

CONTENTS

	Page
Abbreviations.....	(i)
List of Figures.....	(ii)
List of Tables.....	(v)
Abstract.....	(vi)
Abstrak.....	(vii)

CHAPTER 1 INTRODUCTION

1.1 <i>Eurycoma longifolia</i> Jack.....	1
1.1.1 Description and Habitat.....	1
1.1.2 Uses of <i>Eurycoma longifolia</i> in Traditional Medicine.....	3
1.1.3 Biologically Active Compounds of <i>Eurycoma longifolia</i>	4
1.2 Alkaloids.....	5
1.2.1 Definition.....	5
1.2.2 Functions.....	6
1.2.3 Occurrence of Alkaloid-bearing Plants.....	6
1.2.4 Distribution in the Plant.....	7
1.2.5 Isolation of Crude Alkaloids.....	8
1.2.6 Alkaloid-detecting Reagents.....	10
1.3 Synaptosomes.....	12
1.3.1 Formation and Properties.....	12

1.3.2 Preparation of Synaptosomes.....	14
1.4 Dopamine.....	15
1.4.1 Catecholamines.....	15
1.4.2 Principal Dopaminergic Pathways in the Rat Brain.....	16
1.4.3 Synthesis of Dopamine.....	16
1.4.4 Release of Dopamine.....	17
1.4.5 Reuptake and Degradation.....	18
1.4.6 Alterations in Dopaminergic Systems in Disease.....	18
1.5 High Performance Liquid Chromatography (HPLC).....	19
1.5.1 General.....	19
1.5.2 Reversed Phase Chromatography.....	21
1.5.2.1 <i>Mechanism of separation</i>	21
1.5.2.2 <i>Reversed phase HPLC of basic compounds</i>	22
1.5.3 Trace Enrichment.....	24
1.5.4 Absorbance Detectors and Photodiode Array Detectors.....	24
1.5.5 Gradient Separation.....	25
1.5.6 Quantitation.....	27
1.5.7 Column Efficiency.....	27
Objective.....	29

CHAPTER 2 MATERIALS AND METHODS

MATERIALS.....	30
METHODS.....	33
2.1 Extraction of Crude Alkaloids.....	33
2.1.1 Methanolic Extract.....	33
2.1.2 Crude Alkaloid Fraction.....	33

2.2 High Performance Liquid Chromatography (HPLC).....	36
2.2.1 HPLC Systems Used.....	36
2.2.2 Sample Preparation.....	36
2.2.3 Preparation of Mobile Phases.....	37
2.2.4 Isocratic Separations.....	38
2.2.5 Gradient Separations.....	38
2.2.6 Collection of Fractions from Gradient Separation.....	40
2.2.7 Removal of Water from the Collected Fractions.....	41
2.2.7.1 By high performance liquid chromatography (HPLC).....	41
2.2.7.2 By using Sep-Pak cartridges.....	41
2.2.8 Column Efficiency.....	42
2.3 Thin-layer Chromatography (TLC).....	43
2.3.1 Thin-layer Chromatography.....	43
2.3.2 Dragendorff's Spray Reagent.....	43
2.4 Preparation of Synaptosomes.....	44
2.4.1 Solutions.....	44
2.4.2 Preparation of Synaptosomes.....	45
2.4.3 Protein Determination.....	47
2.4.3.1 Preparation of Bradford reagent.....	47
2.4.3.2 Protein assay.....	48
2.5 Assay for the Synaptosomal Release of Tritiated Dopamine.....	49
2.5.1 Pre-loading Synaptosomes with [³H]Dopamine.....	49
2.5.2 Assay for the Release of [³H]Dopamine.....	49
2.6 Assay for the Synaptosomal Uptake of Tritiated Dopamine.....	51
2.7 Scintillation Counting.....	52

2.7.1 Preparation of the Scintillation Cocktail.....	52
2.7.2 Effect of the Concentration of Crude Alkaloids on the Liquid Scintillation Counting of Tritiated Samples.....	53
2.7.3 Preparation of Scintillation Samples.....	54
2.8 Electron Microscopy.....	57

CHAPTER 3 RESULTS AND DISCUSSION

3.1 The Crude Alkaloid Fraction from <i>Eurycoma longifolia</i> Root.....	59
3.1.1 Yield of Crude Alkaloids.....	59
3.1.2 Quenching of Tritium Counts by the Crude Alkaloid Fraction.....	62
3.1.3 The Effect of the Concentration of Crude Alkaloids on the Release of [³ H]Dopamine from Rat Brain Synaptosomes.....	65
3.1.4 The Effect of the Concentration of Crude Alkaloids on the Uptake of [³ H]Dopamine by Rat Brain Synaptosomes.....	69
3.2 Development of an HPLC Method for the Separation of the Crude Alkaloid Fraction from <i>Eurycoma longifolia</i> Root.....	75
3.2.1 Isocratic Separation.....	75
3.2.1.1 Selection of detection wavelength.....	75
3.2.1.2 Percentage of organic modifier in the mobile phase.....	78
3.2.1.3 Type of organic modifier.....	80
3.2.1.4 Comparison of buffered and non-buffered mobile phase.....	84

3.2.2 Gradient Separation.....	84
 3.2.2.1 Aqueous acetonitrile and aqueous methanol	
gradients.....	84
 3.2.2.2 Flow-rate.....	89
 3.2.2.3 Gradient slope.....	91
 3.2.2.4 pH.....	93
 3.2.2.5 Chromatographic conditions used in the collection	
of fractions from the reversed phase HPLC	
separation of the crude alkaloid fraction from	
<u>Eurycoma longifolia root</u>	97
3.2.3 Integration.....	98
3.2.4 Determination of Void Volume and Column Efficiency.....	100
3.3 The Fractions Obtained by Reversed Phase HPLC of the Crude	
Alkaloid Fraction from <i>Eurycoma longifolia</i> Root: Fractions 1 to 5.....	104
3.3.1 Thin-layer Chromatography (TLC).....	104
3.3.2 Summary of the Characteristics of Fractions 1 to 5.....	108
3.3.3 Release of [³H]Dopamine from Rat Brain Synaptosomes	
Incubated with Fractions 1 to 5.....	109
3.3.4 Uptake of [³H]Dopamine by Rat Brain Synaptosomes	
Incubated with Fractions 1 to 5.....	112
3.4 Electron Microscopy.....	115
General Discussion.....	117
Conclusion.....	120
References	