
Table of Contents

Items	Page
Acknowledgement	i
Abstract	iii
Abstrak	vi
List of Abbreviation	x
List of Tables	xiv
List of Figures	xvi
Chapter 1	
Introduction	
Introduction	1
Chapter 2	
Literature Review	
2.1 Introduction	6
2.1.1 <i>Origin of Papaya</i>	6
2.1.2 <i>Classification and Nomenclature</i>	6
2.1.3 <i>Synonyms and Common Names</i>	7
2.1.4 <i>Etymology and Leave Characteristics</i>	7
2.1.5 <i>Plant Description</i>	7
2.1.6 <i>Common Uses</i>	7
2.1.7 <i>Pharmacological Properties of Carica papaya L.</i>	8
2.1.8 <i>Carica papaya L. var Eksotika</i>	9
2.2 Economic Importance of Eksotika	10

2.3 Plant Tissue Culture	10
2.3.1 <i>Introduction</i>	10
2.3.2 <i>Plant Regeneration</i>	11
2.3.2.1 <i>Organogenesis</i>	11
2.3.2.2 <i>Somatic Embryogenesis</i>	12
2.3.2.2.1 <i>Germination of Somatic Embryo</i>	12
2.3.4 <i>Single Cell Culture</i>	13
2.4 Tissue Culture of Papaya	14
2.4.1 <i>Plant Growth Regulators</i>	15
2.4.1.1 <i>Auxins</i>	16
2.4.1.2 <i>Cytokinins</i>	16
2.4.1.3 <i>Interaction between Auxin and Cytokinin</i>	17
2.4.1.4 <i>Gibberellins</i>	18
2.5 Alkaloids	19
2.6 Carpaine	20
2.7 Compound Extraction	22
2.8 Supercritical Fluid Extraction	25
2.9 Chromatography	26
2.9.1 <i>Thin Layer Chromatography</i>	26
2.9.2 <i>Gas Chromatography</i>	27

2.10 Characterization of Extract	28
<i>2.10.1 Mass Spectrometry</i>	28
<i>2.10.2 Magnetic Resonance Spectrometry</i>	29
2.11 Objective of Study	30
Chapter 3	
Materials and Methods	
3.1 Plant Material	31
3.2 Preparation of Culture Medium	31
<i>3.2.1 Embryogenic Callus Induction Medium</i>	31
<i>3.2.2 Liquid Multiplication Medium</i>	32
<i>3.2.3 Germination, Regeneration and Rooting Medium</i>	32
3.3 Sterilization Procedure	32
3.4 Explant Culture	33
<i>3.4.1 Preparation of Explants</i>	33
<i>3.4.2 Callus Induction Medium</i>	33
<i>3.4.3 Establishment of Suspension Cultures</i>	34
3.5 Verification of Embryogenic Callus	34
<i>3.5.1 Histochemical Test</i>	34
<i>3.5.2 Histological Studies</i>	35
3.6 Viability Test	35

3.7 Observation and Growth Measurements	36
3.7.1 <i>Callus Cultures</i>	36
3.7.2 <i>Growth Curve of Suspension Cultures</i>	36
3.7.3 <i>Histological Analysis</i>	37
3.7.4 <i>Viability of Cell Suspension</i>	37
3.7.5 <i>Experimental Replicate</i>	37
3.8 Student 't' Distribution Test	37
3.9 General Remarks for Compound Extraction Experimental Procedure	39
3.10 Plant Material	39
3.11 Sample Extraction	40
3.11.1 <i>Conventional Method</i>	40
3.11.1.1 <i>Mother Plant</i>	40
3.11.1.2 <i>In vitro Regenerant</i>	40
3.11.1.3 <i>Embryogenic Suspension Cells</i>	40
3.11.2 <i>Supercritical Fluid Extraction</i>	42
3.11.2.1 <i>Mother Plant</i>	42
3.11.2.2 <i>In vitro Regenerant</i>	42
3.11.2.3 <i>Embryogenic Suspension Cells</i>	42
3.12 Characterization of Alkaloid Carpaine	43
3.12.1 <i>Melting Point</i>	43
3.12.2 <i>Thin Layer Chromatography</i>	43

3.12.3 Magnetic Resonance Spectrometry	44
3.12.4 Gas Chromatography / Mass Spectrometry	44
Chapter 4	
Results and Observation	
4.1 Induction and Development of Embryogenic Callus	46
4.2 Formation of Somatic Embryos	47
4.3 Embryogenic Cell for Liquid Multiplication Culture	50
4.4 Histochemical Test	53
4.5 Histo-Anatomy Study	54
4.6 Somatic Embryo Multiplication in Liquid Culture	57
4.7 Cell Viability Assessment	62
4.8 Somatic Embryo Germination	64
4.9 Regeneration of Plantlet	67
4.10 Characterization of Alkaloid Carpaine	72
4.10.1 Conventional Method (Acid/Base Extraction)	72
4.10.1.1 Field Grown Plant, In vitro Regenerant and Embryogenic Cells Suspension	72
4.10.2 Supercritical Fluid Extraction	78
4.10.2.1 Field Grown Plant and In vitro Regenerant	78

Chapter 5
Discussion

5.1 Choice of Explants	106
5.2 Choice of Media	106
5.3 Formations of Embryogenic Callus	107
5.4 Verification of Embryogenic Cell	108
5.5 Formations of Somatic Embryo	109
5.6 Somatic Embryogenesis in Suspension Culture	110
5.6.1 <i>Effects of Liquid Multiplication Medium</i>	110
5.6.2 <i>Growth and Multiplications of Cells</i>	111
5.7 Plant Regeneration from Cell Suspension	113
5.8 Characterization of Alkaloid Carpaine	115

Chapter 6
Conclusion

Conclusion	120
------------	-----

Chapter 7
Future Studies

Future Studies	123
----------------	-----

References	xxviii
-------------------	---------------

Appendices	xxxiii
-------------------	---------------

Publications	xlii
---------------------	-------------