | 2.70c | 0e |
| E6F3 | 0a | 2.70b | 27.03b | 54.05c |
| E6F4 | 2.70a | 35.14a | 81.08a | 94.59a |
| E6F5 | 2.70a | 5.41b | 27.03b | 83.78b |
| E6F6 | 0a | 5.41b | -2.70c | 2.70de |
| E6F7 | -2.70a | 0b | 0c | 10.81e |

Values with same letters in each column there are not significantly different at $P$=0.05

**Table 4.15** Allelopathic effects of 7 subfractions from E6 on seedling growth of Barnyardgrass

| Subfrations | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0ab | 0bc | 0cd | 0e | 0ab | 0a | 0cde | 0e |
| E6F1 | -0.62ab | -0.48c | -1.73d | 4.22e | 1.34b | 2.52a | -5.64e | -4.30e |
| E6F2 | -1.77b | 3.79bc | 3.88c | 3.93e | -3.71a | 0.74a | -1.78de | 1.04e |
| E6F3 | 4.99a | 4.03bc | 3.45c | 22.77c | 4.15ab | 0.59a | -2.08de | 24.63c |
| E6F4 | 3.79ab | 26.89a | 79.39a | 95.69a | 0ab | 1.78a | 89.17a | 100a |
| E6F5 | 5.66a | 3.74bc | 19.08b | 53.02b | -9.79c | 4.15a | 20.62b | 43.62b |
| E6F6 | 0.24ab | 4.46bc | 3.64c | 4.03e | -11.42c | -9.94b | 4.30c | -6.82e |
| E6F7 | 5.08a | 5.18b | 3.55c | 9.40d | 0.45ab | 3.41a | 3.41cd | 9.05d |

Values with same letters in each column there are not significantly different at $P$=0.05


**Figure 4.16** Allelopathic effects of subfractions E6F3-E6F5 at concentrations of 125-1,000 ppm on Barnyardgrass

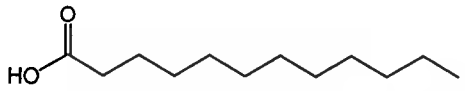
## 4.9 Isolation and Identification of active compounds from fractions E5-E6 and subfractions E6F3-E6F5

The $^1$H NMR data indicated that the active fractions and subfractions E5, E6, E6F3-E6F5 consisted of fatty acids in high yield (data not show). These subfractions were prepared to produce fatty acid methyl esters (FAMEs), identified and analyzed by GC-MS using heptadecanoic acid methyl ester as an internal standard. According to several research studies palmitic acid **10**, γ-linolenic acid **11**, linoleic acid **12**, stearic acid **13**, and oleic acid **14** are the major fatty acids in *S. platensis* [47]. These fatty acids were quantified by GC-MS analysis as shown in Appendix A and Figure A1. However, some of the identified constituents could not be quantified because they were minor compounds. The compound compositions of these subfractions are shown in Table 4.16. Experimental results reported the retention time, % relative amount of peak area, concentration of fatty acids in 1,500 ppm sample solution, formula, molecular weight, and mass fragment ion of compounds.

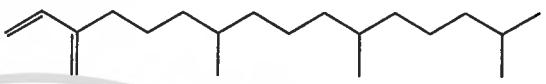
The GC-MS analysis of two active fractions and three active subfractions allowed the identification of 16 known compounds. The fraction E5 is made up of palmitic acid **10** (59.38%) as a major constituent, linoleic acid **12** (13.41%), oleic acid **14** (8.79%), stearic acid **13** (7.26%), dihydroactinidiolide **51** (4.00%), and other minor compounds, palmitoleic acid **15**, pentadecanoic acid **47**, tetradecanoic acid **46**, 4-oxo-β-ionone **52** and γ-linolenic acid **11**, respectively. The fraction E6 consisted of linoleic acid **12** (34.80%) as a major constituent, palmitic acid **10** (34.46%), γ-linolenic acid **11** (11.84%), oleic acid **14** (5.19%), palmitoleic acid **15** (5.15%), stearic acid **13** (2.81%), dihydroactinidiolide **51** (1.75%), 4-oxo-β-ionone **52** (1.46%), pentadecanoic acid **47** (1.13%), and other minor compounds, tetradecanoic acid **41** and 2-ethyl-3-methylmaleimide **50**, respectively.

The GC-MS results of active subfractions, E6F3-E6F5, showed that subfraction E6F3 is made up of palmitic acid **10** (58.71%) as a major constituent, palmitoleic acid **15** (12.82%), oleic acid **14** (11.76%), tetradecanoic acid **46** (3.97%), pentadecanoic acid **47** (2.16%), and other minor compounds, dodecanoic acid **44**, 9-oxonanoic acid **48**, azelaic acid **49**, dihydroactinidiolide **51**, stearic acid **13**, 4-oxo-β-ionone **52** and linoleic acid **12**, respectively. The most active subfraction, E6F4, consisted of linoleic acid **12** (72.19%) as a major constituent, dihydroactinidiolide **51** (8.32%), palmitoleic acid **15** (4.55%), γ-linolenic acid **11** (3.02%), 2-ethyl-3-methylmaleimide **50** (2.90%), 5,8,11-eicosatrienoic acid **53** (2.53%), palmitic acid **10**

(2.29%), 4-oxo-β-ionone **52** (2.15%), and other minor compounds, 9-oxonanoic acid **48**, stearic acid **13**, and oleic acid **14**, respectively. The active subfraction E6F5 consisted of palmitic acid **10** (31.41%) as a major constituent, linoleic acid **12** (31.24%), γ-linolenic acid **11** (23.46%), 5,8,11-eicosatrienoic acid **53** (2.80%), oleic acid **14** (3.16%), palmitoleic acid **15** (2.71%), 4-oxo-β-ionone **52** (1.91%), and other minor compounds, neophytadiene **45**, tetradecanoic acid **46** and stearic acid **13**, respectively.



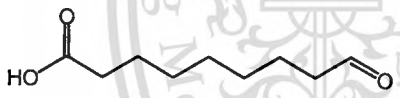
Dodecanoic acid **44**



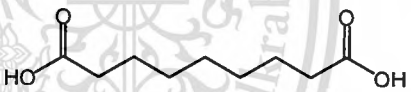
Neophytadiene **45**

Tetradecanoic acid **46**

Pentadecanoic acid **47**

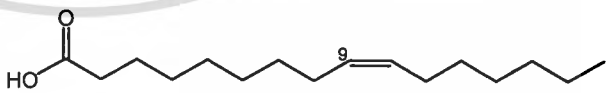


9-Oxonanoic acid **48**



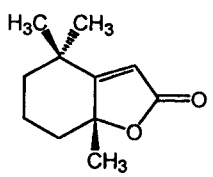
Azelaic acid **49**

Palmitic acid **10**



Palmitoleic acid **15**

2-Ethyl-3-methylmaleimide **50**



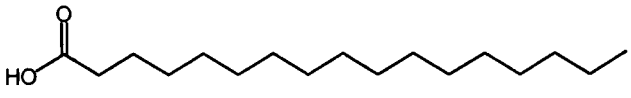
Dihydroactinidiolide **51**

**Table 4.16** Chemical compositions of fractions E5-E6 and subfractions E6F3-E6F5

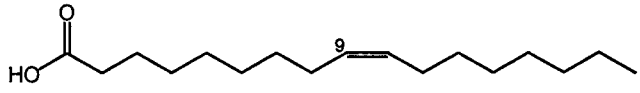
| Compounds | RT | Relative amount % and concentrations (ppm) | | | | | Formula | MW | Fragment ion (m/z) |
|---|---|---|---|---|---|---|---|---|---|
| | | E5 | E6 | E6F3 | E6F4 | E6F5 | | | |
| Methyl dodecanoate | 7.21 | 0 | 0 | 0.55 | 0 | 0 | $C_{13}H_{26}O_2$ | 214 | 214 $[M]^+$, 183, 171, 157, 143, 129, 115, 101, 87, 74, 69, 55 |
| Neophytadiene **45** | 7.96 | 0 | 0 | 0 | 0 | 1.04 | $C_{20}H_{38}$ | 278 | 278 $[M]^+$, 151, 137, 123, 109, 103, 95, 89, 82, 68, 57 |
| Methyl tetradecanoate | 8.28 | 0.95 | 0.95 | 3.97 | 0 | 0.41 | $C_{15}H_{30}O_2$ | 242 | 242 $[M]^+$, 211, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 69, 55 |
| Methyl pentadecanoate | 8.60 | 1.06 | 1.13 | 2.16 | 0 | 0 | $C_{16}H_{32}O_2$ | 256 | 256 $[M]^+$, 225, 213, 199, 185, 171, 157, 143, 129, 111, 101, 87, 74, 69, 55 |
| Methyl-9-oxonanoate | 8.63 | 0 | 0 | 2.04 | 0.46 | 0 | $C_{10}H_{18}O_3$ | 186 | 155, 143, 136, 111, 87, 74, 69, 55 |
| Methyl azelate | 9.15 | 0 | 0 | 1.60 | 0 | 0 | $C_{11}H_{20}O_4$ | 216 | 185 $[M\text{-}OCH_3]^+$, 152, 143, 133, 124, 117, 111, 97, 89, 83, 74, 69, 59, 55 |
| Methyl palmitate | 9.71 | 59.38 (483.58) | 34.46 (191.77) | 58.71 (385.94) | 2.29 (13.59) | 31.41 (229.96) | $C_{17}H_{34}O_2$ | 270 | 270 $[M]^+$, 239, 227, 213, 199, 185, 171, 157, 143,129, 115, 97, 87, 74, 69, 55 |
| Methyl palmitoleate | 9.96 | 3.38 | 5.15 | 12.82 | 4.55 | 2.71 | $C_{17}H_{32}O_2$ | 268 | 268 $[M]^+$, 237, 207, 194, 179, 165, 152, 143, 137, 129, 123, 110, 97, 87, 81, 75, 69, 55 |
| 2-Ethyl-3-methylmaleimide **50** | 10.29 | 0 | 0.44 | 0 | 2.90 | 0 | $C_7H_9O_2N$ | 139 | 139 $[M]^+$, 124, 110, 106, 96, 87, 73, 67, 53 |
| Methyl heptadecanoate (Internal standard) | 10.63 | - | - | - | - | - | $C_{18}H_{36}O_2$ | 284 | 284 $[M]^+$, 253, 241, 227, 213, 199, 185, 157, 143, 129, 97, 87, 74, 55 |
| Dihydroactinidiolide **51** | 11.34 | 4.00 | 1.75 | 1.27 | 8.32 | 0 | $C_{11}H_{10}O_2$ | 180 | 180 $[M]^+$, 165, 152, 137, 124, 111, 95, 89, 81, 77, 73, 67, 55 |

**Table 4.16** Chemical compositions of fractions E5-E6 and subfractions E6F3-E6F5 (Continued)

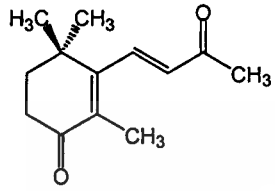
| Compounds | RT | Relative amount % and concentrations (ppm) | | | | | Formula | MW | Fragment ion (m/z) |
|---|---|---|---|---|---|---|---|---|---|
| | | E5 | E6 | E6F3 | E6F4 | E6F5 | | | |
| Methyl stearate | 11.71 | 7.26 (65.30) | 2.81 (17.30) | 1.69 (12.28) | 0.42 (2.73) | 1.87 (15.13) | $C_{19}H_{38}O_2$ | 298 | 298 $[M]^+$, 267, 255, 241, 213, 199, 185, 171, 157, 143, 129, 111, 97, 87, 74, 55 |
| Methyl oleate | 11.98 | 8.79 (75.13) | 5.19 (30.35) | 11.76 (81.16) | 1.17 (7.30) | 3.16 (24.26) | $C_{19}H_{36}O_2$ | 296 | 296 $[M]^+$, 264, 253, 246, 235, 222, 213, 207, 194, 180, 166, 152, 143, 137, 129, 123, 110, 97, 74, 69, 55 |
| 4-Oxo-β-ionone **52** | 12.32 | 0.92 | 1.46 | 1.66 | 2.15 | 1.91 | $C_{13}H_{18}O_2$ | 206 | 206 $[M]^+$, 191, 177, 163, 150, 135, 122, 107, 91, 77, 65, 55 |
| Methyl linoleate | 12.59 | 13.41 (104.26) | 34.80 (184.84) | 1.76 (11.07) | 72.19 (409.15) | 31.24 (218.32) | $C_{19}H_{34}O_2$ | 294 | 294 $[M]^+$, 263, 220, 192, 178, 164, 150, 141, 135, 129, 123, 115, 109, 95, 87, 81, 74, 67,55 |
| Methyl-γ-linolenate | 13.01 | 0.84 (13.12) | 11.84 (125.73) | 0 | 3.02 (34.19) | 23.46 (327.93) | $C_{19}H_{32}O_2$ | 292 | 292 $[M]^+$, 243, 235, 221, 194, 175, 163, 157, 150, 144, 135, 129, 121, 115, 107, 101, 93, 87, 79, 67, 55 |
| Methyl-5,8,11-eicosatrienoate | 16.01 | 0 | 0 | 0 | 2.53 | 2.80 | $C_{21}H_{36}O_2$ | 320 | 320 $[M]^+$, 263, 222, 207, 190, 177, 163, 150, 143, 133, 121, 107, 94, 89, 79, 67, 59 |



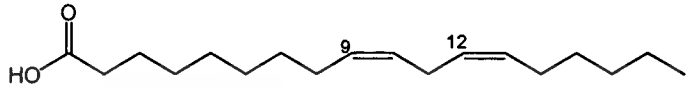
Stearic acid **13**



Oleic acid **14**



4-Oxo-β-ionone **52**



Linoleic acid **12**

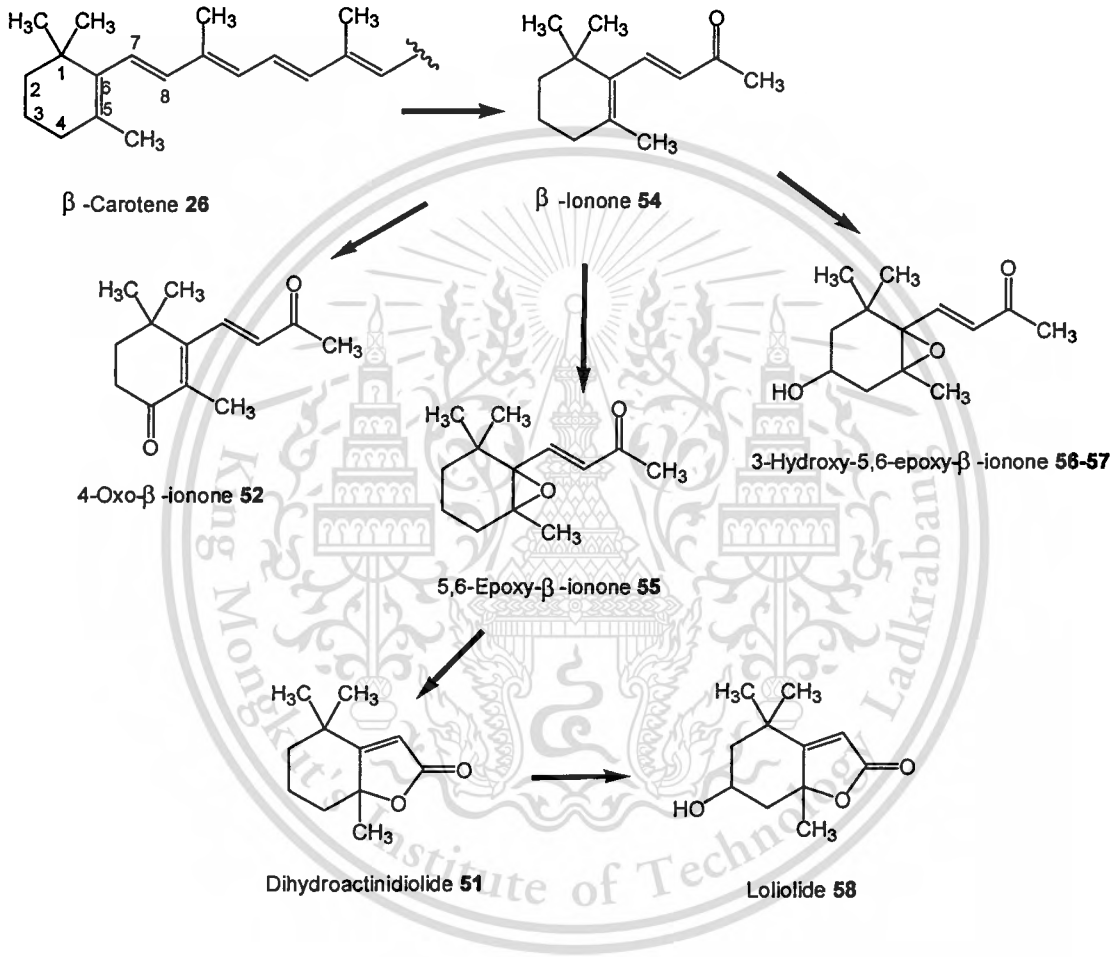
γ-Linolenic acid **11**

5,8,11-Eicosatrienoic acid **53**

The results from the FAMEs analysis found that the active fractions and subfractions contained 4 non-fatty acid compounds, i.e. neophytadiene **45**, 2-ethyl-3-methylmaleimide **50**, dihydroactinidiolide **51** and 4-oxo-β-ionone **52** in minute amounts. It has been reported that neophytadiene **45** has been detected from *S. platensis*, this being a minor component of volatile compounds [119]. The simple alkaloid, 2-ethyl-3-methylmaleimide **50**, had also been detected from ground water [120], caffeinated beverages [121] and essential oil of *V. spiralis* [122]. This compound is a byproduct of the photooxidation of chlorophyll [123].

Dihydroactinidiolide **51** is a $C_{11}$-terpenic lactone was an essential oil that was first isolated from the leaves of *Actinidia polygama* and was active, at low level, toward Felidae animals [124]. It was an important flavour component from tobacco, tea, coffee juice [125], Centaurea [126], and macrophytes [127]. Moreover, it is one of the three components of the pheromone for queen recognition by red fire worker ants, *Soleneopsis invicta* [128] and could be released from an aquatic plant, *Eleocharis coloradoenis*, which had a distinct allelopathy on other

coexisting macrophytes [129-130]. 4-Oxo-β-ionone **52** is a derivative of ionone and an oxidative degration product of β-carotene **26** [131]. It is the minor product from aerobic oxidation process of α-ionone catalyzed by *N*-hydroxyphthalimide combined with acetylacetone cobalt (II) catalyst (NHPI/$(Co(acac)_2)$) [132]. In addition, this compound is also obtained from commercially available β-ionone **54** by a two-step protocol involving selective epoxidation of the free double bond followed by a base catalyzed isomerization [133].



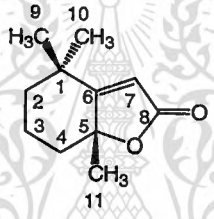

**Scheme 4.2** Degradation products of β-carotene **26**

Both dihydroactinidiolide **51** and 4-oxo-β-ionone **52** are derivatives of β-carotene **26**. Photo-oxidation of carotenoids in living plants (i.e. flowers) which is often responsible for their odor. Many carotenoids and β-carotene **26**, when cleaved, gives rise to various compounds [134-137]. Among them, β-ionone **54**, 5,6-epoxy-β-ionone **55** and dihydroactinidiolide **51** are often associated with fruity, floral and woody notes to varying degrees. Pathways of these production compounds were proposed by Xian et al. in Scheme 4.2 [122]. Enzymatic catalysis was very

important, especially lipoxygenase in the processes of their biosynthesis. The pathway that dihydroactinidiolide **51** was produced through β-ionone **54** had been proposed by Bosser [138]. However, the chemical pathway may involve the formation of 5, 6-epoxy-β-carotene from β-carotene **26** and further cleavage into dihydroactinidiolide **51** [139]. As the derivatives of chlorophyll or carotene, all compounds are secondary metabolites with biological activities from above references.

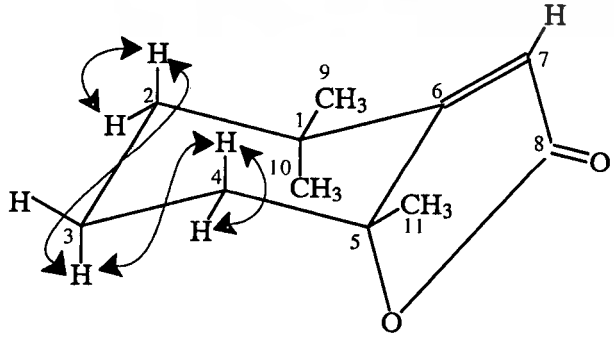
From GC-MS resulted, dihydroactinidiolide **51** and 4-oxo-β-ionone **52** were isolated from subfractions E6F3-E6F5 by flash column chromatography eluted with hexane : EtOAc mixtures of increasing polarity and identified by $^{1}$H NMR, $^{13}$C NMR, 2D NMR (COSY and HETCOR), IR spectroscopy and MS spectrometry as shown below.

**Dihydroactinidiolide 51**




Dihydroactinidiolide **51**

Dihydroactinidiolide **46**: colourless oil, 9.6 mg (0.027% from crude ethyl acetate extract; 2.7 mg from E6F3 210 mg, and 6.9 mg from E6F4 67 mg); $R_f$ 0.39 (hexane: EtOAc, 70 : 30); $[\alpha]_D^{26.9}$ -13.14° (*c* = 0.22, $CDCl_3$), lit. $[\alpha]_D$ -105° (*c* = 0.1, $CHCl_3$) [140]; ESMS (positive mode) *m/z* (% rel. intensity): 383.1 $[2M+Na]^+$(100); IR (Neat) $\nu_{max}$ 2939, 2871, 1753, 1627, 1459, 1389, 1376, 1257, 1226, 1188, 1151, 1123, 1029, 991, 955, 941, 908, 882, 856 $cm^{-1}$; $^{1}$H NMR, $^{13}$C NMR, DEPT 135, COSY and HETCOR were shown in Table 4.17-4.19 and Figure B1-B4.




⟷ COSY

**Table 4.17** $^{13}C$ NMR and DEPT spectroscopic data of dihydroactinidiolide **51** in $CDCl_3$

| $\delta_C$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 182.51 | - | C | C-8 |
| 171.99 | - | C | C-6 |
| 112.38 | Positive | CH | C-7 |
| 87.26 | - | C | C-5 |
| 41.66 | Negative | $CH_2$ | C-2 |
| 40.09 | Negative | $CH_2$ | C-4 |
| 36.50 | - | C | C-1 |
| 29.83 and 24.18 | Positive | $CH_3$ | C-10 and C-9 |
| 24.35 | Positive | $CH_3$ | C-11 |
| 19.65 | Negative | $CH_2$ | C-3 |

**Table 4.18** $^{1}H$- $^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of dihydroactinidiolide **51** in $CDCl_3$

| Positions | $^{1}H$-$^{13}C$ HETCOR | | $^{1}H$-$^{1}H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 36.50 | - | - | - |
| 2 | 41.66 | 1.22-1.34 (1H, *m*, H-2α)<br>1.62-1.72 (1H, *brm*, H-2β) | 1.22-1.34 (H-2α) | 1.62-1.72 (H-2β) and 1.72-1.77 (H-3) |
| 3 | 19.65 | 1.72-1.77 (2H, *brm*, H-3) | 1.72-1.77 (H-3) | 1.46 (H-4α) |
| 4 | 40.09 | 1.46 (1H, *m*, H-4α)<br>2.24 (1H, *dq*, *J* = 12.47, 2.05 Hz, H-4β) | 1.46 (H-4α) | 2.24 (H-4β) |
| 5 | 87.26 | - | - | - |
| 6 | 171.99 | - | - | - |
| 7 | 112.38 | 5.64 (1H, *s*) | - | - |
| 8 | 182.51 | - | - | - |
| 9 and 10 | 24.18<br>29.83 | 1.22 (3H, *s*)<br>1.27 (3H, *s*) | -<br>- | -<br>- |
| 11 | 24.35 | 1.55 (3H, *s*) | - | - |

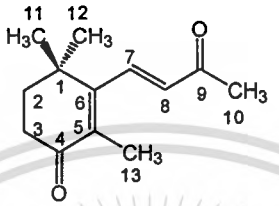
**Table 4.19** $^{1}H$ and $^{13}C$ NMR of dihydroactinidiolide **51** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CDCl_3$) [140] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 36.50 | - | 36.5 | - |
| 2 | 41.66 | 1.22-1.34 (1H, *m*, H-2α)<br>1.62-1.72 (1H, *brm*, H-2β) | 41.6 | 1.22-1.33 (*m*)<br>1.62-1.77 (*m*) |
| 3 | 19.65 | 1.72-1.77 (2H, *brm*, H-3) | 19.6 | 1.62-1.77 (*m*) |
| 4 | 40.09 | 1.46 (1H, *m*, H-4α)<br>2.24 (1H, *dq*, *J* = 12.47, 2.05 Hz, H-4β) | 40.1 | 1.46 (*m*)<br>2.23 (*dq*, *J* = 12.1, 2.5 Hz) |
| 5 | 87.26 | - | 87.2 | - |
| 6 | 171.99 | - | 171.9 | - |
| 7 | 112.38 | 5.64 (1H, *s*) | 112.4 | 5.64 (s) |
| 8 | 182.51 | - | 182.5 | - |
| 9 and | 24.18 | 1.22 (3H, *s*) | 24.2 | 1.20 (*s*) |
| 10 | 29.83 | 1.27 (3H, *s*) | 29.8 | 1.25 (*s*) |
| 11 | 24.35 | 1.55 (3H, *s*) | 24.3 | 1.53 (*s*) |

Dihydroactinidiolide **51**, which was isolated as a colourless oil, had the molecular formula $C_{11}H_{16}O_2$ determined by ESMS *m/z* 383.1 $[2M+Na]^+$ and GC-MS *m/z* 180 $[M]^+$ and required 4 degrees of unsaturation. The characteristic IR absorptions showed the presence of a carbonyl group of α,β-unsaturated ester (1753 $cm^{-1}$), double bond (1627 and 955 $cm^{-1}$) and a C-O stretching of ester (1029 $cm^{-1}$). The $^{13}C$ NMR spectrum, including the DEPT experiment, revealed the presence of one $sp^2$ methine ($\delta_C$ 112.38), three $sp^3$ methylenes ($\delta_C$ 19.65, 40.09 and 41.66), three methyls ($\delta_C$ 24.18, 24.35 and 29.13), and four quaternary carbons ($\delta_C$ 36.50, 87.26, 171.99, and 182.51 (C=O)), and the four degree of unsaturation was fully accounted for by one carbonyl group, one olefin, and two cyclics. The $^{1}H$-$^{13}C$ HETCOR experiment showed one methine proton at $\delta_H/\delta_C$ 5.64/112.38, three methylene protons at $\delta_H/\delta_C$ 1.72-1.77/19.65, 1.46 and 2.24/40.09 and 1.22-1.34 and 1.62-1.72/41.66, three methyl proton at $\delta_H/\delta_C$ 1.22/24.18, 1.55 /24.35 and 1.27/29.13. In the $^{1}H$ NMR and $^{1}H$-$^{1}H$ COSY spectra, the methylene signal at $\delta_H$ 1.22-1.34 (1H, *m*, H-2α) was germinal coupled with a proton at $\delta_H$ 1.62-1.72 (1H, *brm*, H-2β) and vicinal coupled with protons at $\delta_H$ 1.72-1.77 (2H, *brm*, H-3), the methylene signal at $\delta_H$ 1.46 (1H, *m*, H-4α) was germinal coupled with a proton at $\delta_H$ 2.24 (1H, *dq*, *J* = 12.47, 2.05 Hz, H-4β) and

vicinal coupled with protons at $\delta_H$ 1.72-1.77 (2H, *brm*, H-3). Based on these spectroscopic evidences, the carbon signal at $\delta_C$ 36.50 was assigned to tetrasubstituted carbon (C-1), the carbon at $\delta_C$ 87.26 was an oxygenated quaternary carbon (C-5), and the carbon at $\delta_C$ 171.99 was $sp^2$ quaternary carbon (C-6). This compound was determined to be (-)-dihydroactinidiolide **51** [140-142].

**4-Oxo-β-ionone 52**




4-Oxo-β-ionone **52**

4-Oxo-β-ionone **52**: 7.2 mg (0.018% from crude ethyl acetate extract; 3 mg from E6F3 210 mg, 1.4 mg from E6F4 67 mg and 2.8 mg from E6F5 150 mg), waxy solid; $R_f$ 0.31 (hexane : EtOAc, 70 : 30); ESMS (positive mode) *m/z* (% rel. intensity): 207.1 $[M+H]^+$(100); IR (Neat) $\nu_{max}$ 2952, 2924, 2851, 1695, 1672, 1616, 1457, 1362, 1250, 1172, 1110, 1026 and 976 $cm^{-1}$; $^1H$ NMR, $^{13}C$ NMR, DEPT 135, COSY and HETCOR were shown in Table 4.20-4.22 and Figure B5-B8.

**Table 4.20** $^{13}C$ NMR and DEPT spectroscopic data of 4-oxo-β-ionone **52** in $CDCl_3$

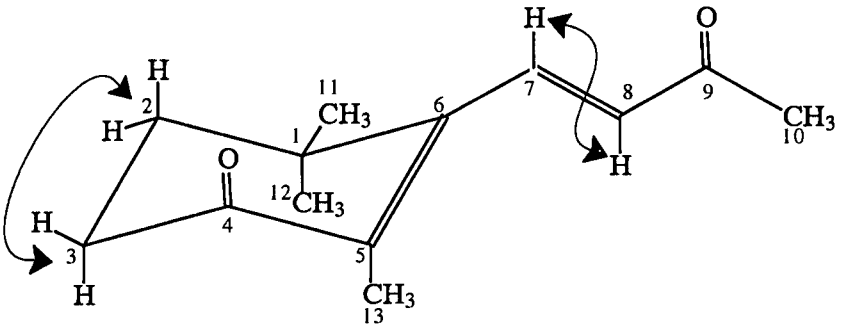
| $\delta_C$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 198.50 | - | C | C-4 |
| 198.00 | - | C | C-9 |
| 157.16 | - | C | C-6 |
| 140.31 | Positive | CH | C-7 |
| 133.57 | Positive | CH | C-8 |
| 131.14 | - | C | C-5 |
| 37.41 | Negative | $CH_2$ | C-2 |
| 35.60 | - | C | C-1 |
| 34.21 | Negative | $CH_2$ | C-3 |
| 27.94 | Positive | $CH_3$ | C-10 |
| 27.35 | Positive | $CH_3$ | C-11 and C-12 |
| 13.43 | Positive | $CH_3$ | C-13 |

**Table 4.21** $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of 4-oxo-β-ionone **52** in $CDCl_3$

| Positions | $^{1}H$-$^{13}C$ HETCOR | | $^{1}H$-$^{1}H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 35.60 | - | - | - |
| 2 | 37.41 | 1.89 (2H, *t*, *J* = 6.85 Hz, H-2) | 1.89 (H-2) | 2.24 (H-3) |
| 3 | 34.21 | 2.54 (2H, *t*, *J* = 6.85 Hz, H-3) | | |
| 4 | 198.50 | - | - | - |
| 5 | 131.14 | - | - | - |
| 6 | 157.16 | - | - | - |
| 7 | 140.31 | 7.24 (1H, *d*, *J* = 16.51 Hz, H-7) | 7.24 (H-7) | 6.19 (H-8) |
| 8 | 133.57 | 6.19 (1H, *d*, *J* = 16.51 Hz, H-8) | | |
| 9 | 198.00 | - | - | - |
| 10 | 27.94 | 2.35 (3H, *s*) | - | - |
| 11 and 12 | 27.35 | 1.19 (6H, *s*) | - | - |
| 13 | 13.43 | 1.80 (3H, *s*) | - | - |

**Table 4.22** $^{1}H$ and $^{13}C$ NMR of 4-oxo-β-ionone **52** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CDCl_3$) [143] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 35.60 | - | 35.62 | - |
| 2 | 37.41 | 1.89 (2H, *t*, *J* = 6.85 Hz, H-2) | 37.65 | 1.70-2.10 (*m*) |
| 3 | 34.21 | 2.54 (2H, *t*, *J* = 6.85 Hz, H-3) | 34.22 | 2.15-2.85 (*m*) |
| 4 | 198.50 | - | 197.76 | - |
| 5 | 131.14 | - | 131.56 | - |
| 6 | 157.16 | - | 157.53 | - |
| 7 | 140.31 | 7.24 (1H, *d*, *J* = 16.51 Hz, H-7) | 140.04 | 7.23 (*d*, *J*~16 Hz) |
| 8 | 133.57 | 6.19 (1H, *d*, *J* = 16.51 Hz, H-8) | 133.77 | 6.16 (*d*, *J*~16 Hz) |
| 9 | 198.00 | - | 196.79 | - |
| 10 | 27.94 | 2.35 (3H, *s*) | 27.65 | 2.36 (*s*) |
| 11 and 12 | 27.35 | 1.19 (6H, *s*) | 27.38 | 1.20 (*s*) |
| 13 | 13.43 | 1.80 (3H, *s*) | 13.20 | 1.81 (*d*, *J*~1 Hz) |




4-Oxo-β-ionone **52**, which was obtained as a waxy solid with the molecular formula $C_{13}H_{18}O_2$ calculated from ESMS *m/z* 207.1 $[M+H]^+$ and GC-MS *m/z* 206 $[M]^+$ and required 5 degrees of unsaturation. Its IR spectrum showed absorption bands due to the carbonyl group of α,β-unsaturated ketone (1695 and 1672 $cm^{-1}$), double bond ( 1616 and 976 $cm^{-1}$). The $^{13}C$ NMR spectrum along with the DEPT experiment, revealed the presence of two $sp^2$ methines ($\delta_C$ 133.57 and 140.31), two $sp^3$ methylenes ($\delta_C$ 34.21 and 37.41), four methyls ($\delta_C$ 13.43, 2x27.35, and 27.94), and five quaternary carbons ($\delta_C$ 35.60, 131.14, 157.16, 198.00 (C=O at C-9) and 198.50 (C=O at C-4)), and the five degrees of unsaturation was fully accounted for by two carbonyl groups, two olefins, and one cyclic. The $^1H$-$^{13}C$ HETCOR experiment showed two methine protons at $\delta_H/\delta_C$ 6.19/133.57 and 7.24/140.31, two methylene protons at $\delta_H/\delta_C$ 1.89/37.41 and 2.54/34.21, four methyl protons at $\delta_H/\delta_C$ 1.19(6H)/27.35(2C), 1.80/13.43 and 2.35/27.94. In the $^1H$ NMR and $^1H$-$^1H$ COSY spectra, the olefinic signal at $\delta_H$ 1.89 (2H, *t*, *J* = 6.85 Hz) was vicinal coupled with protons at $\delta_H$ 2.54 (2H, *t*, *J* = 6.85 Hz), the methine proton at 6.19 (1H, *d*, *J* = 16.51 Hz) was vicinal coupling with a *trans* proton at 7.24 (1H, *d*, *J* = 16.51 Hz). From these spectroscopic evidences, the carbons signal at $\delta_C$ 35.60 was assigned to tetrasubstituted carbon (C-1), the carbon at $\delta_C$ 37.41 and 34.21 were $sp^2$ carbons at C-2 and C-3 respectively, the carbon signals at $\delta_C$ 131.14 and 157.16 were $sp^2$ quaternary carbons at C-5 and C-6 respectively. This compound was determined to be 4-oxo-β-ionone **52** [132, 143].

## 4.10 Allelopathic effects of 6 subfractions from E13 on seed germination and seedling growth

Results from the allelopathic effects of 23 subfractions from ethyl acetate extract on tested plants showed that only subfraction E13 had completely inhibitory effect on Chinese amaranth at a concentration of 2,000 ppm. This active subfraction, 650 mg (100%), was fractionated to 6 subfractions by column chromatography and studied the allelopathic activity on tested plants.

Subfraction E13F1 (38.60 mg, 5.94%) eluted by gradient solvent range (0-6% EtOAc in hexanes), was obtained as a dark green wax.

Subfraction E13F2 (56.40 mg, 8.68%) eluted by gradient solvent range (7-9% EtOAc in hexanes), was obtained as a dark green wax.

Subfraction E13F3 (112.50 mg, 17.31%) eluted by gradient solvent range (10-14% EtOAc in hexanes), was obtained as a dark green wax.

Subfraction E13F4 (88.30 mg, 1.36%) eluted by gradient solvent range (15-19% EtOAc in hexanes), was obtained as a dark green wax.

Subfraction E13F5 (220.80 mg, 34%) eluted by gradient solvent range (20-30% EtOAc in hexanes), was obtained as a dark brown wax.

Subfraction E13F6 (116 mg, 17.85%) eluted by gradient solvent range (31-35% EtOAc in hexanes), was obtained as a dark brown wax.

Total 632.60 mg, 97.32%.

**Chinese amaranth**

Effect of 6 subfractions from E13 on Chinese amaranth as shown in Table 4.23-4.24 found that subfractions E13F2-E13F4 at concentrations of 500-1,000 ppm and E13F6 at a concentration of 1,000 ppm inhibited the germination of seed. Subfraction E13F3 at concentrations of 250-1,000 ppm, E13F2 and E13F4 at concentrations of 500-1,000 ppm reduced shoot length whereas subfraction E13F1 at a concentration of 250 ppm promoted shoot length. Subfractions E13F2-E13F4 at concentrations of 500-1,000 ppm caused the inhibitory effect on root length but subfractions E13F1 at all applied concentrations, E13F2 at a concentration of 125 ppm, E13F4 at concentrations of 125-250 ppm, E13F5 at concentrations of 250-1,000 ppm and E13F6 at concentrations of 125 and 500-1,000 ppm, promoted root length. Subfraction E13F3

had the highest inhibitory effect on Chinese amaranth that at a concentration of 500 ppm showed the completely inhibited on seed germination and seedling growth but other concentrations had no effect (Figure 4.17).

**Table 4.23** Allelopathic effects of 6 subfractions from E13 on seed germination of Chinese amaranth

| Subrations | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0d | 0c |
| E13F1 | 2.94a | 2.94a | 0d | 5.88bc |
| E13F2 | 2.94a | 2.94a | 76.47b | 100a |
| E13F3 | -2.94a | 8.82a | 100a | 100a |
| E13F4 | 0a | 0a | 50c | 97.06a |
| E13F5 | 0a | -2.94a | 2.94d | 2.94bc |
| E13F6 | 2.94a | 2.94a | 2.94d | 11.76b |

Values with same letters in each column there are not significantly different at $P$=0.05

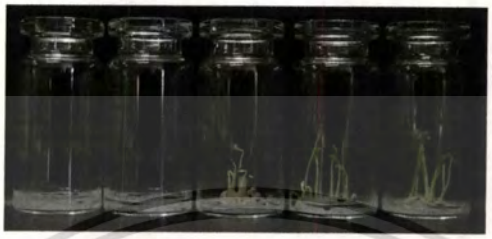
**Table 4.24** Allelopathic effects of 6 subfractions from E13 on seedling growth of Chinese amaranth

| Subfrations | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0ab | 0b | 0d | 0b | 0a | 0a | 0d | 0b |
| E13F1 | -6.25b | -8.54c | 0.15d | 7.93b | -14.57c | -20.29d | -15.13ef | -32.40c |
| E13F2 | 3.66ab | 5.64b | 81.71b | 100a | -15.36c | 0.78a | 86.55b | 100a |
| E13F3 | 5.95a | 22.87a | 100a | 100a | 1.91a | 1.23a | 100a | 100a |
| E13F4 | 3.96ab | 2.90b | 65.24c | 93.90a | -15.70c | -14.69c | 48.99c | 88.79a |
| E13F5 | 4.42ab | 1.68b | 1.68d | 3.96b | 3.36a | -8.41b | -22.53f | -33.97c |
| E13F6 | 2.90ab | 4.88b | 2.13d | 5.34b | -7.06b | -3.92ab | -8.41e | -28.25c |

Values with same letters in each column there are not significantly different at $P$=0.05

E13F2

E13F3



E13F4

1,000 500 250 125 control

**Figure 4.17** Allelopathic effects of subfractions E13F2-E13F4 at concentrations of 125-1,000 ppm on Chinese amaranth

**Barnyardgrass**

The result on Barnyardgrass showed that all the applied concentrations had no allelopathic effects on seed germination. While subfractions E13F1, E13F3 and E13F5 at a concentration of 1,000 ppm and E13F2 at concentrations of 500-1,000 ppm had slightly inhibitory effect on shoot length. Subfractions E13F1 at a concentration of 1,000 ppm and E13F5 at concentrations of 500-1,000 ppm showed the promotion effect on root length but other concentrations had no effect (Table 4.25-4.26).

**Table 4.25** Allelopathic effects of 6 subfractions from E13 on seed germination of Barnyardgrass

| Subfrations | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0a | 0a |
| E13F1 | 0a | 2.70a | -2.70a | 5.41a |
| E13F2 | 0a | 0a | 0a | -2.70a |
| E13F3 | 0a | 0a | 2.70a | 0a |
| E13F4 | 0a | 2.70a | -2.70a | 0a |
| E13F5 | -2.70a | 5.41a | 0a | 0a |
| E13F6 | 2.70a | -2.7027a | -2.70a | -2.70a |

Values with same letters in each column there are not significantly different at $P$=0.05

**Table 4.26** Allelopathic effects of 6 subfractions from E13 on seedling growth of Barnyardgrass

| Subfrations | % Inhibition on shoot length | | | | % Inhibition on root length | | | |
|---|---|---|---|---|---|---|---|---|
| | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0b | 0c | 0a | 0a | 0a | 0ab |
| E13F1 | 4.05a | 2.98a | 3.54ab | 5.26ab | 0.98a | 2.81a | -3.93a | -26.93d |
| E13F2 | 2.51a | 3.58a | 6.75a | 7.45ab | 5.33a | 3.37a | 0a | 0.14ab |
| E13F3 | 2.42a | 3.72a | 2.70ab | 8.66a | 5.05a | 4.07a | 3.37a | 5.05a |
| E13F4 | 3.91a | 3.72a | 2.84ab | 3.071bc | -0.28a | -2.38a | -0.70a | 2.38ab |
| E13F5 | 2.84a | 1.86a | 3.26ab | 5.03ab | 4.21a | -0.28a | -27.07b | -13.88c |
| E13F6 | 3.96a | 1.54a | 2.93ab | 3.12bc | 0.84a | 2.81a | -6.87a | -5.89bc |

Values with same letters in each column there are not significantly different at $P$=0.05

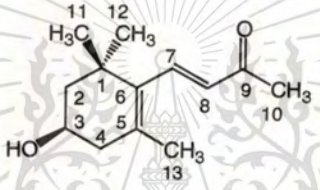
## 4.11 Isolation and identification of active compounds from subfractions E13F2–E13F4

Results from allelopathic assay found that subfractions E13F2-E13F4 showed a high inhibitory activity on Chinese amaranth. These subfractions were mixed and isolated to pure active compounds by flash column chromatography. The structures of the pure compounds were identified by spectroscopic techniques. Percentage of the pure compounds was illustrated in Table 4.27.

**Table 4.27** Pure compounds from E13F2-E13F4

| Compounds and subfractions | Weight (mg) | % |
|---|---|---|
| | 28.20+83+52.60 | |
| E13F2+E13F3+E13F4 | =163.80 | 100 |
| 3-Hydroxy-β-ionone **59** | 27 | 16.56 |
| 3-Hydroxy-5α, 6α-epoxy-β-ionone **56** | 7.50 | 4.58 |
| 3-Hydroxy-5β, 6β-epoxy-β-ionone **57** | 6.90 | 4.21 |
| Loliolide **58** | 35 | 21.37 |

**3-Hydroxy-β-ionone 59**




3-Hydroxy-β-ionone **59**

3-Hydroxy-β-ionone **59**: pale yellow oil, 27 mg (0.076% from crude ethyl acetate); $R_f$ 0.34 (hexane: EtOAc, 50 : 50); $[\alpha]_D^{27.5}$ -40.26° (*c* = 0.23, $CDCl_3$), lit. $[\alpha]_D^{22}$ -140.5° (*c* = 0.1, $CHCl_3$) [144]; ESMS (positive mode) *m/z* (% rel. intensity) 209.1 $[M+H]^+$(100); IR (Neat) $\nu_{max}$ 3436, 2958, 2924, 2862, 1670, 1605, 1457, 1426, 1363, 1256, 1173, 1051 and 978 $cm^{-1}$; $^1H$ NMR, $^{13}C$ NMR, DEPT 135, COSY and HETCOR were shown in Table 4.28-4.30 and Figure B9-B12.

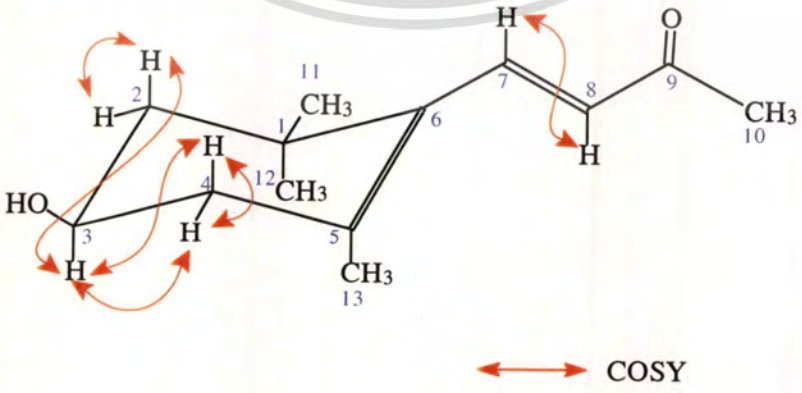


**Table 4.28** $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-β-ionone **59** in $CDCl_3$

| $\delta_C$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 198.52 | - | C | C-9 |
| 142.33 | Positive | CH | C-7 |
| 135.66 | - | C | C-6 |
| 132.39 | Positive | CH | C-8 |
| 132.26 | - | C | C-5 |
| 64.54 | Positive | CH | C-3 |
| 48.44 | Negative | $CH_2$ | C-2 |
| 42.78 | Negative | $CH_2$ | C-4 |
| 36.92 | - | C | C-1 |
| 28.58 and 30.09 | Positive | $CH_3$ | C-11 and C-12 |
| 27.32 | Positive | $CH_3$ | C-10 |
| 21.59 | Positive | $CH_3$ | C-13 |

**Table 4.29** $^1H$-$^{13}C$ HETCOR and $^1H$-$^1H$ COSY correlations of 3-hydroxy-β-ionone **59** in $CDCl_3$

| Positions | $^1H$-$^{13}C$ HETCOR | | $^1H$-$^1H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 36.92 | - | - | - |
| 2 | 48.44 | 1.50 (1H, *t*, *J* = 11.98 Hz, H-2α)<br>1.78-1.82 (1H, *m*, H-2β) | 1.50 (H-2α) | 1.78-1.82 (H-2β) and<br>3.96-4.06 (H-3α) |
| 3 | 64.54 | 3.96-4.06 (1H, *m*, H-3α)<br>1.65 (1H, *br*-OH) | 3.96-4.06 (H-3α) | 2.09 (H-4α) and<br>2.44 (H-4β) |
| 4 | 42.78 | 2.09 (1H, *dd*, *J* = 17.42, 9.40 Hz, H-4α)<br>2.44 (1H, *dd*, *J* = 17.42, 5.51 Hz, H-4β) | 2.09 (H-4α) | 2.44 (H-4β) |
| 5 | 132.26 | - | - | - |
| 6 | 135.66 | - | - | - |
| 7 | 142.33 | 7.21 (1H, *d*, *J* = 16.41 Hz, H-7) | 7.21 (H-7) | 6.12 (H-8) |
| 8 | 132.39 | 6.12 (1H, *d*, *J* = 16.41 Hz, H-8) | | |
| 9 | 198.52 | - | - | - |
| 10 | 27.32 | 2.30 (3H, *s*) | - | - |
| 11 and 12 | 28.58<br>30.09 | 1.12 (3H, *s*)<br>1.11 (3H, *s*) | -<br>- | -<br>- |
| 13 | 21.59 | 1.78 (3H, *s*) | - | - |

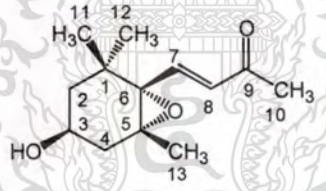
**Table 4.30** $^{1}H$ and $^{13}C$ NMR of 3-hydroxy-β-ionone **59** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CDCl_3$) [144] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 36.92 | - | 36.9 | - |
| 2 | 48.44 | 1.50 (1H, *t*, *J* = 11.98 Hz, H-2α)<br>1.78-1.82 (1H, *m*, H-2β) | 48.4 | 1.49 (*dd*, *J*= 12.0, 12.0 Hz)<br>1.79 (*ddd*, *J*= 12.0, 3.5, 2.0 Hz) |
| 3 | 64.54 | 3.96-4.06 (1H, *m*, H-3α)<br>1.65 (1H, *br*-OH) | 64.6 | 4.01 (*dddd*, *J*= 12.0, 10.0, 5.6, 3.5 Hz) |
| 4 | 42.78 | 2.09 (1H, *dd*, *J* = 17.42, 9.40 Hz, H-4α)<br>2.44 (1H, *dd*, *J* = 17.42, 5.51 Hz, H-4β) | 42.8 | 2.09 (*dd*, *J*= 17.3, 10.0 Hz)<br>2.43 (*ddd*, *J*= 17.3, 5.6, 2.0 Hz) |
| 5 | 132.26 | - | 132.2 | - |
| 6 | 135.66 | - | 135.7 | - |
| 7 | 142.33 | 7.21 (1H, *d*, *J* = 16.41 Hz, H-7) | 142.3 | 7.21 (*d*, *J*= 16.4 Hz) |
| 8 | 132.39 | 6.12 (1H, *d*, *J* = 16.41 Hz, H-8) | 132.4 | 6.11 (*d*, *J*= 16.4 Hz) |
| 9 | 198.52 | - | 198.4 | - |
| 10 | 27.32 | 2.30 (3H, *s*) | 27.3 | 2.30 (*s*) |
| 11 and 12 | 28.58<br>30.09 | 1.12 (3H, *s*)<br>1.11 (3H, *s*) | 28.6<br>30.1 | 1.11 (*s*)<br>1.12 (*s*) |
| 13 | 21.59 | 1.78 (3H, *s*) | 21.6 | 1.78 (*s*) |

3-Hydroxy-β-ionone **59**, which was obtained as a pale yellow oil with the molecular formula $C_{13}H_{20}O_2$ calculated from ESMS *m/z* 209.1 $[M+H]^+$ and required 4 degrees of unsaturation. Its IR spectrum showed absorption bands due to hydroxyl group (3436 $cm^{-1}$), the carbonyl group of α,β-unsaturated ketone (1670 $cm^{-1}$), double bond ( 1605 and 978 $cm^{-1}$) , and a C-O stretching of hydroxyl group (1051 $cm^{-1}$). The $^{13}C$ NMR spectrum along with the DEPT experiment, revealed the presence of two $sp^2$ methines ($\delta_C$ 132.39 and 142.33), one $sp^3$ methine ($\delta_C$ 64.54), two $sp^3$ methylenes ($\delta_C$ 42.78 and 48.44), four methyls ($\delta_C$ 21.19, 27.32, 28.58 and 30.09), and four quaternary carbons ($\delta_C$ 36.92, 132.26, 135.66, and 198.58 (C=O)), and the four degree of unsaturation was fully accounted for one carbonyl group, two olefins, and one cyclic. The $^{1}H$-$^{13}C$ HETCOR experiment showed three methine protons at $\delta_H$/ $\delta_C$ 3.96-4.06/64.54,

6.12/132.39 and 7.21/142.33, two methylene protons at $\delta_H/\delta_C$ 1.50 and 1.78-1.82/48.44, and 2.09 and 2.44/42.78, and four methyl protons at $\delta_H/\delta_C$ 1.11/30.09, 1.12/28.58, 1.78/21.51 and 2.30/27.32. In the $^1$H NMR and $^1$H-$^1$H COSY spectra, the mehtylene protons at $\delta_H$ 1.50 (1H, *t*, *J* = 11.98 Hz, H-2α) was germinal coupled with a proton at $\delta_H$ 1.78-1.82 (1H, *m*, H-2β) and vicinal coupled with proton at $\delta_H$ 3.96-4.06 (1H, *m*, H-3α), the secondary hydroxyl methine proton at 3.96-4.06 (1H, *m*, H-3α) was vicinal coupled with two protons at $\delta_H$ 2.09 (1H, *dd*, *J* = 17.42, 9.4 Hz, H-4α) and 2.44 (1H, *dd*, *J* = 17.42, 5.51Hz, H-4β), the mehtylene protons at $\delta_H$ 2.09 (1H, *dd*, *J* = 17.42, 9.4 Hz, H-4α) was vicinal coupled with a proton at $\delta_H$ 2.44 (1H, *dd*, *J* = 17.42, 5.51Hz, H-4β), the methine proton at 6.12 (1H, *d*, *J* = 16.41 Hz, H-8) was vicinal coupled with a *trans* proton at 7.21 (1H, *d*, *J* = 16.41 Hz, H-7). The hydroxyl proton was observed at $\delta_H$ 1.65 (1H, *br*-OH). From these spectroscopic evidences, the carbon signal at $\delta_C$ 36.92 was assigned to tetrasubstituted carbon (C-1), the carbon signal at $\delta_C$ 64.54 was oxygenated tertiary carbon (C-3), the carbon signals at $\delta_C$ 132.26 and 135.66 were $sp^2$ quaternary carbon at C-5 and C-6, respectively. This compound was determined to be *R*-(-)-3-hydroxy-β-ionone **59** [144-145].

**3-Hydroxy-5α, 6α-epoxy-β-ionone 56**




3-Hydroxy-5α, 6α-epoxy-β-ionone **56**

3-Hydroxy-5α, 6α-epoxy-β-ionone **56**: colourless oil, 7.5 mg (0.021% from crude ethyl acetate); $R_f$ 0.34 (hexane : EtOAc, 40 : 60); $[\alpha]_D^{27.2}$ -63.87° (*c* = 0.23, $CDCl_3$), lit. $[\alpha]_D^{25}$ -43.7° (*c* = 0.39, $CH_2Cl_2$) [146]; ESMS (positive mode) *m/z* (% rel. intensity): 225 $[M+H]^+$(100); IR (Neat) $\nu_{max}$ 3440, 2957, 2926, 2868, 1692, 1674, 1625, 1457, 1426, 1379, 1363, 1258, 1181, 1146, 1051, 1029 and 987 $cm^{-1}$; $^1$H NMR, $^{13}$C NMR, DEPT 135, COSY and HETCOR as shown in Table 4.31-4.33 and Figure B13-B16.

**Table 4.31** $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$

| $\delta_c$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 197.38 | - | C | C-9 |
| 142.35 | Positive | CH | C-7 |
| 132.63 | Positive | CH | C-8 |
| 69.49 | - | C | C-6 |
| 67.24 | - | C | C-5 |
| 64.02 | Positive | CH | C-3 |
| 46.68 | Negative | $CH_2$ | C-2 |
| 40.60 | Negative | $CH_2$ | C-4 |
| 35.12 | - | - | C-1 |
| 29.35 | Positive | $CH_3$ | C-11 |
| 28.29 | Positive | $CH_3$ | C-10 |
| 25.00 | Positive | $CH_3$ | C-12 |
| 19.87 | Positive | $CH_3$ | C-13 |

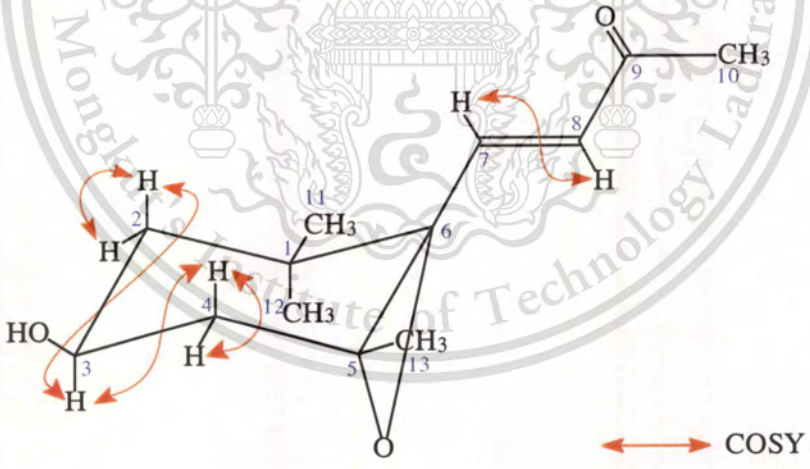


**Table 4.32** $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$

| Positions | $^{1}H$-$^{13}C$ HETCOR | | $^{1}H$-$^{1}H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 35.12 | - | - | - |
| 2 | 46.68 | 1.24 (1H, *dd*, *J* = 13.19, 2.66 Hz, H-2α)<br>1.62-1.67 (1H, *m*, H-2β) | 1.24 (H-2α) | 1.62-1.67 (H-2β)and<br>3.86-3.95 (H-3α) |
| 3 | 64.02 | 3.86-3.95 (1H, *m*, H-3α)<br>1.60-1.70 (1H, *brm*-OH) | 3.86-3.95 (H-3α) | 1.65-1.69 (H-4α) |
| 4 | 40.60 | 1.65-1.69 (1H, *m*, H-4α)<br>2.40 (1H, *ddd*, *J* = 14.35, 5.01,<br>1.59 Hz, H-4β) | 1.65-1.69 (H-4α) | 2.40 (H-4β) |
| 5 | 67.24 | - | - | - |
| 6 | 69.49 | - | - | - |
| 7 | 142.35 | 7.03 (1H, *d*, *J* = 15.59 Hz, H-7) | 7.03 (H-7) | 6.29 (H-8) |
| 8 | 132.63 | 6.29 (1H, *d*, *J* = 15.59 Hz, H-8) | 6.29 (H-8) | 7.03 (H-7) |
| 9 | 197.38 | - | - | - |
| 10 | 28.29 | 2.29 (3H, *s*) | - | - |
| 11 | 29.35 | 1.19 (3H, *s*) | - | - |
| 12 | 25.00 | 0.98 (3H, *s*) | - | - |
| 13 | 19.87 | 1.19 (3H, *s*) | - | - |

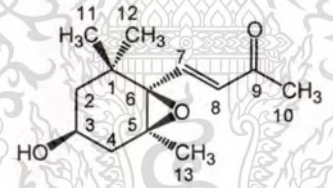
**Table 4.33** $^{1}H$ and $^{13}C$ NMR of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CDCl_3$) [146] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 35.12 | - | 35.3 | - |
| 2 | 46.68 | 1.24 (1H, *dd*, *J* = 13.19, 2.66 Hz, H-2α)<br>1.62-1.67 (1H, *m*, H-2β) | 40.8 | 1.60 (*dd*, *J*= 12.2, 4.5 Hz)<br>1.30 (*dd*, *J*= 12.2, 10.2 Hz) |
| 3 | 64.02 | 3.86-3.95 (1H, *m*, H-3α)<br>1.60-1.70 (1H, *brm*-OH) | 64.2 | 3.90 (*m*) |
| 4 | 40.60 | 1.65-1.69 (1H, *m*, H-4α)<br>2.40 (1H, *ddd*, *J* = 14.35, 5.01, 1.59 Hz, H-4β) | 47 | 1.65 (*dd*, *J*= 11.5, 9.2 Hz)<br>2.39 (*dd*, *J*= 9.2, 5.2 Hz) |
| 5 | 67.24 | - | 67.5 | - |
| 6 | 69.49 | - | 69.7 | - |
| 7 | 142.35 | 7.03 (1H, *d*, *J* = 15.59 Hz, H-7) | 142.6 | 7.03 (*d*, *J*= 15.5 Hz) |
| 8 | 132.63 | 6.29 (1H, *d*, *J* = 15.59 Hz, H-8) | 132.8 | 6.29 (*d*, *J*= 15.5 Hz) |
| 9 | 197.38 | - | 197.6 | - |
| 10 | 28.29 | 2.29 (3H, *s*) | 28.5 | 2.28 (3H, *s*) |
| 11 | 29.35 | 1.19 (3H, *s*) | 29.6 | 1.20 (3H, *s*) |
| 12 | 25.00 | 0.98 (3H, *s*) | 25.2 | 0.98 (3H, *s*) |
| 13 | 19.87 | 1.19 (3H, *s*) | 20 | 1.56 (3H, *s*) |

3-Hydroxy-5α, 6α-epoxy-β-ionone **56**, which was isolated as a colourless oil with the molecular formula $C_{13}H_{20}O_3$ calculated from ESMS *m/z* 225 $[M+H]^+$ and required 4 degrees of unsaturation. Its IR spectrum showed absorption bands due to hydroxyl group (3440 $cm^{-1}$), the carbonyl group of α,β-unsaturated ester (1674 $cm^{-1}$), double bond ( 1625 and 987 $cm^{-1}$) and a C-O stretching of hydroxyl group (1051 $cm^{-1}$). The $^{13}C$ NMR spectrum along with the DEPT experiment, revealed the presence of two $sp^2$ methines ($\delta_C$ 132.63 and 142.35), one $sp^3$ methine ($\delta_C$ 64.02), two $sp^3$ methylenes ($\delta_C$ 40.06 and 46.68), four methyls ($\delta_C$ 19.87, 25.00, 28.29 and 29.35), and four quaternary carbons ($\delta_C$ 35.12, 67.24, 69.49 and 197.38 (C=O)), and the four degree of unsaturation was fully accounted for by one carbonyl group, one olefin, and two cyclics. The $^{1}H$-$^{13}C$ HETCOR experiment showed three methine protons at $\delta_H$/ $\delta_C$ 3.86-

3.95/64.02, 6.29/132.63 and 7.03/142.35, two methylene protons at $\delta_H/\ \delta_C$ 1.24 and 1.62-1.67/46.68, and 1.65-1.69 and 2.40/40.60, and four methyl protons at $\delta_H/\ \delta_C$ 0.98/25.00, 1.19/19.87, 1.19/29.35 and 2.29/28.29. In the $^1$H NMR and $^1$H-$^1$H COSY spectra, the mehtylene protons at $\delta_H$ 1.24 (1H, *dd*, $J$ = 13.19, 2.66 Hz, H-2α) was germinal coupled with a proton at $\delta_H$ 1.62-1.67 (1H, *m*, H-2β) and vicinal coupled with the secondary hydroxyl methine proton at $\delta_H$ 3.86-3.95(1H, *m*, H-3α), the mehtylene protons at $\delta_H$ 1.65-1.69 (1H, *m*, H-4α) was vicinal coupled with proton at $\delta_H$ 2.40 (1H, *ddd*, $J$ = 14.35, 5.01, 1.59 Hz, H-4β) and vicinal coupled with proton at $\delta_H$ 3.86-3.95(1H, *m*, H-3α), and the methine proton at $\delta_H$ 6.29 (1H, *d*, $J$ = 15.59 Hz, H-8) was vicinal coupled with a *trans* proton at 7.03 (1H, *d*, $J$ = 15.59 Hz, H-7). The hydroxyl proton was observed at $\delta_H$ 1.60-1.70 (1H, *brm*-OH). From these spectroscopic evidences, the carbon signal at $\delta_C$ 35.12 was assigned to tetrasubstituted carbon (C-1), the carbon at $\delta_C$ 64.02 was tertiary carbon-bond hydroxyl group (C-3), the carbon signals at $\delta_C$ 67.24 and 69.49 were oxygenated quaternary carbon of epoxide at C-5 and C-6, respectively. This compound was determined to be (-)-3-hydroxy-5α, 6α-epoxy-β-ionone **56** [146-147]

**3-Hydroxy-5β, 6β-epoxy-β-ionone 57**




3-Hydroxy-5β, 6β-epoxy-β-ionone **57**

3-Hydroxy-5β, 6β-epoxy-β-ionone **57**: colourless oil, 6.9 mg (0.020% from crude ethyl acetate); $R_f$ 0.32 (hexnae : EtOAc, 40 : 60); $[\alpha]_D^{27.4}$ +4.08° ($c$ = 0.13, $CDCl_3$), Lit. (acetate derivative at C-3) $[\alpha]_D^{22}$ +3.7° ($c$ = 0.6, $CHCl_3$) [148]; ESMS (positive mode) *m/z* (% rel. intensity): 225.1 $[M+H]^+$(100); IR (Neat) $\nu_{max}$ 3434, 2957, 2926, 2868, 1692, 1673, 1626, 1456, 1426, 1379, 1363, 1257, 1172, 1125, 1040 and 983 $cm^{-1}$; $^1$H NMR, $^{13}$C NMR, DEPT 135, COSY, HETCOR, HMBC and NOESY were shown in Table 4.34-4.36 and Figure B17-B22.

**Table 4.34** $^{13}C$ NMR and DEPT spectroscopic data of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$

| $\delta_C$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 197.21 | - | C | C-9 |
| 141.02 | Positive | CH | C-7 |
| 133.13 | Positive | CH | C-8 |
| 71.00 | - | C | C-6 |
| 66.00 | - | C | C-5 |
| 63.73 | Positive | CH | C-3 |
| 43.51 | Negative | $CH_2$ | C-2 |
| 38.92 | Negative | $CH_2$ | C-4 |
| 34.86 | - | - | C-1 |
| 28.49 | Positive | $CH_3$ | C-10 |
| 26.84 | Positive | $CH_3$ | C-11 |
| 25.78 | Positive | $CH_3$ | C-12 |
| 20.98 | Positive | $CH_3$ | C-13 |

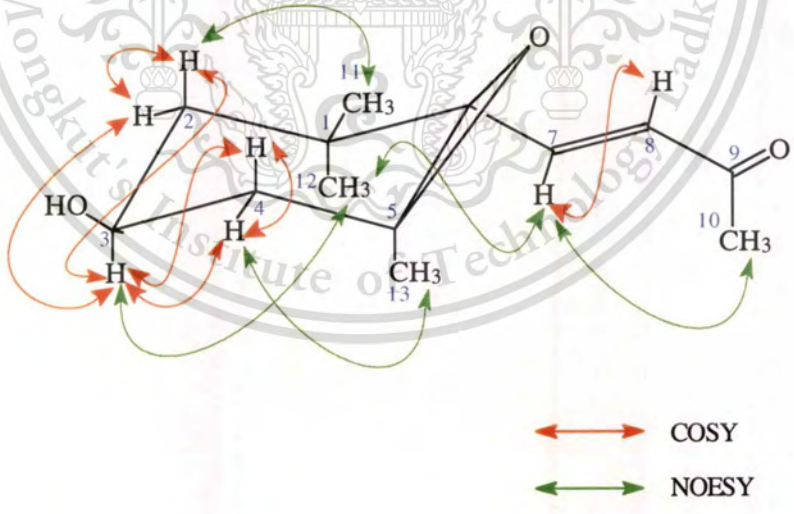


**Table 4.35** $^{1}H$-$^{13}C$ HETCOR correlations in $CDCl_3$ and $^{1}H$-$^{1}H$ COSY correlations in $CD_3COCD_3$ of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**

| Positions | $^{1}H$-$^{13}C$ HETCOR | | $^{1}H$-$^{1}H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 34.86 | - | - | - |
| 2 | 43.51 | 1.56-1.64 (1H, *m*, H-2α)<br>1.37 (1H, *dd*, *J* = 12.98, 3.56 Hz, H-2β) | H-2α | H-2β and H-3α |
| 3 | 63.73 | 3.85-3.95 (1H, *m*, H-3α)<br>1.56-1.64 (1H, *brm*-OH) | H-3α | H-2β, H-4α and H-4β |
| 4 | 38.92 | 1.89 (1H, *dd*, *J* = 14.68, 8.55 Hz, H-4α)<br>2.23 (1H, *dd*, *J* = 14.68, 6.48 Hz, H-4β) | H-4α | H-4β |
| 5 | 66.00 | - | - | - |
| 6 | 71.00 | - | - | - |
| 7 | 141.02 | 6.98 (1H, *d*, *J* = 15.63 Hz, H-7) | H-7 | H-8 |
| 8 | 133.13 | 6.31 (1H, *d*, *J* = 15.63 Hz, H-8) | | |
| 9 | 197.21 | - | - | - |
| 10 | 28.49 | 2.28 (3H, s) | - | - |
| 11 | 26.84 | 1.00 (3H, s) | - | - |
| 12 | 25.78 | 1.21 (3H, s) | - | - |
| 13 | 20.98 | 1.19 (3H, s) | - | - |

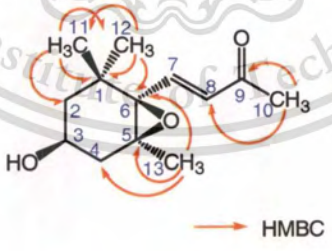


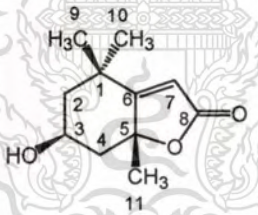
**Table 4.36** $^{1}H$ and $^{13}C$ NMR of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CD_3OD$) [149] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 34.86 | - | 35.1 | - |
| 2 | 43.51 | 1.56-1.64 (1H, *m*, H-2α)<br>1.37 (1H, *dd*, *J* = 12.98, 3.56 Hz, H-2β) | 40.5 | 2.38 (*dd*, *J*= 14.3, 5.1 Hz)<br>1.64 (*dd*, *J*= 14.3, 8.8 Hz) |
| 3 | 63.73 | 3.85-3.95 (1H, *m*, H-3α)<br>1.56-1.64 (1H, *brm*-OH) | 64 | 3.9 (*ddd*, *J*= 8.8, 5.1, 4.9 Hz) |
| 4 | 38.92 | 1.89 (1H, *dd*, *J* = 14.68, 8.55 Hz, H-4α)<br>2.23 (1H, *dd*, *J* = 14.68, 6.48 Hz, H-4β) | 46.6 | 1.62 (*dd*, *J*= 14.5, 1.9 Hz)<br>1.27 (*dd*, *J*= 14.5, 4.9 Hz) |
| 5 | 66.00 | - | 69.2 | - |
| 6 | 71.00 | - | 77.2 | - |
| 7 | 141.02 | 6.98 (1H, *d*, *J* = 15.63 Hz, H-7) | 142.4 | 7.0 (*d*, *J*= 15 Hz) |
| 8 | 133.13 | 6.31 (1H, *d*, *J* = 15.63 Hz, H-8) | 132.6 | 6.3 (*d*, *J*= 15 Hz) |
| 9 | 197.21 | - | 197.5 | - |
| 10 | 28.49 | 2.28 (3H, *s*) | 29.3 | 2.27 (3H, *s*) |
| 11 | 26.84 | 1.00 (3H, *s*) | 24.9 | 1.18 (3H, *s*) |
| 12 | 25.78 | 1.21 (3H, *s*) | 19.8 | 0.96 (3H, *s*) |
| 13 | 20.98 | 1.19 (3H, *s*) | 28.3 | 1.24 (3H, *s*) |

3-Hydroxy-5β, 6β-epoxy-β-ionone **57**, which was isolated as a colourless oil with the molecular formula $C_{13}H_{20}O_3$ calculated from ESMS *m/z* 225.1 $[M+H]^+$ and required 4 degrees of unsaturation. Its IR spectrum showed absorption bands due to hydroxyl group (3434 $cm^{-1}$), the carbonyl group of α,β-unsaturated ester (1673 $cm^{-1}$), double bond ( 1626 and 983 $cm^{-1}$) and a C-O stretching of hydroxyl group (1040 $cm^{-1}$). The $^{13}C$ NMR spectrum along with the DEPT experiment, revealed the presence of two $sp^2$ methines ($\delta_C$ 133.13 and 141.02), one $sp^3$ methine ($\delta_C$ 63.73), two $sp^3$ methylenes ($\delta_C$ 38.92 and 43.51), four methyls ($\delta_C$ 20.98, 25.78, 26.84 and 28.49), and four quaternary carbons ($\delta_C$ 34.86, 66.00, 71.00 and 197.21 (C=O)), and the four degree of unsaturation was fully accounted for by one carbonyl group, one olefin, and two

cyclics. The $^{1}H$-$^{13}C$ HETCOR experiment showed three methine protons at $\delta_H$/ $\delta_C$ 3.85-3.95 /63.73, 6.31/133.13 and 6.98/141.02, two methylene protons at $\delta_H$/ $\delta_C$ 1.37 and 1.56-1.64/43.51, and 1.89 and 2.23/38.92, and four methyl protons at $\delta_H$/ $\delta_C$ 1.00/26.84, 1.19/20.98, 1.21/25.78 and 2.28/28.49. In order to confirm, the structure of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** was studied by the $^{1}H$-$^{1}H$ COSY, HMBC and NOESY experiments. The $^{1}H$-$^{1}H$ COSY spectrum indicated the correlation from H-2α to H-2β and H-3; H-3 to H-2β, H-4α and H-4β; H-4α to H-4β and H-7 to H-8, respectively. The HMBC spectrum illustrated the correlations from H-10 to C-8 and C-9; H-11 to C-1, C-2, C-6 and C-12; H-12 to C-1, C-2, C-6 and C-11; H-13 to C-4, C-5 and C-6. The relative stereochemistry of compound **57** was also confirmed by the NOESY correlations which demonstrated cross peak from H-2α to H-11; H-2β to H-13; H-3 to H-12; H-7 to H-10 and H-12. The hydroxyl proton was observed at $\delta_H$ 1.56-1.64 (1H, *brm*-OH). From these spectroscopic evidences, the carbon signal at $\delta_C$ 34.86 was assigned to tetrasubstituted carbon (C-1), the carbon at $\delta_C$ 63.73 was tertiary carbon-bond hydroxyl group (C-3), the carbon signals at $\delta_C$ 66.00 and 71.00 were oxygenated quaternary carbon of epoxide at C-5 and C-6 respectively. This compound was determined to be (+)-3-hydroxy-5β, 6β-epoxy-β-ionone **57** [148-150].

**Loliolide 58**




Loliolide **58**

Loliolide **58**: needle solid (crytalyzed from hexane/EtOAc) 35 mg (0.099% from crude ethyl acetate); mp. 152-153 °C, lit. mp. 150.5-151.5°C [151] ; $R_f$ 0.30 (hexane : EtOAc, 30 : 70); $[\alpha]_D^{27.5}$ -35.67° (c 0.58, $CDCl_3$), lit. $[\alpha]_D^{21}$ -103.13° (*c* 0.79, $CHCl_3$) [151]; ESMS (positive mode) *m/z* (% rel. intensity): 415 $[2M+Na]^+$(100); IR (Neat) $\nu_{max}$ 3435, 2974, 2946, 2923, 2879, 2851, 1731, 1720, 1621, 1446, 1420, 1373, 1272, 1166, 1099, 1023, 964, 867, 780, 682 and 609 $cm^{-1}$; $^{1}H$ NMR, $^{13}C$ NMR, DEPT 135, COSY and HETCOR were shown in Table 4.37-4.39 and Figure B23-B26.

**Table 4.37** $^{13}C$ NMR and DEPT spectroscopic data of loliolide **58** in $CDCl_3$

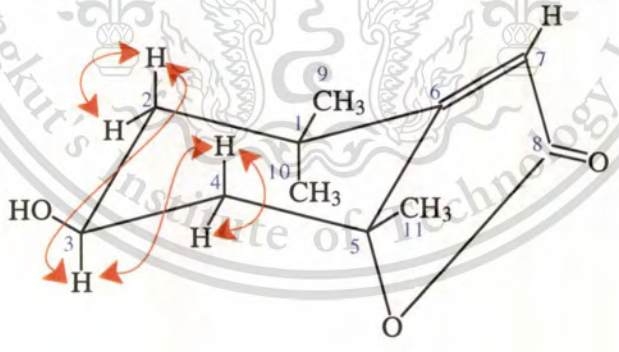
| $\delta_C$ (ppm) | DEPT 135 | Assignment | Positions |
|---|---|---|---|
| 182.79 | - | C | C-8 |
| 172.08 | - | CH | C-6 |
| 112.79 | Positive | CH | C-7 |
| 86.92 | - | C | C-5 |
| 66.67 | Positive | C | C-3 |
| 47.33 | Negative | CH | C-2 |
| 45.66 | Negative | $CH_2$ | C-4 |
| 35.98 | - | $CH_2$ | C-1 |
| 30.67 | Positive | - | C-10 |
| 27.03 | Positive | $CH_3$ | C-11 |
| 26.50 | Positive | $CH_3$ | C-9 |

**Table 4.38** $^{1}H$-$^{13}C$ HETCOR and $^{1}H$-$^{1}H$ COSY correlations of loliolide **58** in $CDCl_3$

| Positions | $^{1}H$-$^{13}C$ HETCOR | | $^{1}H$-$^{1}H$ COSY | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_H$ (ppm) | Proton correlated with $\delta_H$ (ppm) |
| 1 | 35.98 | - | - | - |
| 2 | 47.33 | 1.52 (1H, *dd*, *J* = 14.67, 3.6 Hz, H-2α)<br>2.00 (1H, *dt*, *J* = 14.67, 2.51 Hz, H-2β) | 1.52 (H-2α) | 2.00 (H-2β) and 4.31-4.34 (H-3α) |
| 3 | 66.67 | 4.31-4.34 (1H, *m*, H-3α)<br>1.95 (1H, *br*-OH) | 4.31-4.34 (H-3α) | 1.79 (H-4α) |
| 4 | 45.66 | 1.79 (1H, *dd*, *J* = 13.97, 3.9 Hz, H-4α)<br>2.48 (1H, *dt*, *J* = 13.97, 2.35 Hz, H-4β) | 1.79 (H-4α) | 2.48 (H-4β) |
| 5 | 86.92 | - | - | - |
| 6 | 172.08 | - | - | - |
| 7 | 112.79 | 5.69 (1H, *s*) | - | - |
| 8 | 182.79 | - | - | - |
| 9 | 26.50 | 1.47 (3H, *s*) | - | - |
| 10 | 30.67 | 1.27 (3H, *s*) | - | - |
| 11 | 27.03 | 1.79 (3H, *s*) | - | - |

**Table 4.39** $^{1}H$ and $^{13}C$ NMR of loliolide **58** in $CDCl_3$ compared to literature data

| Positions | Experimental data ($CDCl_3$) | | Lit. ($CDCl_3$) [152] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern | $\delta_C$ (ppm) | $\delta_H$ (ppm) and splitting pattern |
| 1 | 35.98 | - | 35.9 | - |
| 2 | 47.33 | 1.52 (1H, *dd*, *J* = 14.67, 3.6 Hz, H-2α)<br>2.00 (1H, *dt*, *J* = 14.67, 2.51 Hz, H-2β) | 47.0 | 1.51 (*dd*, *J*= 14.6, 3.6 Hz)<br>1.96 (*dt*, *J*= 14.6, 2.8 Hz) |
| 3 | 66.67 | 4.31-4.34 (1H, *m*, H-3α)<br>1.95 (1H, *br*-OH) | 66.5 | 4.30 (*m*) |
| 4 | 45.66 | 1.79 (1H, *dd*, *J* = 13.97, 3.9 Hz, H-4α)<br>2.48 (1H, *dt*, *J* = 13.97, 2.35 Hz, H-4β) | 45.3 | 1.76 (*dd*, *J*= 13.9, 4.1 Hz)<br>2.44 (*ddd*, *J*= 13.9, 2.7 Hz) |
| 5 | 86.92 | - | 86.6 | - |
| 6 | 172.08 | - | 171.8 | - |
| 7 | 112.79 | 5.69 (1H, *s*) | 112.6 | 5.67 (*s*) |
| 8 | 182.79 | - | 182.4 | - |
| 9 | 26.50 | 1.47 (3H, *s*) | 26.1 | 1.45 (*s*) |
| 10 | 30.67 | 1.27 (3H, *s*) | 30.3 | 1.25 (*s*) |
| 11 | 27.03 | 1.79 (3H, *s*) | 26.7 | 1.76 (*s*) |




COSY

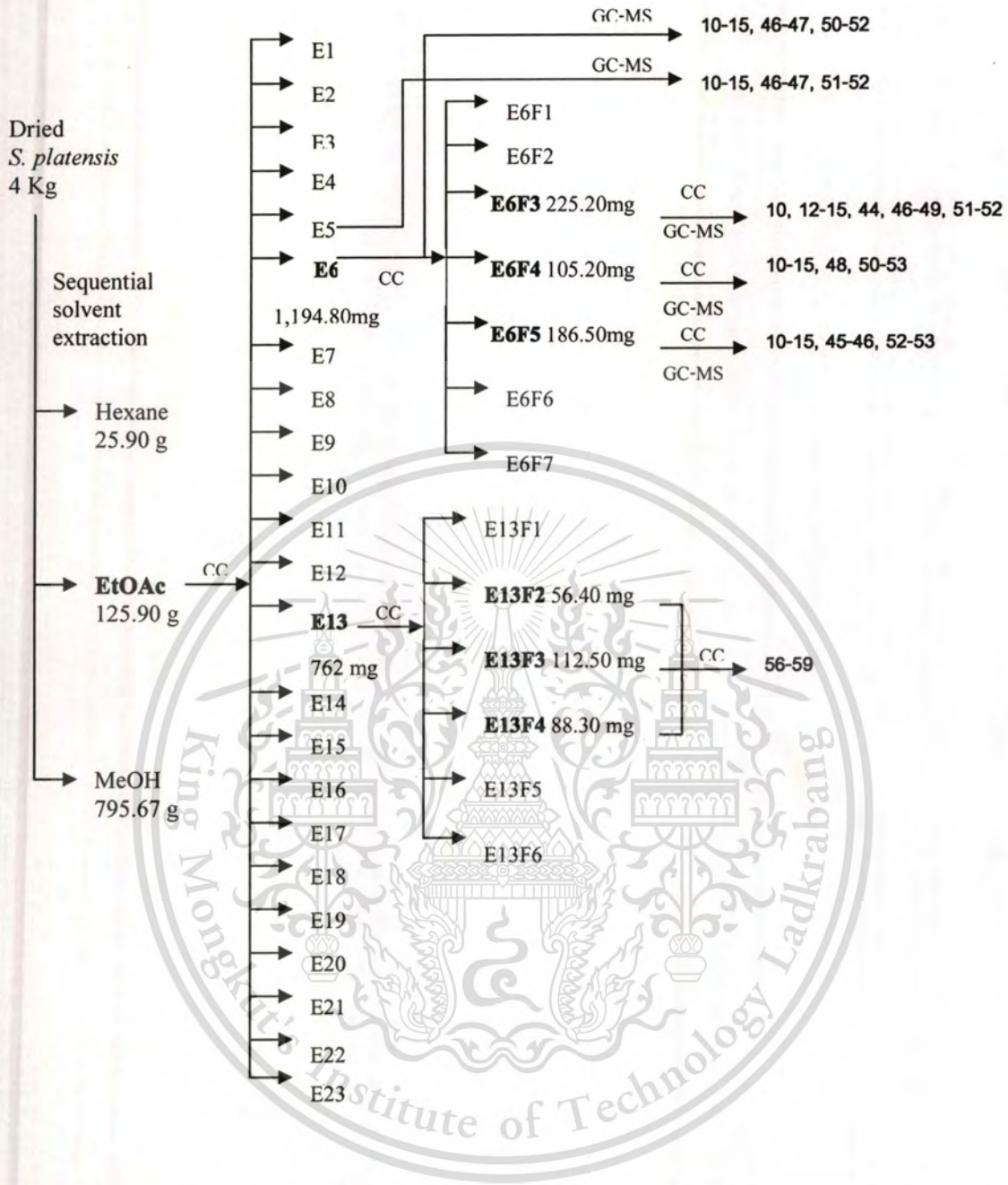
Loliolide **58**, which was obtained as a needle solid with the molecular formula $C_{11}H_{16}O_3$ calculated from ESMS *m/z* 415 $[2M+Na]^+$ and required 4 degrees of unsaturation. Its IR spectrum showed absorption bands due to hydroxyl group (3435 $cm^{-1}$), the carbonyl group of α, β-unsaturated ester (1720 $cm^{-1}$), double bond ( 1621 and 964 $cm^{-1}$) and a C-O stretching of ester (1023 $cm^{-1}$). The $^{13}C$ NMR spectrum, including the DEPT experiment, exhibited the presence of

one $sp^2$ methine ($\delta_C$ 112.79), one $sp^3$ methine ($\delta_C$ 66.67), two $sp^3$ methylenes ($\delta_C$ 45.66 and 47.33), three methyls ($\delta_C$ 26.50, 27.03 and 30.67), and four quaternary carbons ($\delta_C$ 35.98, 86.92, 172.08, and 182.79 (C=O)), and the four degrees of unsaturation as fully accounted for by one carbonyl group, one olefin, and two cyclics. The $^1H$-$^{13}C$ HETCOR experiment showed two methine protons at $\delta_H/\delta_C$ 4.31-4.34 /66.67 and 5.69/112.79, two methylene protons at $\delta_H/\delta_C$ 1.52 and 2.00/47.33, and 1.79 and 2.48/45.66, and three methyl protons at $\delta_H/\delta_C$ 1.27/30.67, 1.47/26.50 and 1.79/27.03. In the $^1H$ NMR and $^1H$-$^1H$ COSY spectra, the mehtylene protons at $\delta_H$ 1.52 (1H, *dd*, $J$ = 14.67, 3.6 Hz, H-2α) was germinal coupled with a proton at $\delta_H$ 2.00 (1H, *dt*, $J$ = 14.67, 2.51 Hz, H-2β) and vicinal coupled with proton at $\delta_H$ 4.31-4.34 (1H, *m*, H-3α), and the mehtylene protons at $\delta_H$ 1.79 (1H, *dd*, $J$ = 13.97, 3.9 Hz, H-4α) was germinal coupled with a proton at $\delta_H$ 2.48 (1H, *dt*, $J$ = 13.97, 2.35 Hz, H-4β) and vicinal coupled with proton at $\delta_H$ 4.31-4.34 (1H, *m*, H-3α). The hydroxyl proton was observed at $\delta_H$ 1.95 (1H, *brm*-OH). From these spectroscopic evidences, the carbon signal at $\delta_C$ 35.98 was assigned to tetrasubstituted carbon (C-1), the carbon at $\delta_C$ 66.67 was tertiary carbon-bond hydroxyl group (C-3), the carbon at $\delta_C$ 86.92 was oxygenated quaternary carbon (C-5), and the carbon at $\delta_C$ 171.99 was $sp^2$ quaternary carbon (C-6). This compound was determined to be (-)-loliolide **58** [140, 142, 151-156].

All the four compounds thus identified, which were isolated from subfractions E13F2-E13F4, were derivatives of carotenoids. 3-Hydroxy-β-ionone **59** had already been isolated and detected from quince fruits [145], tobacco plants [157], *Vitis vinifera* Cv. Riesling leaves [158] and *Bunias orientalis* leaves [159]. It was synthesized by DDQ oxidation of 3-hydroxy-β-ionol [150b]. 3-Hydroxy-β-ionone **59** has been reported as a potent growth inhibitor by Kato-Noguchi. [145].

3-Hydroxy-5α, 6α-epoxy-β-ionone **56** was a natural norisoprenoid that isolated from leaves of *Acanthosyris paulo-alvinii* Barroso (Santhalaceae) [146] and aerial parts of *Beta vulgaris* var. *cicla* [147]. 3-Hydroxy-5β, 6β-epoxy-β-ionone **57** was reported at the first time as natural product from sunflower leaves cv. Peredovick [149], and it had been isolated from leaves of a large natural tree from Southeast Asia, *Tectona grandis* [160]. These two isomers had been synthesized from 3-hydroxy-β-ionone **59** [148, 150a-b]. Moreover, Isoe et al. reported that the isolated 3-hydroxy-5, 6-epoxy-β-ionone (**56** or **57**) still is an unknown stereochemistry as one of the photo-oxygenation products of zeaxanthin **27** [161].

Loliolide **58** has also been isolated from higher plants [152] and marine algae [162]. It is well-known to have immunosuppressive, germination inhibitory and antirepellent activities [163-165]. Recently, this compound has been isolated from the brown alga *Undaria pinnatifida* [153], *Sargassum thunbergii* [155] and *Cladostephus spongiosus* f. *verticillatus* (Heterokonta, Sphacelariales) [156]. The pathways of these isolated compounds were proposed by Xian et al. [122] including the isolated 3-hydroxy-5, 6-epoxy-β-ionone (**56** or **57**) [161]. The chemical compositions of *S. platensis* consisted of carotenoids [44] as summarized in Scheme 4.2. Hence, these isolated compounds may be degraded from many carotenoids such as myxoxanthophyll, zeaxanthin **27**, canthaxanthin **28**, violaxanthin, echinenone, 3'-hydroxyechinenone, β-cryptoxanthin, and oscillaxanthin etc. Flow chart 4.1, shown below, illustrated the isolated of active compounds from crude ethyl acetate extract.




**Flow chart 4.1** The extract of allelochemical compounds from E5-E6, E6F3-E6F5 and E13F2-E13F4

## 4.12 Allelopathic effects of pure compounds from subfractions E6F3-E6F5 and E13F2-E13F4

The active subfractions of the ethyl acetate extract were isolated and characterized the pure compounds by spectroscopic techniques. These isolated compounds were divided into 2 groups, fatty acids and norisoprenoids, respectively. Commercial fatty acids were selected as representative of natural fatty acids from *S. platensis*. The allelopathic assay was performed at concentrations of 62.5-1,000 ppm. Seeds of Chinese amaranth and Barnyardgrass were used to assess the inhibitory effect of isolated compounds germination, with distilled water served as control.

### 4.12.1 Fatty acids

The major fatty acids consisted of fractions E5-E6 and subfractions E6F3-E6F5, namely palmitic acid **10**, stearic acid **13**, oleic acid **14**, linoleic acid **12,** and γ-linolenic acid **11**. The allelopathic effects of these fatty acids are described below.

**Chinese amaranth**

The results in Table 4.40 showed that linoleic acid **12** and γ-linolenic acid **11** at concentrations of 500-1,000 ppm were highly toxic on seed germination of Chinese amaranth. Both of fatty acids completely inhibited at a concentration of 1,000 ppm. The effect on seedling growth found that both compounds inhibited shoot length at concentrations of 500-1,000 ppm, and root length at concentrations of 250-1,000 ppm. In contrast, palmitic acid **10**, stearic acid **13** and oleic acid **14** promoted the shoot and root length of Chinese amaranth (Figure 4.18).

**Table 4.40** Allelopathic effects of commercial fatty acids from E6F3-E6F5 on seed germination and seedling growth of Chinese amaranth

| Fatty acids | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|
| | % Inhibition on seed germination | | | | |
| Control | 0a | 0a | 0a | 0b | 0b |
| Palmitic acid **10** | 5a | 2.50a | 5a | 0b | 2.50b |
| Stearic acid **13** | 5a | 5a | 5a | 2.50b | 0b |
| Oleic acid **14** | 2.50a | 0a | 2.50a | 2.50b | 2.50b |
| Linoleic acid **12** | 0a | 0a | 0a | 25a | 100a |
| γ-Linolenic acid **11** | 2.50a | 2.50a | 5a | 20a | 100a |
| | % Inhibition on shoot length | | | | |
| Control | 0ab | 0a | 0a | 0b | 0b |
| Palmitic acid **10** | -15.51d | -15.27bc | -14.08b | -25.54c | -21.72c |
| Stearic acid **13** | -6.92bc | -21.72c | -38.90c | -38.19d | -30.31d |
| Oleic acid **14** | -3.58bc | -13.37b | -5.25ab | -19.81c | -21.72c |
| Linoleic acid **12** | 5.01a | -9.07b | -11.69b | 37.95a | 100a |
| γ-Linolenic acid **11** | -10.74cd | -20.76c | -12.41b | 42.72a | 100a |
| | % Inhibition on root length | | | | |
| Control | 0a | 0a | 0b | 0c | 0b |
| Palmitic acid **10** | -0.67ab | -5.73b | -8.77c | -4.55c | -2.87b |
| Stearic acid **13** | -7.59bc | -22.60c | -28.67d | -32.38d | -22.77d |
| Oleic acid **14** | -28.50d | -26.14c | -28.16d | -40.98e | -14.17c |
| Linoleic acid **12** | -3.88ab | 4.05a | 9.95a | 82.97a | 100a |
| γ-Linolenic acid **11** | -11.80c | -35.92d | 14.17a | 70.32b | 100a |

Values with same letters in each column there are not significantly different at $P$=0.05

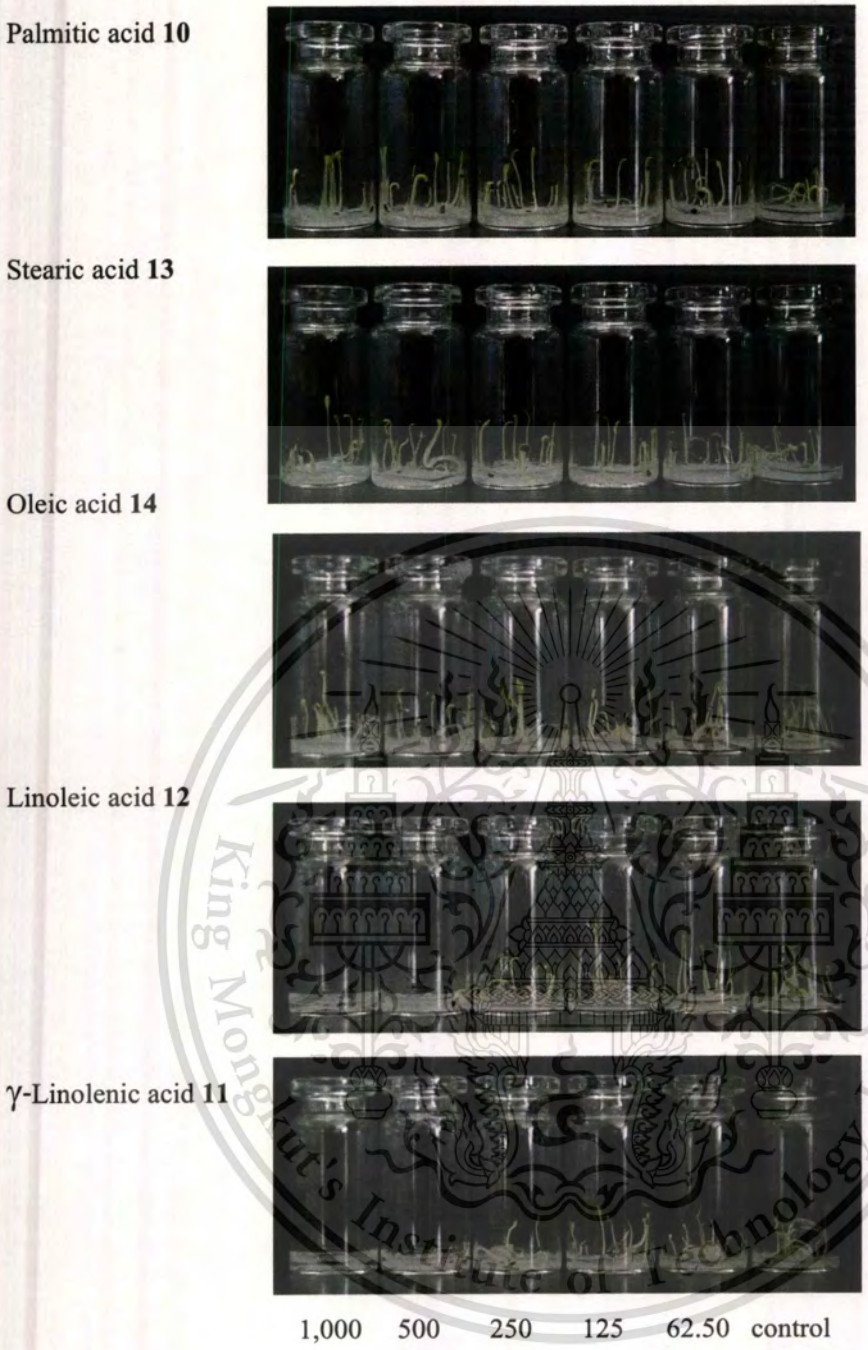



**Figure 4.18** Allelopathic effects of commercial fatty acids at concentrations of 62.5-1,000 ppm on Chinese amaranth

**Barnyardgrass**

As shown in Table 4.41, linoleic acid **12** and γ-linolenic acid **11** exhibited significant on germination of Barnyardgrass seed at concentrations of 500-1,000 ppm. As for seedling growth, oleic acid **14** at a concentration of 1,000 ppm had a slightly inhibitory effect on shoot length. Linoleic acid **12** and γ-linolenic acid **11** at concentrations of 500-1,000 ppm reduced shoot and

root length of Barnyardgrass by a moderately inhibition percentage while other concentrations had no effect on seedling growth. As the effect on Chinese amaranth, palmitic acid **10**, stearic acid **13** and oleic acid **14** did not show any inhibitory effect on the growth of seedling but exhibited the promotion effect in weak percentage on seedling growth of Barnyardgrass (Figure 4.19).

**Table 4.41** Allelopathic effects of commercial fatty acids from E6F3-E6F5 on seed germination and seedling growth of Barnyardgrass

| | % Inhibition on seed germination | | | | |
|---|---|---|---|---|---|
| Fatty acids | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0a | 0b | 0b |
| Palmitic acid **10** | 0a | 0a | 0a | -2.70b | 0b |
| Stearic acid **13** | -2.70a | 0a | 0a | -2.70b | 0b |
| Oleic acid **14** | 0a | 0a | 2.86a | 2.86b | 0b |
| Linoleic acid **12** | 0a | -2.70a | 0a | 16.22a | 56.76a |
| γ-Linolenic acid **11** | 0a | 0a | 0a | 17.14a | 60a |
| | % Inhibition on shoot length | | | | |
| Control | 0ab | 0a | 0a | 0c | 0d |
| Palmitic acid **10** | -2.38bc | -4.81b | -5.35b | -2.85c | -2.73d |
| Stearic acid **13** | -5.82c | -4.87b | -4.81b | -9.80d | -3.62d |
| Oleic acid **14** | -2.10bc | -0.37a | -0.80a | -0.93c | 4.57c |
| Linoleic acid **12** | 0.12ab | 2.67a | 0.06a | 9.27b | 45.63a |
| γ-Linolenic acid **11** | 3.46a | 3.40a | 0.74a | 14.03a | 38.13b |
| | % Cnhibition on root length | | | | |
| Control | 0a | 0a | 0ab | 0b | 0b |
| Palmitic acid **10** | -0.67ab | -5.73bc | -8.77c | -4.55c | -2.87b |
| Stearic acid **13** | 1.85a | 0.67a | -1.18b | -16.86e | -21.75c |
| Oleic acid **14** | -5.49b | -8.16c | -9.89c | -8.95d | -4.08b |
| Linoleic acid **12** | 0.17a | 1.01a | 3.37a | 19.06a | 91.06a |
| γ-Linolenic acid **11** | -1.88ab | -2.35ab | -2.83b | 20.25a | 95.76a |

Values with same letters in each column there are not significantly different at $P$=0.05

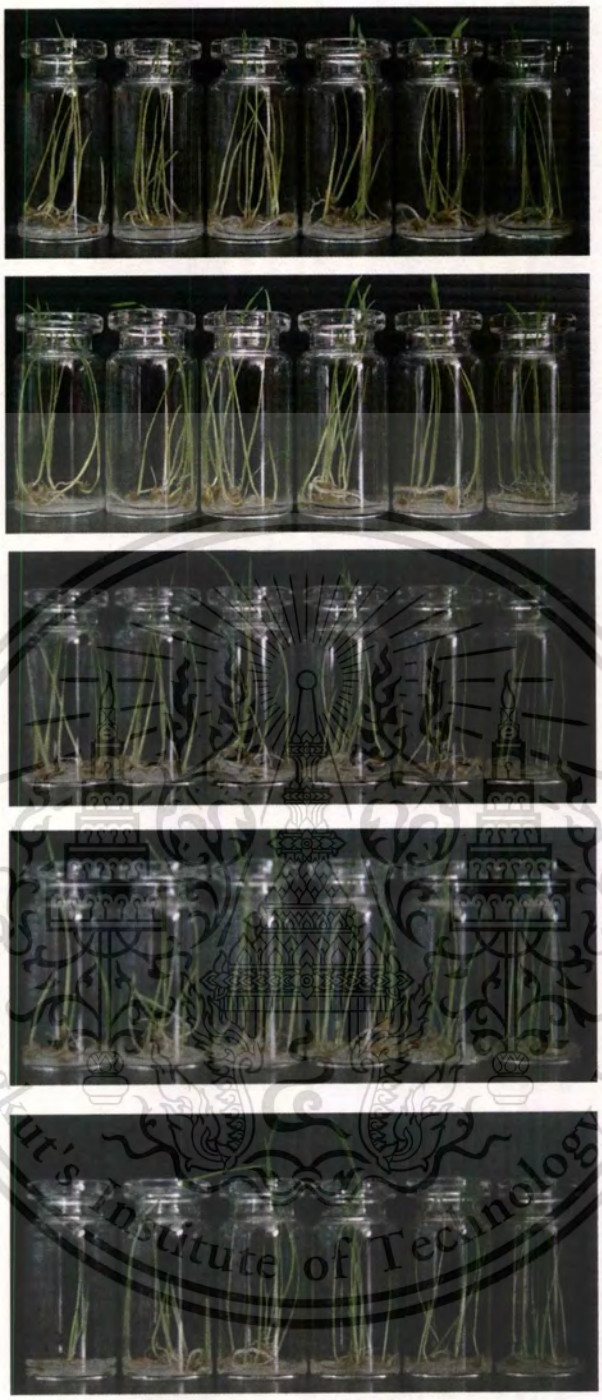
Palmitic acid **10**

Stearic acid **13**

Oleic acid **14**

Linoleic acid **12**

γ-Linolenic acid **11**

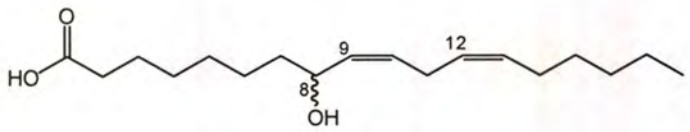


1,000 500 250 125 62.50 control

**Figure 4.19** Allelopathic effects of commercial fatty acids at concentrations of 62.5-1,000 ppm on Barnyardgrass

The results of the experiment revealed that fatty acids were exhibited an allelopathic role onto tested plants. The allelopathic effects of fatty acids have been reported for a along time, as in the following:

In 1985, Bowers et al. [166] isolated alleloachemical, laetisaric acid **60** (Z,Z-9,12-8-hydroxyoctadecadienoic acid) from fungus, *Laetisaria arvalis*. This acid inhibited growth of *Phytium ultimum* by 50% at a concentration of 22 μg/mL.



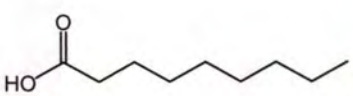

Laetisaric acid **60**

In 1988, Kakisawa et al. [12] isolated an allelopathic substance from the brown algae, *Chadosiphon okamuranus*, and identified it as 6Z,9Z,12Z,15Z- octadecatetraenoic acid **61**, which is capable of inhibiting the growth of the red algae, *Porphyra yezoensis*, and microalga, *Heterosigma akashiwo*.


Octadeca-6,9,12,15-tetraenoic acid **61**

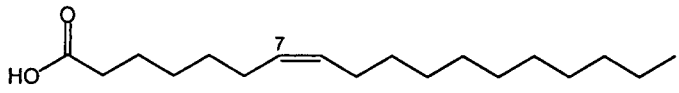
Gallardo-Williams et al. [167] isolated phytotoxic compounds from aqueous and leachates of cattails (*Typha domingensis*). These compounds were identified as essential fatty acids (linoleic acid **12** and α–linolenic acid **16**) and phenolic compounds. The two essential fatty acid compounds were the major components in active fractions, comprising more than 80% of the total isolated material. These fractions inhibited the germination of lettuce seeds by 80-90%.

Jin et al. [168] studied the allelopathic potential of *Echhornia crassipes* root extract on *Chlorella* sp. and *Scendesmus obliquus*. The results found that the active fraction was composed of saturated fatty acid and pelargonic acid **62** ($C_9H_{18}O_2$). These fractions can cause the removal rate of Chlorophyll-a **25** by 95.3%.




Pelargonic acid **62**

Ko et al. [169] studied the allelopathy of rice husk on Barnyardgrass and found that the strongest inhibitory effect was caused by a fraction composed of 9-octadecenoic acid **14**, 7-octadecenoic acid **63**, 5,8,11-heptadecatriecenoic acid **64** an androstan-17-one. This fraction completely inhibited seed germination of Barnyardgrass at a concentration of 200 ppm and had the minimum inhibition concentration (MIC) at 50 ppm.



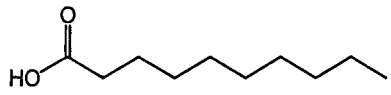

7-Octadecenoic acid **63**


5,8,11-heptadecatriecenoic acid **64**

In 2005, Alamsjah et al. [170] studied the algicidal activity of the green algae, *Ulva fascica*, and isolated three active compounds which identified to be polyunsaturated fatty acids, namely hexadeca-4,7,10,13-tetraenoic acid **65** (HDTA), octadeca-6,9,12,15-tetraenoic acid **61** (ODTA), and α-linolenic acid **16**. These polyunsaturated fatty acids showed potent algicidal activity against red-tide phytoplankton *Heterosigma akshiwo* (LC50 1.35 , 0.83 and 1.13 μg/mL for compounds **65, 61** and **16** respectively).


Hexadeca-4,7,10,13-tetraenoic acid (HDTA) **65**

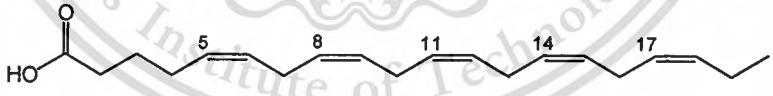
In 2006, Xuan et al. [171] identified phytotoxic substances from the early growth of Barnyardgrass root exudates. They found that the active fractions consisted of two phenolic derivatives, four derivatives of phthalic acid, a benzoic acid derivative, derivatives of decane, derivative of acenaphthene, two lactones and three long chain fatty acids, including decanoic acid **66**, myristic acid **46** and stearic acid **13**. At a concentration of 100 ppm, these three fatty acids showed the allelopathic activity against the seed germination and seedling growth of Barnyardgrass approximately 4-20%.



Decanoic acid **66**

The allelopathic activity of commercial fatty acids revealed that unsaturated fatty acids showed a higher inhibitory effect on tested plants than saturated fatty acids. These results are in agreement with several researches, for examples, Spruell [172] studied the activity of three $C_{18}$ fatty acids against a number of algae and zooplankton. He found that the three acids, linolenic acid **11**, linoleic acid **12** and oleic acid **14**, reduced the growth of *Haematoccus lacustris*, *Synechococcus leopolienesis*, and *Botrydiopsis alpine* by 50% of control growth in concentrations below 7 ppm.

In 1988, Kakisawa et al. [12] studied the allelopathic activity of octadeca-6,9,12,15-tetraenoic acid **61** (ODTA), arachidonic acid **19**, 5,8,11,14-ecosapentaenoic acid (EPA) **67**, γ-linolenic acid **11**, linoleic acid **12**, oleic acid **14**, ODTA methyl ester, methyl linolenate, ODTA sodium salt, sodium linolenate, trilinolenine, sodium laurylbenzenesulfonate, and Tween 80 against *Heterosigma akashiwo*. They found that arachidonic acid **19**, EPA **67** exhibit similar activity to ODTA **61**. The sodium salt of ODTA is as effective as free ODTA **61**. Less unsaturated fatty acids exhibit weaker activities. The ester of unsaturated fatty acids also exhibited weaker activities than that found in the corresponding free acids. Sodium laurylbenzenesulfinate and Tween 80 also showed activity, but to a lesser degree.




5,8,11,14,17-Ecosapentaenoic acid (EPA) **67**

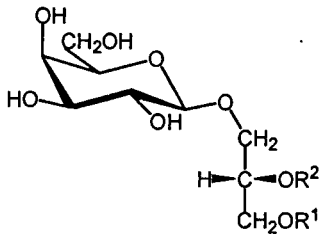
Aliotta et al. [173] isolated allelochemical compounds from *Typha latifolia* L. that consisted of three steroids and three fatty acids (α-linolenic acid **16**, linoleic acid **12** and unidentified C18:2 fatty acid). They found that the most effective fatty acid against algae growth was α-linolenic acid **16**.

Murakami et al. [174] studied the allelopathic activity of crude acetone extract and methanol extract of *P. tenue*, on its own culture. Results found that the crude acetone extract exhibited the growth inhibitory activity. Then, they separated the extract by column

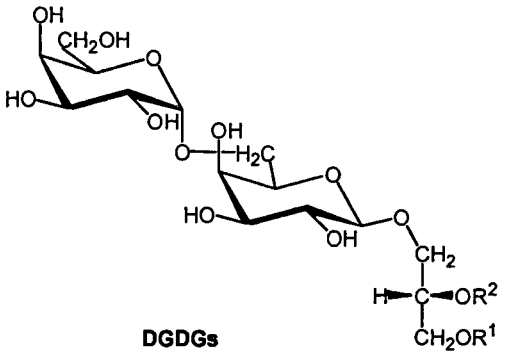
chromatography which the extract gave a potent growth-inhibiting substance. After analysis the active substance by GC-MS found that this substance was a mixture of methyl myristate, methyl palmitate, methyl palmitoleate, methyl oleate, methyl *cis*-vaccenate, methyl linoleate, and methyl linolenate in a ratio of 5 : 4 : 5 : 4 : 1: 47 : 36. The allelopathic assayed of active substance and authentic fatty acids found that the mixture of the fatty acid substance consist of MIC at 0.5 ppm, unsaturated fatty acids such as palmitoleic acid **15**, oleic acid **14**, and *cis*-vaccenic acid **68** were inhibitory at concentrations of 2.5, 1.0, and 5.0 ppm, respectively. The higher unsaturated fatty acids, linoleic acid **12** and linolenic acid **11** had the highest inhibitory at a concentration of 0.5 ppm, while saturated ones were inactive even at a concentration of 100 ppm.


*cis*-Vaccenic acid **68**

In 1991, Murakami et al. [175] extracted the lyophilized cells of *P. tenue* by the Bligh-Dyer method. The resulting extract was successively subjected to silica gel column chromatography to obtain monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) fractions. These two fractions were further saparated by reversed-phase HPLC to furnish 17 derivative compounds **69-76, 78-87** and a mixture of **77a-77b** and **88a-88b**. Then these seventeen compounds were assayed the antialgal activity on *P. tenue* at concentrations of 1-100 ppm, and determined an activity by $IC_{50}$ value. The results found that the lower polarity compounds (MGDG derivatives) had an inhibitory effect whereas the higher polarity compounds (DGDG derivatives) showed no inhibitory activity. Particularly, MGDG containing unsaturated fatty acids, such as linoleic acid **12** and linolenic acids **11** or **16**, exhibited more potent growth inhibitory activity than those which possessed saturated fatty acids.



MGDGs



DGDGs

**69**: $R^1$ = linolenoyl; $R^2$ = myristoyl ($IC_{50}$ 26 ppm)

**70**: $R^1$ = linolenoyl; $R^2$ = palmitelaidoyl (32 ppm)

**71**: $R^1$ = linoleoyl; $R^2$ = myristoyl (45 ppm)

**72**: $R^1$ = palmitolepyl; $R^2$ = myristoyl (>100 ppm)

**73**: $R^1$ = oleoyl; $R^2$ = myristoyl (>100 ppm)

**74**: $R^1$ = linoleoyl; $R^2$ = palmitoyl (27 ppm)

**75**: $R^1$ = palmitoyl; $R^2$ = myristoyl (>100 ppm)

**76**: $R^1$ = linolenoyl; $R^2$ = linolenoyl (18 ppm)

**77a**: $R^1$ = linoleoyl; $R^2$ = palmitoyl

**77b**: $R^1$ = linoleoyl; $R^2$ = palmitelaidoyl

**78**: $R^1 = R^2 = H$

**79**: $R^1$ = linolenoyl; $R^2$ = myristoyl (>100 ppm)

**80**: $R^1$ = linoleoyl; $R^2$ = myristoyl (>100 ppm)

**81**: $R^1$ = palmitoleoyl; $R^2$ = myristoyl (>100 ppm)

**82**: $R^1$ = oleoyl; $R^2$ = myristoyl (>100 ppm)

**83**: $R^1$ = palmitoyl; $R^2$ = myristoyl (>100 ppm)

**84**: $R^1$ = linolenoyl; $R^2$ = linoleoyl (>100 ppm)

**85**: $R^1$ = linolenoyl; $R^2$ = linolenoyl (>100 ppm)

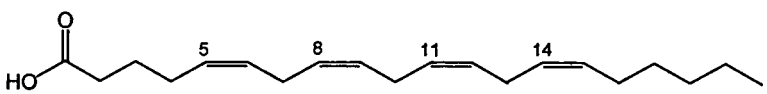
**86**: $R^1$ = linoleoyl; $R^2$ = linoleoyl (>100 ppm)

**87**: $R^1$ = linoleoyl; $R^2$ = palmitoyl (>100 ppm)

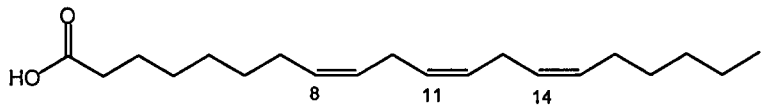
**88a**: $R^1$ = linolenoyl; $R^2$ = palmitoyl

**88b**: $R^1$ = linoleoyl; $R^2$ = palmitelaidoyl

Suzuki et al. [176] isolated two fatty acids, palmitic acid **10** and (5Z, 8Z, 11Z, 14Z, 17Z)-eicosapentaenoic acid **67**, from the red algae, *Neodilsea yendoana* by preperative TLC. Then these two acids and four commercially available unsaturated fatty acids were tested the growth-inhibitory activity on the red algae, *Monostroma oxyspermum*. The MIC were found that the more polar acid **67** showed allelopathic activity (1 μg/mL), 5,8,11,14-eicosatetraenoic acid **89** (arachidonic acid) (1 μg/mL), 8,11,14-eicosatrienoic acid **90** (dihomo-γ-linolenic acid) (10 μg/mL), 6,9,12,15-octadecatetraenoic acid **61** (1 μg/mL), and γ-linolenic acid **11** (1 μg/mL), while palmitic acid **10** proved inactive.



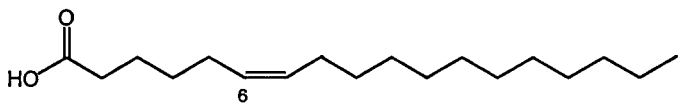
5,8,11,14-Ecosatetraenoic acid **89**



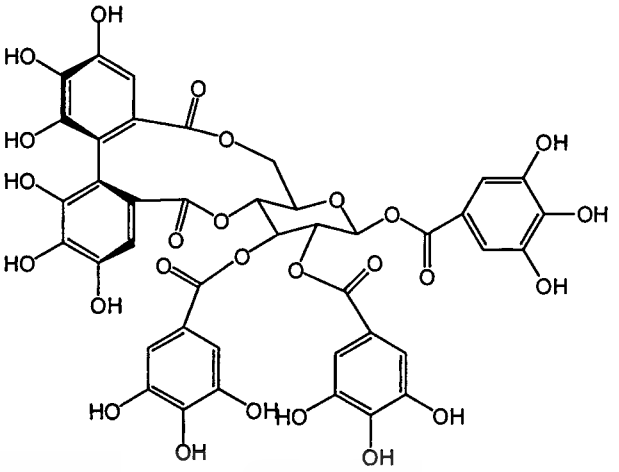

8,11,14-Eicosatrienoic acid **90**

In 2007, Alamsjah et al. [177] determined the toxic effect of α-linolenic acid **16** and linoleic acid **12**, that isolated from *Ulva fasciata*, on red-tide phytoplankton, *Heterosigma akashiwo*. These two acids had lethal concentration ($LC_{50}$) of 0.58 (for α-linolenic acid **16**) and 1.91 (for linoleic acid **12**), respectively. They then suggested that α-linolenic acid **16** had a higher toxicity than linoleic acid **12** due to a chemical structure feature of α-linolenic acid **16** such as the number of unsaturated double bond.

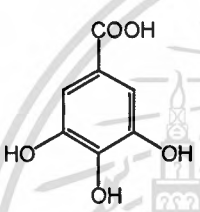
Furthermore, Nakai et al. [178] studied the anti-cyanobacterial activity of fatty acids, that released from *Myriophyllum spicatum*. The results found that nonanoic **62** had the strongest inhibitory effect, 6-*cis*-octadecenoic **91** and 9-*cis*-octadecenoic acid **14** significantly inhibited the growth of *M. aeruginosa*, while tetradecanoic acid **46**, hexadecanoic acid **10**, octadecanoic acid **13** did not show any effect. A few previous studies of allelochemicals released by *M. spicatum* identified polyphenol (eugeniin **92**, gallic acid **93**, and ellagic acid **94** [179]; pyrogallic acid **95**, gallic acid **93**, and ellagic acid **94** and (+)-catechin **96** [180]), whereas this study showed that *M. spicatum* released not only polyphenols but also fatty acids. The $EC_{50}$ of polyphenols for *M. aeruginosa* are 1.5 mg/L for euginiin **92** [181], 0.7 mg/L for pyrogallic acid **95**, 1.0 mg/L for gallic acid **93**, 5.5 mg/L for (+)-catechin **96**, and 5.1 mg/L for ellagic acid **94** [180]. Thus, compared with these polyphenols, nonanoic acid **62** was the most inhibitory against *M. aeruginosa*. However, the total inhibitory effect of the *M. spicatum* culture solution could not be accounted for by nonanic acid **62**, because the apparent concentration of nonanoic acid **62** in the *M. spicatum* culture solution was 50 μg/L, much lower than the $EC_{50}$. They suggested that because polyphenols and fatty acids have different chemical properties which may result in the different growth inhibition modes, it is reasonable to except that their cyanobacterial growth inhibition activities may be additive or synergistic.



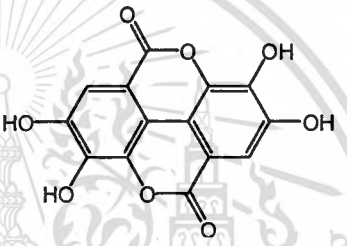

6-*cis*-Octadecenoic acid **91**



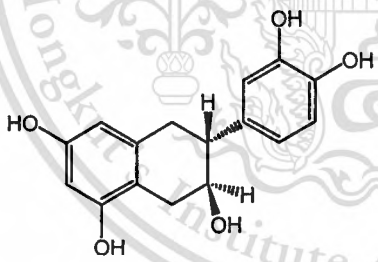
Eugeniin **92**



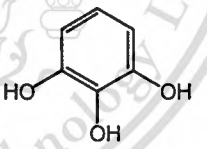
Gallic acid **93**



Ellagic acid **94**



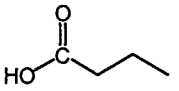
(+)-Catechin **96**



Pyrogallic acid (PA) **95**

In 1980, McCracken et al. [182] showed experimentally that anti-algal effects of fatty acids were proportional to the number of unsaturated linkages by assaying 7 fatty acids extracted from the green alga *Chlamydomonas*, whereas Nakai et al. [180] confirmed that octadecenoic acids, **14** and **91,** not octadecanoic acid **13** significantly inhibited the growth of *M. aeruginosa*. In addition, the inhibitory effect of 9-*cis*-octadecenoic acid **14** was stronger than 6-*cis*-octadecenoic **91**. Among the four saturation fatty acids, **10, 13, 41** and **62,** that they identified, nonanoic acid **62**, which has the shortest carbon chain, was the only one demonstrating significant growth

inhibition of *M. aeruginosa* at 0.5 mg/L. Takamura et al. [183] confirmed that butyric acid **97**, another saturated fatty acid which a short carbon chain, inhibited maximum growth of *Microcystis novacekii* by 64% of the control at 8.8 mg/L. From these results, Nakai et al. [178] suggested that (i) length of carbon chain, (ii) number of unsaturated linkages, and (iii) positions of any double bonds may affect the anticyanobacterial activity of fatty acids.




Butyric acid **97**

### 4.12.2 $C_{11}$ and $C_{13}$ norisoprenoids

Six norisoprenoids were isolated from 6 active subfractions, E6F3-E6F5 and E13F2-E13F4, by flash column chromatography. These compounds had different allelopathic effects on tested plants. The results were shown in Table 4.42-4.43.

**Chinese amaranth**

The results from Table 4.42 showed that dihydroactinidiolide **51**, 3-hydroxy-β-ionone **59**, 3-hydroxy-5α, 6α-epoxy-β-ionone **56** at concentrations of 250-1,000 ppm, and 4-oxo-β-ionone **52**, 3-hydroxy-5β, 6β-epoxy-β-ionone **57** and loliolide **58** at concentrations of 500-1,000 ppm were highly toxic on seed germination of Chinese amaranth, particularly compounds **51**, **56** and **57** showed completely inhibition at a concentration of 500 ppm. The effect on seedling growth found that compounds **51**, **56**, **57** and **59** at concentrations of 125-1,000 ppm, and compounds **52** and **58** at concentrations of 500-1,000 ppm had an inhibitory effect on shoot length of Chinese amaranth. As for root length, it was found that compounds **51** and **56** at concentrations of 125-1,000 ppm, compounds **57**, **58** and **59** at concentrations of 250-1,000 ppm, and compounds **52** at concentrations of 500-1,000 ppm inhibited the root length of Chinese amaranth. It was noted that compound (-)-**56** had more effect on Chinese amaranth than compound (+)-**57** (Figure 4.20).

**Table 4.42** Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on seed germination and seedling growth of Chinese amaranth

| Norisoprenoids | % Inhibition on seed germination | | | | |
|---|---|---|---|---|---|
| | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0c | 0d | 0 |
| Dihydroactinidiolide **51** | 0a | 6.06a | 54.55a | 100a | 100 |
| 4-Oxo-β-ionone **52** | 0a | 0a | 7.5c | 85b | 100 |
| 3-Hydroxy-β-ionone **59** | 0a | 0a | 15.38b | 89.74b | 100 |
| **56** | 0a | 0a | 22.5b | 100a | 100 |
| **57** | 0a | 0a | 7.5c | 100a | 100 |
| Loliolide **58** | 0a | 2.56a | 0c | 28.21c | 100 |
| | % Inhibition on shoot length | | | | |
| Control | 0a | 0e | 0e | 0d | 0 |
| Dihydroactinidiolide **51** | 3.96a | 9.35cd | 64.03a | 100a | 100 |
| 4-Oxo-β-ionone **52** | -21.71d | 2.94de | 3.92e | 84.31b | 100 |
| 3-Hydroxy-β-ionone **59** | -7.76b | 14.35bc | 17.88d | 85.65b | 100 |
| **56** | -0.24a | 33.33a | 45.99b | 100a | 100 |
| **57** | -0.49a | 18.25b | 28.95c | 100a | 100 |
| Loliolide **58** | -16.42c | -31.62f | -26.47f | 26.47c | 100 |
| | % Inhibition on root length | | | | |
| Control | 0a | 0c | 0e | 0d | 0 |
| Dihydroactinidiolide **51** | -0.46a | 13.36b | 66.06a | 100a | 100 |
| 4-Oxo-β-ionone **52** | 0.13a | -3.32cd | -6.78f | 84.04b | 100 |
| 3-Hydroxy-β-ionone **59** | -30.18c | -5.92d | 6.71d | 83.23b | 100 |
| **56** | -1.34a | 17.45a | 25.37c | 100a | 100 |
| **57** | -11.95b | -3.76cd | 9.26d | 100a | 100 |
| Loliolide **58** | 2.52a | -0.92c | 52.75b | 77.64c | 100 |

Values with same letters in each column there are not significantly different at $P$=0.05

Dihydroactinidiolide **51**

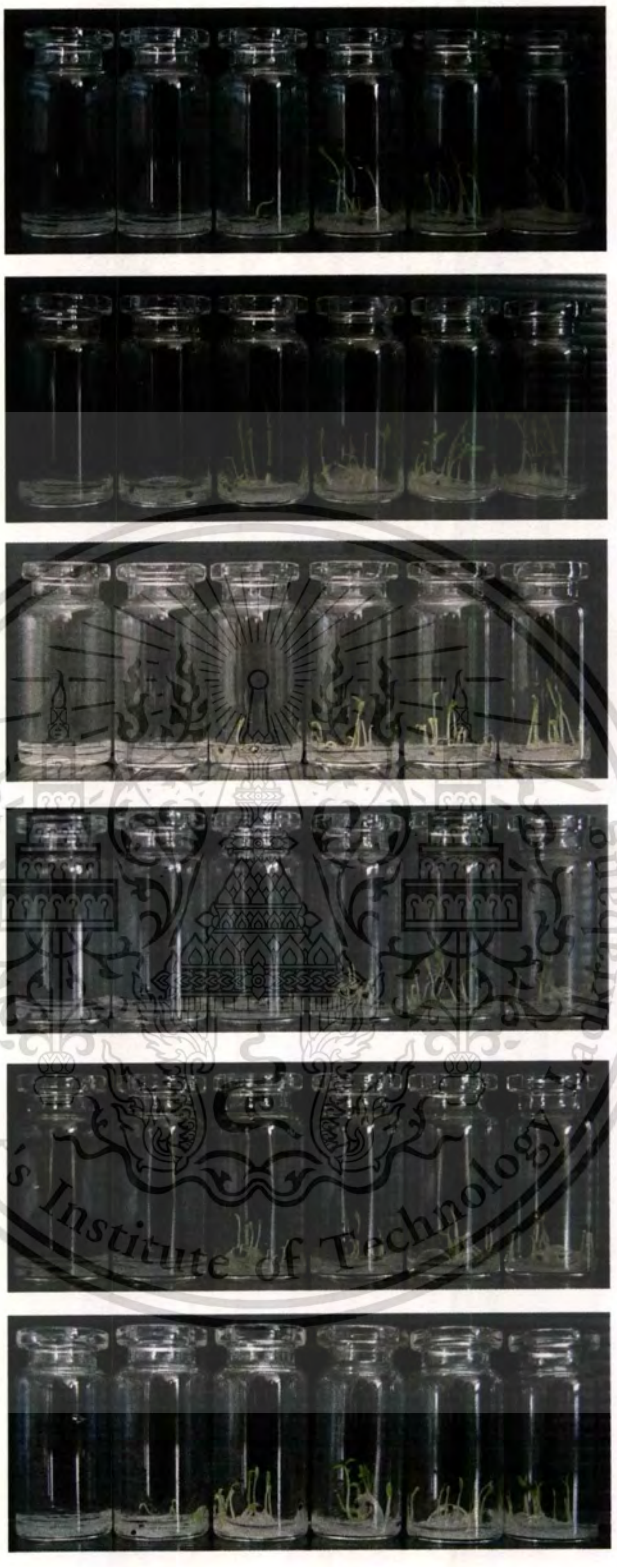
4-Oxo-β-ionone **52**

3-Hydroxy-β-ionone **59**

3-Hydroxy-5α, 6α-epoxy-β-ionone **56**

3-Hydroxy-5β, 6β-epoxy-β-ionone **57**

Loliolide **58**



1,000 500 250 125 62.50 Control

**Figure 4.20** Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F3-E6F5 and E13F2-E13F4 on Chinese amaranth

**Table 4.43** Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on seed germination and seedling growth of Barnyardgrass

| Norisoprenoids | % Inhibition on seed germination | | | | |
|---|---|---|---|---|---|
| | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
| Control | 0a | 0a | 0b | 0b | 0d |
| Dihydroactinidiolide **51** | 0a | 3.03a | 12.12a | 54.55a | 100a |
| 4-Oxo-β-ionone **52** | 0a | 0a | 6.06ab | 42.42a | 78.79b |
| 3-Hydroxy-β-ionone **59** | 0a | -2.78a | 2.78b | 2.78b | 16.67c |
| Loliolide **58** | 2.86a | 2.86a | 0b | 5.71b | 5.71d |
| | % Inhibition on shoot length | | | | |
| Control | 0b | 0c | 0c | 0e | 0d |
| Dihydroactinidiolide **51** | 13.02a | 19.23a | 45.82a | 71.65a | 100a |
| 4-Oxo-β-ionone **52** | -1.04b | -2.37c | -1.96c | 18.01b | 83.83b |
| 3-Hydroxy-β-ionone **59** | -0.70b | 6.79b | 7.54b | 14.56c | 36.31c |
| Loliolide **58** | -1.04b | -0.46c | -0.17c | 5.81d | 42.15c |
| | % Inhibition on root length | | | | |
| Control | 0b | 0b | 0d | 0c | 0d |
| Dihydroactinidiolide **51** | 38.20a | 51.26a | 75.42a | 92.13a | 100a |
| 4-Oxo-β-ionone **52** | 1.21b | 2.77b | 15.74b | 52.08b | 85.29b |
| 3-Hydroxy-β-ionone **59** | -15.67c | 3.30b | 7.22c | 52.37b | 79.18b |
| Loliolide **58** | 1.019b | -9.85c | -21.90e | -4.24c | 43.97c |

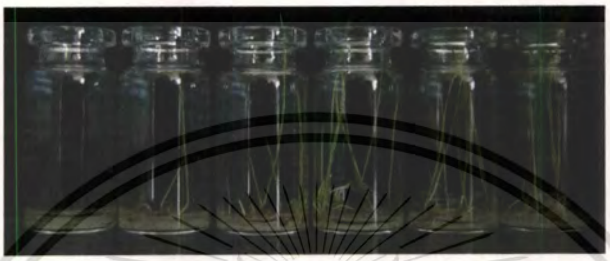
Values with same letters in each column there are not significantly different at $P$=0.05

**Barnyardgrass**

The allelopathic assay on Barnyardgrass was studied using 4 compounds, **51, 52, 58** and **59**. Compounds **56** and **57** were not study because they were minor compounds and yielded in low percentage. As shown in Table 4.43, the results on the seed germination of compounds showed that compound **51** at concentrations of 250-1,000 ppm, compound **52** at concentrations of 500-1,000 ppm, and compound **59** at a concentration of 1,000 ppm had an inhibitory effect on seed germination of Barnyardgrass. In particular, compound **51** at the highest concentration exhibited complete inhibition on seed germination of Barnyardgrass. The other concentrations of compounds **52**, **58** and **59** showed no inhibitory effect on seed germination of Barnyardgrass. The effect on seedling growth found that all the applied concentrations of compounds **51**,

compound **59** at concentrations of 125-1,000 ppm, and compounds **52** and **58** at concentrations of 500-1,000 ppm inhibited shoot length of Barnyardgrass, in contrast, other concentrations had no effect. As for root length, it was found that all the applied concentrations of compounds **51**, compounds **52** and **59** at concentrations of 250-1,000 ppm, and compound **58** at a concentration of 1,000 ppm inhibited root length of Barnyardgrass (Figure 4.21).

Dihydroactinidiolide **51**



4-Oxo-β-ionone **52**

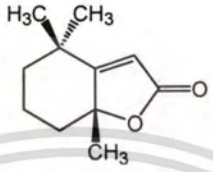
3-Hydroxy-β-ionone **59**

Loliolide **58**

1,000 500 250 125 62.50 Control

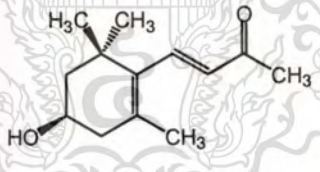
**Figure 4.21** Allelopathic effects of $C_{11}$ and $C_{13}$ norisoprenoids from E6F4-E6F5 and E13F2-E13F4 on Barnyardgrass

The allelopathic effects of norisoprenoids, particularly dihydroactinidiolide **51**, have been reported, with the first report being published in 1980 by Stevens and Merrill [184]. They isolated this compound from the spikerush plant and tested inhibitory activity against reddish seed germination. They found that after 48 hrs, a 50% reduction in germination occurs at a concentration of 5 ppm while at a concentration >50 ppm, no germination occurred even after 5 days.



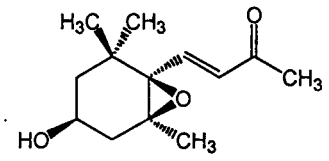

Dihydroactinidiolide **51**

In 1993, Kato-Noguchi et al. [144] isolated a growth inhibitor, *R*-(-)-3-hydroxy-β-ionone **59**, from light-grown dwarf bean shoots (*Phaseolus vulgaris* cv Morocco). They found that this compound inhibited the growth of hypocotyls of lettuce seedlings and hypocotyls segments of dwarf bean seedlings at concentrations greater than 0.3μM, and that its inhibitory activity was higher than that of abscisic acid (ABA).



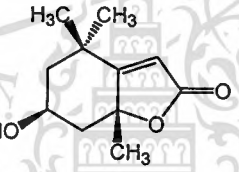

*R*-(-)-3-Hydroxy-β-ionone **59**

Kurokawa et al. [185] isolated allechemical, 3-hydroxy-5,6-epoxy-β-ionone (**56** or **57**), from the leaves of *Athyrium yokoscense*. This compound showed an inhibitory activity against lettuce seed germination. Macías et al. [149] also reported the isolation, structural elucidation and allelopathic bioassays of 13 compounds from the sunflower cultivar cv. Peredovick. After studied the effect of a series of aqueous solutions, ranged between $10^{-4}$-$10^{-9}$ M, of isolation compounds on the germination, root and shoot length of *Lactuca sativa*, *Lepidium sativum* seedling (dicotyledons), *Hordeum vulgare* and *Allium cepa* (monocotyledons). They found that compound **57** showed the most relevant observed effects over monocotyledons species.

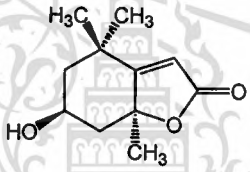


3-Hydroxy-5β, 6β-epoxy-β-ionone **57**

In 1999, Macías et al. [186] studied the allelopathic effects of twelve phenolics, two loliolides (isololiolide **98** and loliolide **58**), a diterpene and a cyclitol isolated from the MeOH extract of sweetclover, *Melilotus messanensis*, on germination and growth of the dicotyledons *Lactuca sativa* cvs. Roman and Nigra and *Lycopersicum esculentum* and the monocotyledons *Allium cepa* and *Hordeum vugare*. They found that both loliolide **58** and isololiolide **98** also inhibited the germination of tomato (*L. esculentum*; **58**: -16%, $10^{-5}$ M, -19% $10^{-9}$ M, -19% $10^{-8}$ M; **98**: -27%, $10^{-5}$ M, -28%, $10^{-6}$ M) and promoted the germination of barley (*H. vulgare*; **58**: 29%, $10^{-5}$ M, 39%, $10^{-7}$ M; **98**: 20%, $10^{-5}$ M, 23%, $10^{-8}$ M).



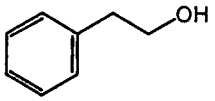
Loliolide **58**



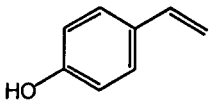
Isololiolide **98**

Kato et al. [187] reported the allelopathic activity of four natural compounds, 2-phenylethyl alcohol **99**, 4-vinylphenol **100**, 2-methoxy-4-vinylphenol **101** and dihydroactinidiolide **51**, isolated from wheat (*Triticum aestivum*) husk, one authentic compound, 1-phenylethyl alcohol **102**, and one synthetic compound, tetrahydroactinidiolide **103** on three tested wheats, *Lancer*, *RL4137* and *Menyou*. They found that all natural products, particularly dihydoactinidiolide **51**, possessed a high potency of inhibition activity. It was revealed that the related tetrahydroactinidiolide **103** was as active as the original natural product **51** whereas the activity of compound **102** was much higher than that of compounds **99-101**. However, the absolute configuration of compound **51**, which has molecular asymmetry, could not be determined due to the minute amounts of available materials in the study. They synthesized both enatiomers of compounds **51** and **103** and purchased commercial D- and L- **102**, and submitted them to a germination assay for evaluation of each enantiomer. The results showed that D-, L-, and DL- forms of each compound **51**, **102** and **103**, revealing no noticeable difference in

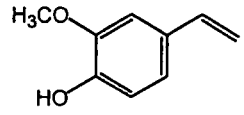
inhibition activities among these three forms. Furthermore, they studied the inhibitory activity of the mixture between compounds **101** and **103**. The results found the synergistic relations between these active compounds, and the strongest activity was observed at a ratio of 1:1. Similarly relations were seen between compound **51** and aromatic compounds **99-102**.



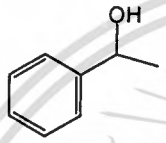

2-Phenylethyl alcohol **99**



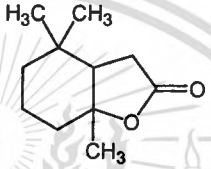

4-Vinylphenol **100**




2-Methoxy-4-vinylphenol **101**

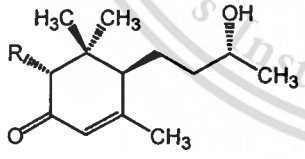



1-Phenylethyl alcohol **102**



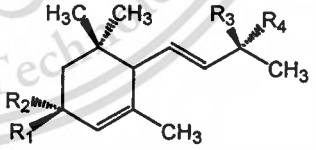

Tetrahydroactinidiolide **103**

D'Abrosca et al. [146] isolated twelve $C_{13}$ norisoprenoids, **56**, **58** and **104-114**, from the leaves of *Cestrum parqui* (Solanaceae). All compounds, with exception of **111**, were tested the allelopathic activity on seed germination and seedling growth of *Lactuca sativa* L. Results found that with exception of $C_{13}$ norisoprenoid **107**, the compounds had no effect on germination, but they had a moderate inhibitory effect on root and shoot growth of lettuce.




**104** R = OH

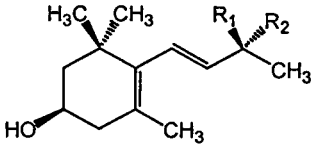
**105** R = H




**106** $R_1$ = H, $R_2$ = OH, $R_3$, $R_4$ = O

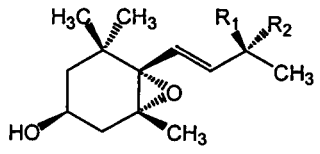
**107** $R_1$, $R_2$ = O, $R_3$ = H, $R_4$ = OH

**108** $R_1$, $R_3$ = H, $R_2$, $R_4$ = OH



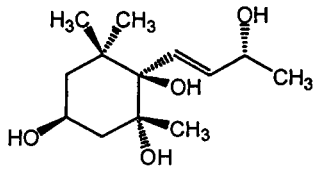

**109** $R_1$ = H, $R_2$ = OH

**58** $R_1$, $R_2$ = O

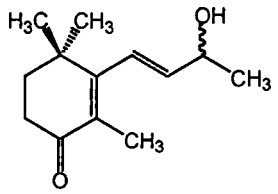



**56** $R_1$, $R_2$ = O

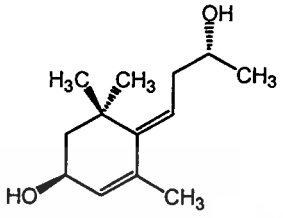
**110** $R_1$ = H, $R_2$ = OH



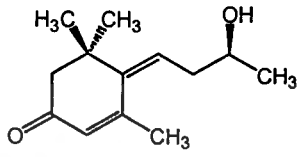
**111**



**112**

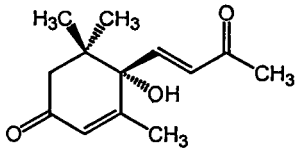


**113**



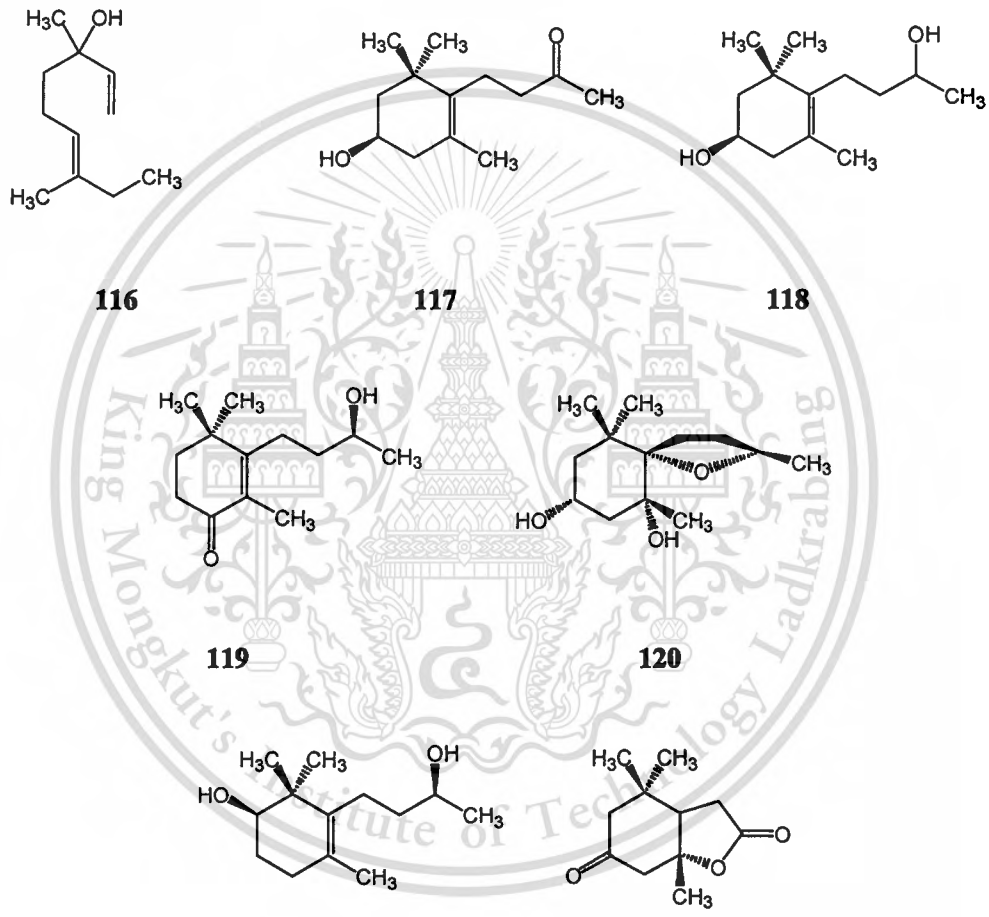
**114**

Xian et al. [122] studied the allelopathic potential of partitioned fractions (petroleum ether, EtOAc, $CHCl_3$, and *n*-BuOH, respectively) from leaves of submerged macrophyte, *Vallisneria spiralis* Linn. Antialgal activities showed that the strongest inhibitory effect of chloroform extract whose percentage inhibition of growth rate was up to 91% (at 66 mg/ mL). The chloroform extract was fractionated to seven fractions (A-G) and the allelopathic activities were studied. Results found that fraction A showed the strongest inhibitory activity on growth of algae. The further separation of A was processed by column chromatography and 3 subfractions were obtained followed; A1-A3 and antialgal activities showed that the growth of algae was fully inhibited at a concentration of 66 mg/mL for A1 and A3. After analyzing the active subfractions by high resolution GC-MS, they found that subfractions A1 consisted of 2-ethyl-3-methylmaleimide **50** (77.10%), dihydroactinidiolide **51** (18.90%), and 4-oxo-β-ionone **52** (4.00%), subfraction A3 consisted of 3-hydroxy-5, 6-epoxy-β-ionone **59** (38.30%), loliolide **58** (10.10%), and 6-hydroxy-3-oxo-α-ionone **115** (44.50%), and unknown (7.10%, MW 289), respectively.



6-Hydroxy-3-oxo-α-ionone **115**

In 2008 Macías et al. [160] isolated allelochemical compounds consisted of monoterpene **116**, apocarotenoids, **56, 115, 117-119**, and compounds **120-122** from leaves of *Tectona grandis*. After studying the allelopahic activity of isolated compounds **115-122** on wheat coleoptile elongation of monocotyledons *Allium cepa* L. (onion) and *Trititum aestivum* L. (wheat), and dicotyledons *Lactuca sativa* L. (lettuce), *Lepidium sativum* L. (cress), and *Lycopersicum esculentum* L. (tomato) they found that the most acvtive compounds were compounds **117-118** and **122**, respectively.




The results of present study, the allelopathic activity of subfractions from E6, commercial fatty acids, isolated dihydroactinidiolide **51** (DAD), and 4-oxo-β-ionone **52**, DAD **51** showed the highest activity. These compounds exhibited activity similar to those observed in subfraction E6F4, but the major fatty acids, linoleic acid **12**, showed a slightly low activity. This may be due to synergistic of all compounds or the mixtures such as fatty acids and fatty acids or

fatty acids and 2-ethyl-3-methylmaleimide **50** fatty acids and 4-oxo-β-ionone **52** or fatty acids and DAD **51** because the synergistic between aromatic compounds and DAD **51** or aromatic compounds and fatty acids has already been reported [178, 180, 187].

A significant inhibitory effect of aqueous extract, crude organic extracts and isolated pure compounds on the tested plant seeds was observed on roots more than shoots because roots were present in direct contact with inhibitors [6]. At the higher concentration the extracts and pure compounds showed an inhibitory effect, but at the lower concentration it promoted growth. These results are in agreement with several research studies that some allelochemicals can show the promotion effect at low concentration [188-189]. Although the detailed mechanism of allelopathic action of the extracts and active compounds on tested plants is unknown and the typically resulted from the combined action of allelochemicals group, which collectively interfere with several physiological processes [25b], the results of this research indicate that the reduction in root length may be due to block of gibberellins and indol acetic acid functions [29, 190].

A comparison the effect on Barnyardgrass and Chinese amaranth, found that the extracts and pure compounds had an inhibitory effect on Chinese amaranth higher than Barnyardgrass. It has been reported that the mass and size of seeds affect on allelopathic activity [191] that the large seed more tolerance than small seed. The effects of allelochemical compounds from *S. platensis* against on tested plants in this study were similar to that observation. It should be noted that Barnyardgrass possessed stronger resistance against phytotoxins released by plants other than paddy weeds [192-193].

## 4.13 Synergistic phenomenon of linoleic acid 12 and dihydroactinidiolide 51

Results from the allelopathic activity of subfraction E6F4 and major components, linoleic acid **12** (72.19%) and dihydroactinidiolide **51** (8.32%), showed that the most active subfraction had an allelopathic effect closely related to dihydroactinidiolide **51**. In contrast, the major component, linoleic acid **12**, showed the lower activity that may occur due to synergistic effect among these compounds. In order to investigate the synergistic effect, these two compounds were mixed into 5 ratios and the allelopathic effects on Chinese amaranth studied at concentrations of 62.5-500 ppm which the results were campared with both of pure compounds.

The results on seed germination found that all of 5 ratios and pure dihydroactinidiolide **51** had an inhibitory effect on seed germination of Chinese amaranth at concentrations of 250-500

ppm and completely inhibited it at a concentration of 500 ppm. In contrast, pure linoleic acid **12** exhibited a weak inhibitory effect at a concentration of 500 ppm but the other concentrations had no effect. At a concentration of 250 ppm the 3 mixtures of linoleic acid **12** : dihydroactinidiolide **54** in the ratios of 7:3, 5:5 and 3:7 showed a higher inhibitory activity than dihydroactinidiolide **51**. The effect of the ratio of 1:9 was similar to that observed with pure compound **51**; however the effect of the ratio of 9:1 was different from subfraction E6F4. It has been noticed that the synergistic effect occurred from other minor compounds in subfraction E6F4, such as 2-ethyl-3-methymaleimide **50** and 4-oxo-β-ionone **52**. These observation results indicated that the synergistic phenomenon was appeared in the mixtures of the highest active subfraction, E6F4 (Table 4.44, Figure 4.22-23).

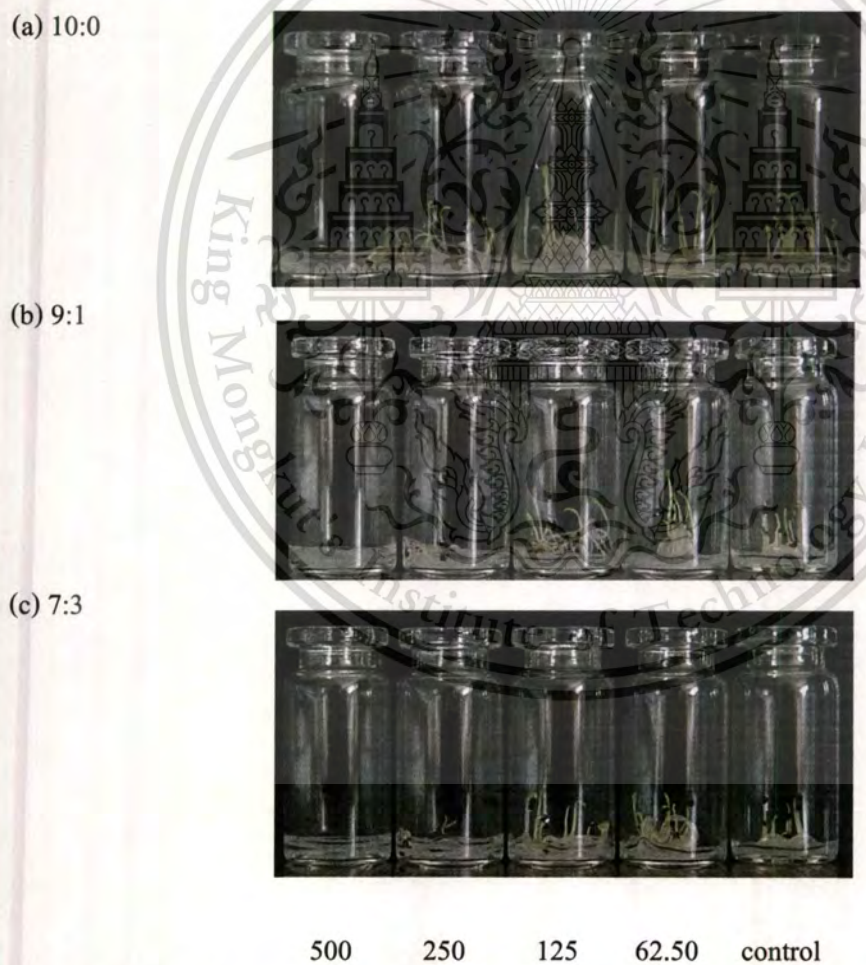
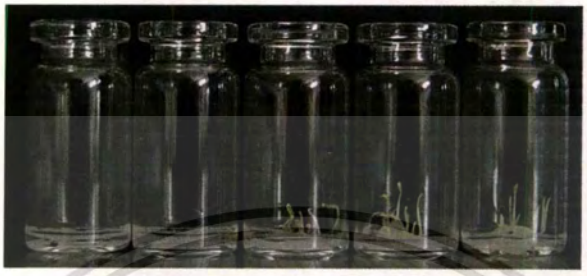



**Figure 4.22** The allelopathic effects of 5 mixtures of linoleic acid **12**: DAD **51** in ratios of 9:1 (b), 7:3 (c), 5:5 (d), 3:7 (f) and 1:9 (g), and both of pure compounds, (a) and (h), at concentrations of 62.5-500 ppm on Chinese amaranth.

(d) 5:5

(e) 3:7



(f) 1:9

(g) 0:10

500 250 125 62.50 control

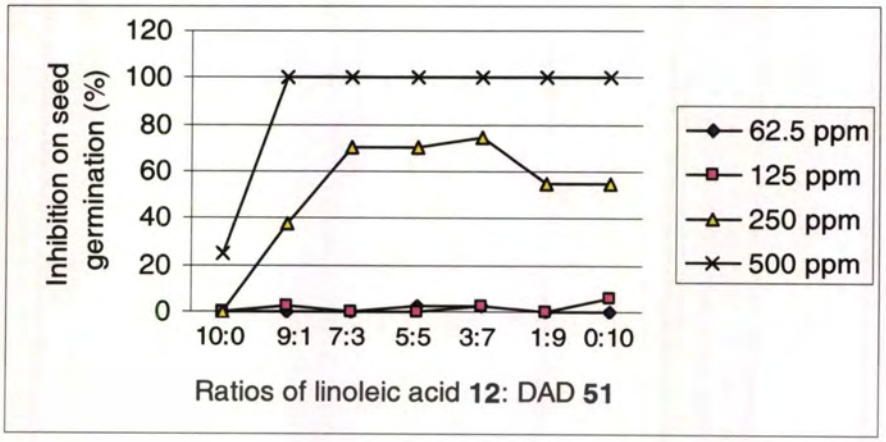
**Figure 4.22** The allelopathic effects of 5 mixtures of linoleic acid **12**: DAD **51** in ratios of 9:1 (b), 7:3 (c), 5:5 (d), 3:7 (f) and 1:9 (g), and both of pure compounds, (a) and (h), at concentrations of 62.5-500 ppm on Chinese amaranth (Continued)

**Table 4.44** Allelopathic effects of a mixture between linoleic acid **12** and DAD **51** on seed germination and seedling growth of Chinese amaranth.

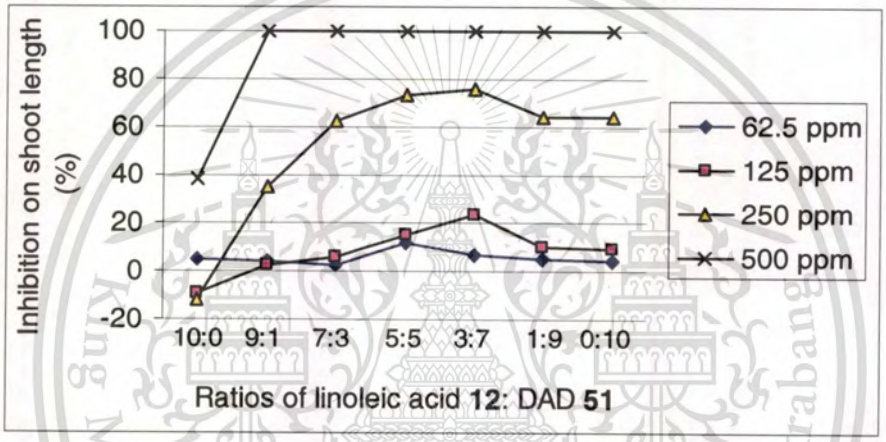
| Ratio of linoleic acid **12** : DAD **51** | % Inhibition on seed germination | | | |
|---|---|---|---|---|
| | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm |
| Control | 0a | 0a | 0d | 0c |
| 10:0 | 0a | 0a | 0d | 25b |
| 9:1 | 0a | 2.50a | 37.50c | 100a |
| 7:3 | 0a | 0a | 70a | 100a |
| 5:5 | 2.50a | 0a | 70a | 100a |
| 3:7 | 2.50a | 2.50a | 75a | 100a |
| 1:9 | 0a | 0a | 55b | 100a |
| 0:10 | 0a | 6.06a | 54.55b | 100a |
| | % Inhibition on shoot length | | | |
| Control | 0b | 0e | 0d | 0c |
| 10:0 | 5.01ab | -9.07f | -11.69e | 37.95b |
| 9:1 | 3.88b | 2.58de | 35.14c | 100a |
| 7:3 | 2.33b | 5.94cd | 62.27b | 100a |
| 5:5 | 11.63a | 15.25b | 73.13a | 100a |
| 3:7 | 6.98ab | 23.26a | 75.97a | 100a |
| 1:9 | 4.65ab | 10.08bc | 64.08b | 100a |
| 0:10 | 3.96b | 9.35c | 64.03b | 100a |
| | % Inhibition on root length | | | |
| Control | 0ab | 0f | 0f | 0c |
| 10:0 | -3.88b | 4.05e | 9.95e | 82.97b |
| 9:1 | 1.58a | 5.03de | 36.35d | 100a |
| 7:3 | -3.88b | 7.90d | 70.40b | 100a |
| 5:5 | -3.02b | 21.98b | 78.59a | 100a |
| 3:7 | 0ab | 27.59a | 77.73a | 100a |
| 1:9 | -1.44ab | 20.11b | 66.09c | 100a |
| 0:10 | -0.46ab | 13.36c | 66.06c | 100a |

Values with same letters in each column there are not significantly different at $P$=0.05

(a)




(b)




(c)

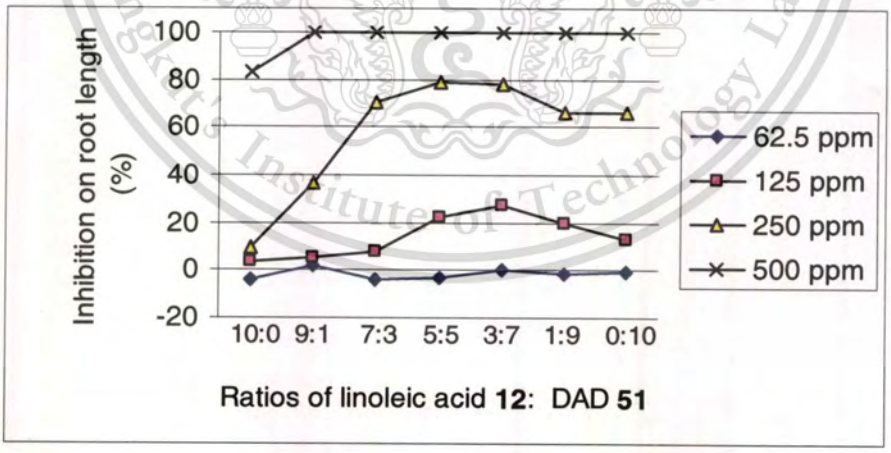



**Figure 4.23** The allelopathic effects of 5 mixtures of linoleic acid **12** : DAD **51** and both of pure compounds at concentrations of 62.5-500 ppm on Chinese amaranth (a) inhibition on seed germination, (b) inhibition on shoot length and (c) inhibition on root length.

In Table 4.44, the results on shoot length showed that pure linoleic acid **12** at a concentration of 500 ppm, mixed in a ratio of 9:1 at concentrations of 250-500 ppm, mixtures in the ratios of 7:3, 3:7, 1:9 and pure dihidroactinidiolide **51** at concentrations of 125-500 ppm, and the mixture in a ratio of 5:5 at all applied concentrations had an inhibitory effect on shoot length of Chinese amaranth. In particular, the mixture in the ratios of 5:5 and 3:7 exhibited higher inhibitory activity than did pure dihydroactinidiolide **51**. The other concentrations had no inhibitory effect but linoleic acid **12** at concentrations of 125-250 ppm promoted shoot length of Chinese amaranth. As for root length, it was found that all mixtures and pure compounds at concentrations of 125-500 ppm inhibited the root length of Chinese amaranth. The mixtures in the ratios of 5:5 and 3:7 showed the highest activity but at the lowest tested concentration had no effect (Figure 4.22-23).

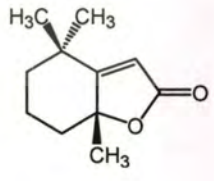
The results indicated that linoleic acid **12** and dihydroactinidiolide **51** exhibited the synergistic phenomenon. The most active was the mixture of linoleic acid **12** : dihydroactinidiolide **51** in the ratios of 5:5 and 3:7. The 2 mixtures inhibited the seed germination and seedling growth of Chinese amaranth to a higher level than both of pure compounds.

# CHAPTER 5

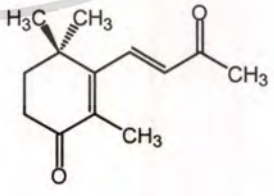
# CONCLUSIONS

In this research, extracts from *S. platensis* were tested on dicotyledon, Chinese amaranth, and monocotyledon, Barnyardgrass, to evaluate their influence on the seed germination and seedling growth. The assays were run by petri dish test and vial test methods in laboratory conditions. The results found that aqueous extract had the strongest inhibitory effect on test species. C-phycocyanin (C-PC) **29**, the major biological active compound in aqueous extract, exhibited higher inhibitory effect on Chinese amaranth than Barnyardgrass. In order to confirm, the activity of the aqueous extract and C-PC **29**, the allelopathic effects of the denatured C-PC and hot aqueous extract were also studied. The results indicated that the inhibitory effect of hot aqueous extract and the denatured C-PC exhibited similar properties to that of the aqueous extract and C-PC **29**.

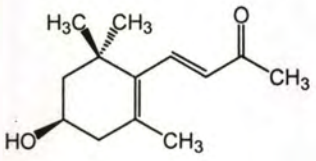
Further study was performed with crude hexane, ethyl acetate and methanol extracts. The crude ethyl acetate extract showed the highest inhibitory activity. Purification and identification, the major active compound from the ethyl acetate extract was linoleic acid **12.** Apart from fatty acid, the extract also contained of nonfatty acid that play an allelochemicals role, for examples, 2-ethyl-3-methylmaleimide **50** and six norisoprenoids : dihydroactionidiolide **51**, 4-oxo-β-ionone **52**, 3-hydroxy-β-ionone **59**, 3-hydroxy-5α, 6α-epoxy-β-ionone **56**, 3-hydroxy-5β, 6β-epoxy-β-ionone **57**, and loliolide **58** in small amount. These nonfatty acid compounds are reported here for the first time from a cyanobacteria, *S. platensis*.



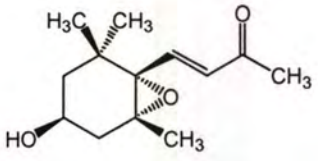

Dihydroactinidiolide **51**



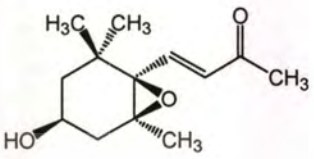

4-Oxo-β-ionone **52**



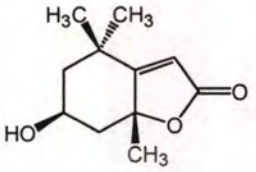
3-Hydroxy-β-ionone **59**



3-Hydroxy-5α, 6α-epoxy-β-ionone **56**



3-Hydroxy-5β, 6β-epoxy-β-ionone **57**



Loliolide **58**

These data confirm that the allelopathic activity of the extracts, authentic or isolated compounds from *S. platensis* depends on the following;

-Tested plants species: Tested dicotyledon (smaller seed) exhibited a higher sensitivity than monocotyledon (larger seed) or Barnyardgrass possessed greater resistance against isolated allelochemicals than Chinese amaranth.

-Synergistic effect: The mixture of linoleic acid **12** : dihydroactinidiolide **51** at ratios of 5:5 and 3:7 exhibited the highest allelopathic effect on Chinese amaranth compare to when using pure compounds.

-Concentrations: At high concentrations had a high inhibitory effect while at low concentration had a slight promotion effect.

-Nature of allelochemicals: Dihydroactinidiolide **51** is the strongest allelochemical.

-Contact zone of tested plants to allelochemicals: The root is presented in direct contact with allelochemicals then these compounds inhibit root length more than shoot length.

**5.1 Suggestions**

The observation results found that aqueous extract had a high allelopathic activity on tested plants. This extract not only composed of C-PC **29** but also composed of several biological active compounds. The isolation and identification of active compounds from aqueous extract should be considered and studied in detail for their effects on plant growth. Other studies should be conducted with the same plants, used in this study, in pot and field conditions.

# REFERENCES


[1] Patterson D.T. In : Duke S.O., Editor. **Weed Physiology**. vol. 1, Boca Raton, Florida : CRC Press. 1985.

[2] Macías F.A. In : Inderjit, Dakshini K.M.M. and Einhellig F.A., Editors. **ACS Symposium Series 582. Allelopathy : Organisms, Processes and Applications**. Washington DC : American Chemical Society. 1995. pp. 310–329.

[3] Inderjit, Dakshini K.M.M. and Foy C.L. **Principles and Practices in Plant Ecology: Allelochemical Interactions.** Boca Raton, Florida: CRC Press . 1999.

[4] Dayan F.E., Romagni J.G, Tellez M.R., Rimando A.M. and Duke S.O. "Managing Weeds with Natural Products." **Pestic. Outlook**, vol. 5, 1999. pp. 185–188.

[5] Putnam A.R. "Allelopathic Chemicals: Nature's Herbicides in Action." **Chem. Eng. News.** vol. 61, no. 14, April 4 1983. pp. 34–45.

[6] Rice E.L. **Allelopathy**. 2nd ED. New York : Academic. 1984.

[7] Putnam A.R. and Tang C.-S. In: Putnam A.R. and Tang C.-S., Editors. **The Science of Allelopathy**. New York : John Wiley and Sons. 1986. pp. 1–22.

[8] Aldrich R.J. In : Waller G.R., Editor. **Allelochemicals: Role in Agriculture and Forestry.** vol. 330. Washington DC : American Chemical Society. 1987. pp. 300-312.

[9] Rice E.L. In : Waller G.R., Editor. **Allelochemicals: Role in Agriculture and Forestry.** vol. 330. Washington DC : American Chemical Society. 1987. pp. 8-32.

[10] Liu D.L. and Lovett J.V. "Biologically Active Secondary Metabolites of Barley. II. Phytotoxicity of Barley Allelochemicals." **J. Chem. Ecol.**, vol. 19, no. 10, 1993. pp. 2231-2244.

[11] Shamsher S.N. **Allelopathy Update vol. 2 : Basic and Applied Aspects**. New Hampshire : Science Publishers, Inc. 1999.

[12] Kakisawa H., Asari F., Kusumi T., Toma T., Sakurai T., Oohusa T., Hara Y. and Chihara M. "An Allelopathic Fatty Acid from the Brown Alga *Cladosiphon okamuranus*" **Phytochemistry**, vol. 27, no. 3, 1988. pp. 731-735.

[13] Jüttner F., Todorova A.K., Walch N. and Philipsborn W.V. "Nostocyclamide M: a Cyanobacterial Cyclic Peptide with Allelopathic Activity from *Nostoc* 31." **Phytochemistry,** vol. 57, no. 4, 2001. pp. 613-619.

[14] Smith G.D. and Doan N.T. "Cyanobacterial Metabolites with Bioactivity Against Photosynthesis in Cyanobacteria, Algae and Higher Plants." **J. Appl. Phycol.,** vol. 11, no. 4, 1999. pp. 337-344.

[15] Blinkova L.P., Gorobets O.B. and Baturo A.P. "Biological Activity of *Spirulina*." **Zh. Mikrobiol. Epidemiol. Immunobiol.,** vol. 2, 2001. pp. 114-118.

[16] Chamorro G., Salazar M., Favila L. and Bourges H. "Pharmacology and Toxicology of the Alga Spirulina." **Rev. Invest. Clin.,** vol. 48, no. 5, 1996. pp. 389-399. (in Spanish)

[17] Bhat V.B. and Madyastha K.M. "C-Phycocyanin : A Potent Peroxyl Radical Scavenger *in Vivo* and *in Vitro*." **Biochem. Biophys. Res. Commun.**, vol. 275, no. 1, 2000. pp. 20-25.

[18] Romay C. and GonzÁlez R. "Phycocyanin is an Antioxidant Protector of Human Erythrocytes Against Lysis by Peroxyl Radicals." **J. Pharm. Pharmacol.**, vol. 52, no. 4, 2000. pp. 367-368.

[19] Romay C., Ledon N. and GonzÁlez R. "Further Studies on Anti-Inflammatory Activity of Phycocyanin in Some Animal Models of Inflammation." **Inflamm. Res.**, vol. 47, no. 8, 1998. pp. 334-338.

[20] Pang Q.S., Guo B.J. and Ruan J.H. "Enhancement of Endonuclease Activity and Repair DNA Synthesis by Polysaccharide of *Spirulina platensis*." **Acta. Genet. Sinica. (Chin. J. Genet.)**, vol. 15, no. 5, 1998. pp. 374-381. (in Chinese)

[21] Qureshi M.A., Garlich J.D. and Kidd M.T. "Dietary *Spirulina platensis* Enhances Humoral and Cell-Mediated Immune Functions in Chickens." **Immunopharmacol. Immunotoxical.**, vol. 18, no. 3, 1996. pp. 465-476.

[22] Chotsang P., Mongkolaussavaratana T., Laosinwattana C., Ruangsomboon S., and Charoenying P. "Inhibitory Effect of *Spirulina platensis* Extracts on Seed Germination and Seedling Growth of Chinese mustard." **Laos Journal on Applied Science** : Special Issue, vol. 1, no. 1, 2006. pp. 360-364.

[23] Molisch H. **Der Einfluβ einer Pflanze auf die andere-allelopathie**. Germany. Jena : G. Fischer. 1937. (in German)

[24] Torrest A., Oliva R. M., Castellano D. and Cross P. **First World Congress on Allelopathy. A Science of the Future.**, SAI (University of Cadiz). Spain, Cadiz. 1996. pp.278.

[25] (a) Einhellig F.A. In : Inderjit Dakshini K.M.M. and Einhellig F.A., Editors. **Allelopathy-Current Status and Future Goals. In Allelopathy : Organisms, Processes and Applications.** ACS Symposium Series 582. Washington DC : American Chemical Society. 1995. pp. 1-24.

(b) Einhellig F.A. In : Inderjit Dakshini K.M.M. and Einhellig F.A., Editors. **Mechanisms of Action of Allelochemicals in Allelopathy. In Allelopathy : Organisms, Processes and Applications**. ACS Symposium Series 582. Washington, DC: American Chemical Society. 1995. pp. 96-116.

[26] Corcuera L.J. "Biochemical Basis for the Resistance of Barley to Aphids. Review Article Number 78." **Phytochemistry**, vol. 33, no. 4, 1993. pp. 741-747.

[27] Gershenzon J. In Bernays E. A., Editor. **Insect-Plant Interactions**. vol. 5, Boca Raton, Florida : CRC Press. 1994. pp. 105-173.

[28] Berg B. and Staaf H. "Decomposition Rate and Chemical Changes of Scots Pine Needle Litter. II. Influence of Chemical Composition." **Ecol. Bull.**, vol. 32, 1980. pp. 373-390.

[29] Mandava N.B. In Thompson A.C., Editor. **The Chemistry of Allelopathy.** Symposium Series 268, Washington DC : AmericanChemical Society. 1985. pp. 33-54.

[30] Chick T.A. and Kielbaso J.J. "Allelopathy as an Inhibition Factor in Ornamental Tree Growth : Implications from the Literature." **J. Arboric.**, vol. 24, no. 5, 1998. pp. 274-279.

[31] Fisher N.H., Williamson G.B., Weidenhamer J.D. and Richardson D.R. "In Search of Allelopathy in the Florida Scrub : the Role of Terpenoids." **J. Chem. Ecol.**, vol. 20, no. 6, 1994. pp. 1355-1380.

[32] Moral R.D. and Muller C.H. "The Allelopathic Effects of *Eucalyptus camaldulensis*." **Am. Midl. Nat.**, vol. 83, no. 1, 1970. pp. 254-282.

[33] Kumari A. and Kohli R.K. "Autotoxicity of Ragweed Parthenium (*Parthenium hysterophorus* L.)." **Weed Sci.**, vol. 35, 1987. pp. 629-635.

[34] Molina A., Reigosa M.J. and Carballeira A. "Release of Allelochemical Agents from Litter, Throughfall, and Topsoil in Plantations of *Eucalyptus globulus* Labill. in Spain." **J. Chem. Ecol.**, vol. 17, no. 1, 1991. pp. 147-160.

[35] Macías F.A., Simonet A.M., and Esteban M.D. "Potential Allelopathic Lupane Triterpenes from Bioactive Fractions of *Melilotus messanensis*." **Phytochemistry**, vol. 36, no. 6, 1994. pp. 1369-1379.

[36] AlSaadawi I.S., Al-Uquili J.K., Al-Hadithy S.M. and Alrubeaa A.J. "Effect of Gamma Irradiation on Allelopathic Potential of *Sorghum bicolor* Against Weeds and Nitrification." **J. Chem. Ecol.**, vol. 11, no. 12, 1985. pp. 1737-1745.

[37] Weston L.A., Harmon R. and Mueller S. "Allelopathic Potential of Sorghum-Sudangrass Hybrid (Sudex)." **J. Chem. Ecol.**, vol. 15, no. 6, 1989. pp. 1855-1865.

[38] Yu J.Q. and Matsui Y. "Phytotoxic Substances in Root Exudates of Cucumber (*Cucumis sativus* L.)." **J. Chem. Ecol.**, vol. 20, no. 1, 1994. pp. 21-31.

[39] Einhellig F.A. In Waller G.R., Editors. **Allelochemical: Role in Agriculture and Forestry**. vol. 330, Washington DC : American Chemical Society. 1987. pp. 343-357.

[40] Goel U. "Allelopathic Effect of Weeds Associated with Gram (*Cicer arietinum* Linn.) on Its Seed Germination and Seedling Growth." **Acta Bot. Ind.**, vol. 15, no. 1, 1987. pp. 129-130.

[41] Mason-Sedun W., Jessop R.S. and Lovett J.V. "Differential Phytotoxicity Among Species and Cultivars of the Genus *Brassica* to Wheat. I. Laboratory and Field Screening of Species." **Plant Soil**, vol. 93, no. 1, 1986. pp. 3-16.

[42] Blum U. and Shafer S.R. "Microbial Populations and Phenolic Acids in Soils." **Soil Biol. Biochem.**, vol. 20, no. 6, 1988. pp. 793-800.

[43] Macías F.A., Varela R.M., Simonet A.M., Cutler H.G., Cutler S.J., Dugan F.M. and Hill R.A. "Novel Bioactive Breviane Spiroditerpenoids from *Penicillium brevicompactum* Dierckx." **J. Org. Chem.**, vol. 65, no. 26, 2000. pp. 9039-9046.

[44] Vonshak, A. ***Spirulina platensis* (*Arthrospira*) : Physiology, Cell-biology and Biotechnology**. London : Taylor and Francis, 1997.

[45] Al-Homaidan A.A. "Large-Scale Cultivation of *Spirulina* in Saudi Arabia." **Saudi. J. Biol. Sci.**, vol. 8, no. 2, 2002. pp. 13-23. (In Arabic with English Summary)

[46] Ciferri O. "*Spirulina*, the Edible Microorganism." **Microbiol. Rev.**, vol. 47, no. 4, 1983. pp. 551-578.

[47] Babadzhanov A.S., Abdusamatova N., Yusupova F.M., Faizullaeva N. and Mezhlumyan L. G. "Chemical Composition of *Spirulina platensis* Cultivated in Uzbekistan." **Chem. Nat. Comp.**, vol. 40, no. 3, 2004. pp. 276-279.

[48] Shekharam K.M., Venkataraman L.V. and Salimath P.V. "Carbohydrate Composition and Characterization of Two Unusual Sugars from the Blue-green Algae *Spirulina platensis*." **Phytochemistry**, vol. 26, no. 8, 1987. pp. 2267-2269.

[49] Richmond A. In : Mann N. and Carr N.G., Editors. **Mass Culture of Cyanobacteria. *Photosynthetic prokaryotes.* 2nd ED**. New York and London : Plenum Press. pp. 181-210, 1992.

[50] Pugh N., Ross S.A., ElSohly H.N., ElHosly M.A. and Pasco D.S. "Isolation of Three Weight Polysaccharide Preparations with Potent Immunostimulatory Activity from *Spirulina platensis, Aphanizomenon flos-aguae* and *Chlorella pyrenoidosa*." **Planta Med.,** vol. 67, no. 8, 2001. pp. 737-742.

[51] Tokusoglu Ö. and Üunal M.K. "Biomass Nutrient Profiles of Three Microalgae : *Spirulina platensis, Chlorella vulgaris*, and *Isochrisis galbana*." **J. Food Sci.,** vol. 68, no. 4, 2003. pp. 1144-1148.

[52] Ayehunie S., Belay A., Baba T.W. and Ruprecht R.M. "Inhibition of HIV-1 Replication by an Aqueous Extract of *Spirulina platensis* (*Arthrospira platensis*)." **J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.,** vol. 18, no. 1, 1998. pp. 7-12.

[53] Khan M., Shobha J.C., Mohan I.K., Naidu M.U., Sundaram C., Singh S., Kuppumary P. and Kutala V.K. "Protective Effect of *Spirulina* Against Doxorubicin-Induced Cardiotoxicity." **Phytother. Res.,** vol. 19, no. 12, 2005. pp. 1030-1037.

[54] Wang Y., Chang C.F., Chou J., Chen H.-L., Deng X., Harvey B.K., Cadet J.L. and Bickford P.C. "Dietary Supplementation with Blueberries, Spinach, or *Spirulina* reduces Ischemic Brain Damage." **Exp. Neurol.**, vol. 193, no. 1, 2005. pp. 75-84.

[55] Gemma C., Mesches M.H., Sepesi B., Choo K., Holmes D.B. and Bickford P.C. "Diets Enriched in Foods with High Antioxidant Activity Reverse Age-Induced Decreases in Cerebellar Beta-Adrenergic Function and Increases in Proinflammatory Cytokines." **J. Neurosci.**, vol. 22, no. 14, 2002. pp. 6114-6120.

[56] Chen L.L., Zhang S.F., Huang D.N., Tan J.Q. and He S.H. "Experimental Study of *Spirulina platensis* in Treating Allergic Rhinitis in Rats." **J. Cent. South Univ. Med. Sci.,** vol. 30, no. 1, 2005. pp. 96-98. (in Chinese)

[57] Misbahuddin M., Islam AZM M., Khandker S., and Islam N. "Efficacy of *Spirulina* Extract Plus Zinc in Patients of Chronic Arsenic Poisoning: a Randomized Placebo-Controlled Study." **Clin. Toxicol.**, vol. 44, no. 2, 2006. pp. 135-141.

[58] Simpore J., Zongo F., Kabore F., Dansou D., Bere A., Nikiema J-B, Pignatelli S., Binodi D. M., Ruberto G. and Musumeci S. "Nutrition Rehabilitation of HIV-Infected and HIV-Negative Undernourished Children Utilizing *Spirulina*." **Ann. Nutr. Metab.**, vol. 49, no. 6, 2005. pp. 373-380.

[59] Mao T.K., Van de Water J. and Gershwin M.E. "Effects of a *Spirulina*-Based Dietary Supplement on Cytokine Production from Allergic Rhinitis Patients." **J. Med. Food,** vol. 8, no. 1, 2005. pp. 27-30.

[60] Harder R. "Ernahrungsphysiologische Untersuchungen an Cyanophyceen, hauptsachlich. dem Endophytischen *Nostoc punctiforme*." **Z. Bot.,** vol. 9, 1917. pp. 145-242. (in German)

[61] Akehurst S.C. "Observations on Pond Life, with Special Reference to the Possible Explanation for Effects Noted Between Various Species of Grasses and Legumes." **J. Am. Soc. Agron.**, vol. 31, 1931. pp. 982-985.

[62] Lefevre M. and Nisbet M. "Sur la Secretion par Certaines Especes. d'Algues de Substances Inhibitrices d'autres Especes d'Algues." **C.R. Acad. Sci.**, vol. 226. 1948. pp. 107-109. (in France)

[63] Fogg G.E and Westlake D.F. "The Importance of Extracellular Products of Algae in Fresh Water." **Int. Ver. Theor. Angew. Limnol. Mitt.**, vol. 12, 1955. pp. 219-232.

[64] Martin D.F., Kutt E.C. and Kim Y.S. "Use of a Multiple Diffusion Chamber Unit in Culture Studies. Application to *Gomphosphaeria aponina*." **Environ. Lett.**, vol. 7, no. 1, 1974. pp. 39-46.

[65] Pignatello J.J., Porwoll J., Carlson R.E., Xavier A., Gleason F.K. and Wood J.M. "Structure of the Antibiotic Cyanobacterin, a Chlorine Containing γ-Lactone from the Freshwater Cyanobacterium *Scytonema hofmanni*." **J. Org. Chem.**, vol. 48, no. 22, 1983. pp. 4035-4038.

[66] Mason C.P., Edwards K.R., Calson R.E., Pignatello J., Gleason F.K. and Wood J.M. "Isolation of Chlorine-Containing Antibiotic from the Freshwater Cyanobacterium *Scytonema hofmanni*." **Science**, vol. 215, no. 4531, 1982. pp. 400-402.

[67] Gleason F.K., Baxa C.A. "Activity of the Natural Algicide, Cyanobacterin, on Eukaryotic Microorganisms." **FEMS Microbiol. Lett.**, vol. 33, no. 1, 1986. pp. 85-88.

[68] Murakami N., Imamura H., Sakakibara J. and Yamada N. "Seven New Monogalactosyl Diacylglycerols Isolated from the Axenic Cyanobacterium *Phormidium tenue* .sup.1." **Chem. Pharm. Bull.**, vol. 38, no. 12, 1990. pp. 3497-3499.

[69] Gross E.M., Wolk C.P. and Jüttner F. "Fischerellin, a New Allelochemical from the Freshwater Cyanobacterium *Fischerella muscicola*." **J. Phycol.** vol. 27, no. 6, 1991. pp. 686-692.

[70] Hagmann L. and Jüttner F. "Fischerellin A, a Novel Photosystem-II-Inhibiting Allelochemical of the Cyanobacterium *Fischerella muscicola* with Antifungal and Herbicidal Activity." **Tetrahedron Lett.**, vol. 37, no. 36, 1996. pp. 6539-6542.

[71] Bagchi S.N., Chauhan V.S., Marwah J.B. "Effect of an Antibiotic from *Oscillatoria late-virens* on Growth, Photosynthesis, and Toxicity of *Microcystis aeroginosa*." **Curr. Microbiol.**, vol. 26, no. 4, 1993. pp. 223-228.

[72] Bagchi S.N. "Structure and Site of Action of an Algicide from a Cyanobacterium, *Oscillatoria late-virens*." **J. Plant. Physiol.**, vol. 146, no. 3, 1995. pp. 372-374.

[73] Papke U., Gross E.M. and Francke W. "Isolation, Identification and Determination of the Absolute Configuration of Fischerellin B a New Algicide from the Freshwater Cyanobacterium *Fischerella muscicola* (Thuret)." **Tetrahedron Lett.**, vol. 38, no. 3, 1997. pp. 379-382.

[74] Schlegel I., Doan N.T., Chazal N.D. and Smith G.D. "Antibiotic Activity of New Cyanobacterial Isolates from Austria and Asia Against Green Algae and Cyanobacteria." **J. Appl. Phycol.**, vol. 10, no.5, 1999. pp. 471-479.

[75] Pushparaj B., Pelosi E. and Jütter F. "Toxicological Analysis of the Marine Cyanobacterium *Nodularia Harveyana*." **J. Appl. Phycol.**, vol. 10, no. 6, 1998. pp. 527-530.

[76] Doan N.T., Rickards R.W., Rothschild J.M., Smith G.D. "Allelopathic Actions of the Alkaloid 12-*Epi*-Hapalindole E Isonitrile and Calothrixin A from Cyanobacteria of the

Genera *Fischerella* and *Calothrix*." **J. Appl. Phycol.**, vol. 12, no. 3-5, 2000. pp. 409-416.

[77] Singh D., Tyagi M.B., Kumar A., Thakur J.K. and Kumar A. "Antialgal Activity of a Hepatotoxin-Producing Cyanobacterium, *Microcystis aeruginosa*." **World J. Microb. Biot.**, vol. 17, no.1, 2001. pp. 15-22.

[78] Hirata K., Yoshitomi S., Dwi S., Iwabe O., Mahakhant A., Polchai J. and Miyamoto K. "Bioactivities of Nostocine A Produced by a Freshwater Cyanobacterium *Nostoc spongiaeforme* TISTR 8619." **J. Biosci. Bioeng.**, vol. 95, no. 5, 2003. pp. 512-517.

[79] Suikkanen S., Fistarol G.O. and Granéli E. "Allelopathic Effects of the Baltic Cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermanii* on Algal Monocultures." **J. Exp. Mar. Biol. Ecol.**, vol. 308, no. 1, 2004. pp. 85-101.

[80] Zarrouk C. 1966. **Contribution à l'étude Dune Cyanophycée. Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthèse de *Spirulina maxima***. Ph.D. thesis, University of Paris, France, 45 pp. (In French)

[81] Rito-Palomares M., Nunez L. and Amador D. "Practical Application of Aqueous Two-Phase Systems for the Development of a Prototype Process for C-Phycocyanin Recovery from *Spirulina maxima*." **J. Chem. Technol. Biotechnol.**, vol. 76, no. 12, 2001. pp. 1273-1280.

[82] Bennett A., Bogorad, L. "Complementary Chromatic Adaptation in a Filamentous Blue-Green Alga." **J. Cell Biol.**, vol. 58, 1973. pp. 419-435.

[83] Noguchi Y., Ishii A., Matsushima A., Haishi D., Yasumuro K., Moriguchi T., Wada T., Kodera Y., Hiroto M., Nishimura H., Sekine M. and Inada Y. "Isolation of Biopterin-α-glucoside from *Spirulina* (*Arthrospira*) *platensis* and Its Physiologic Function." **Ma. Biotechnol.**, vol. 1, no. 2, 1999. pp. 207-210.

[84] Harwood L.M., Moody C.J. and Percy J.M. **Experimental Organic Chemistry Standard and Microscale**. 2nd ED. London : Blackwell Science Ltd. 1999.

[85] http://www.chem.agilent.com/Library/applications/5989-3760EN.pdf

[86] Berns D.S. and MacColl R. "Phycocyanin in Physical–Chemical Studies." **Chem. Rev.**, vol. 89, no. 4, 1989. pp. 807–825.

[87] Edwards M.R., MacColl R. and Eisele L.E. "Some Physical Properties of an Unusual C-Phycocyanin Isolated from a Photosynthetic Thermophile." **Biochim. Biophys. Acta**, vol. 1276, no. 1, 1996. pp. 64–70.

[88] Edwards M.R., Hauer C., Stack R.F., Eisele L.E. and MacColl R. "Thermophilic C-Phycocyanin : Effect of Temperature, Monomer Stability, and Structure." **Biochim. Biophys. Acta2-Bioenerg.**, vol. 1321, no. 2, 1997. pp. 157–164.

[89] Szalontai B., Gombos Z., Csizmadia V., Bagyinka C. and Lutz M. "Structure and Interactions of Phycocyanobilin Chromophores in Phycocyanin and Allophycocyanin from an Analysis of Their Resonance Raman Spectra." **Biochemistry**, vol. 33, no. 39, 1994. pp. 11823–11832.

[90] Bermejo R. Minor Thesis, Faculty of Pharmacy, University of Granada, Granada, Spain, 1994.

[91] Gantt E. "Phycobilisomes." **Ann. Rev. Plant Physiol.**, vol. 32, 1981. pp. 327-347.

[92] Glazer A.N. In: Hatch M.D and Boardman N.K., Editors (4th ED). **The Biochemistry of Plants**. New York : Academic Press, vol. 8, 1981. pp. 51-96.

[93] Benedetti S., Benvenuti F., Pagliarani S., Francogli S., Scoglio S. and Canestrari, F., "Antioxidant Properties of a Novel Phycocyanin Extract from the Blue-Green Alga *Aphanizomenon flos-aquae*." **Life Sci.**, vol. 75, no. 19, 2004. pp. 2353–2362.

[94] Farooq S.M., Asokan D., Sakthivel R., Kalaiselvi P. and Varalakshmi P. "Salubrious Effect of C-Phycocyanin Against Oxalate-Mediated Renal Cell Injury." **Clin. Chim.Acta**, vol. 348, no. 1-2, 2004. pp. 199–205.

[95] Pardhasaradhi B.V.V., Ali A.M., Kumari A.L., Reddanna P. and Khar A. "Phycocyanin-Mediated Apoptosis in AK-5 Tumor Cells Involves Down-Regulation of Bcl-2 and Generation of ROS." **Mol. Cancer Ther.**, vol. 2, no. 11, 2003. pp. 1165–1170.

[96] Reddy C.M., Bhat V.B., Kiranmai G., Reddy M.N., Reddanna P. and Madyastha K.M., "Selective Inhibition of Cyclooxygenase-2 by C-Phycocyanin, a Biliprotein from *Spirulina platensis*." **Biochem. Biophys. Res. Commun.**, vol. 277, no. 3, 2000. pp. 599–603.

[97] Romay Ch., Gonzalez R., Ledon N., Remirez D. and Rimbau V. "C-Phycocyanin: a Biliprotein with Antioxidant, Anti-Inflammatory and Neuroprotective Effects." **Curr. Protein Pept.**, vol. 4, no. 3, 2003. pp. 207–216.

[98] Subhashini J., Mahipal S.V.T., Reddy M.C., Reddy M.M., Rachamallu A. and Reddanna P. "Molecular Mechanisms in C-Phycocyanin Induced Apoptosis in Human Chronic Myeloid Leukemia Cell Line-K562." **Biochem. Pharmacol.**, vol. 68, no. 3, 2004. pp. 453–462.

[99] Jørgensen E.G. "Antibiotic Substances from Cells and Culture Solutions of Unicellular Algae with Special Reference to Some Chlorophyll Derivatives." **Physiologia Plantarum**, vol. 15, 1962. pp. 530-545.

[100] Ricci P., Bonnet P., Huet J.C., Sallantin M., Beauvais-Cante F., Bruneteau M., Billard V., Michel G. and Pernollet J.C. "Structure and Activity of Proteins from Pathogenic Fungi *Phytopthora* Eliciting Necrosis and Acquired Resistance in Tobacco." **Eur. J. Biochem.**, vol. 183, no. 3, 1989. pp. 555-563.

[101] Kamoun S., Young M., Glascock C.B. and Tyler B.M. "Extracellular Protein Elicitors from *Phytopthora*: Host-Specificity and Induction of Resistance to Bacterial and Fungal Phytopthogens." **Mol. Plant Microbe. Interact.**, vol. 6, no. 1, 1993. pp. 15-25.

[102] Bourke A.T.C. and Hawes R.B. "Freshwater Cyanobacteria (Blue-Green Algae) and Human Health." **Med. J. Aust.**, vol. 1, no. 11, 1983. pp. 491-492.

[103] MacCol R. and Guard-Friar D. **Phycobililiproteins**. Boca Raton, Florida : CRC Press. 1987.

[104] MacColl R., Kapoor S., Montellese D.R., Kukadia S. and Eisele L.E. "Bilin Chromophores as Reporters of Unique Protein Conformations of Phycocyanin 645." **Biochemistry**, vol. 35, no. 48, 1996. p. 15436-15439.

[105] Sarada R., Pillai M.G. and Revishankar G.A. "Phycocyanin from *Spirulina* sp. : Influence of Processing of Biomass on Phycocyanin Yield, Analysis of Efficacy of Extraction Methods and Stability Studies on Phycocyanin." **Process Biochem.**, vol. 34, no. 8, 1999. pp. 795-801.

[106] Pasco D.S., Pugh N., Ross S. and Elsohly M. **Potent Immunostimulants from Microalgae**. International Patent, WO/2002/004000. 17 January 2002.

[107] Edwards M.R., MacColl R. and Eisele L.E. "Some Physical Properties of an unusual C-Phycocyanin Isolated from a Photosynthetic Thermophile." **Biochim. Biophys. Acta.**, vol. 1276, no. 1, 1996. pp. 64-70.

[108] Antelo F.S., Costa J.A.V. and Kalil S.J. "Thermal Degradation Kinetics of the Phycocyanin from *Spirulina platensis*." **Biochem. Eng. J.**, vol. 41, no. 1, 2008. pp. 43-47.

[109] Zhou Z-P., Liu L-N., Chen X-L., Wang J-X., Chen M., Zhang Y-Z. and Zhou B-C. "Factors that Effect Antioxidant Activity of C-Phycocyanins from *Spirulina platensis*." **J. Food Biochem.**, vol. 29, no. 3, 2005. pp. 313-322.

[110] Han B.-H., Pyo M.-K. and Yang S.J. **Denatured *Spirulina* and Manufacturing Method Thereof**. International Patent, WO 03/080811 A1. 2 October 2003.

[111] O'Ćarra P., Murphy R. F. And Killilea S.D. "The Native Forms of the Phycobilin Chromophores of Algal Biliproteins." **Biochem. J.**, vol. 187, no. 2, 1980. pp. 303-309.

[112] Macias F.A. Marin D., Oliveros-Bastidas A., Varela R.M., Simonent A.M., Carrera C. and Molinillo J.M.G. "Allelopathy as a New Strategy for Sustainable Ecosystems Development." **Biol. Sci. Space.,** vol. 17, no. 1, 2003. pp. 18-23.

[113] Macias F.A., Oliveros-Bastidas A., Marin D., Castellano D., Simonet A.M. and Molinillo J.M.G. "Degradation Studies on Benzoxazinoids. Soil Degradation Dynamics of (2*R*)-2-*O*-β-D-Glucopyranosyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and Its Degradation Products, Phytotoxic Allelochemicals from Gramineae." **J. Agric. Food Chem.**, vol. 53, no. 3, 2005. pp. 554-561.

[114] Gagliardo R.W. and Chilton W.S. "Soil Transformation of 2(3*H*)-Benzoxazolone of Rye into Phototoxic 2-Amino-3*H*-phenoxazin-3-one." **J. Chem. Ecol.**, 18, no. 10, 1992. pp. 1683-1691.

[115] Niemeyer H.M. "Hydroxamic Acids (4-Hydroxy-1,4-benzoxazin-3-ones), Defence Chemicals in The Gramineae." **Phytochemistry**, vol. 27, no. 11, 1988. pp. 3349-3358.

[116] Yue Q., Bacon C.W. and Richardson M.D. "Biotransformation of 2-Benzoxazolinone and 6-Methoxy-benzoxazolinone by *Fusarium moniliforme*." **Phytochemistry**, vol. 48, no.3, 1998. pp. 451-454.

[117] Zikmundová M. Dandrarov K., Hesse M. and Werner C. "Hydroxylated 2-Amino-3*H*-phenoxazin-3-one Derivatives as Products of 2-Hydroxy-1,4-benzoxazin-3-one (HBOA) Biotransformation by *Chaetosphaeria* sp., an Endophytic Fungus from *Aphelandra tetragona*." **Z. Naturforsch.**, vol. 57c, no. 7-8, 2002. pp. 660-665.

[118] Weaver R.J. **Plant Growth Substances in Agriculture**. San Francisco, California : W.H. Freeman and Company. 1972. 594 pp.

[119] Ozdemier G., Karabay N.U., Dalay M.C. and Pazarbasi, B. "Antibacterial Activity of Volatile Component and Various Extracts of *Spirulina platensis*." **Phytother. Res.**, vol. 18, no. 9, 2004. pp. 754-757.

[120] Guzzella L., and Soraa S. "Mutagenic Activity of Lake Water Samples Used as Drinking Water Resources in Northern Italy." **Wat. Res.**, vol. 32, no. 6, 1998. pp. 1733-1742.

[121] Lee K., Aoki H. and Shibamoto T. "Antioxidative Activities of Aroma Extracts Isolated from Various Beans." **ACS Symp. Ser.** (Caffeinated Bevarages), vol. 754, 2000. pp. 279-287.

[122] Xian Q., Chen H., Liu H., Zou H. and Yin D. "Isolation and Identification of Antialgal Compounds from the Leaves of *Vallisneria spiralis* L. by Activity-Guided Fractionation." **Environ. Sci. Pollut. Res.**, vol. 13, no. 4, 2006. pp. 233-237.

[123] Dembitsky V.Y., Shkrob I and Lev O. "Occurrence of Volatile Nitrogen-Containing Compounds in Nitrogen-Fixing Cyanobacterium *Aphanizomenon flos-aquae*." **J. Chem. Ecol.**, vol. 26, no. 6, 2000. pp. 1359-1366.

[124] Sakan T., Isoe S. and Hyeon S.B. "The Structure of Actinidiolide, Dihydroactinidiolide and Actinidol." **Tetrahedron Lett.**, vol. 8, no. 17, 1967. pp. 1623-1627.

[125] Buckingham J. and Bradley H. **Dictionary of natural products**. Second Supplement, vol. 9, Cambridge : The University Press. pp. 144.

[126] Senatore F., Arnold N.A. and Bruno M. "Volatile Components of *Centaurea eryngioides* Lam. and *Centaurea iberica* Trev. Var. *hermonis* Boiss. Lam., Two Asteraceae Growing Wild in Lebanon." **Nat. Proc. Res.**, vol. 19, no. 8, 2005. pp. 749-754(6).

[127] Bankova V., Stefanov K., Dimitrovea-Konaklieva S., Keremedchieva G., Frette X., Nikolova C., Kujumgiev A. and Popov S. "Secondary Metabolites and Lipids in *Chara globularis* Thuill." **Hydrobiolgia**, vol. 457, no. 1-3, 2001. pp. 199-203.

[128] Rocca J.R., Tumlinson J.H., Glancey B.M. and Lofgren C.S. "The Queen Recognition Pheromone of *Solenopsis invicta*, Preparation of (*E*-6-(1-pentenyl)-2H-pyran-2-one." **Tetrahedron Lett.**, vol. 24, no. 18, 1983. pp. 1889- 1892.

[129] Stevens K.L. and Merrill G.B. "Dihydroactinidiolide-A Potent Growth Inhibitor from *Eleocharis coloradoensis* (Spikerush)." **Cell Mol. Life Sci.** (CMLS), (Historical Archive), vol. 37, no. 11, 1981. pp. 1133.

[130] Ashton F.M., Di Tomaso J.M. and Anderson L.W.J. "Spikerush (*Eleocharis* sp.) : A Source of Allelopathic for the Control of Undesirable Aquatic Plants." **ACS Sym. Ser.**, vol. 68, 1985. pp. 401-414.

[131] Mordi R.C. and Walton J.C. "Oxidative Degradation of β-Carotene and β-Apo-8'-carotenol." **Tetrahedron**, vol. 49, 1993. pp. 911-928.

[132] Tang R.-R., Zhou Y.-P., Gong N.-H. And Zhao Y. "Aerobic Oxidation Behavior of α-Ionone Catalyzed by N-Hydroxyphthalimide Combined with Acetylacetone Cobalt(II)." **J. Cent. South Univ. Technol.**, vol. 15, no. 4, 2008. pp. 474-478.

[133] Khodabocus A. and Beebeejaun B. "Convenient Acid Catalysed Etherification of Hydroxyionone." **Sci. Technol.**, vol. 5, 2000. pp. 21-24.

[134] Isoe S., Hyeon S.B. and Sakan T. "Photo-Oxygenation of Carotenoids. I. The Formation of Dihydroactinidiolide and β-Ionone from β-Carotene." **Tetrahedron Lett.**, vol. 10, no. 4, 1969. pp. 279-281.

[135] Enzell C. "Biodegradation of Carotenoids and Important Route to Aroma Compounds." **Pure and Appl. Chem.**, vol. 57, no. 5, 1985. pp. 693-700.

[136] Mordi R.C., Walton J.C., Burton G.W., Hughes L., Ingold K.U. and Lindsay D.A. "Exploratory Study of β-Carotene Autoxidation." **Tetrahedron Lett.**, vol. 3, no. 33, 1991. pp. 4203-4206.

[137] Baumes R., Wirth J., Bureau S., Gunata Y. and Razungles A. "Biogeneration of $C_{13}$-Norisoprenoids Compounds: Experiments Supportive for an Apo-Carotenoid Pathway in Grapevines." **Anal. Chim. Acta**, vol. 458, no. 1, 2002. pp. 3-14.

[138] Bosser A., Paplorey E. and Belin J. "A Simple Way to (±)-Dihydroactinidiolide from β-Ionone Related to the Enzymic Cooxidation of β-Carotene in Aqueous Solution." **Biotechnol. Prog.**, vol. 11, no. 6, 1995. pp. 689-692.

[139] Waché Y., Bosser-Deratuld A., Lhuguenot J.-C and Belin J.-M. "Effect of *sis/trans* Isomerism of β- Carotene on the Ratios of Volatile Compounds Produced During Oxidative Degration." **J. Agric. Food Chem.**, vol. 51, no. 7, 2003. pp. 1984-1987.

[140] Yao S., Johannsen M., Hazell R.G. and Jørgensen K.A. "Total Synthesis of (*R*)-

Dihydroactinidiolide and (*R*)-Actinidiolide Using Asymmetric Catalytic Hetero-Diels-Alder Methodology." **J. Org. Chem.**, vol. 63, no. 1, 1998. pp. 118-121.

[141] Mori K. and Khlebnikov V. "Carotenoids and Degraded Carotenoids, VIII-Synthesis of (+)-Dihydroactinidiolide, (+)-and (-)-Actinidiolide, (+)-and (-)-Loliolide as well as (+)- and (-)-Epiloliolide." **Liebigs Ann. Chem.**, vol. 1993, no. 1, 1993. pp. 77-82.

[142] Eidman K.F. and MacDougall B.S. "Synthesis of Loliolide, Actinidiolide, Dihydroactinidiolide, and Aeginetolide via Cerium Enolate Chemistry." **J. Org. Chem.**, vol. 71, no. 5, 2006. pp. 9513-9516.

[143] Becher E., Albrecht R., Bernhard K, Leuenberger H.G.W., Mayer H., Müller R.K., Schüep W. and Wangner H.P. "Synthesis of Astaxanthin from β-Ionone. I. Enantiomeric $C_{15}$-Wittig Salts by Chemical and Microbial Resolution of (±)-3-Acetoxy-4-oxo-β-ionone." **Helv. Chim. Acta.**, vol. 64, n. 7, 1981. pp. 2419-2435.

[144] Kato-Noguchi H., Kosemura S., Yamamura S. and Hasegawa K. "A Growth Inhibitor, *R*-(-)-3-Hydroxy-β-ionone, from Light-growth Shoots of a Dwarf Cultivar of *Phaseolus vulgaris*." **Phytochemistry**, vol. 33, no. 3, 1993. pp. 553-555.

[145] Gueldner A. and Winterhalter P. "Structures of Two New Ionone Glycosides from Quince Fruit (*Cydonia oblonga* Mill.)." **J. Agric. Food Chem.**, vol. 39, no. 12, 1991. pp. 2142-2146.

[146] D'Abrosca B., DellaGreca M., Fiorentino A., Monaco P., Oriano P. and Temussi F. "Structure Elucidation and Phytotoxicity of $C_{13}$ *nor*-Isoprenoids from *Cestrum parqui*" **Phytochemistry**, vol. 65, no. 4, 2004, pp. 497-505.

[147] Kim I., Chin Y.-W., Lim S.W., Kim Y.C. and Kim J. "Norisoprenoids and Hepatoprotective Flavone Glycosides from the Aerial Parts of *Beta vulgaris* var. *cicla*." **Arch. Pharm. Res.**, vol. 27, no. 6, 2004. pp. 600-603.

[148] Mori K. "Synthesis of Optically Active Grasshopper Ketone and Dehydrovomifoliol as a Synthetic Support for the Revised Absolute Configuration of (+)-Abscisic Acid." **Tetrahedron**, vol. 30, no. 9, 1974. pp. 1065-1072.

[149] Macías F.A, Oliva R.M, Varela R.M., Torres A. and Molinillo J.M.G. "Allelochemicals from Sunflower Leaves cv. Peredovick." **Phytochemistry**, vol. 52, no. 4, 1999. pp. 613-621.

[150] a. Mori K. "Synthesis of The Optically Active Dehydrovomifoliol. A Synthetic Proff of the Absolute Configuration of (+)-Abscisic Acid." **Tetrahedron Lett.**, vol. 14, no. 28, 1973. pp. 2635-2638.

b. Mori K. "Synthesis of dl-3-Isobutyroxy-β-ionone and dl-Dehydrovomifoliol." **Agric. Biol. Chem.**, vol. 37, no. 12, 1973. pp. 2899-2905.

[151] Kuba M., Furuichi N. and Katsumura S. "Stereocontrolled Syntheses of Carotenoid Oxidative Metabolites, (-)-Loliolide, (-)-Xanthoxin, and Their Stereoisomers." **Chem. Lett**, vol. 31, no. 12, 2002. pp. 1248-1249.

[152] Fernandez I., Pedro J.R. and Vidal R. "Norisoprenoids from *Centaurea aspera* and *C. salmantica*." **Phytochemistry**, vol. 34, no. 3, 1993. pp. 733-736.

[153] Kimura J. and Maki N. "New Loliolide Derivatives from the Brown Alga *Undaria pinnatifida*." **J. Nat. Prod.**, vol. 65, no. 1, 2002. pp. 57-58.

[154] Kato T., Imai T., Kashimura K., Saito N. and Masaya K. "Germination Response in Wheat Grains to Dihydroactinidiolide, a Germination Inhibitor in Wheat Husks, and Related Compounds." **J. Agric. Food Chem.**, vol. 51, no. 8, 2003. pp. 2161-2167.

[155] Park K.E., Kim Y.A., Jung H.A., Lee H.J., Ahn J.-W., Lee B.-J. and Seo Y. "Three Norisoprenoids from the Brown Alga *Sagassum thunbergii*." **J. Kor. Chem. Soc.**, vol. 48, no. 4, 2004. pp. 394-398.

[156] Hattab M.E., Culioli G., Valls R., Richou M. and Piovetti L. "Apo-fucoxanthinoids and Loliolide from the Brown Alga *Cladostephus spongiosus* f. *verticillatus* (Heterokonta, Sphacelariales)." **Biochem. System. Ecol.**, vol. 36, no. 5-6, 2008. pp. 447-451.

[157] Fujimori T., Kasuga R., Noguchi M. and Kaneko H. "Isolation of *R*-(-)-3-Hydroxy-β-ionone from Burley Tobacco." **Agric. Biol. Chem.**, vol. 38, no. 4, 1974. pp. 891-892.

[158] Skouroumounis G.K. and Winterhalter P. "Glycosidically Bound Norisoprenoids from *Vitis vinifera* Cv. Riesling Leaves." **J. Agric. Food Chem**, vol. 42, no. 5, 1994. pp. 1068-1072.

[159] Dietz H. and Winterhalter P. "Phytotoxic Constituents from *Bunias orientalis* Leaves." **Phytochemistry**, vol. 42, no. 4, 1996. pp. 1005-1010.

[160] Macías F.A, Lacret R., Varela R.M., Nogueiras C. and Molinillo J.M.G. "Bioactive Apocarotenoids from *Tectona grandis*." **Phytochemistry**, vol. 69, no. 15, 2008. pp. 2708-2715.

[161] Isoe S., Hyeon S.B., Katsumura S. and Sakan T. "Photo-oxygenation of Carotenoids. II. The Absolute Configuration of Loliolide and Dihydroactinidiolide." **Tetrahedron Lett.**, vol. 13, no. 25, 1972. pp. 2517-2520.

[162] Hodges R. and Porte A.L. "The Structure of Loliolide. A Terpene from *Lolium perenne*." **Tetrahedron**, vol. 20, no. 6, 1964, pp. 1463-1467.

[163] Okunade A.L. and Wiemer D.F. "(-)-Loliolide, an Ant-Repellent Compound from *Xanthoxyllum setulosum*." **J. Nat. prod.**, vol. 48, no. 3, 1985. pp. 472-473.

[164] Okada N., Shirata K., Niwano M., Koshino H. and Uramoto M. "Immunosuppressive Activity of a Monoterpene from *Eucommia ulmoides*." **Phytochemistry**, vol. 37, no. 1, 1994. pp. 281-282.

[165] Kuniyoshi M. "Germination Inhibitors from the Brown Alga *Sargassum crassifolium* (*Phaeophyta, Sargassaceae*)." **Bot. Mar.**, vol. 28, no. 11, 1985. pp. 501-503.

[166] Bowers W.S., Hoch H.C., Evans P.H. and Katayama M. "Thallphytic Allelopathy: Isolation and Identification of Laetisaric Acid." **Science**, vol. 32, no. 4746, 1985. pp. 105-106.

[167] Gallardo-Williams M.T., Geiger C.L., Pidala J.A. and Martin D.F. "Essential Fatty Acids and Phenolic Acids from Extracts and Leachates of Southern Cattail (*Typha domingensis* P.)." **Phytochemistry**, vol. 59, no. 3, 2002. pp. 305-308.

[168] Jin Z.H., Zhuang Y.Y., Dai S.G. and Li T.L. "Isolation and Identification of Extracts of *Eichhornia crassipes* and Their Allelopathic Effects on Algae." **Bull. Environ. Contam. Toxicol.**, vol. 71, no. 5, 2003. pp. 1048-1052.

[169] Ko J., Eom S.H., Kim M.J., Yu C.Y. and Lee Y.S. "Allelopathy of Rice Husk on Barnyardgrass." **J. Agron.**, vol.4, no. 4, 2005. pp. 288-292.

[170] Alamsjah M.A., Hirao S., Ishibashi F. and Fujita Y. "Isolation and Structure Determination of Algicidal Compounds from *Ulva fasciata*." **Biosci. Biotechnol. Biochem.**, vol. 69, no. 11, 2005. pp. 2186-2192.

[171] Xuan T.D., Chung III M., Khanh T.D. and Tawata S. "Identification of Phytotoxic Substances from Early Growth of Barnyard Grass (*Echinochloa crusgalli*) Root Exudates." **J. Chem. Ecol.**, vol. 32, no. 4, 2006. pp. 895-906.

[172] Spruell J. A. "Response of Algae and Zooplankton to $C_{18}$ Fatty Acids of *Chlamydomonas reinhardtii*." **Hydrobiologia**, vol. 114, no. 1, 1984. pp. 9-12

[173] Aliotta G., Della Greca M., Monaco P., Pinto G., Pollio A. and Previtera L. "In Vitro Algal Growth Inhibition by Phytotoxins of *Typha latifolia* L." **J. Chem. Ecol.**, vol. 16, no. 9, 1990. pp. 2637-2646.

[174] Murakami N., Yamada N. and Sakakibara J. "An Autolytic Substance in a Freshwater Cyanobacterium *Phormidium tenue*." **Chem. Pharm. Bull.**, vol. 38, no. 3, 1990. pp. 812-814.

[175] Murakami N., Morimoto T., Imamura H., Ueda T., Nakai S., Sakakibara J. and Yamada N. "Studies on Glycolipids. III. Glyceroglycolipids from an Axenically Cultured Cyanobacterium, *Phormidium tenue*." **Chem. Pham. Bull.**, vol. 39, no. 9, 1991. pp. 2277-2281.

[176] Suzuki M., Wakana I., Denboh T. and Tatewaki M. "An Allelopathic Polyunsaturated Fatty Acid from Red Algae." **Phytochemistry**, vol. 43, no. 1, 1996. pp. 63-65.

[177] Alamsjah M.A., Ishibe K., Kim D., Yamaguchi K., Ishibashi F., Fujita Y. and Oda T. "Selective Toxic Effects of Polyunsaturated Fatty Acids Derived from *Ulva fasciata* on Red Tide Phytoplankter Species." **Biosci. Biotechnol. Biochem.**, vol. 71, no. 1, 2007. pp. 265-268.

[178] Nakai S., Yamada S. and Hosomi M. "Anti-Cyanobacterial Fatty Acids Released from *Myriophyllum spicatum*." **Hydrobiologia**, vol. 543, no. 1, 2005. pp. 71-78.

[179] Gross E.M., Mayer H. and Schilling G. "Release and Ecological Impact of Algicidal Hydrolysable Polyphenols in *Myriophyllum spicatum*." **Phytochemistry**, vol. 41, no. 1, 1996. pp. 133-138.

[180] Nakai S., Inoue Y., Hosomi M. and Murakami A. "*Myriophyllum spicatum*-Released Allelopathic Polyphenols Inhibiting Growth of Blue-Green Algae *Mycrocystis aeruginosas*." **Wat. Res.**, vol. 34, no. 11, 2000. pp. 3026-3032.

[181] Saito K., Matsumoto M., Sekine T. and Murokoshi I. "Inhibitory Substances from *Myriophyllum brasiliense* on Growth of Blue-Green Algae." **J. Nat. Prod.**, vol. 52, no. 6, 1989. pp.1221-1226.

[182] McCracken M.D., Middaugh R.E. and Middaugh R.S. "A Chemical Characterization of an Algal Inhibitor Obtained from *Chlamydomonas*." **Hydrobiologia**, vol. 70, no. 3, 1980. pp. 271-276.

[183] Takamura N., Chino M., Osada A., Nishihara H. and Yagi O. "Growth Response of *Mycrocystis* and *Oscillatoria* to Various Organic Compounds and Growth Inhibition by

L-Lysine and L-Histidine." **Environmental Science**, vol. 12, no. 3, 1999. pp. 329- 337. (In Japanese)

[184] Stevens K.L. and Merrill G.B. "Growth Inhibitors from Spikerush" **J. Agric. Food Chem.**, vol. 28, no. 3, 1980. pp. 644-646.

[185] Kurokawa M., Hirose T., Sugata Y., Tokumasu M., Hiraga Y. and Suga T. "3-Hydroxy-5,6-epoxy-β-ionone as Germination Inhibitory Active Constituent in *Athyrium yokoscense.*" **Nat. Prod. Res.**, vol. 12, no. 1, 1998. pp. 35-40.

[186] Macías F.A, Simonet A.M., Galindo J.C.G. and Castellano D. "Bioactive Phenolics and Polar Compounds from *Melitotus messanensis.*" **Phytochemistry**, vol. 50, no. 1, 1999. pp. 35-46.

[187] Kato T., Saito N., Kashimura K., Shinohara M., Kurahashi T. and Taniguchi K. "Germination and Growth Inhibitors from Wheat (*Triticum aestivum* L.) Husks." **J. Agric. Food Chem.**, vol. 50, no. 22, 2002. pp. 6307-6312.

[188] Li H.-H., Inoue M., Nishimura H., Mizutani J. and Tsuzuki E. "Interactions of *trans*-Cinnamic Acid, its Related Phenolic Allelochemicals, and Abscisic Acid in Seedling Growth and Seed Germination of Lettuce." **J. Chem. Ecol.**, vol. 19, no. 8, 1993. pp. 1775-1787.

[189] Canals R.M., Emeterio L.S. and Peralta J. "Autotoxicity in *Lolium rigidum*: Analyzing the Role of Chemically Mediated Interactions in Annual Plant Populations." **J. Theor. Biol.**, vol. 235, no. 3, 2005. pp. 402-407.

[190] Rizvi S.J.H., Tahir M., Rizvi V., Kohli R.K. and Ansari A. "Allelopathic Interactions in Agroforestry Systems." **Crit. Rev. Plant Sci.**, vol. 18, no. 6, 1999. pp. 773-796.

[191] Burgos N.R. and Talbert R.E. "Differential Activity of Allelochemicals from *Secale cereale* in Seedling Bioassays." **Weed Sci.**, vol 48, no. 3, 2000. pp. 302-310.

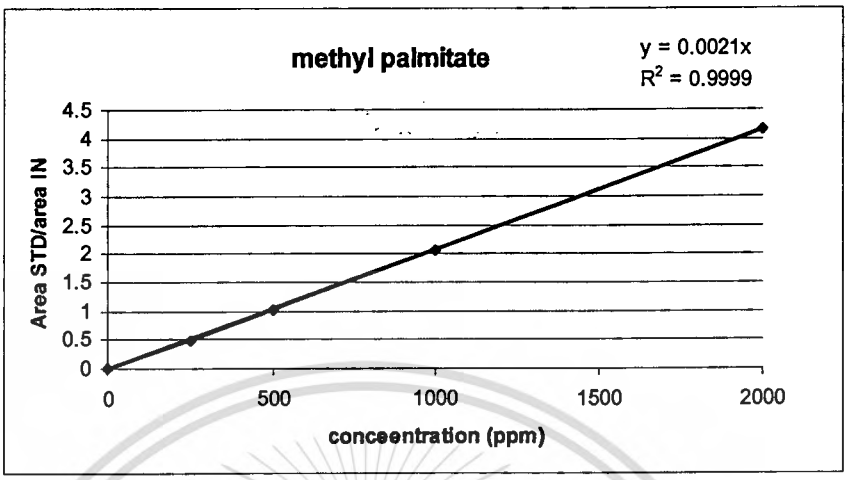
[192] Khanh T.D., Hong N.H., Nhan D.Q., Kim S.L., Chung I.M. and Xuan T.D. "Herbicidal Activity of *Stylosanthes guianensis* and its Phytotoxic Components." **J. Agoon. Crop Sci.**, vol. 192, no. 6, 2006. pp. 427-433.

[193] Sangthong T. "**Herbicidal Potential of Odorine from *Aglaia Odorata* Lour. Leaves.**" M. Sc. Thesis of King Mongkut' s Institute of Technology Ladkrabang. 2008.

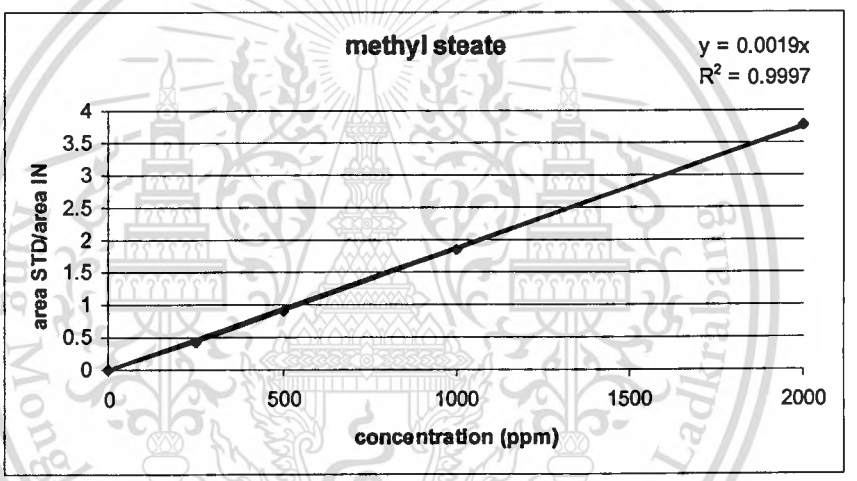
# APPENDICES

## Appendix A: Calibration curve of standard fatty acids

(a) Methyl palmitate




(b) Methyl stearate




(c) Methyl oleate

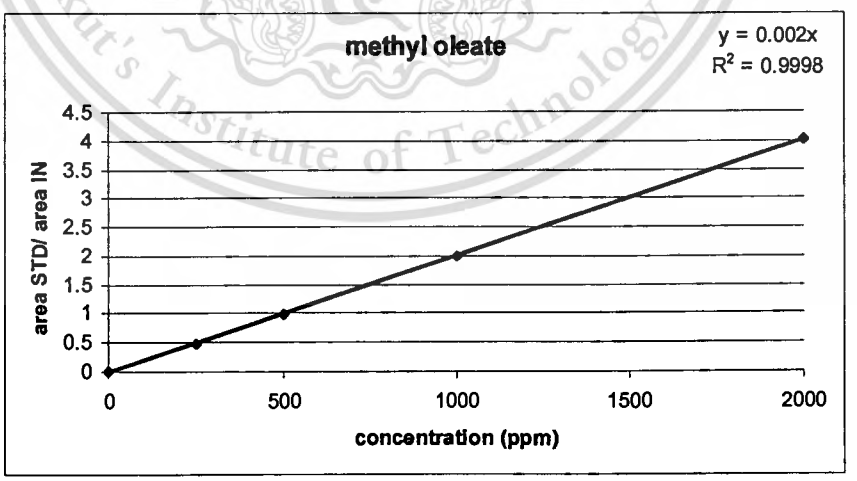
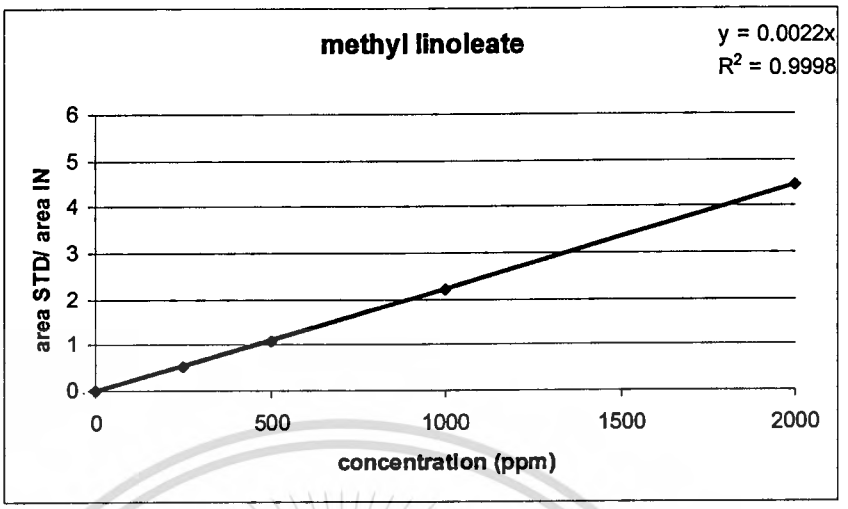



**Figure A1** Internal standard calibration curve for (a) methyl palmitate, (b) methyl stearate, (c) methyl oleate, (d) methyl linoleate and (e) methyl-γ-linolenate

(d) Methyl linoleate




(e) Methyl-γ-linolinate

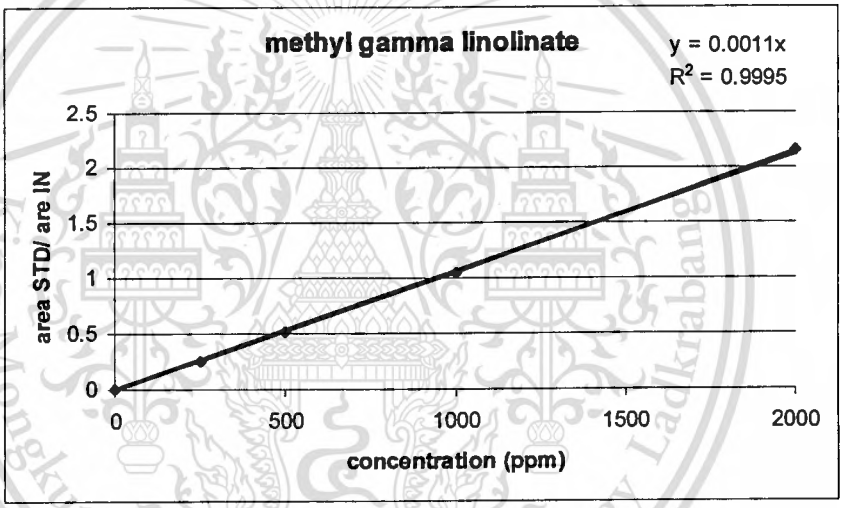



**Figure A1** Internal standard calibration curve for (a) methyl palmitate, (b) methyl stearate, (c) methyl oleate, (d) methyl linoleate and (e) methyl-γ-linolenate (Continued)

Calibration curve: Y = area STD/ area IN

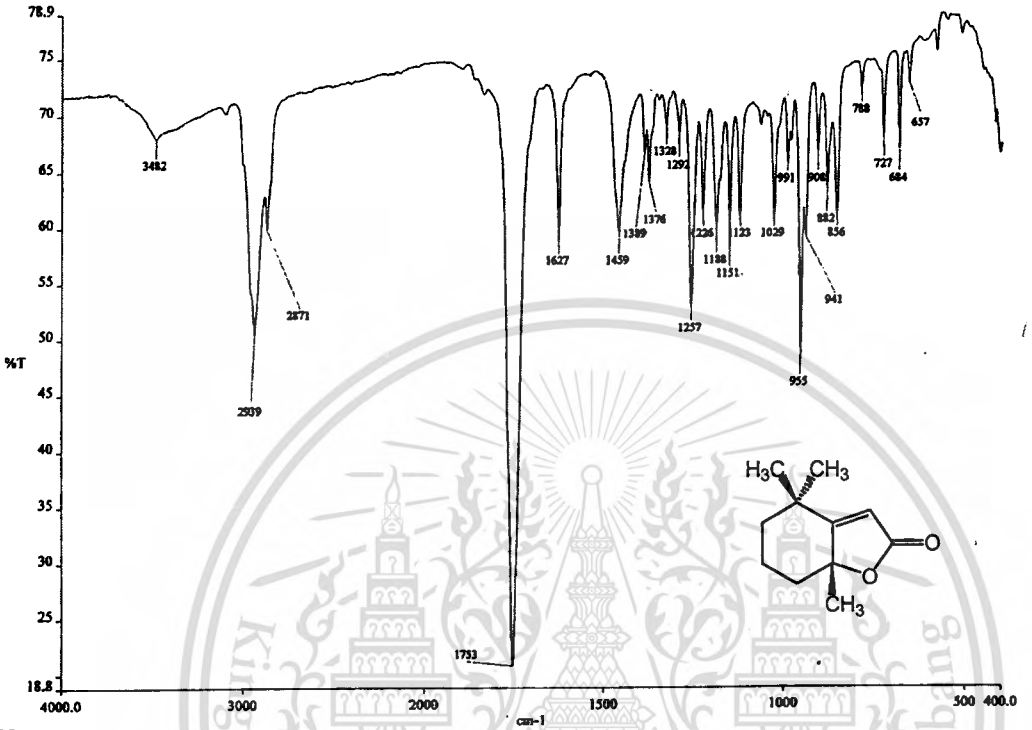
X = concentration (ppm)

STD = standard fatty acid methyl esters

IN = internal standard (methyl heptadecanoate)

## Appendix B: IR, ESMS and NMR spectra of alleochemical compounds

(a) IR




(b) MS

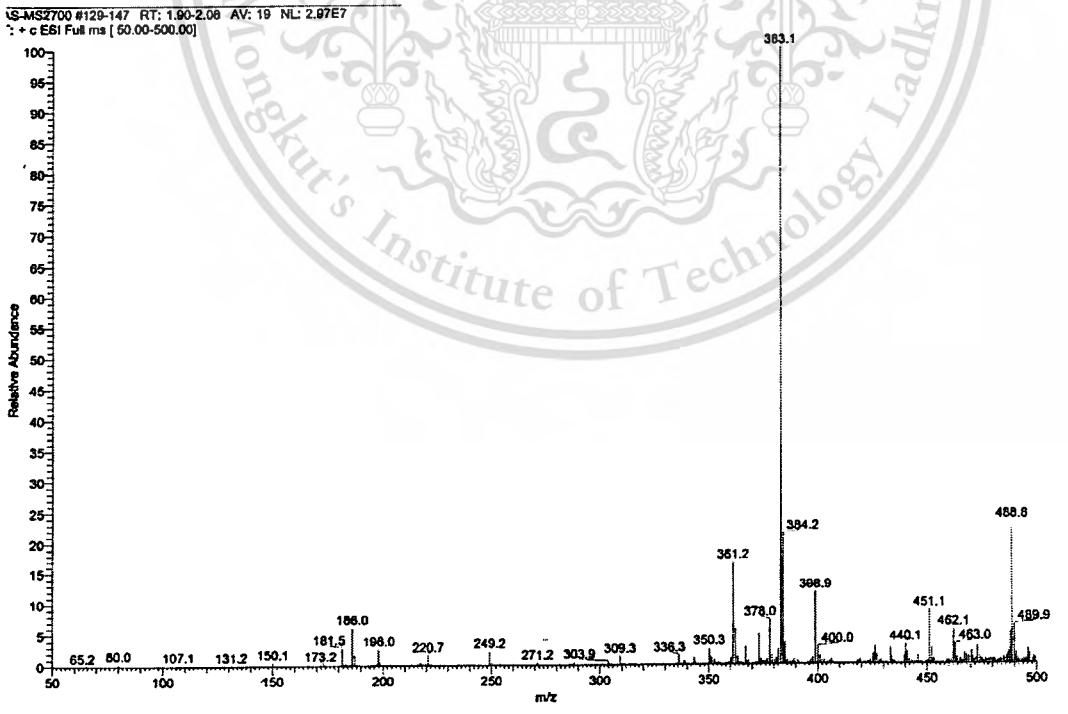
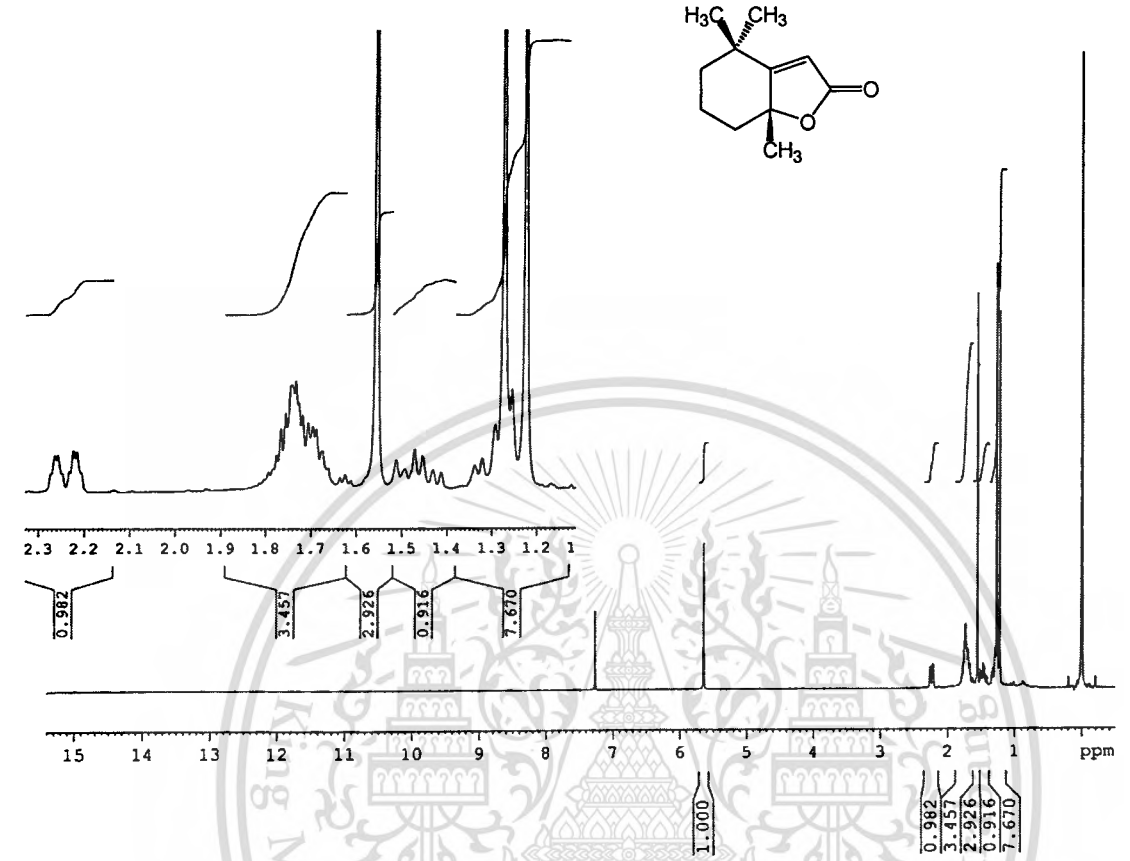



**Figure B1** (a) IR and (b) ESMS spectra of dihydroactinidiolide **51**

(a) $^{1}$H NMR




(b) $^{13}$C NMR

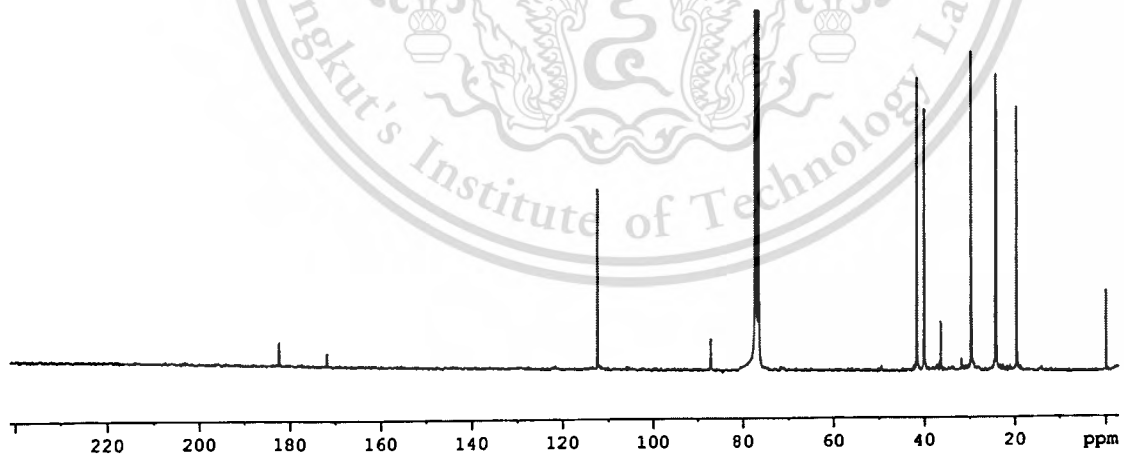
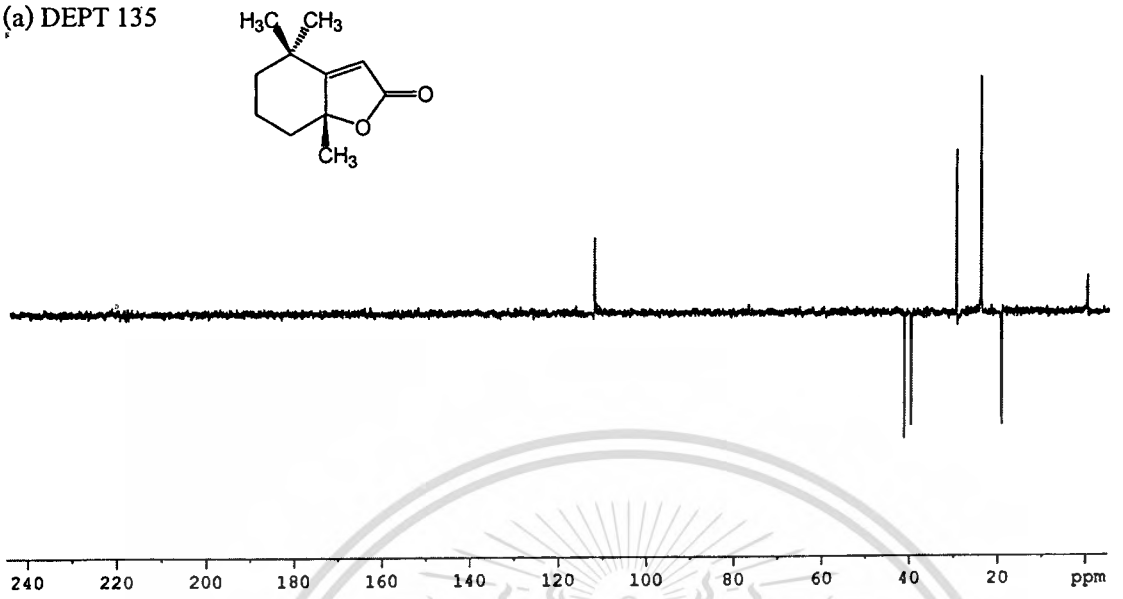



**Figure B2** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of dihydroactinidiolide **51** in $CDCl_3$

(a) DEPT 135




(b) COSY

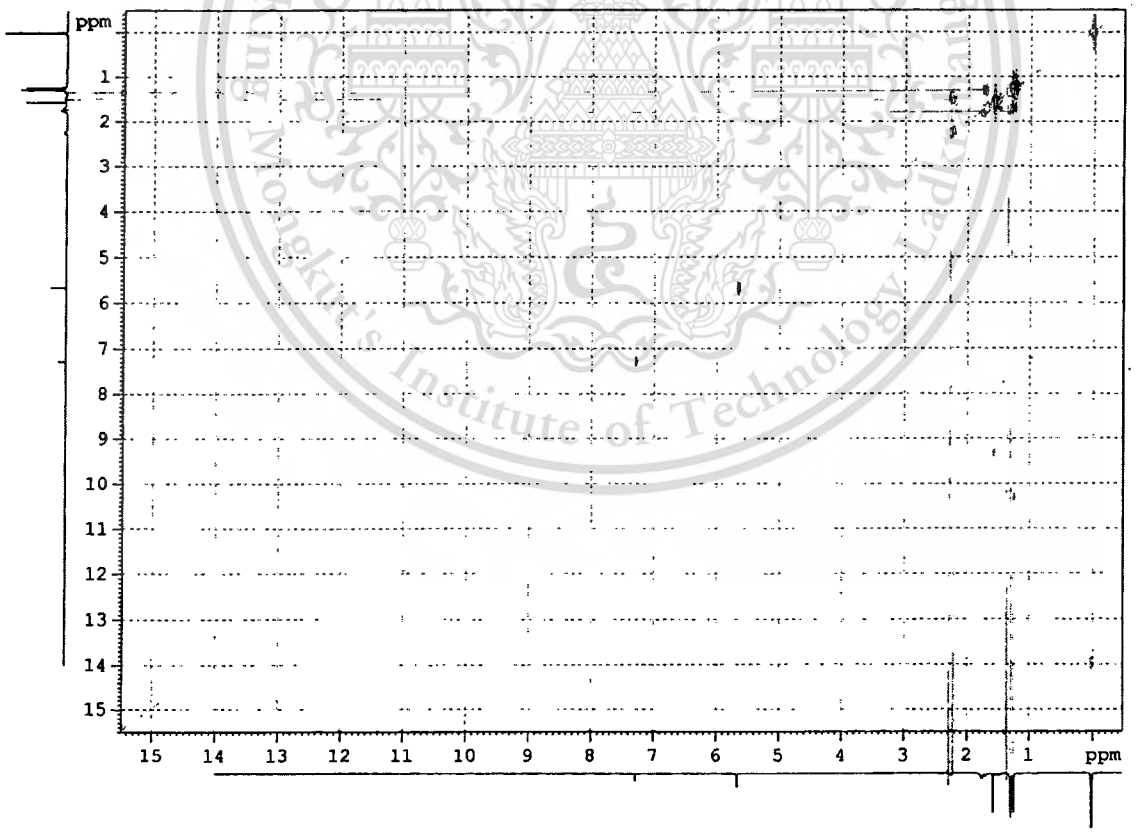
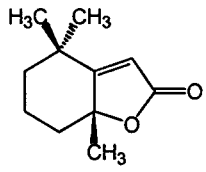



**Figure B3** (a) DEPT 135 and (b) COSY spectra of dihydroactinidiolide **51** in $CDCl_3$



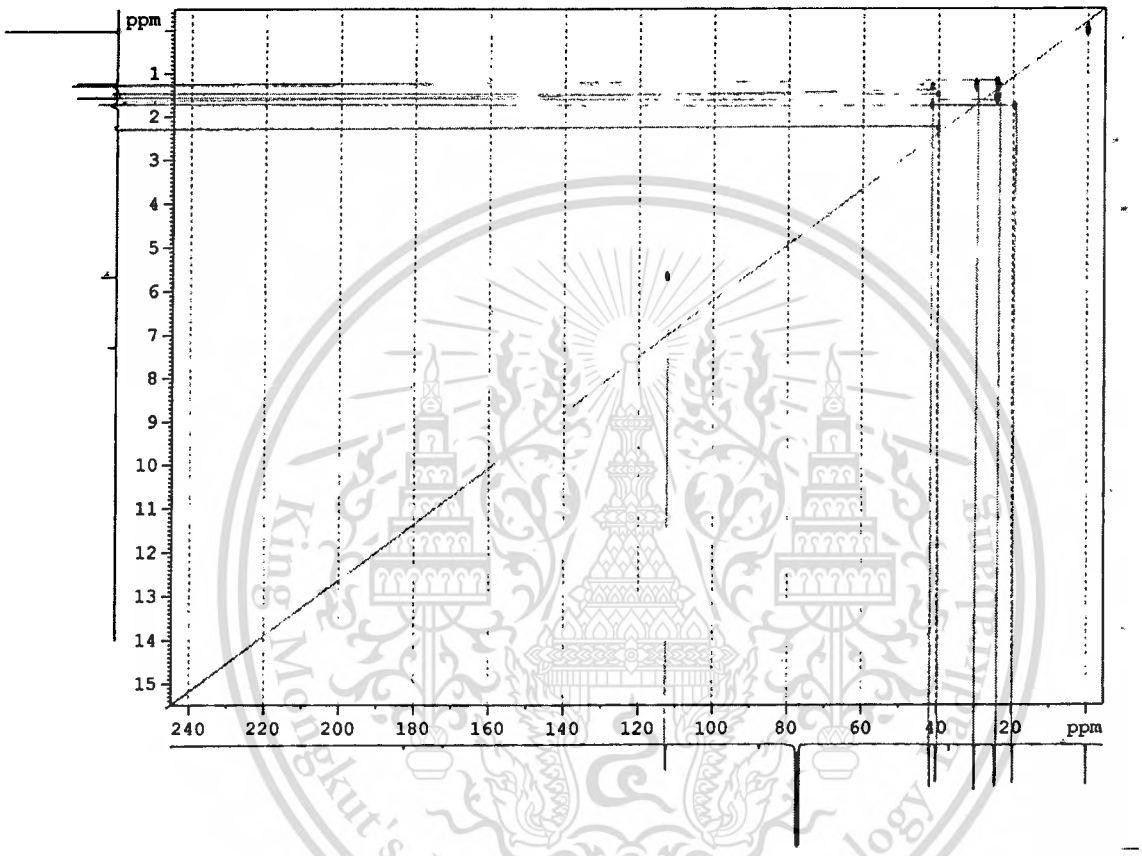
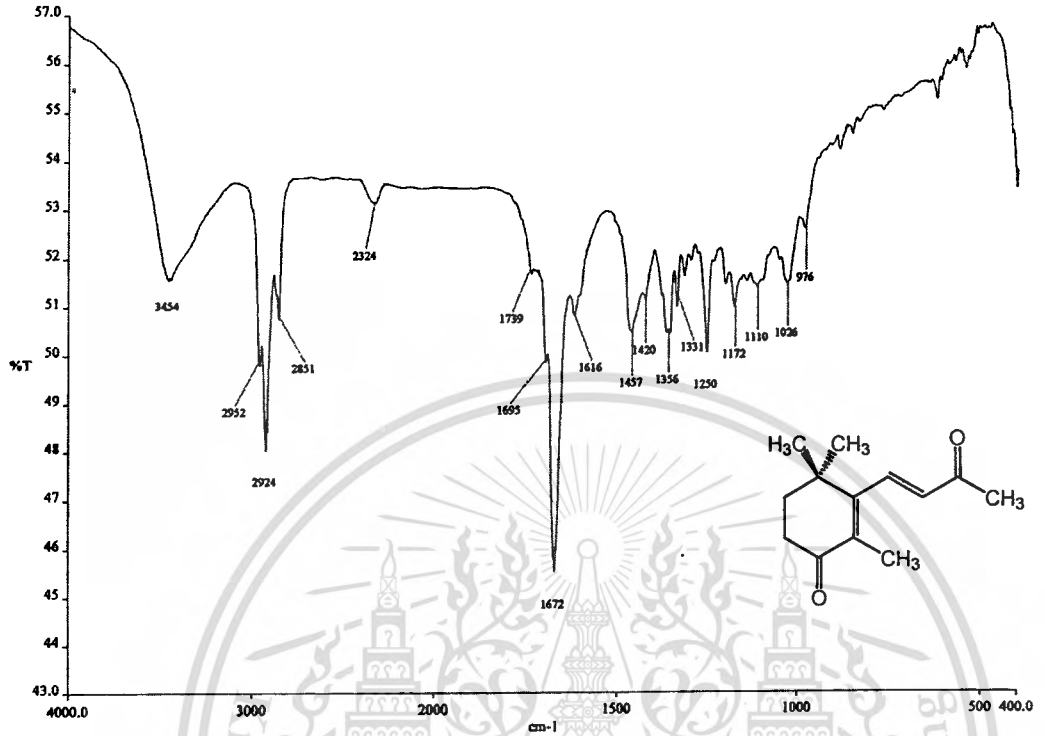




**Figure B4** HETCOR spectrum of dihydroactinidiolide **51** in $CDCl_3$

(a) IR




(b) MS

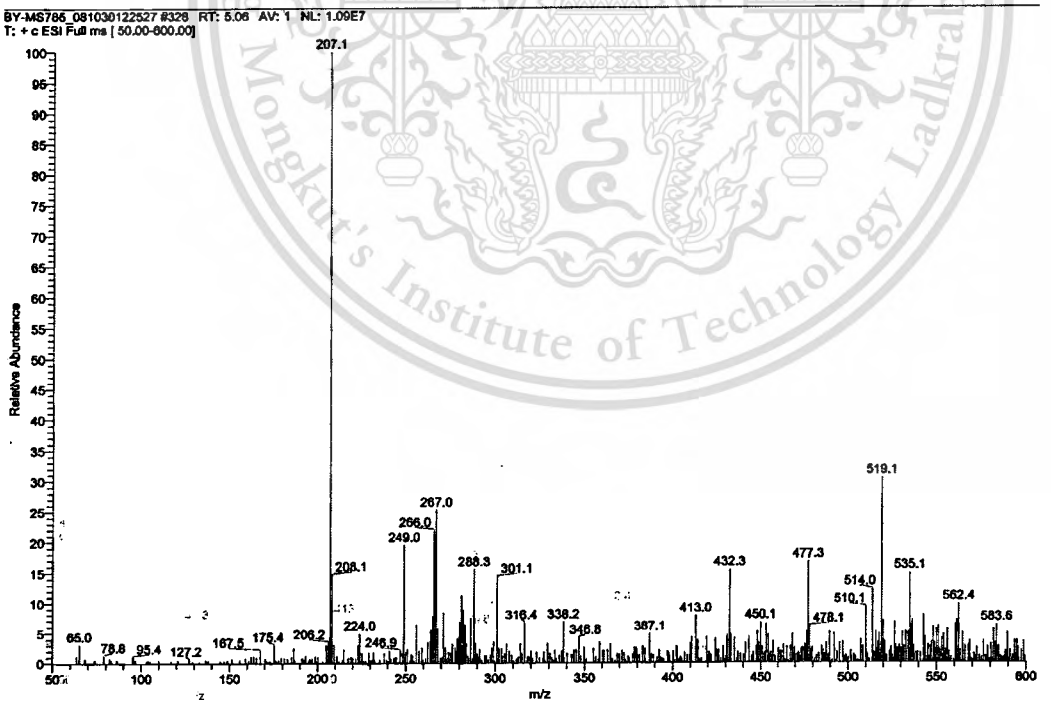



**Figure B5** (a) IR and (b) ESMS spectra of 4-oxo-β-ionone **52**

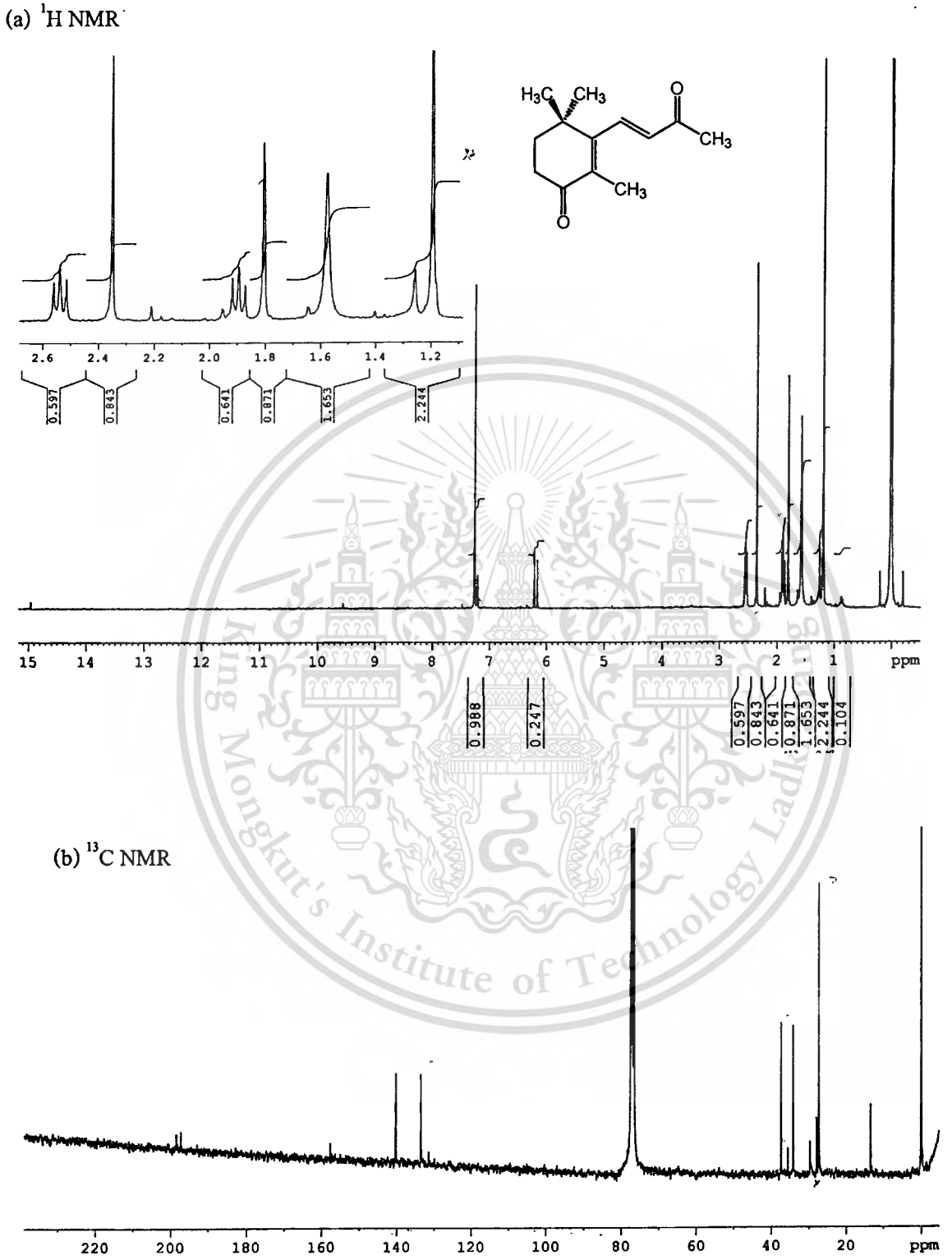
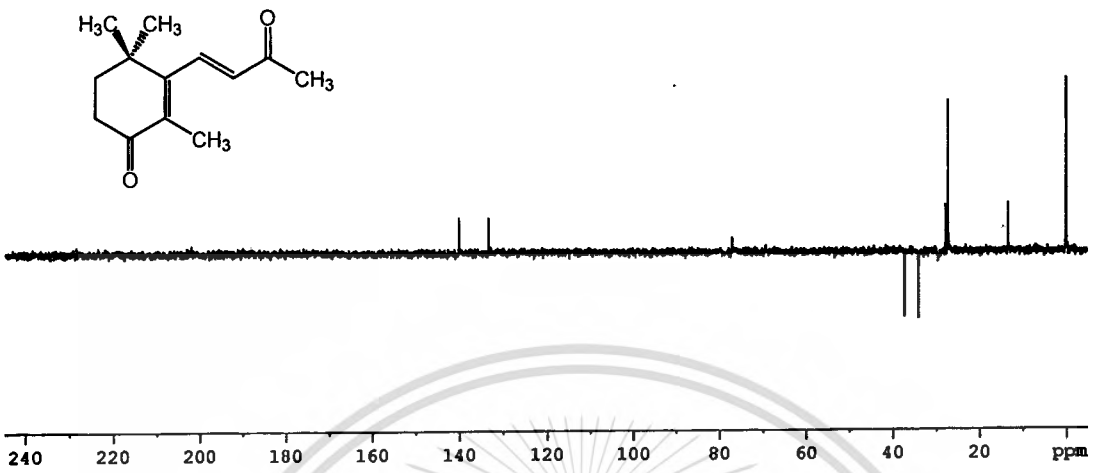



**Figure B6** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 4-oxo-β-ionone **52** in $CDCl_3$

(a) DEPT 135




(b) COSY

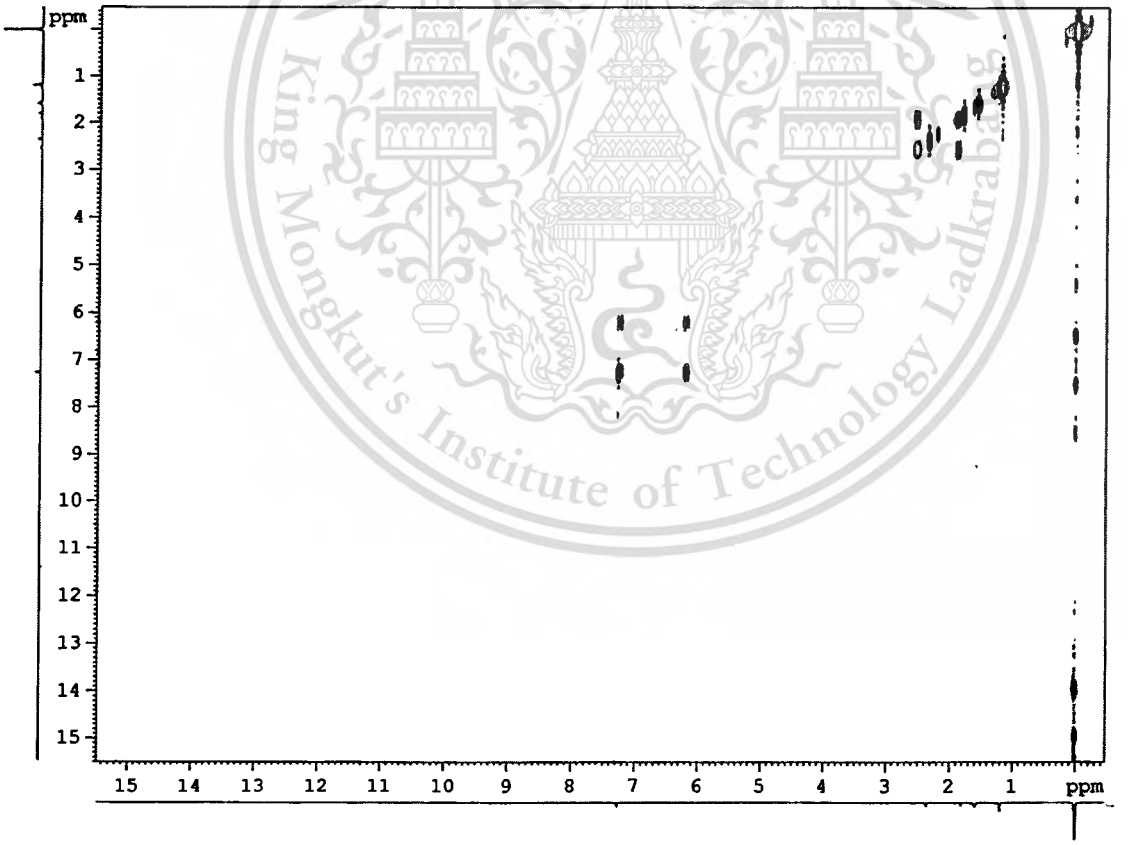
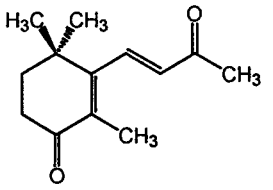



**Figure B7** (a) DEPT 135 and (b) COSY spectra of 4-oxo-β-ionone **52** in $CDCl_3$



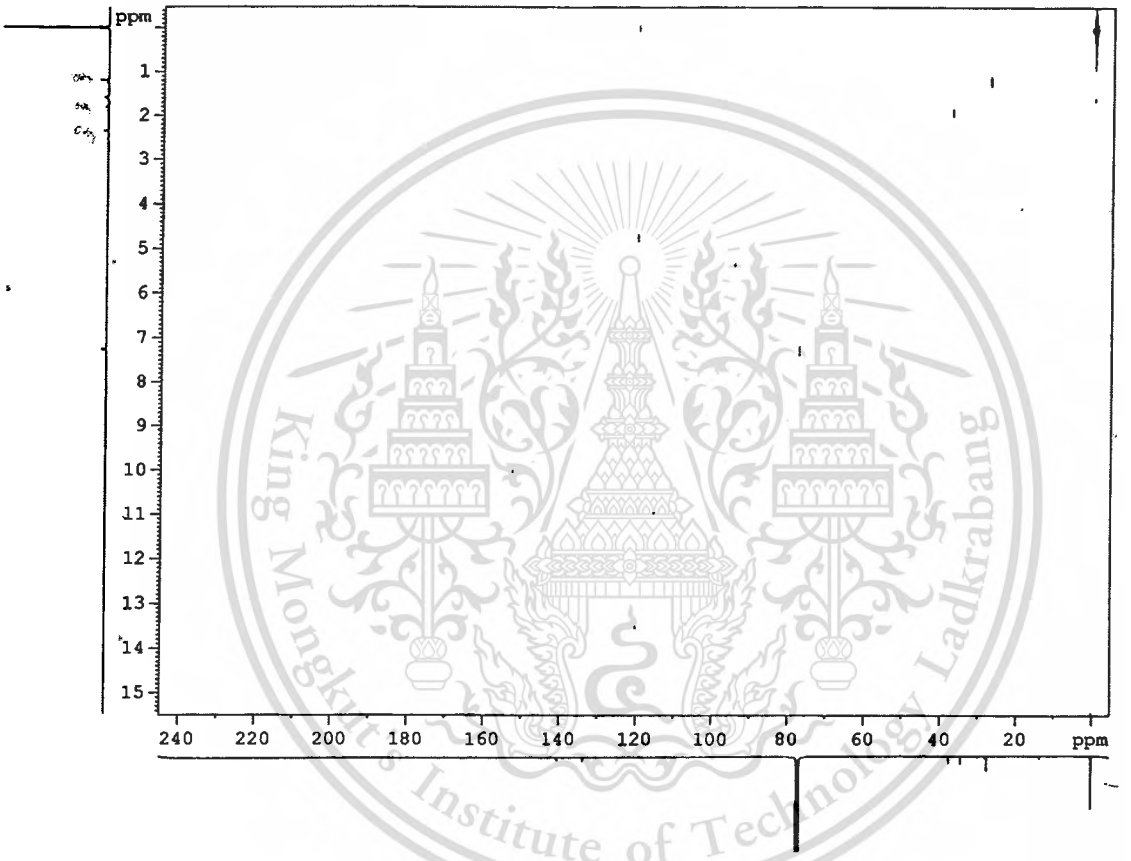
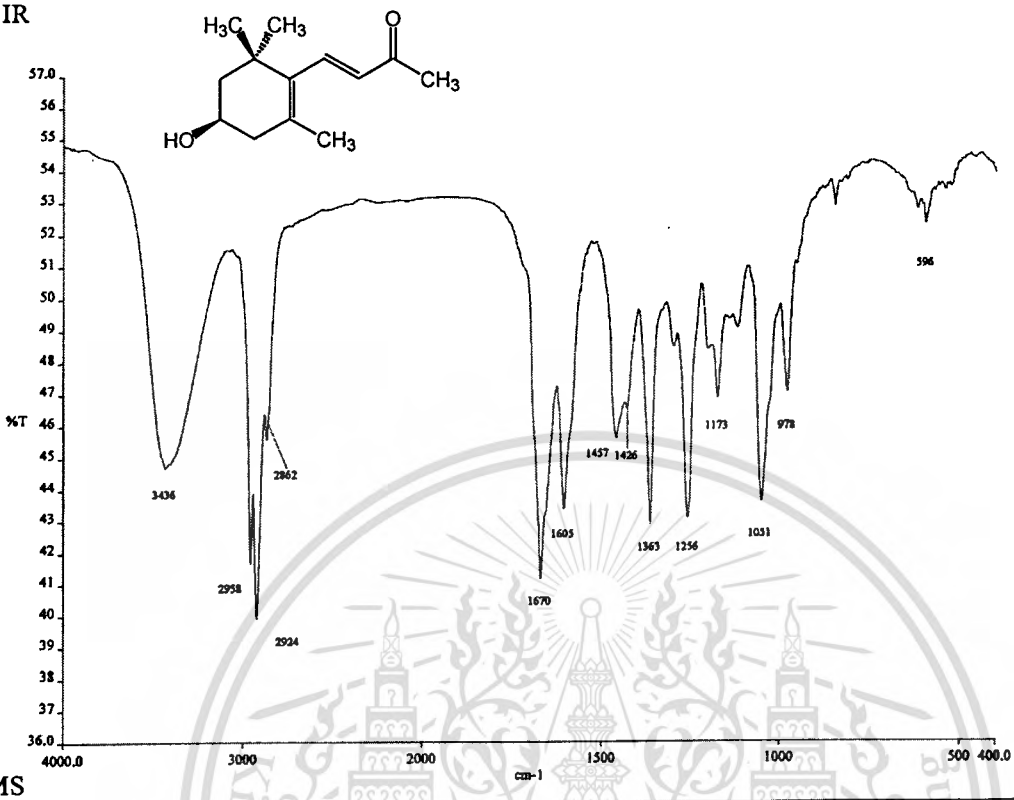




**Figure B8** HETCOR spectrum of 4-oxo-β-ionone **52** in $CDCl_3$

(a) IR




(b) MS

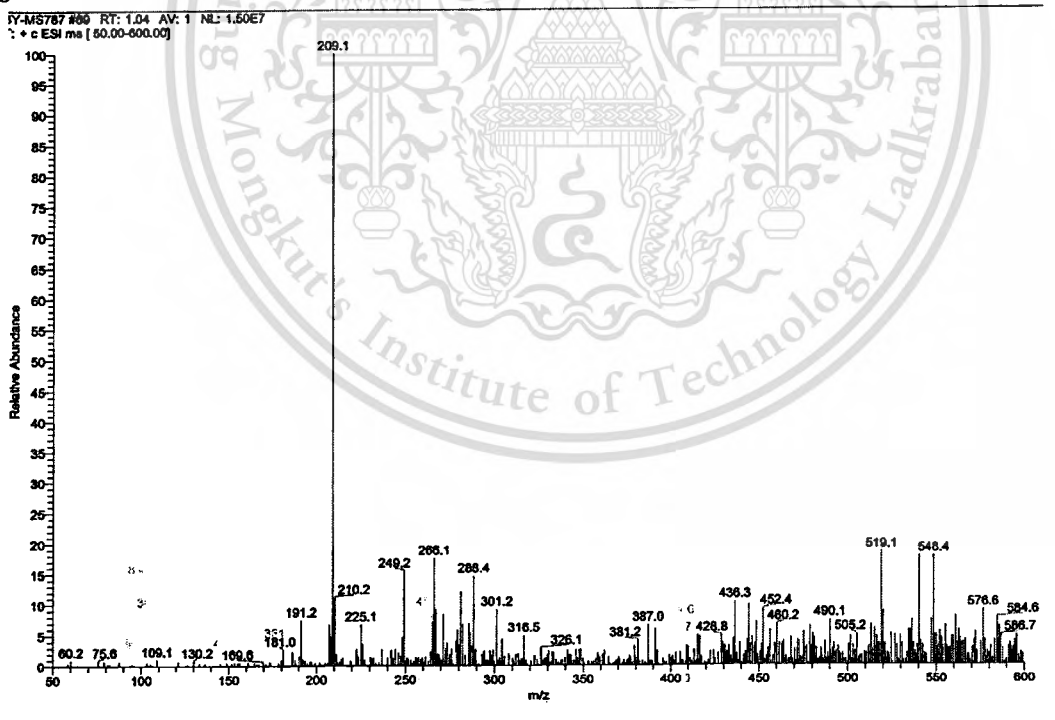
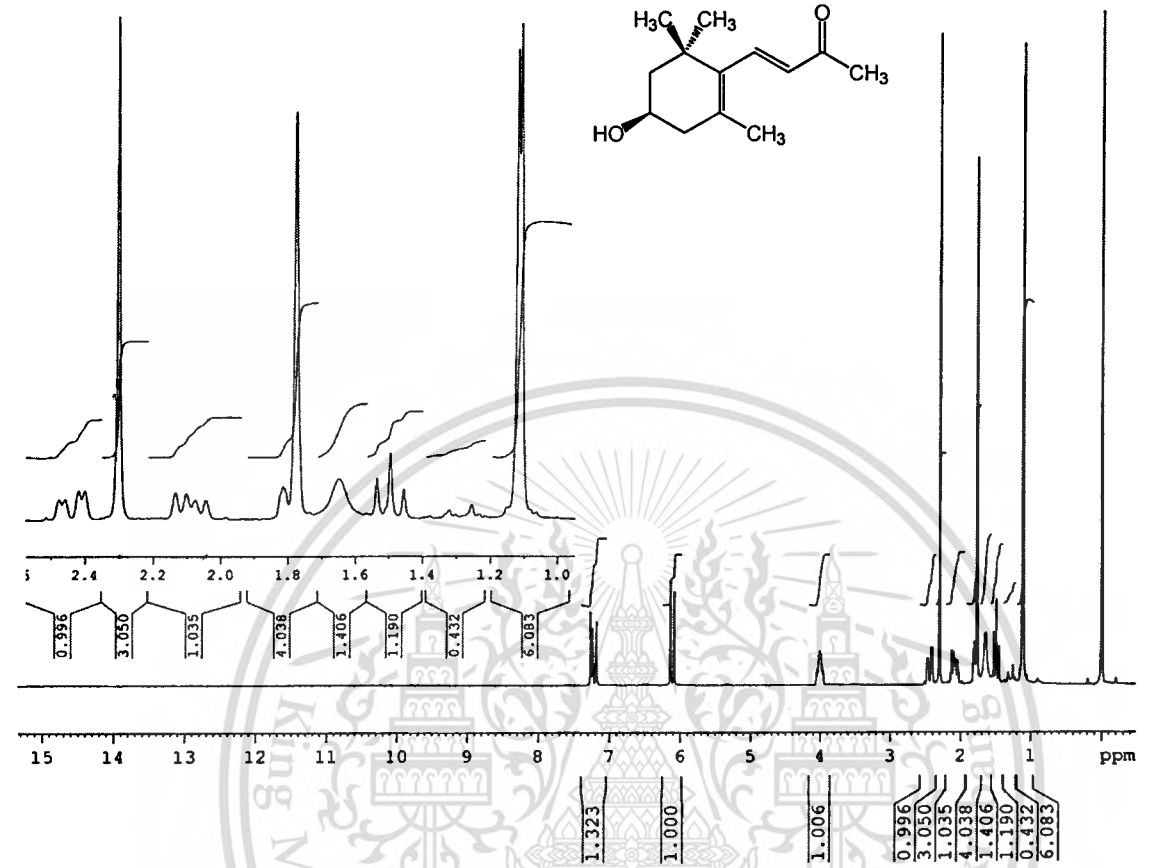



**Figure B9** (a) IR and (b) ESMS spectra of 3-hydroxy-β-ionone **59**

(a) $^{1}$H NMR




(b) $^{13}$C NMR

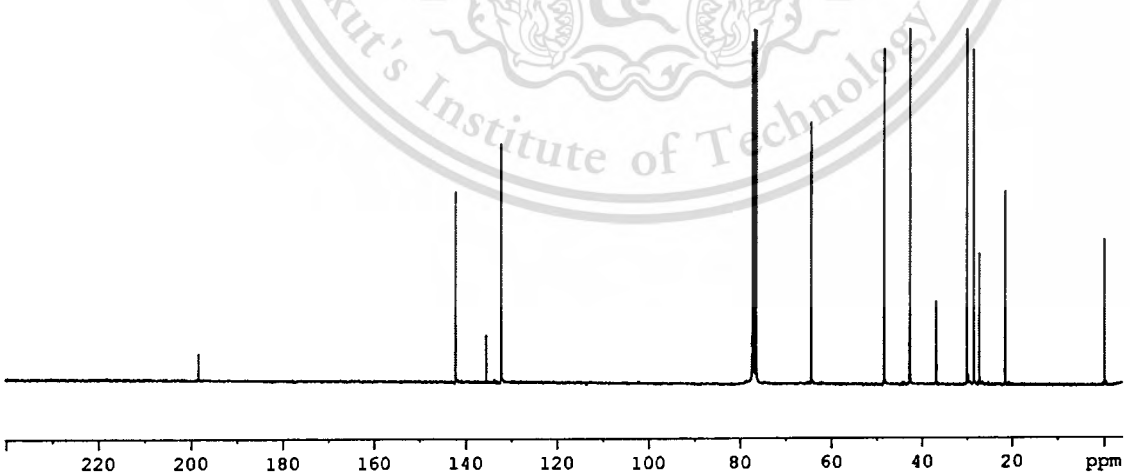
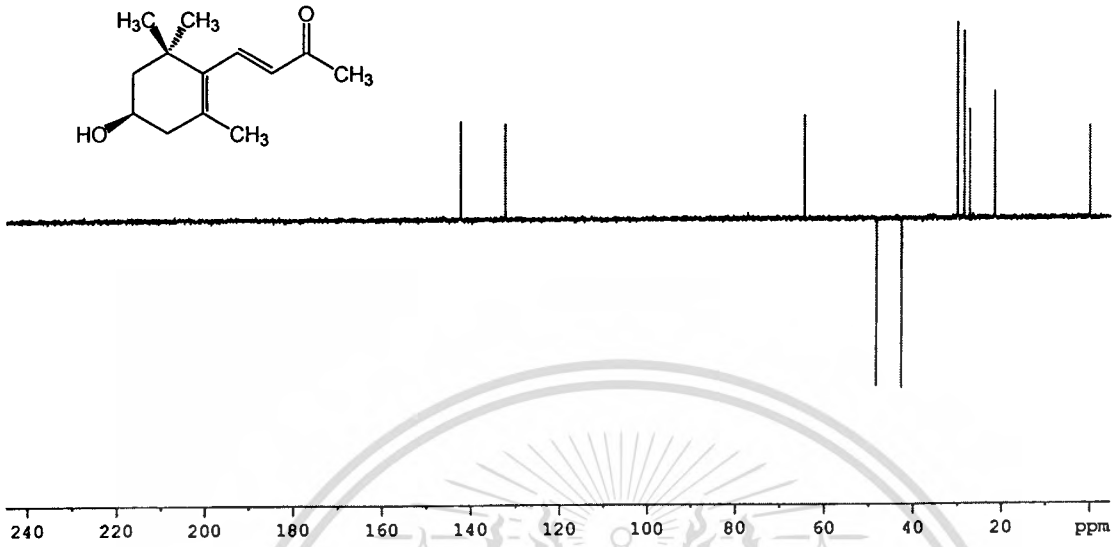



**Figure B10** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 3-hydroxy-β-ionone **59** in $CDCl_3$

(a) DEPT 135




(b) COSY

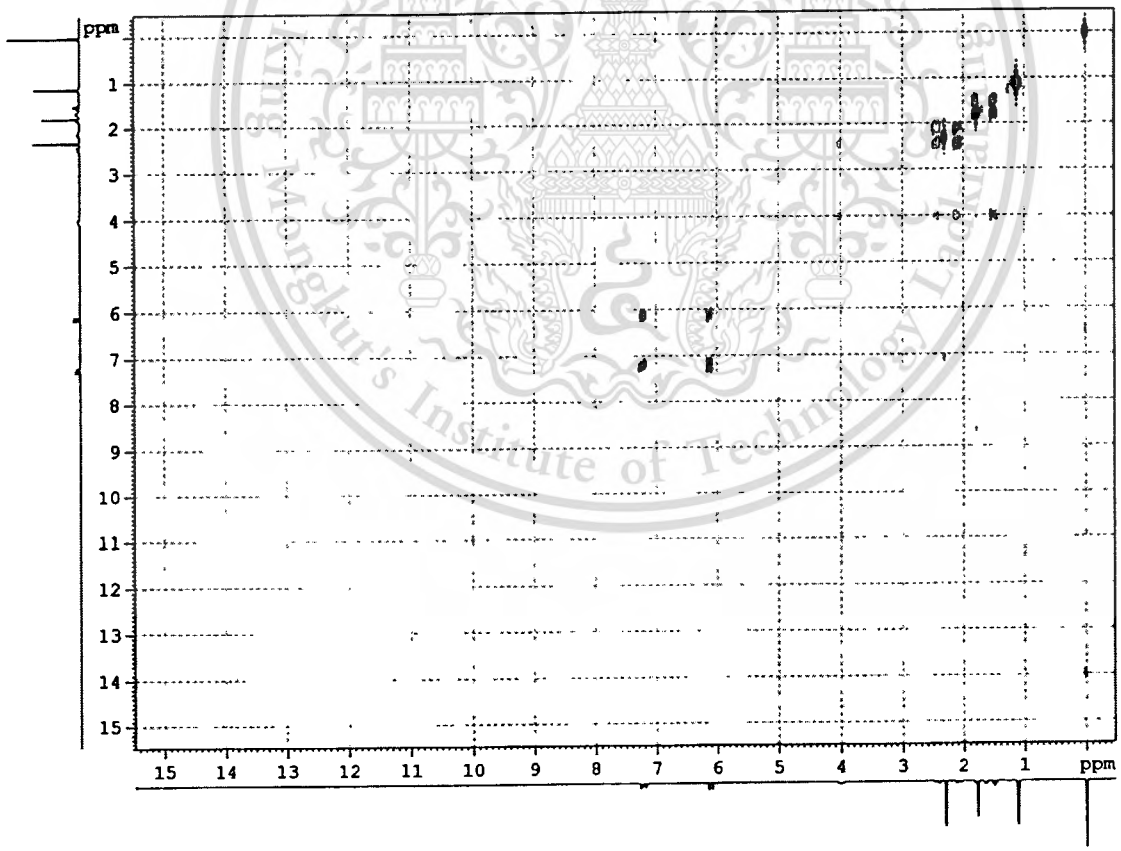
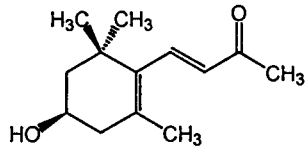



**Figure B11** (a) DEPT 135 and (b) COSY spectra of 3-hydroxy-β-ionone **59** in $CDCl_3$



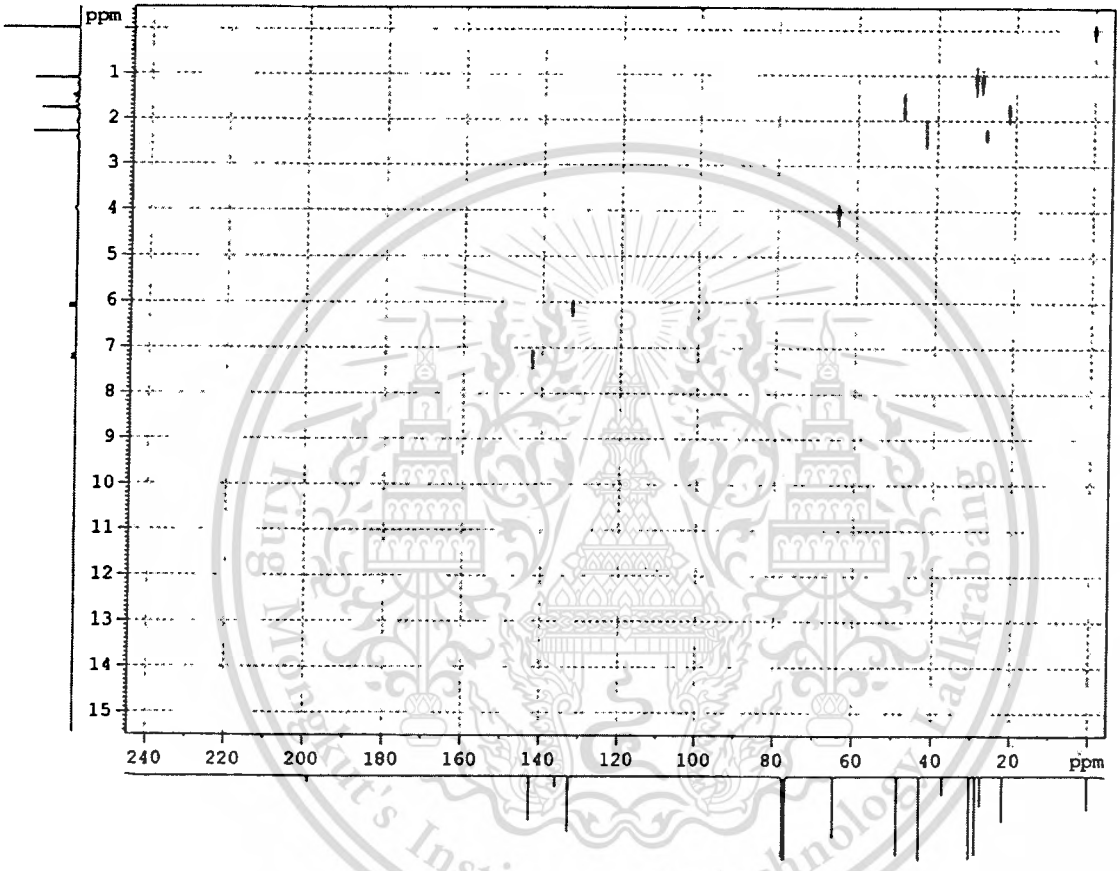
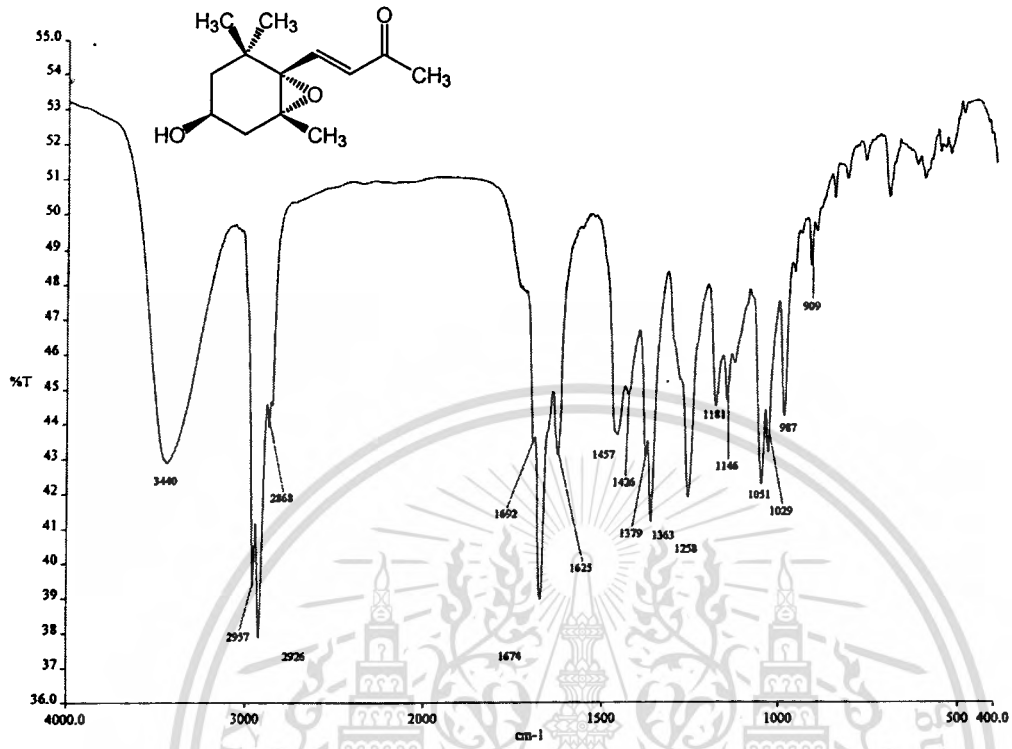




**Figure B12** HETCOR spectrum of 3-hydroxy-β-ionone **59** in $CDCl_3$

(a) IR




(b) MS

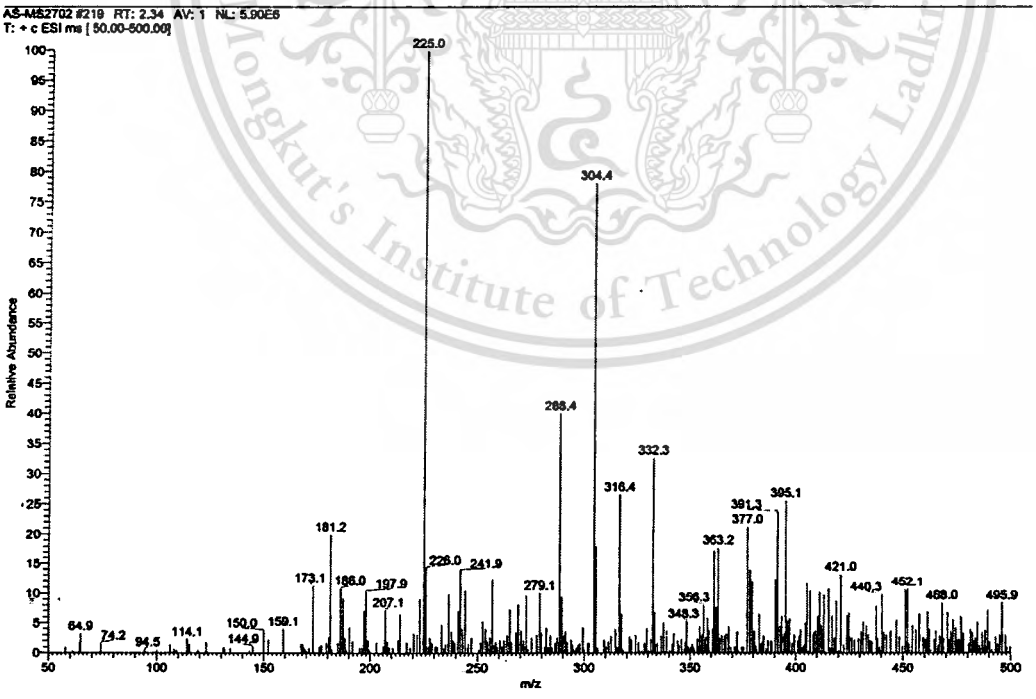
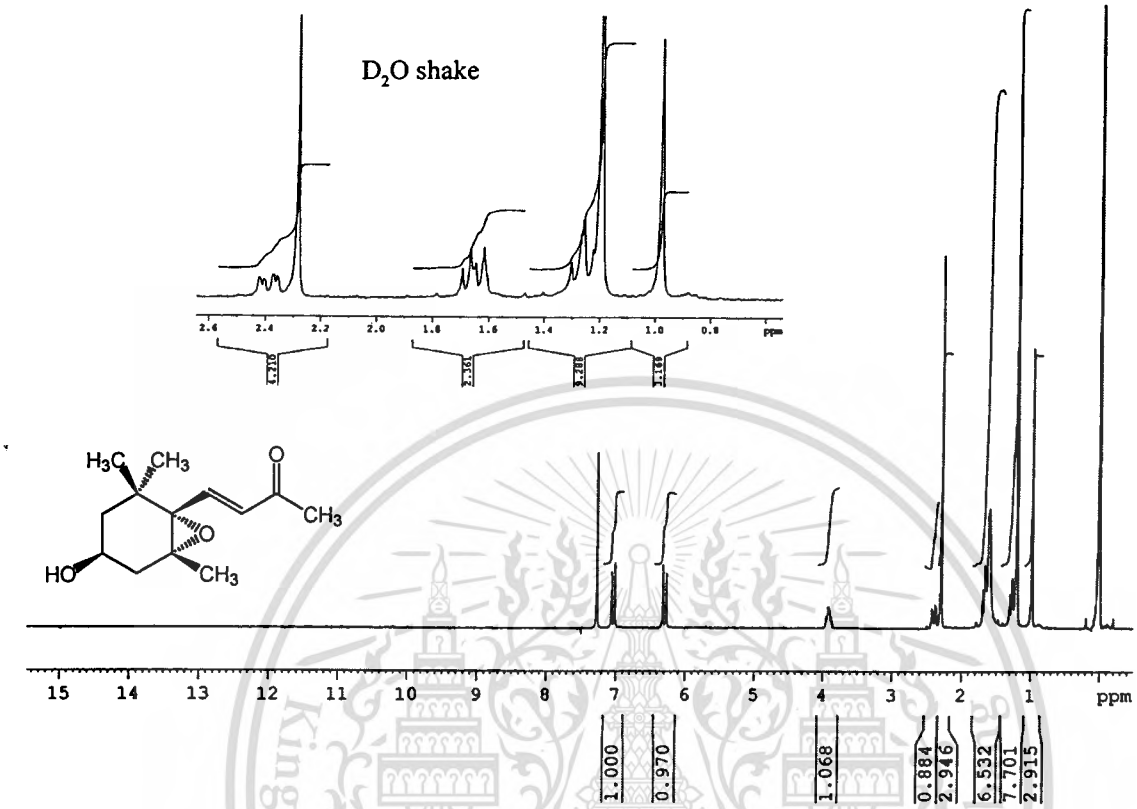



**Figure B13** (a) IR and (b) ESMS spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56**

(a)$^{1}$H NMR




(b) $^{13}$C NMR

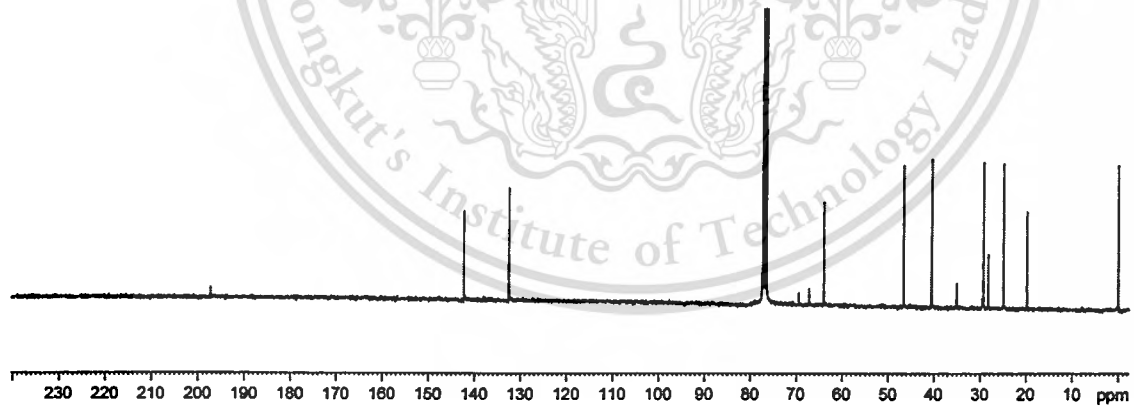
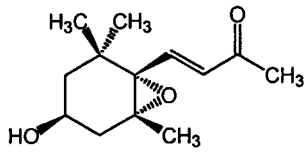
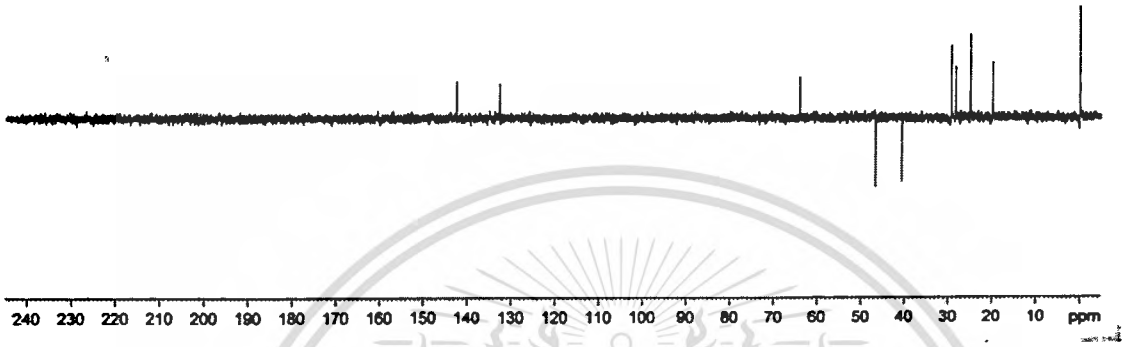



**Figure B14** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$

(a) DEPT 135




(b) COSY

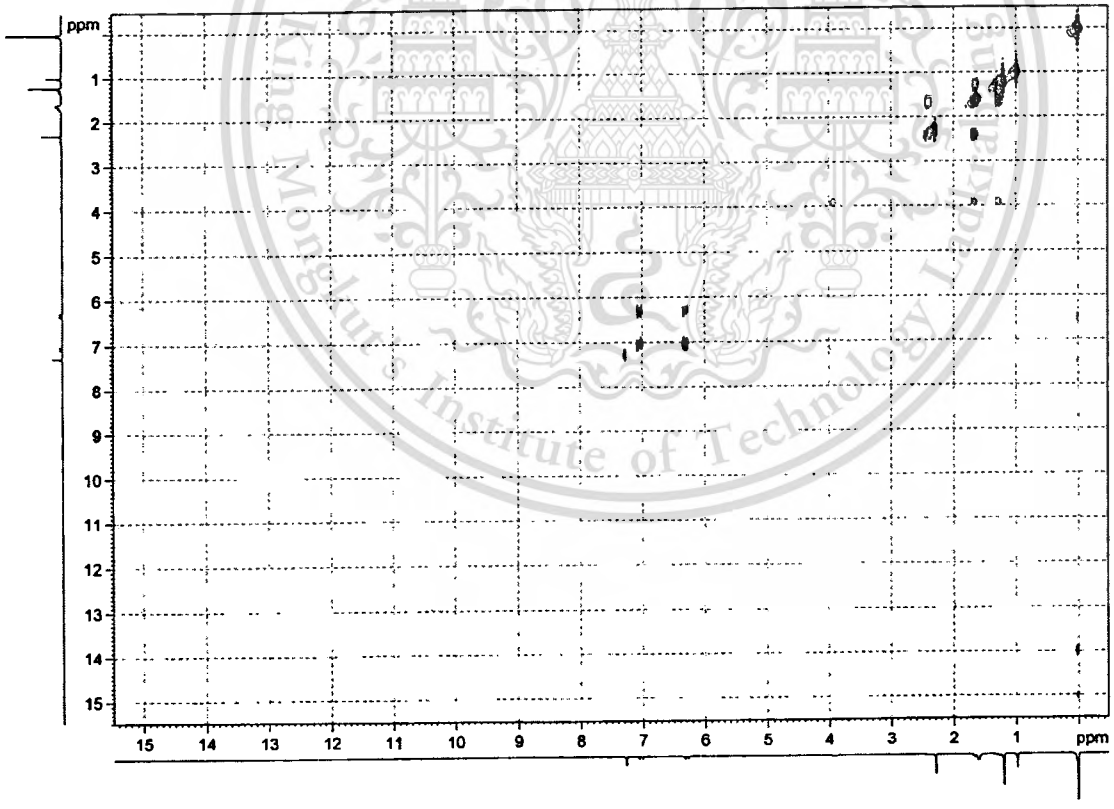
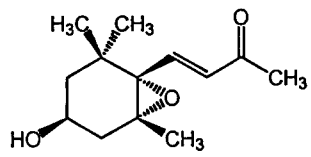



**Figure B15** (a) DEPT 135 and (b) COSY spectra of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$



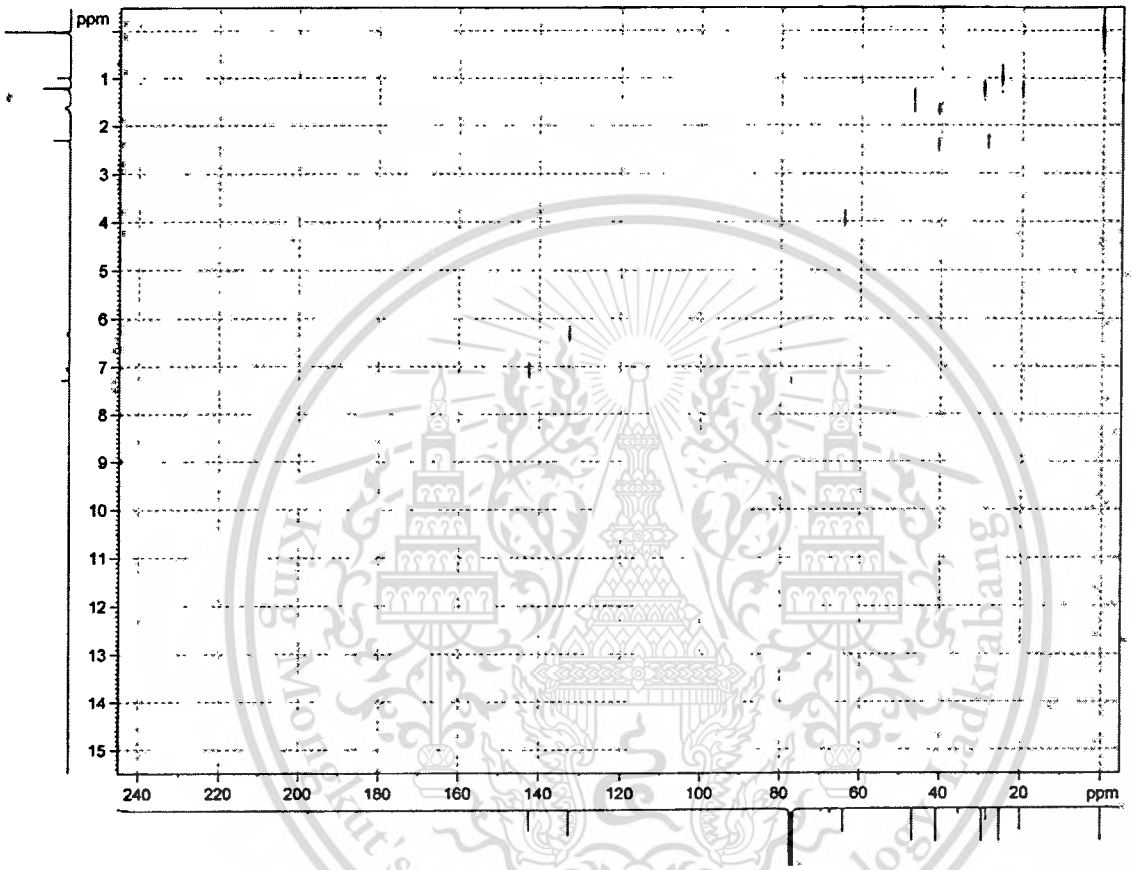
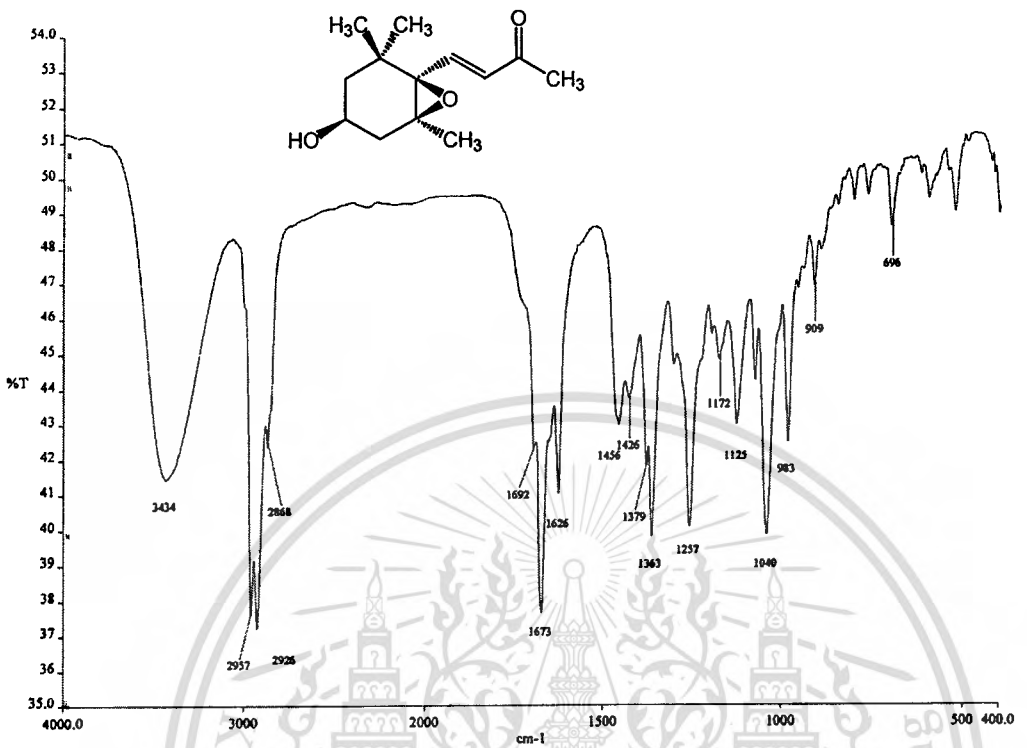




**Figure B16** HETCOR spectrum of 3-hydroxy-5α, 6α-epoxy-β-ionone **56** in $CDCl_3$

(a) IR




(b) MS

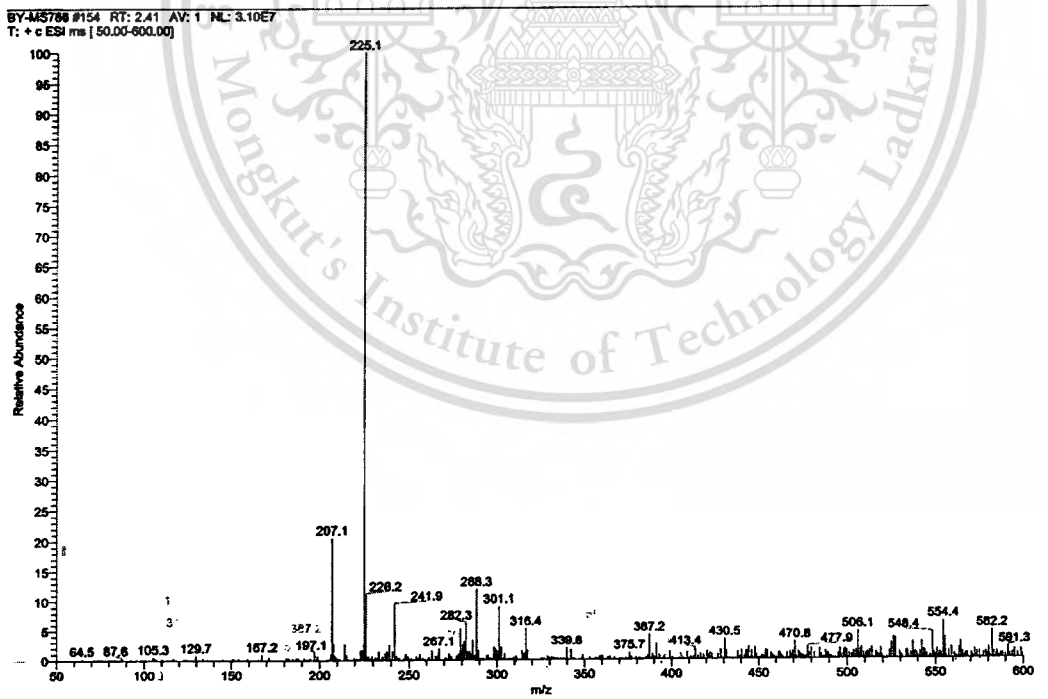



**Figure B17** (a) IR and (b) ESMS spectra of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**

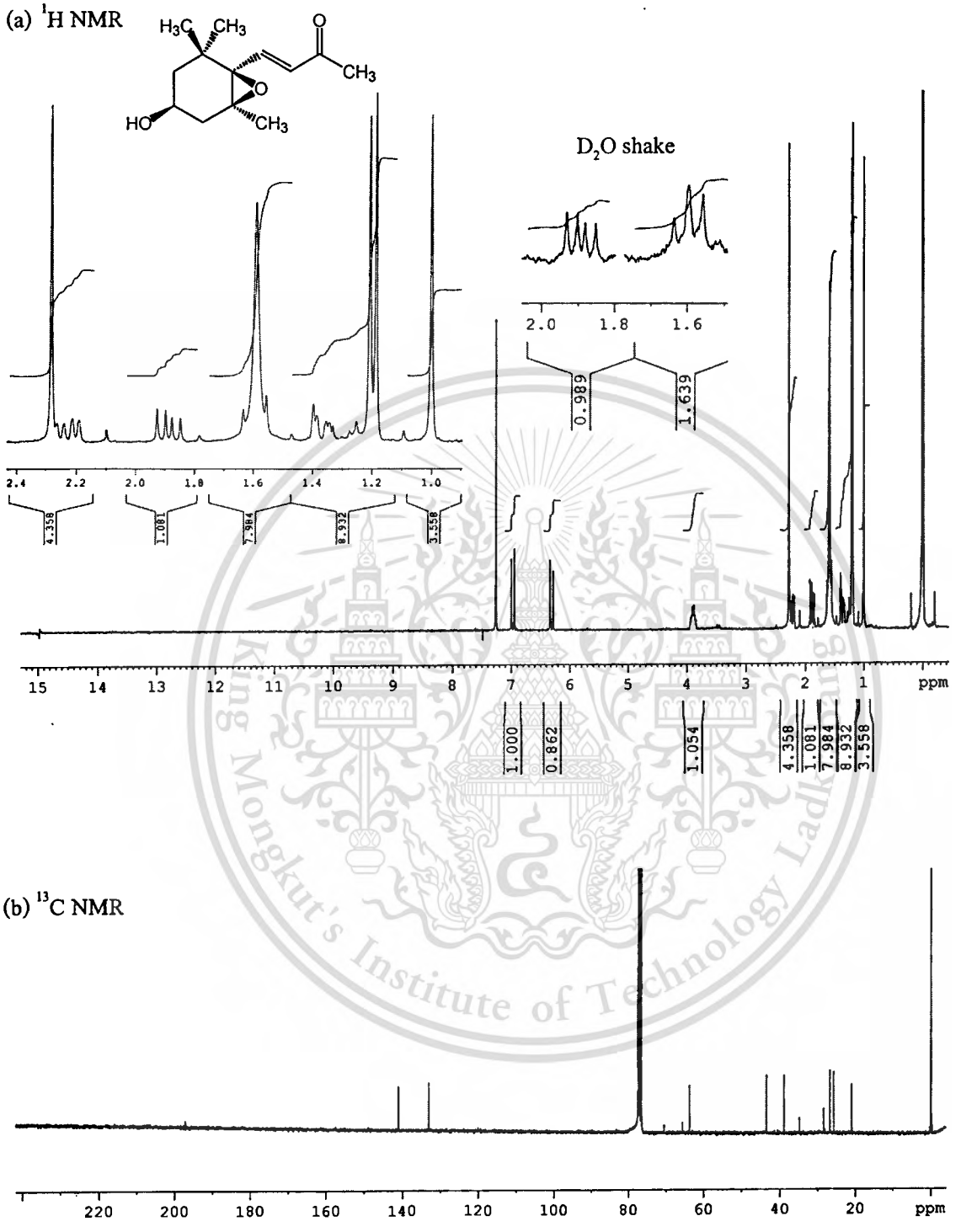
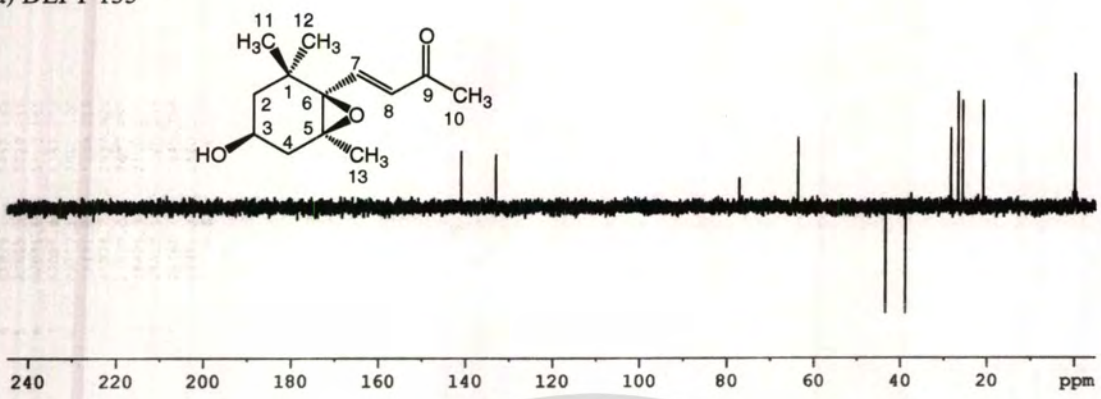



**Figure B18** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$

(a) DEPT 135




(b) COSY

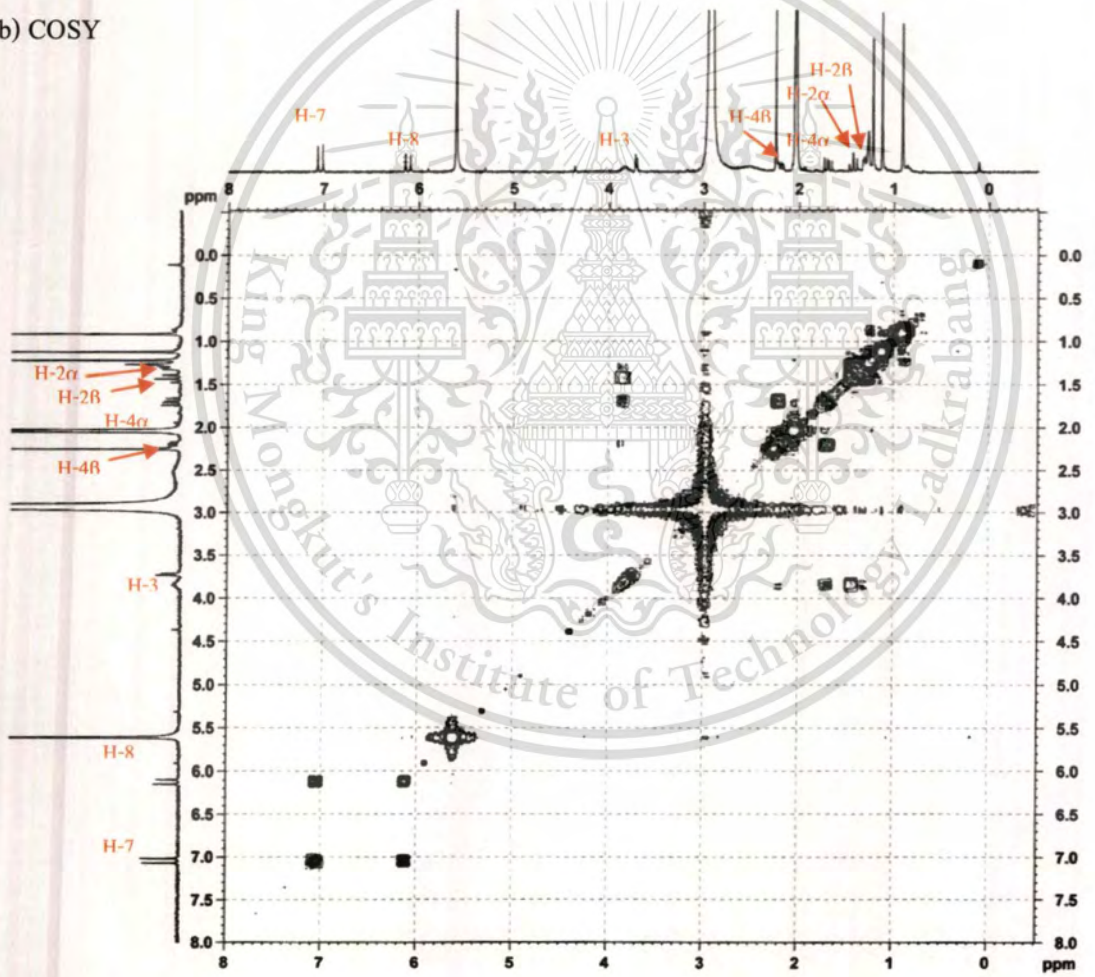
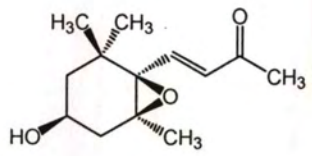



**Figure B19** (a) DEPT 135 spectrum in $CDCl_3$ and (b) COSY spectrum in $CD_3COCD_3$ of 3-hydroxy-5β, 6β-epoxy-β-ionone **57**



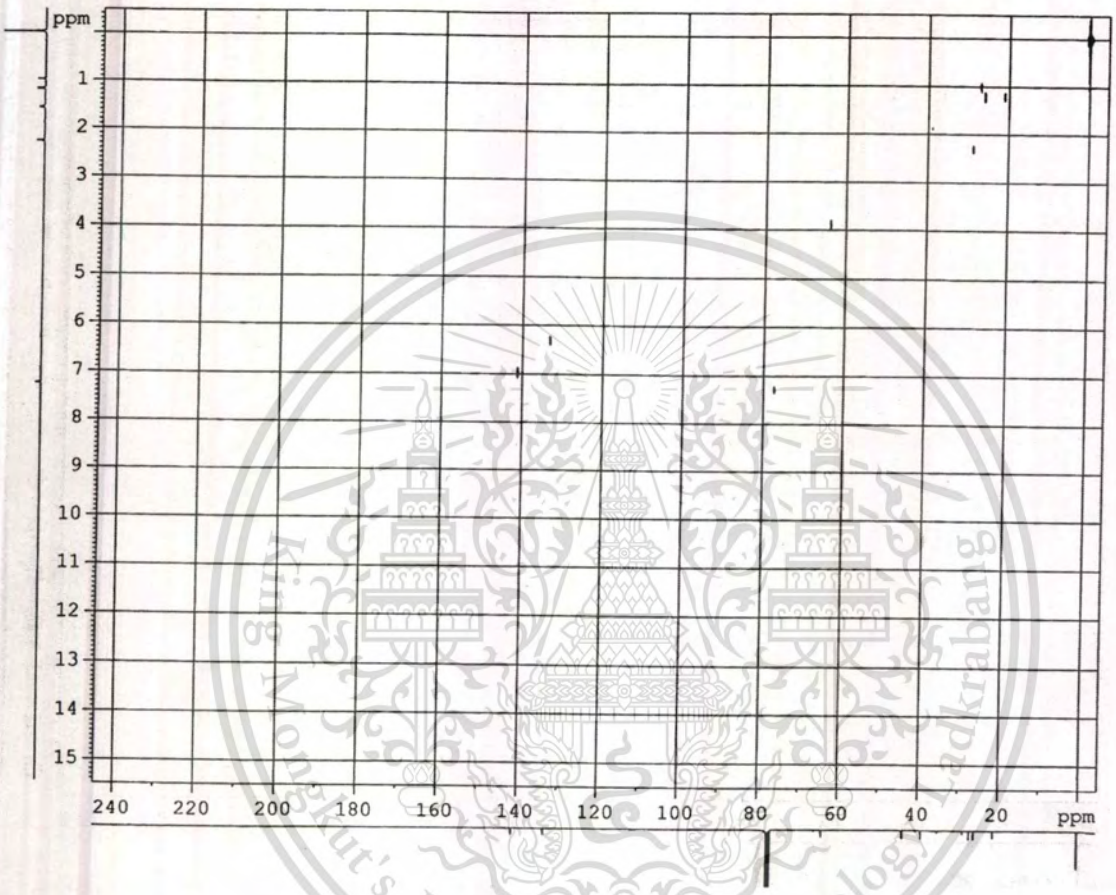




**Figure B20** HETCOR spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CDCl_3$

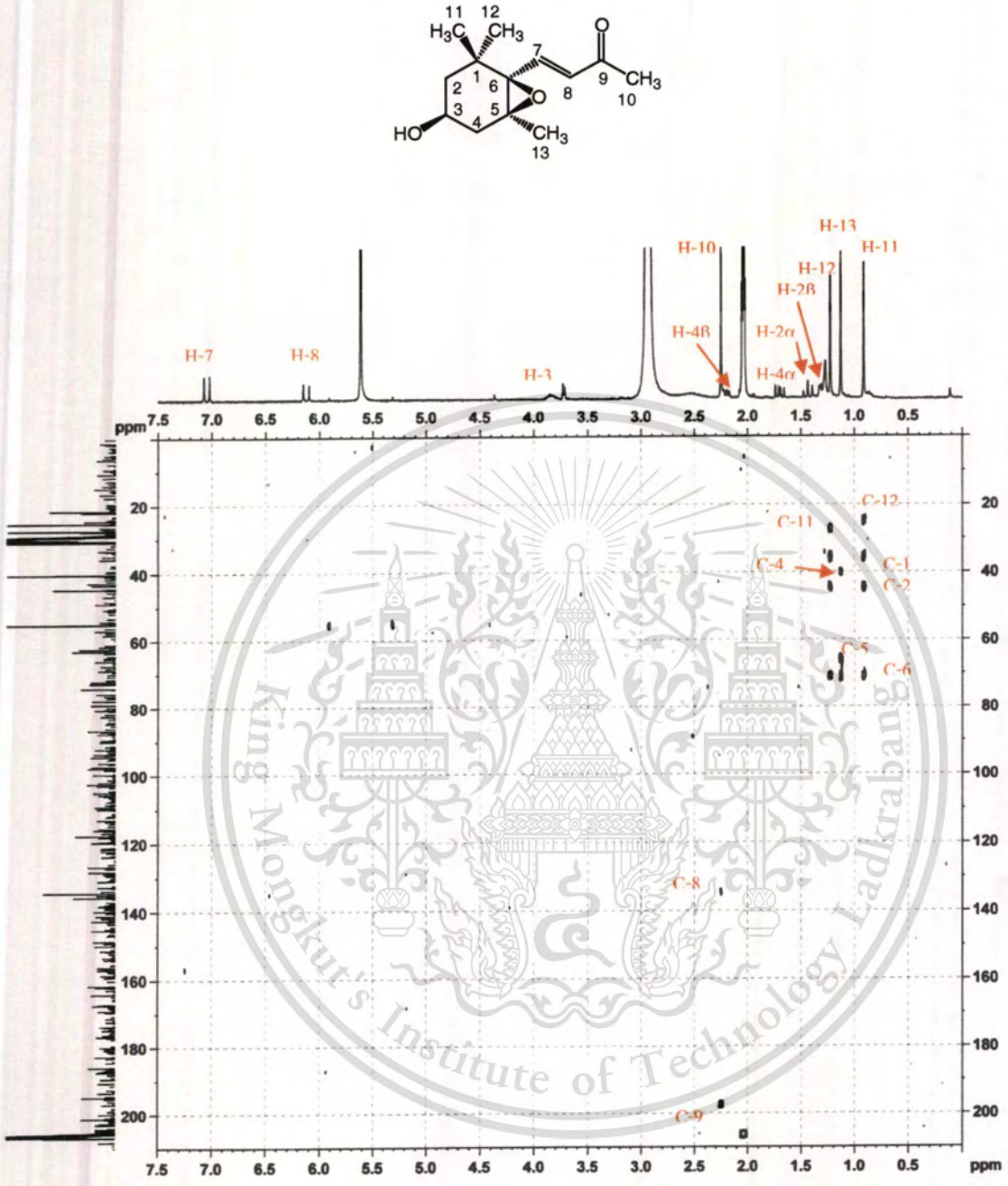
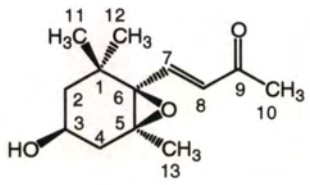



**Figure B21** HMBC spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CD_3COCD_3$



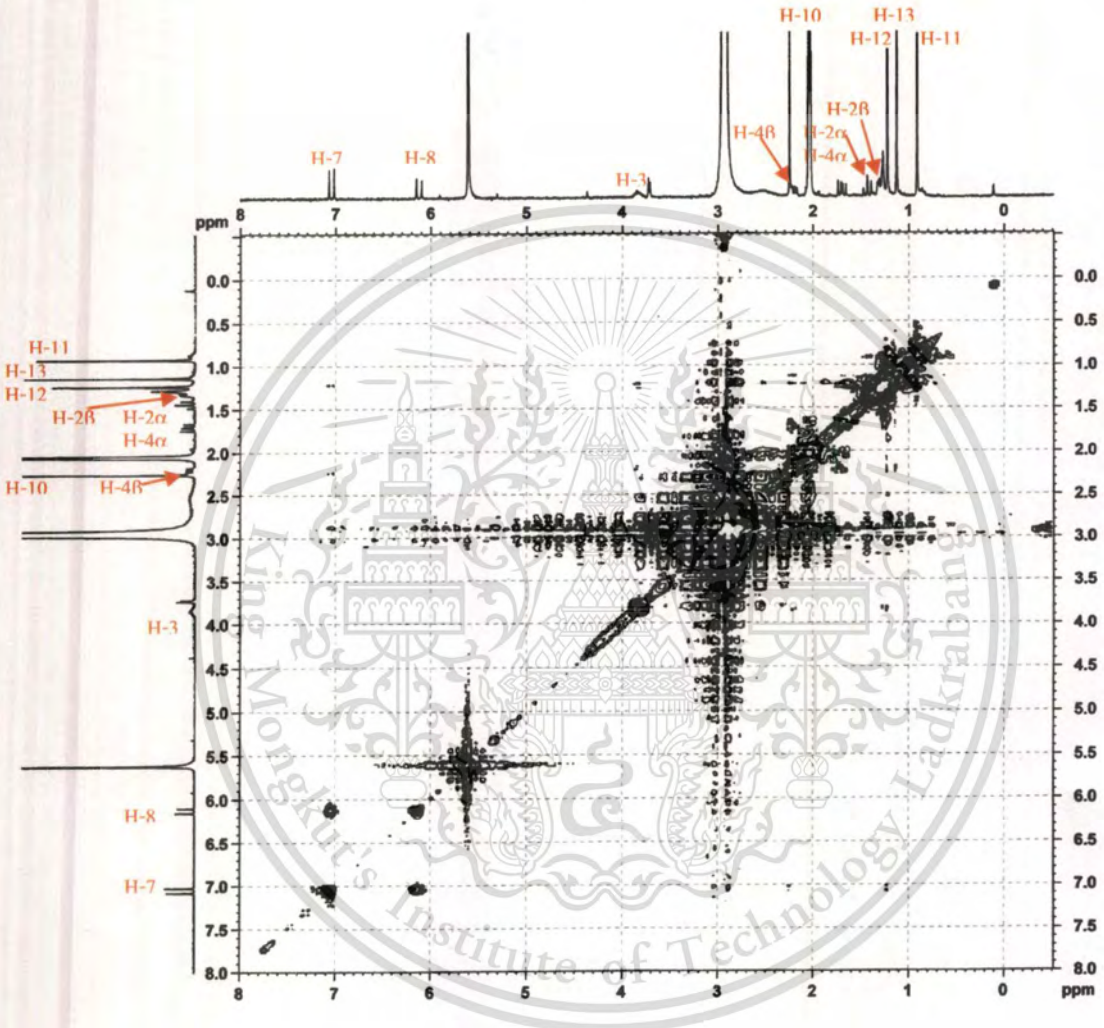
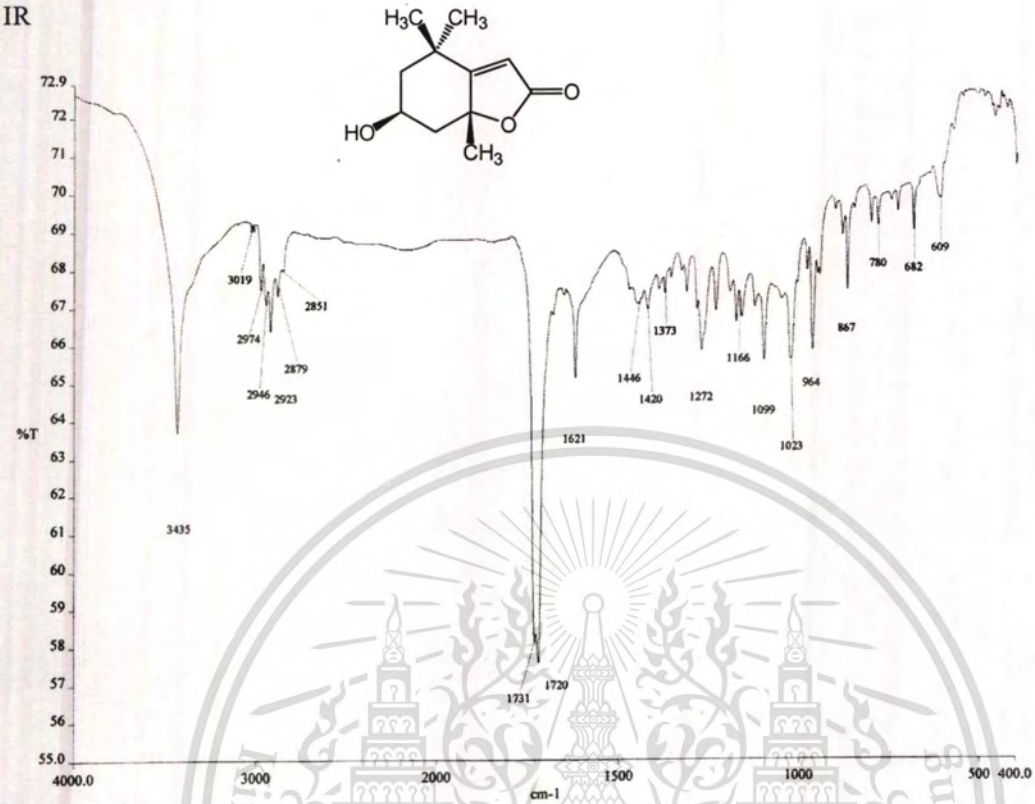




**Figure B22** NOESY spectrum of 3-hydroxy-5β, 6β-epoxy-β-ionone **57** in $CD_3COCD_3$

(a) IR




(b) MS

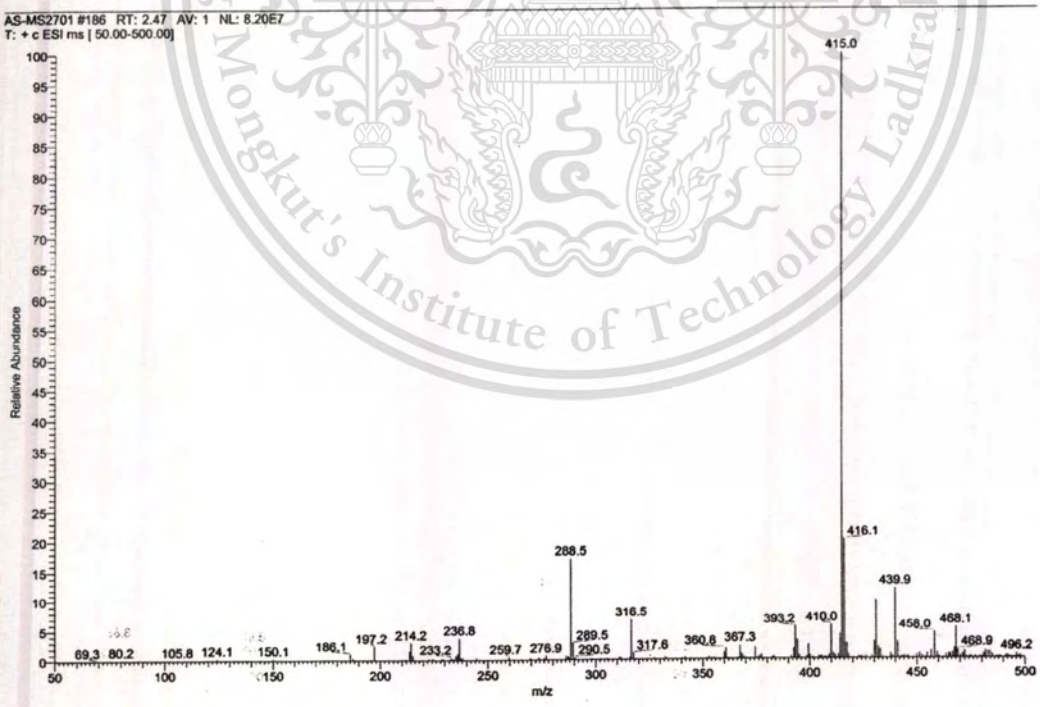



**Figure B23** (a) IR and (b) ESMS spectra of loliolide **58**

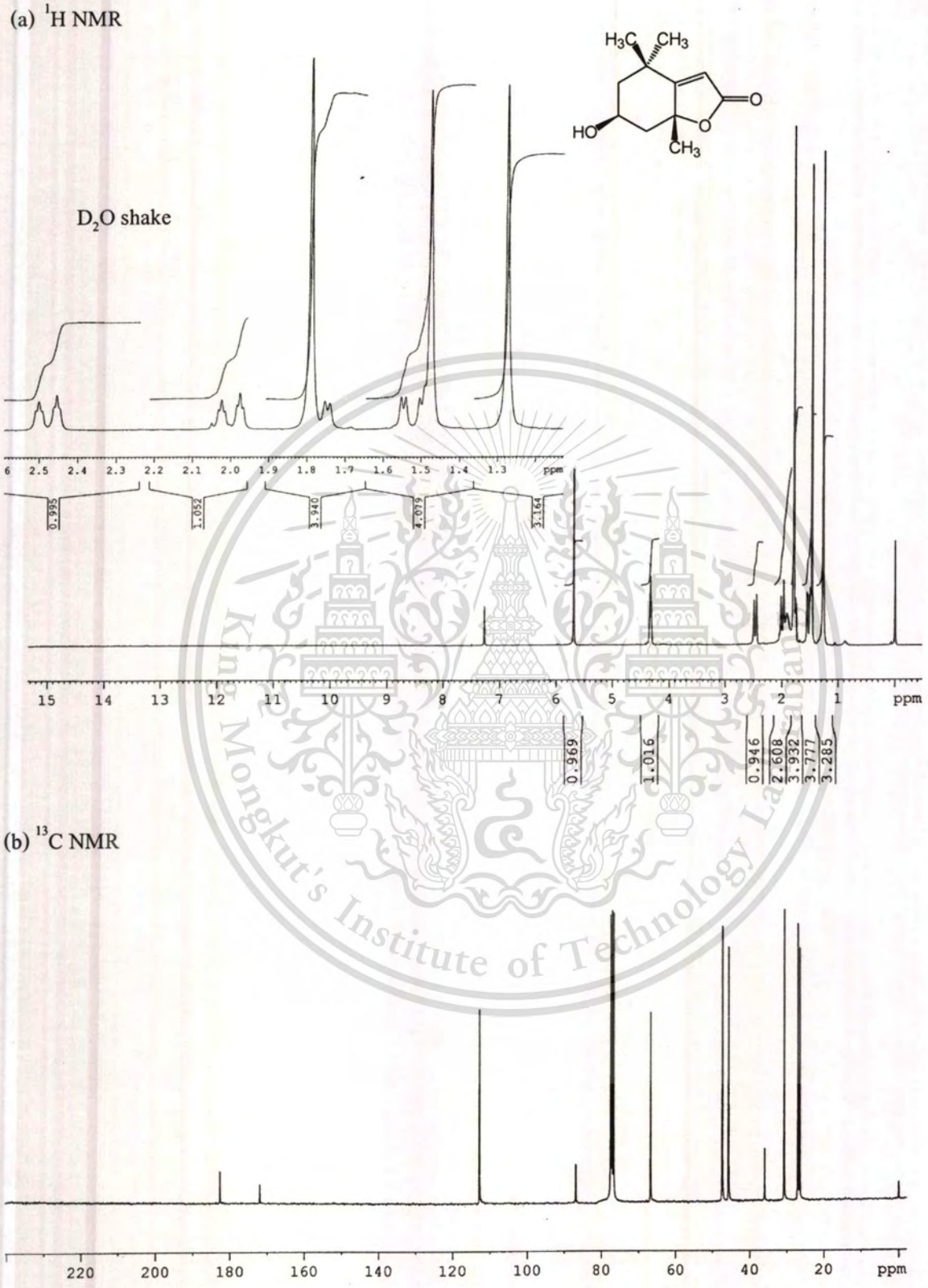
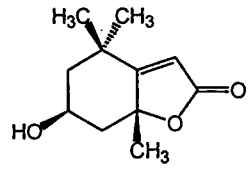



**Figure B24** (a) $^{1}$H NMR and (b) $^{13}$C NMR spectra of loliolide **58** in $CDCl_3$

(a) DEPT 135







(b) COSY

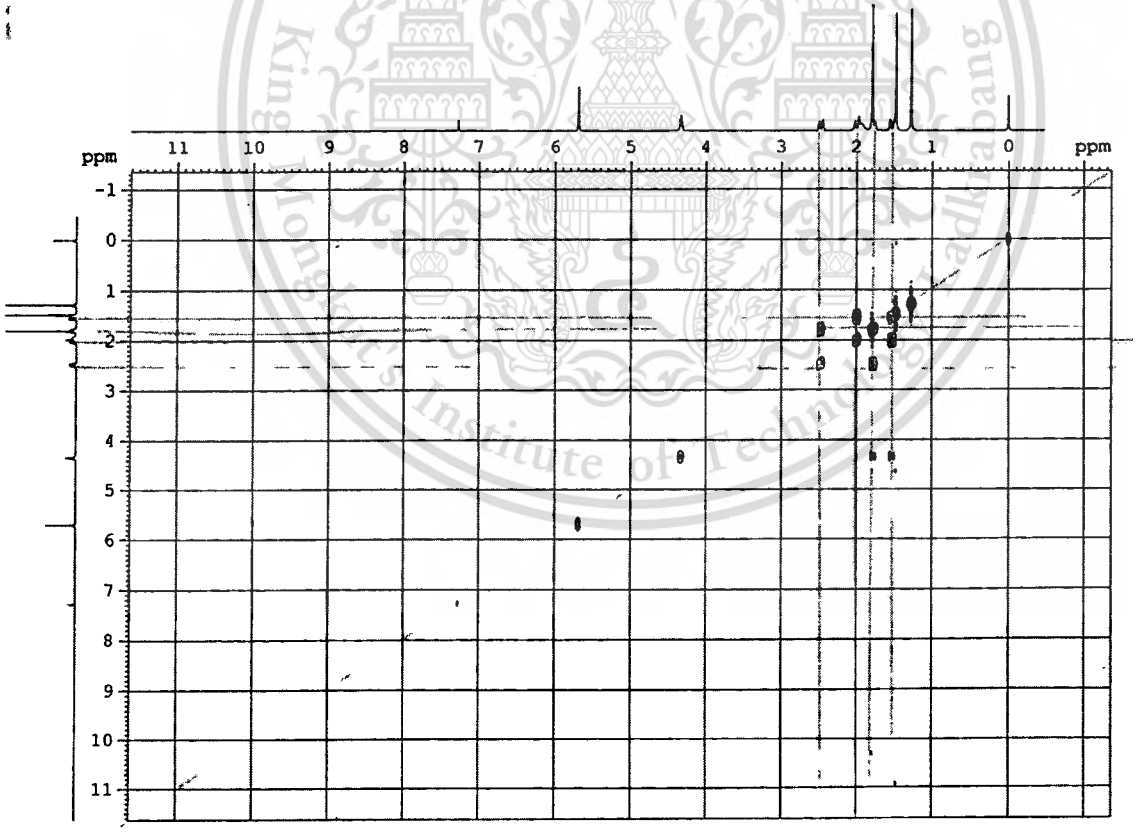



**Figure B25** (a) DEPT 135 and (b) COSY spectra of loliolide **58** in $CDCl_3$

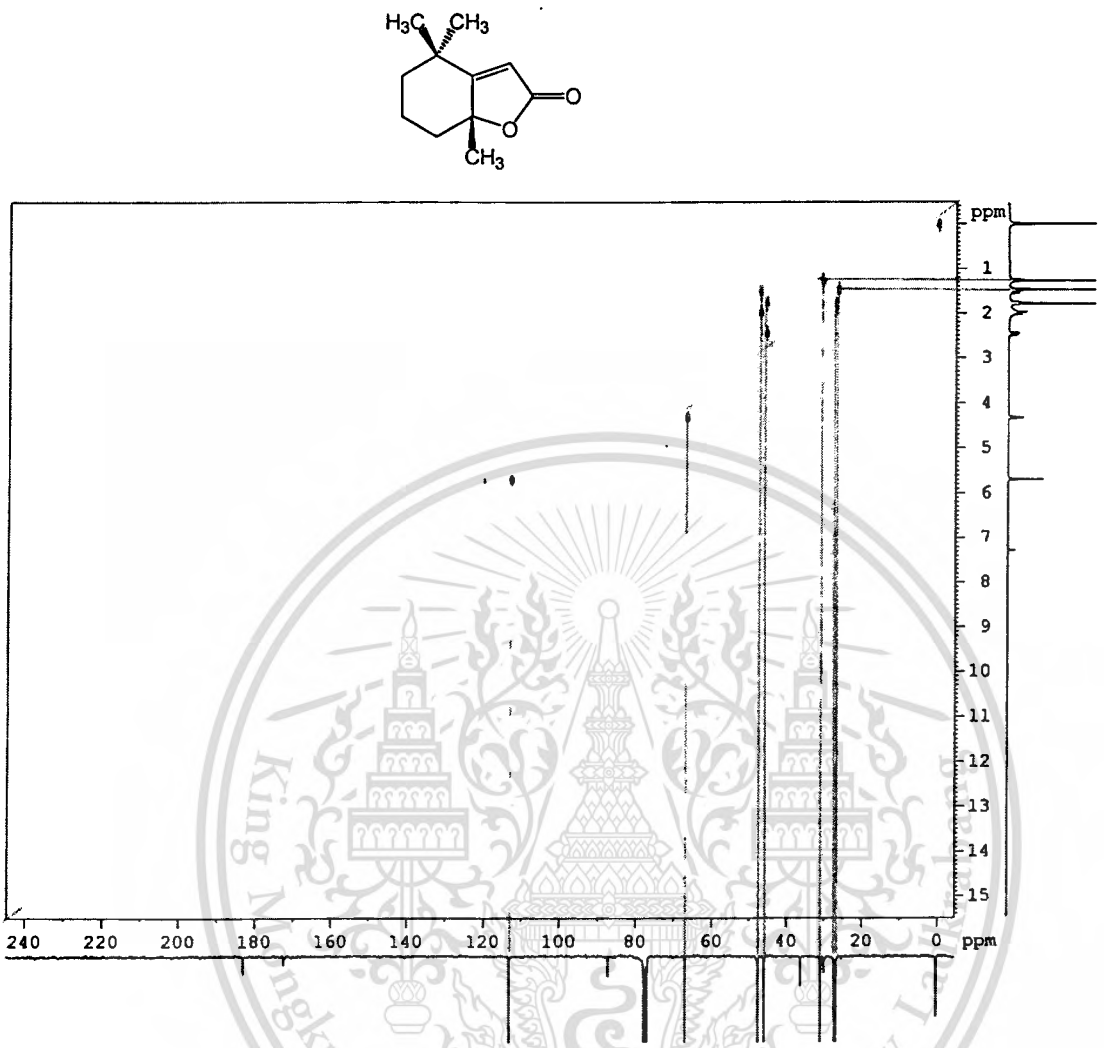



**Figure B26** HETCOR spectrum of loliolide **58** in $CDCl_3$

# BIOGRAPHY

| | |
|---|---|
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