## **CONTENTS**

	Page
บทคัดย่อ	(3)
Abstract	(5)
Acknowledgement	(7)
Contents	(8)
List of Tables	(11)
List of Figures	(12)
Abbreviations and symbols	(14)
Chapter	
1 Introduction	1
2 Literature review	4
2.1 Botanical aspect of Senna alata (L.) Roxb.	4
2.2 Chemical constituents of Senna alata	6
2.3 Biosynthesis of anthraquinone	9
2.4 Biological activity	12
2.4.1 Senna alata crude extracts	12
2.4.2 Aloe-emodin	14
2.4.3 Chrysophanol	15
2.4.4 Emodin	16
2.4.5 Rhein	18
2.5 Anthraquinone production by plant cell and tissue cultures	20

# **CONTENTS (Continued)**

		Page
3	Materials and Methods	24
	3.1 Materials	24
	3.1.1 Plant materials	24
	3.1.2 Chemicals	24
	3.2 Methods	24
	3.2.1 Preparation of Senna alata plantlets	24
	3.2.2 Medium preparation	25
	3.2.3 Selection of high yielding plants	25
	3.2.3.1 Preparation of Senna alata leaf extracts	25
	3.2.3.2 Quantitative analysis of rhein and aloe-emodin content	27
	3.2.4 Establishment of Senna alata tissue cultures	27
	3.2.5 Medium manipulation	28
	3.2.6 Determination of anthraquinone formation	28
	3.2.6.1 Plant tissue culture harvesting	28
	3.2.6.2 Sample preparation	28
	3.2.6.3 Determination of anthraquinones	28
	3.2.7 Time course of growth and anthraquinone production of	30
	Senna alata cell culture	
	3.2.8 Determination of hydroxyanthracene derivatives	30

# **CONTENTS** (Continued)

		Page
1	Results and Discussion	32
	4.1 Selection of high yielding plants	32
	4.2 Establishment of Senna alata tissue cultures	40
	4.3 Anthraquinone formation in Senna alata root and cell cultures	43
	4.4 Effect of medium manipulation on anthraquinone production in	46
	Senna alata cultures	
	4.5 Determination of anthraquinone content	49
	4.6 Time courses of growth and anthraquinone production	56
	4.7 Determination of anthraquinone glycosides in Senna alata cell culture	59
5	Conclusions	60
Bibliography		62
v	Vitae Vitae	85

## LIST OF TABLES

<b>Fable</b>		Page
2.1	Chemical constituents of various parts of Senna alata	6
2.2	Examples of anthraquinone production by plant tissue cultures	22
3.1	Inorganic salt and vitamin composition of B5 medium	26
3.2	Gradient profile of the mobile phase	29
4.1	Aloe-emodin and rhein content in the leaves of plantlets	37
4.2	Chrysophanol and emodin content in Senna alata tissue culture	43
4.3	Emodin and chrysophanol content in different types and	47
	concentrations of growth regulators	
4.4	Anthraquinone content in tissue cultures, leaves and roots of	53
	Senna alata	
4.5	Fresh and dry weight of Senna alata cell biomass	57
4.6	Emodin and chrysophanol content accumulated in Senna alata	57
	cell biomass	

## LIST OF FIGURES

Figure		Page
2.1	Senna alata (L.) Roxb	5
2.2	Chemical structures of anthraquinones	9
2.3	Biogenesis pathway of anthraquinones	11
3.1	Gradient profile of the mobile phase	30
4.1	Senna alata plantlets grown in solid B5 basal medium	33
4.2	HPLC-chromatograms of the authentic anthraquinones and	34
	Senna alata plantlet leaf extract	
4.3	Calibration curves of aloe-emodin and rhein	35
4.4	Aloe-emodin and rhein content in the leaves of	36
	Senna alata plantlets	
4.5	The first and the third subculture of Senna alata root culture	41
4.6	Senna alata root culture (the 7 <sup>th</sup> subculture) grown in	42
	liquid B5 medium supplemented with 0.5 mg/l NAA and	
	1.0 mg/l kinetin	
4.7	Senna alata cell culture (the 12 <sup>th</sup> subculture)grown in	42
	liquid B5 medium supplemented with 0.5 mg/l NAA and	
	1.0 mg/l kinetin	
4.8	HPLC-chromatograms of the authentic anthraquinones and	44
	Senna alata root culture extract	
4.9	UV absorption spectra of authentic anthraquinones and	45
	the compounds obtained from Senna alata root culture extract	
	·	(12)

# LIST OF FIGURES (Continued)

Figure		Page
4.10	Senna alata cell cultures grown in liquid B5 medium	47
	supplemented with NAA 0.5 mg/l	
4.11	Senna alata cell cultures in hormone-free liquid B5 medium	48
4.12	Senna alata cell cultures in liquid B5 medium	48
	supplemented with kinetin 0.5 mg/l	
4.13	HPLC chromatograms of authentic anthraquinones and	51
	Senna alata extract	
4.14	Calibration curve of aloe-emodin and rhein	54
4.15	Calibration curve of emodin and chrysophanol	55
4.16	Growth curves and production curve of cell suspension cultures	58

#### ABBREVIATIONS AND SYMBOLS

B5 = Gamborg's B5 medium

HPLC = high performance liquid chromatography

NAA = 1-naphthalene acetic acid

rpm = round per minute

RT = retention time