

**BIOLOGICAL ACTIVITY OF *HEDYOTIS* SPP. AND CHEMICAL  
CONSTITUENTS OF *HEDYOTIS CAPITELLATA***

**By**

**ROHAYA AHMAD**

**Thesis Submitted to the School of Graduate Studies,  
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**Chairman: Professor Md. Nordin Hj.Lajis, PhD**

**Institute: Bioscience**

*Hedyotis* (Family, Rubiaceae) is a genus of erect decumbent or climbing herbs. The genus consists of some 180 species. They grow well on dry and sandy soil, along rivers and coasts and in the forests. There are 35 species recorded in Malaysia. Most of the species possess medicinal properties and are used by the Malay as well as the Chinese communities.

The methanolic extracts of seven *Hedyotis* species including *H. capitellata* (stems, leaves and roots), *H. dichotoma* (aerial parts and roots), *H. verticillata* (leaves and stems), *H. herbacea* (aerial parts), *H. pinifolia* (aerial parts), *H. corymbosa* (aerial parts) and *H. nudicaulis* (aerial parts) were screened for antioxidant, radical-scavenging, anti-inflammatory, cytotoxic as well as anti-bacterial properties using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA), the diphenylpicryl hydrazyl (DPPH), the Griess assay, the MTT assay and the disc diffusion methods, respectively. The results showed that all of the extracts tested possess strong antioxidant potential. However, they are poor radical scavengers and nitric oxide

inhibitors. They are also found to be weakly cytotoxic and possess weak to moderate antibacterial properties. On the basis of the screening results and literature review, *H. capitellata* (stems) was selected for further phytochemical study.

Phytochemical investigation on the active fraction of the stems of *H. capitellata* plant yielded fifteen compounds. The structure of the compounds was elucidated based on spectroscopic techniques and comparison with literature values. Eight compounds are *new* furanoanthraquinones named capitellataquinone A-G and *epi*-capitellataquinone E. A new anthraquinone, 2,8-dihydroxy-1-methoxyanthraquinone together with rubiadin, alizarin 1-methyl ether, anthragallol 2-methyl ether, digiferruginol and scopoletin were also isolated. From the roots of the plant, lucidin 3-*O*- $\beta$ -glucoside was also isolated. The isolation of an anthraquinone glycoside is a first for the genus.

Antioxidant assays on ten compounds including capitellataquinones A, B, E, F, *epi*-capitellataquinone E, rubiadin, alizarin 1-methyl ether, anthragallol 2-methyl ether, digiferruginol and scopoletin showed that capitellataquinone A and scopoletin possess strong antioxidant properties with percent inhibition of 94-96% compared to quercetin (98%). MTT cytotoxic assays of the same compounds tested showed weak cytotoxicity to most cell-lines with IC<sub>50</sub> values of 24-40  $\mu$ g/ml. However, alizarin 1-methyl ether was found to be selectively cytotoxic against MDA-MB-231 with an IC<sub>50</sub> value of 8  $\mu$ g/ml.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Doktor Falsafah

**KEAKTIFAN BIOLOGI SPESIES *HEDYOTIS* DAN KONSTITUEN KIMIA  
*HEDYOTIS CAPITELLATA***

Oleh

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*Hedyotis* (Famili Rubiaceae) ialah genus tumbuhan herba yang tumbuh menegak atau memanjat. Genus ini terdiri daripada 180 spesies. Ia tumbuh dengan baik di tanah yang kering dan berpasir, di tepi pantai dan di dalam hutan. Terdapat 35 spesies yang direkodkan di Malaysia. Kebanyakan daripadanya mempunyai nilai perubatan dan digunakan oleh kaum Melayu dan Cina.

Ekstrak metanol tujuh spesies *Hedyotis* termasuk *H. capitellata* (daun, batang dan akar), *H. dichotoma* (atas dan akar), *H. verticillata* (atas dan batang), *H. herbacea* (atas), *H. pinifolia* (daun), *H. corymbosa* (atas) dan *H. nudicaulis* (atas) disaring untuk sifat antioksidan, anti-inflamasi, sitotoksik dan anti-bakteria menggunakan biocerakanan ferrik tiosianat (FTC) dan asid tiobarbiturik (TBA), kaedah difenilpikrilhidrazil (DPPH), kaedah Griess, kaedah MTT dan kaedah pembauran cakera, masing-masing. Keputusan biocerakanan menunjukkan bahawa kesemua ekstrak mempunyai potensi antioksidan yang kuat. Walau bagaimana pun, kesemuanya merupakan pemerangkap radikal dan perencat nitrik oksida yang lemah. Mereka juga didapati mempunyai sifat sititoksik yang sederhana serta

mempunyai sifat antibakteria lemah hingga sederhana. Berdasarkan keputusan penyaringan dan kajian literatur, *H. capitellata* (batang) telah dipilih untuk fitokimia.

Penyelidikan fitokimia ke atas fraksi aktif batang *H. capitellata* menghasilkan lima belas sebatian. Struktur kesemua sebatian dikenalpasti berdasarkan kaedah spektroskopi dan perbandingan dengan literatur. Lapan sebatian merupakan furanoantrakuinon baru yang dinamakan capitellataquinone A-G dan *epi*-capitellataquinone E. Satu antrakuinon baru, 2,8-dihidroksi-1-metoksiantrakuinon bersama dengan rubiadin, alizarin 1-metil eter, antragalol 2-metil eter, digiferuginol dan skopoletin juga berjaya dipencilkan. Daripada bahagian akar tumbuhan tersebut, lucidin 3-*O*- $\beta$ -glukosida telah dipencilkan. Ini adalah kali pertama anthrakuinon glikosida dipencilkan daripada genus ini.

Cerakinan antioksidan terhadap sepuluh sebatian termasuk capitellataquinon A, B, E, F, *epi*-capitellataquinon E, rubiadin, alizarin 1-metil ether, anthragalol 2-metil ether, digiferruginol dan skopoletin menunjukkan bahawa hanya capitellataquinon A dan skopoletin menunjukkan sifat antioksidan yang baik dengan peratus hindaran sebanyak 94-96% berbanding dengan quercetin (98%). Cerakinan sitotoksik MTT keatas sebatian yang sama menunjukkan sifat sitotoksik yang lemah terhadap kebanyakan titisan sel dengan nilai IC<sub>50</sub> antara 24-40  $\mu$ g/ml. Walau bagaimana pun, alizarin 1-metil eter didapati sitotoksik secara selektif terhadap MDA-MB-231 dengan nilai IC<sub>50</sub> 8  $\mu$ g/ml.

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I certify that an Examination Committee met on 22<sup>nd</sup> February 2005 to conduct the final examination of Rohaya Ahmad on her Doctor of Philosophy thesis entitled “Biological Activity of *Hedyotis* spp. and Chemical Constituents of *Hedyotis capitellata*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.

---

**ROHAYA AHMAD**

Date:



## TABLE OF CONTENTS

	<b>Page</b>
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENT	vi
APPROVAL	
viii	
DECLARATION	x
LIST OF TABLES	xv
LIST OF FIGURES	
xvii	
LIST OF SCHEMES	
xxii	
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS	
xxiii	

### CHAPTER

#### 1 INTRODUCTION

<b>1</b>		
1.1	General Introduction	1
1.2	From Plants to Drugs	5
1.3	Selection of Medicinal Plants	7
	1.3.1 Chemotaxonomy	7
	1.3.2 Ethnomedical Information	8
	1.3.3 Field Observations	9
	1.3.4 Random Collection	9
1.4	Bioactivity-guided Isolation	9
	Antioxidant Activity and Chemical Assays	11
1.5	Chemical Screening	12
1.6	The Genus <i>Hedyotis/Oldenlandia</i>	16
1.7	Objectives of Research	16

#### 2 LITERATURE REVIEW

	<b>18</b>	
2.1	Evaluation of Antioxidant Potential of Natural Products	18
	2.1.1 Measurement of Lipid Oxidation	19
	2.1.2 Radical-Scavenging of Stable DPPH Radical	19
2.2	Evaluation of Anti-inflammatory Activity related to Nitric Oxide Inhibition	20
	Colorimetric Nitric Oxide Assay (Griess Assay)	20
2.3	Evaluation of anti-cancer activity in natural products	21
	<i>In-vitro</i> anti-cancer screening	22
2.4	Evaluation of Antimicrobial Activity in Natural Products	23
2.5	Family Rubiaceae	24

2.6	Phytochemical Studies on Plants of the Genus <i>Hedyotis</i>	25
2.6.1	Medicinal Properties of <i>Hedyotis</i> species	25
2.6.2	Structural Diversity in the Genus	29
2.6.3	Alkaloids	30
2.6.4	Anthraquinones	33
2.6.5	Flavonoids	35
2.6.6	Iridoids	37
2.6.7	Triterpenoids and Sterols	42
2.6.8	Lignans	44
2.6.9	Other Compounds	46
2.7	Pharmacological Activities and Other Bioactivity Studies	47
2.7.1	Biological Activities of Crude Extracts	47
2.7.2	Biological Activities of Pure Constituents	48
2.8	Structure-Activity Relationships of Some Bioactive Compounds in the Genus <i>Hedyotis</i>	51
2.8.1	Antioxidant Activity	51
2.8.2	Neuroprotective activity	52
2.9	Biogenesis of Anthraquinones, Iridoids and Indole Alkaloids found in <i>Hedyotis</i>	54
2.9.1	Biosynthesis of Anthraquinones	55
2.9.2	Biosynthesis of Iridoid Glycosides	57
2.9.3	Biosynthesis of Indole Alkaloids	59

### 3 RESEARCH EXPERIMENTAL

#### 60

3.1	General Instrumentation	60
3.2	Chromatographic Methods	60
3.3	Solvents	62
3.4	Plant Material	62
3.5	Extraction and Fractionation of Crude Extracts of <i>H. capitellata</i>	62
3.5.1	Extraction of plant and fractionation of crude extract for bioassay	62
3.5.2	Extraction of plant for phytochemical investigation	63
3.6	Isolation of Compounds	65
3.6.1	Compounds <b>80-92 and 77</b>	65
3.6.2	Compound <b>93</b>	65
3.7	Spectral Characterization of Compounds	68
3.8	Bioassay Methods	77
3.8.1	Bacteria	77
3.8.2	Antibacterial bioassay (disc diffusion method)	77
3.8.3	Antioxidant Activity	78
3.8.4	Culture of Cells	79
3.8.5	MTT Assay for Cytotoxic Activity	80
3.8.6	Griess assay for nitric oxide inhibitory activity	81

<b>4</b>	<b>BIOLOGICAL ACTIVITIES OF CRUDE EXTRACTS and BIOASSAY-GUIDED ISOLATION OF BIOACTIVE COMPOUNDS from <i>H. CAPITELLATA</i></b>	<b>83</b>
4.1	Bioactivity of Crude Extracts of <i>Hedyotis</i> species	83
4.1.1	Antioxidant Activity	83
4.1.2	Anti-inflammatory Activity related to nitric oxide inhibition	88
4.1.3	Cytotoxic Activity	90
4.1.4	Antibacterial Activity	90
4.2	Selection of <i>Hedyotis</i> species	92
4.3	Fraction of <i>H. capitellata</i> stems	92
4.3.1	Antioxidant Activity	92
4.3.2	Antibacterial Activity	93
4.4	Bioassay of Subfractions	95
<b>5</b>	<b>CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF COMPOUNDS FROM <i>H. CAPITELLATA</i> STEMS</b>	<b>97</b>
5.1	Characterisation of Compounds	97
5.1.1	Capitellataquinone A ( <b>80</b> )	97
5.1.2	Capitellataquinone B ( <b>81</b> )	110
5.1.3	Capitellataquinone C ( <b>82</b> )	121
5.1.4	Capitellataquinone D ( <b>83</b> )	128
5.1.5	Capitellataquinone E ( <b>84</b> )	136
5.1.6	<i>epi</i> -Capitellataquinone E ( <b>85</b> )	149
5.1.7	Capitellataquinone F ( <b>86</b> )	156
5.1.8	Capitellataquinone G ( <b>87</b> )	163
5.1.9	Scopoletin ( <b>77</b> )	174
5.1.10	Rubiadin ( <b>88</b> )	178

5.1.11	Alizarin 1-methyl ether (89)	184
5.1.12	Anthragallol 2-methyl ether (90)	191
5.1.13	Digiferruginol (91)	199
5.1.14	2,8-dihydroxy-1-methoxyanthraquinone (92)	203
5.1.15	Lucidin 3- <i>O</i> - $\beta$ -glucoside (93)	211
5.2	Chemotaxonomic Significance of <i>H. capitellata</i>	220
5.3	Biological Activity of Compounds	220
5.3.1	Antioxidant Activity	220
5.3.2	Anti-inflammatory Activity related to nitric oxide inhibition	225
5.3.3	Cytotoxic Activity	225
5.4	Structure-Activity Relationships of Anthraquinones from <i>H. capitellata</i>	231
5.4.1	Antioxidant Activity	231
5.4.2	Cytotoxic Activity	233
5.5	Proposed Biosynthetic Pathway of Capitellataquinone A	234

<b>6</b>	<b>CONCLUSION</b>
	<b>236</b>
	<b>BIBLIOGRAPHY</b>
	<b>242</b>
	<b>APPENDICES</b>
<b>250</b>	<b>BIODATA OF THE AUTHOR</b>
	<b>251</b>



## LIST OF TABLES

<b>Table</b>	<b>Page</b>	
1.1	Some tropical plants yielding clinically useful drugs	4
1.2	Simple bioassays for phytochemical laboratories	11
2.1	Medicinal Uses of Some <i>Hedyotis</i> Species	28
2.2	Alkaloids from <i>Hedyotis</i> and structure of borreverine	32
2.3	Anthraquinones from <i>Hedyotis</i>	34
2.4	Flavonoids from <i>Hedyotis</i>	36
2.5	New Iridoids from <i>Hedyotis</i>	39-40
2.6	Common Iridoids from <i>Hedyotis</i>	41
2.7	Some Triterpenoids from <i>Hedyotis</i>	43
2.8	Lignans and Sesquilignans from <i>H. lawsoniae</i>	45
2.9	Other Compounds in <i>Hedyotis</i>	46
4.1	Comparison of Absorbance and Percent Inhibition from FTC and TBA Antioxidant assays	86
4.2	Radical scavenging activity of the methanolic extracts of some <i>Hedyotis</i> species	87
4.3	Percent nitric oxide inhibition of methanolic extracts of some <i>Hedyotis</i> species	89
4.4	CD <sub>50</sub> (µg/ml) values for methanolic extracts of <i>Hedyotis</i> species towards CEM-SS cell-line	90
4.5	Inhibition zones (mm) of methanolic extracts of some <i>Hedyotis</i> species as measured by the disc diffusion method	91
4.6	Inhibition zones (mm) of fractions of <i>H. capitellata</i> stems as measured by the disc diffusion method	93
5.1	HMQC and HMBC Correlations for Capitellataquinone A (DMSO- <i>d</i> <sub>6</sub> )	100
5.2	HSQC and HMBC Correlations for Capitellataquinone B (acetone- <i>d</i> <sub>6</sub> )	113
5.3	HMQC and HMBC Correlations for Capitellataquinone C (DMSO- <i>d</i> <sub>6</sub> )	122
5.4	HMQC and HMBC Correlations for Capitellataquinone D (acetone- <i>d</i> <sub>6</sub> )	130
5.5	HMQC and HMBC Correlations for Capitellataquinone E (acetone- <i>d</i> <sub>6</sub> )	140
5.6	HSQC and HMBC Correlations for <i>epi</i> -Capitellataquinone E (acetone- <i>d</i> <sub>6</sub> )	151

5.7	HSQC and HMBC Correlations for Capitellataquinone F (acetone- <i>d</i> <sub>6</sub> )	158
5.8	HMQC and HMBC Correlations for Capitellataquinone G (DMSO- <i>d</i> <sub>6</sub> )	165
5.9	<sup>1</sup> H (acetone- <i>d</i> <sub>6</sub> , 500 MHz) NMR chemical shifts of Capitellataquinone A-G and <i>epi</i> -Capitellataquinone E	172
5.10	<sup>13</sup> C (acetone- <i>d</i> <sub>6</sub> , 125 MHz) NMR chemical shifts of Capitellataquinone A-G	173
5.11	HMQC and HMBC correlations for Rubiadin (acetone- <i>d</i> <sub>6</sub> )	180
5.12	HMQC and HMBC correlations for Alizarin 1-methyl ether (CDCl <sub>3</sub> )	186
5.13	HSQC and HMBC correlations for Anthragallol 2-methyl ether (CDCl <sub>3</sub> )	193
5.14	<sup>1</sup> H NMR chemical shifts for Digiferruginol (CDCl <sub>3</sub> )	200
5.15	HMQC and HMBC Correlations for 2,8-dihydroxy-1-methoxyanthraquinone (CDCl <sub>3</sub> )	205
5.16	<sup>1</sup> H and <sup>13</sup> C NMR assignment of Lucidin 3- <i>O</i> - $\beta$ -glucoside (DMSO- <i>d</i> <sub>6</sub> )	213
5.17	Comparison of percent inhibition from FTC and TBA antioxidant assays for selected compounds from <i>H. capitellata</i>	222
5.18	IC <sub>50</sub> values of some anthraquinones from <i>H. capitellata</i> against selected cell lines	227

## LIST OF FIGURES

Figure		Page
2.1	Some <i>Hedyotis</i> species	26
2.2	“Bai Hua She She Cao” and <i>Hedyotis</i> Capsules	27
2.3	Biosynthetic pathway leading to anthraquinones in the Rubiaceae	56
2.4	Biosynthetic pathway for rubiaceaceous ridoid glycosides	58
2.5	Proposed biosynthetic pathway for some indole alkaloids from <i>H. capitellata</i>	59
4.1	Antioxidant activity of methanolic extracts of some <i>Hedyotis</i> species as measured by the FTC method (final sample concentration, 0.02% w/v)	84
4.2	Antioxidant activity of methanolic extracts of some <i>Hedyotis</i> species as measured by the TBA method (final sample concentration, 0.02% w/v)	84
4.3	Radical-scavenging activity of methanolic extracts of some <i>Hedyotis</i> species	86
4.4	Antioxidant activity of <i>H. capitellata</i> fractions as measured by the FTC method (final sample concentration, 0.02% w/v).	94
4.5	Antioxidant activity of <i>H. capitellata</i> fractions as measured by the TBA method (final sample concentration, 0.02% w/v).	94
4.6	Antioxidant activity of subfractions of DCM fraction of <i>H. capitellata</i> stems as measured by the FTC method	96
5.1	Selected HMBC Correlations for Capitellataquinone A	99
5.2	EIMS Spectrum of Capitellataquinone A	101
5.3	<sup>1</sup> H NMR Spectrum of Capitellataquinone A (in DMSO- <i>d</i> <sub>6</sub> )	102
5.4	<sup>13</sup> C NMR Spectrum of Capitellataquinone A (in DMSO- <i>d</i> <sub>6</sub> )	103
5.5	DEPT 135 Spectrum of Capitellataquinone A	104
5.6	FGCOSY Spectrum Capitellataquinone A	105
5.7	Expanded FGCOSY Spectrum of Capitellataquinone A	106
5.8	FGHMQC Spectrum of Capitellataquinone A	107
5.9	FGHMBC Spectrum of Capitellataquinone A	108
5.10	Expanded FGHMBC Spectrum of Capitellataquinone A	109

5.11	<i>trans</i> and <i>cis</i> configuration of of Capitellataquinone B	112
5.12	Selected HMBC Correlations for Capitellataquinone B	112
5.13	EIMS Spectrum of Capitellataquinone B	114
5.14	<sup>1</sup> H NMR Spectrum of Capitellataquinone B	115
5.15	FGCOSY Spectrum of Capitellataquinone B	116
5.16	NOESY Spectrum of Capitellataquinone B	116
5.17	<sup>13</sup> C NMR Spectrum of Capitellataquinone B	117
5.18	FGHSQC Spectrum of Capitellataquinone B	118
5.19	FGHMBC Spectrum of Capitellataquinone B (Expansion 1)	119
5.20	FGHMBC Spectrum of Capitellataquinone B (Expansion 2)	120
5.21	Selected HMBC Correlations for Capitellataquinone C	123
5.22	EIMS Spectrum of Capitellataquinone C	124
5.23	<sup>1</sup> H NMR Spectrum of Capitellataquinone C	125
5.24	FGHMQC Spectrum of Capitellataquinone C	126
5.25	Expanded FGHMQC Spectrum of Capitellataquinone C	126
5.26	FGHMBC Spectrum of Capitellataquinone C	127
5.27	Selected HMBC Correlations for Capitellataquinone D	129
5.28	EIMS Spectrum of Capitellataquinone D	131
5.29	FDMS Spectrum of Capitellataquinone D	131
5.30	<sup>1</sup> H NMR Spectrum of Capitellataquinone D	132
5.31	<sup>1</sup> H NMR Spectrum of Capitellataquinone D (Expansion 1)	133
5.32	<sup>1</sup> H NMR Spectrum of Capitellataquinone D (Expansion 2)	133
5.33	HMQC Spectrum of Capitellataquinone D (Expansion 1)	134
5.34	HMBC Spectrum of Capitellataquinone D (Expansion 2)	134
5.35	HMBC Spectrum of Capitellataquinone D (Expansion 1)	135
5.36	HMBC Spectrum of Capitellataquinone D (Expansion 2)	135
5.37	Selected HMBC Correlations for Capitellataquinone E	137
5.38	Newman projection along C-2'-C-1' bond and structure of capitellataquinone E	138

5.39	EIMS Spectrum of Capitellataquinone E	141
5.40	<sup>1</sup> H NMR Spectrum of Capitellataquinone E	142
5.41	Expanded <sup>1</sup> H NMR Spectrum of Capitellataquinone E	143
5.42	<sup>13</sup> C NMR Spectrum of Capitellataquinone E (in CDCl <sub>3</sub> )	144
5.43	<sup>13</sup> C NMR Spectrum of Capitellataquinone E (in acetone- <i>d</i> <sub>6</sub> )	145
5.44	FGHSQC Spectrum of Capitellataquinone E	146
5.45	FGHMBC Spectrum of Capitellataquinone E	147
5.46	FGCOSY Spectrum of Capitellataquinone E	148
5.47	NOE-1D Spectrum of Capitellataquinone E	148
5.48	Newman projection along C-2'-C-1' bond and structure of <i>epi</i> -capitellataquinone E	150
5.49	EIMS Spectrum of <i>epi</i> -Capitellataquinone E	152
5.50	FABMS Spectrum of <i>epi</i> -capitellataquinone E	152
5.51	<sup>1</sup> H NMR Spectrum of <i>epi</i> -capitellataquinone E	153
5.52	FGCOSY Spectrum of <i>epi</i> -capitellataquinone E	154
5.53	Expanded FGCOSY Spectrum of <i>epi</i> -capitellataquinone E	154
5.54	Expanded FGHMBC Spectrum of <i>epi</i> -capitellataquinone E	155
5.55	Selected HMBC Correlations for Capitellataquinone F	157
5.56	EIMS Spectrum of Capitellataquinone F	159
5.57	FABMS Spectrum of Capitellataquinone F	159
5.58	<sup>1</sup> H NMR Spectrum of Capitellataquinone F	160
5.59	FGCOSY Spectrum of Capitellataquinone F	161
5.60	Expanded FGCOSY Spectrum of Capitellataquinone F	161
5.61	FGHMBC Spectrum of Capitellataquinone F (Expansion 1)	162
5.62	FGHMBC Spectrum of Capitellataquinone F (Expansion 2)	162
5.63	Selected HMBC Correlations for Capitellataquinone G	165
5.64	LCMS Spectrum of Capitellataquinone G	166
5.65	<sup>1</sup> H NMR Spectrum of Capitellataquinone G	167
5.66	<sup>1</sup> H NMR Spectrum (presaturated) of Capitellataquinone G	168

5.67	<sup>13</sup> C NMR Spectrum of Capitellataquinone G	169
5.68	FGCOSY Spectrum of Capitellataquinone G	170
5.69	FGHMBC Spectrum of Capitellataquinone G	171
5.70	EIMS Spectrum of Scopoletin	175
5.71	FGCOSY Spectrum of Scopoletin	175
5.72	<sup>1</sup> H NMR Spectrum of Scopoletin	176
5.73	<sup>13</sup> C NMR Spectrum of Scopoletin	177
5.74	EIMS Spectrum of Rubiadin	181
5.75	FGCOSY Spectrum of Rubiadin	181
5.76	<sup>1</sup> H NMR Spectrum of Rubiadin	182
5.77	<sup>13</sup> C NMR Spectrum of Rubiadin	183
5.78	EIMS Spectrum of Alizarin 1-methyl ether	187
5.79	FGCOSY Spectrum of Alizarin 1-methyl ether	187
5.80	<sup>1</sup> H NMR Spectrum of Alizarin 1-methyl ether	188
5.81	<sup>13</sup> C NMR Spectrum of Alizarin 1-methyl ether	189
5.82	Expanded FGHMBC Spectrum of Alizarin 1-methyl ether	190
5.83	EIMS Spectrum of Anthragallol 2-methyl ether	194
5.84	FGCOSY Spectrum of Anthragallol 2-methyl ether	194
5.85	<sup>1</sup> H NMR Spectrum of Anthragallol 2-methyl ether	195
5.86	<sup>13</sup> C NMR Spectrum of Anthragallol 2-methyl ether	196
5.87	Expanded HSQC Spectrum of Anthragallol 2-methyl ether	197
5.88	Expanded HMBC Spectrum of Anthragallol 2-methyl ether	198
5.89	EIMS Spectrum of Digiferruginol	201
5.90	<sup>1</sup> H NMR Spectrum of Digiferruginol	202
5.91	FDMS Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	206
5.92	EIMS Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	206
5.93	<sup>1</sup> H NMR Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	207
5.94	<sup>13</sup> C NMR Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	208

5.95	Expanded FGHMQC Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	209
5.96	FGHMBC Spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	210
5.97	Expanded FGHMBC spectrum of 2,8-dihydroxy-1-methoxyanthraquinone	210
5.98	Selected HMBC Correlations for Lucidin 3- <i>O</i> - $\beta$ glucoside	213
5.99	FABMS Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	214
5.100	EIMS Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	215
5.101	<sup>1</sup> H NMR Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	216
5.102	<sup>13</sup> C NMR Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	217
5.103	FGHMQC Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	218
5.104	FGHMBC Spectrum of Lucidin 3- <i>O</i> - $\beta$ glucoside	219
5.105	Antioxidant Activity of Selected Compounds as measured by the FTC method (final sample concentration, 300 $\mu$ M)	223
5.106	Antioxidant Activity of Selected Compounds as measured by the TBA method	224
5.107	IC <sub>50</sub> values of some anthraquinones against selected cell-lines	228
5.108	Caov-3 cells before treatment (control)	229
5.109	Caov-3 cells after treatment with 10 $\mu$ g/ml of rubiadin	229
5.110	Caov-3 cells at 50% of control after treatment with 30 $\mu$ g/ml rubiadin	230
5.111	Caov-3 cells after treatment with 40 $\mu$ g/ml of rubiadin	230

## LIST OF SCHEMES

<b>Scheme</b>		<b>Page</b>
1.1	Procedure for obtaining the active principles from plants and use of LC-hyphenated techniques as strategic analytical tools during the isolation process	6
1.2	Approaches to bioassay-directed natural product development	14
1.3	A typical bioactivity-guided isolation scheme	15
2.1	Steps associated with MTT Assay	23
3.1	Flowchart for column chromatography of chloroform extract of <i>H. capitellata</i> stems (Column 1)	64
3.2	Flowchart for column chromatography of Fraction B of Column 1 (Column 2)	67
5.1	Proposed biosynthetic pathway for Capitellataquinone A	235