

CONTENTS

	Page
CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATION AND SYMBOLS	xv
CHAPTER	
1 INTRODUCTION	1
1.1 General introduction	1
1.2 Review of literatures	4
1.2.1 Pesticides	4
1.2.2 Pesticide formulation	5
1.2.3 <i>Stemona</i> plant	9
1.2.4 <i>Stemona</i> alkaloids	18
2 EXPERIMENTS	23
2.1 Plant material	23
2.2 Chemicals	24
2.3 Extraction, isolation and identification	24
2.4 HPLC analysis	25
2.4.1 Chromatographic conditions	25
2.4.2 Standard and sample solutions	25
2.5 Method development and validation	26
2.5.1 Specificity	26
2.5.2 Linearity and range	27
2.5.3 Precision	27
2.5.4 Accuracy	28
2.6 Extraction of pyridostemin by maceration using organic solvents	28
2.7 Preparation of partially purified <i>S. curtisii</i> extract	28
2.8 Chemical stability of partially purified <i>S. curtisii</i> extract	28
2.8.1 Accelerated stability study	28
2.8.2 Photostability study	29
2.9 Preparation of water dispersible granules containing <i>S. curtisii</i> extract	29

CONTENTS (CONTINUED)

	Page
2.10 Evaluation of physicochemical properties of water dispersible granules containing <i>S. curtisii</i> extract	30
2.10.1 Pyridostemin content	30
2.10.2 Disintegration time	30
2.10.3 Friability	30
2.10.4 Flowability	31
2.10.5 Viscosity	31
2.10.6 pH	31
2.11 Stability of water dispersible granules containing <i>S. curtisii</i> extract	31
2.11.1 Chemical stability of water dispersible granules containing <i>S. curtisii</i> extract	32
2.11.2 Physical stability of water dispersible granule containing <i>S. curtisii</i> extract	32
2.12 Preparation of emulsifiable concentrate containing <i>S. curtisii</i> extract	32
2.13 Evaluation of physicochemical properties of emulsifiable concentrate containing <i>S. curtisii</i> extract	33
2.13.1 Pyridostemin content	33
2.13.2 Particle size	33
2.13.3 Viscosity	33
2.13.4 pH	34
2.14 Stability of emulsifiable concentrate containing <i>S. curtisii</i> extract	34
2.14.1 Chemical stability of emulsifiable concentrate containing <i>S. curtisii</i> extract	34
2.14.2 Physical stability of emulsifiable concentrate containing <i>S. curtisii</i> extract	34
3 RESULTS AND DISCUSSIONS	35
3.1 The chemical components in <i>S. curtisii</i>	35
3.2 Validation of HPLC method for analysis of pyridostemin in extract	40
3.2.1 Specificity	42
3.2.2 Linearity and range	46

CONTENTS (CONTINUED)

	Page
3.2.3 Precision	47
3.2.4 Accuracy	48
3.3 Comparison of pyridostemin content in <i>S. curtisii</i> extract obtained by maceration using different solvents	49
3.4 Determination of pyridostemin content in crude extract and partially purified extract	49
3.5 Stability of pyridostemin in partially purified extract	50
3.6 Formulation development of <i>S. curtisii</i> extract	54
3.6.1 Development of water dispersible granules containing <i>S. curtisii</i> extract	54
3.6.2 Development of emulsifiable concentrate containing <i>S. curtisii</i> extract	60
3.7 Stability of developed formulations	66
3.7.1 Chemical stability of developed formulation containing <i>S. curtisii</i> extract	66
3.7.2 Physical stability of developed formulation containing <i>S. curtisii</i> extract	74
4 CONCLUSION	78
BIBLIOGRAPHY	81
APPENDIX	99
A	100
B	107
VITAE	112

LIST OF TABLES

Table	Page
1. Pesticides as classified by their target species	4
2. Classification of pesticide formulations	6
3. Chemical constituents of <i>Stemona</i> spp.	10
4. Toxicity (LD ₅₀) and growth inhibition (EC ₅₀) of thirteen <i>Stemona</i> alkaloids against neonate larvae of <i>Spodoptera littoralis</i>	21
5. Contact toxicity against <i>S. littoralis</i> compared to commercial pyrethrum extract	22
6. ¹ H NMR spectral data of pyridostemin (500 MHz, CDCl ₃) compared with reference of pyridostemin (500 MHz, CDCl ₃)	38
7. RSD (%) of intra-day repeatability and intermediate precision studies	48
8. Recovery (%) of pyridostemin spiked in crude extract at various concentration	48
9. Pyridostemin content in <i>S. curtisii</i> extract obtained by maceration using different solvent	49
10. Stability rate constants (<i>k</i>) for pyridostemin in partially purified extract	51
11. Compositions of blank water dispersible granules and their physical properties	55
12. Viscosity of solution of 1 %w/v blank water dispersible granules in water	56
13. Comparison of physical properties of the water dispersible granules containing partially purified <i>S. curtisii</i> extract between formula 9 and 10	58
14. Physical properties of water dispersible granules containing partially purified <i>S. curtisii</i>	59
15. Disintegration time of water dispersible granules containing partially purified <i>S. curtisii</i> and the physical properties of suspension after dilution	59
16. Appropriate formula of emulsifiable concentrate	63
17. Physical properties of emulsion prepared from emulsifiable concentrate containing <i>S. curtisii</i> extract	65
18. Stability rate constants for pyridostemin in water dispersible granules	68
19. Stability rate constants for pyridostemin in emulsifiable concentrate	68
20. Frequency factor (<i>A</i>) and activation energy (<i>E_a</i>) for pyridostemin calculated from Arrhenius equation	69

LIST OF TABLES (CONTINUED)

Table	Page
21. Pyridostemin remaining (%) after storage for 1 month at ambient temperature and predicted $t_{90\%}$ of the developed formulations containing <i>S. curtisii</i> extract	70
22. Physical properties of water dispersible granules containing partially purified <i>S. curtisii</i> extract after storage at ambient temperature	75
23. Physical properties of the water dispersible granules containing partially purified <i>S. curtisii</i> extract after storage at 4 °C	75
24. Physical properties of the emulsifiable concentrate containing <i>S. curtisii</i> extract after storage at ambient temperature	76
25. Physical properties of emulsifiable concentrate containing <i>S. curtisii</i> extract after storage at 4 °C	76

LIST OF FIGURES

Figure	Page
1. <i>Stemona curtisii</i> Hook f.	2
2. Stichoneurine type alkaloids	19
3. Croomine type alkaloids	19
4. Protostemonine type alkaloids	20
5. Roots and rhizome of <i>S. curtisii</i>	23
6. Dried root powder of <i>S. curtisii</i>	23
7. Structure of pyridostemin	35
8. FTIR spectrum of pyridostemin	36
9. ¹ H NMR of pyridostemin	37
10. Mass spectrum of pyridostemin	39
11. UV spectrum of pyridostemin	41
12. HPLC chromatograms of pure pyridostemin and <i>S. curtisii</i> extract using C18 reverse phase column with acetonitrile-water-triethylamine (30:70:0.12, v/v/v) using a PDA detector (300 nm); flow rate 1.25 ml/min; injection volume 20 µl	42
13. Absorption spectra of the pyridostemin peak at retention time of 11.05, 11.20, and 11.32 min	43
14. HPLC chromatograms of <i>S. curtisii</i> extract in acid hydrolysis, base hydrolysis, oxidation, and reduction conditions for 24 hours	44
15. Predicted degradation products of pyridostemin by acid-base hydrolysis and oxidation reactions	45
16. Calibration curve of pyridostemin	47
17. First-order plots of degradation of pyridostemin at 45, 60, and 70 °C (75 %RH)	51
18. Arrhenius plot of pyridostemin in partially purified extract	52
19. Pyridostemin remaining (%) when storage in light and dark at ambient temperature (30±2 °C) conditions	53
20. Appearance of water dispersible granules containing <i>S. curtisii</i> crude extract and partially purified <i>S. curtisii</i> extract	56

LIST OF FIGURES (CONTINUED)

Figure	Page
21. Appearance of suspension of 1 %w/v water dispersible granules containing partially purified <i>S. curtisii</i> extract after dilution and stand for 12 hours	60
22. Ternary phase diagram of the mixtures consisting of soybean oil, Tween 80, and Span 80	62
23. Appearance of emulsifiable concentrate containing <i>S. curtisii</i> extract	64
24. Appearance of emulsion of 1 %w/v emulsifiable concentrate containing <i>S. curtisii</i> extract after dilution and stand for 12 hours	64
25. First-order plots of degradation of pyridostemin in water dispersible granules at 45, 60, and 70 °C (75 %RH)	67
26. First-order plots of degradation of pyridostemin in emulsifiable concentrate at 45, 60, and 70 °C (75 %RH)	67
27. Arrhenius plots of pyridostemin in partially purified extract, water dispersible granules and emulsifiable concentrate	69
28. Pyridostemin remaining (%) in water dispersible granules after storage at 4 °C and ambient temperature for 3 months	72
29. Pyridostemin remaining (%) in the developed emulsifiable concentrate containing after storage at 4 °C and ambient temperature for 3 months	72
30. Pyridostemin remaining (%) in the developed water dispersible granules and the developed emulsifiable concentrate after storage at ambient temperature for 3 months	73
31. The oil droplet size between 10 th and 90 th percentile of resultant 1 %w/v emulsions from emulsifiable concentrate containing <i>S. curtisii</i> extract after storage at 4 °C and ambient temperature	77
32. Structure of lactose	100
33. Structure of Tween 80	101
34. Structure of PVP	103
35. Structure of sodium alginate	105
36. Structure of BHT	107
37. Structure of Span 80	108

LIST OF ABBREVIATIONS AND SYMBOLS

A	= frequency factor
BHT	= butylated hydroxytoluene
brs	= broad singlet
°C	= degree celcius
CDCl₃	= deuteriochloroform
CMEC	= carboxylmethylethylcellulose
cm⁻¹	= reciprocal centimeter (wave number)
cm²	= centimeter squared
cps	= centipoises
d	= doublet
dd	= doublet of doublets
dm²	= decimeter squared
g	= gram
E_a	= activation energy
ED₅₀	= effective concentration 50
ED₉₀	= effective concentration 90
EIMS	= electron impact mass spectroscopy
k	= stability rate constant
kJmol⁻¹	= kilo joule per mole
¹H NMR	= proton nuclear magnetic resonance
HPLC	= high-performance liquid chromatography
HPMC	= hydroxypropylmethylcellulose
Hz	= hertz
IC₅₀	= inhibition concentration 50
IR	= infrared
J	= coupling constant
LD₅₀	= lethal concentration 50
ln c	= logarithm of the percentage remaining amount of pyridostemin
ln c₀	= logarithm of the percentage remaining amount of pyridostemin at t = 0
m	= multiplet
mg	= milligram

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

MHz	= megahertz
min	= minute
mg	= milligram
ml	= milliliter
mm	= millimeter
MS	= mass spectroscopy
m/z	= a value of mass divided by charge
μg	= microgram
μl	= microliter
μm	= micrometer
μM	= micromolar
N	= normality (the number of equivalents per liter of solution)
nm	= nanometer
PDA	= photodiode array
ppm	= part per million
PEG	= polyethyleneglycol
PVP	= polyvinylpyrrolidone
quin	= quintet
R	= gas constant
RH	= relative humidity
rpm	= revolution per minute
r^2	= coefficient of determination
RSD	= relative standard deviation
s	= singlet
SD	= standard deviation
T	= absolute temperature in Kelvins
T_g	= glass transition
t	= triplet
t	= time
$t_{1/2}$	= half-life
$t_{90\%}$	= shelf-life

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

ν_{\max}	= maximum absorption frequency
v/v	= volume by volume
w/v	= weight by volume
w/w	= weight by weight
δ	= chemical shift relative to tetramethylsilane