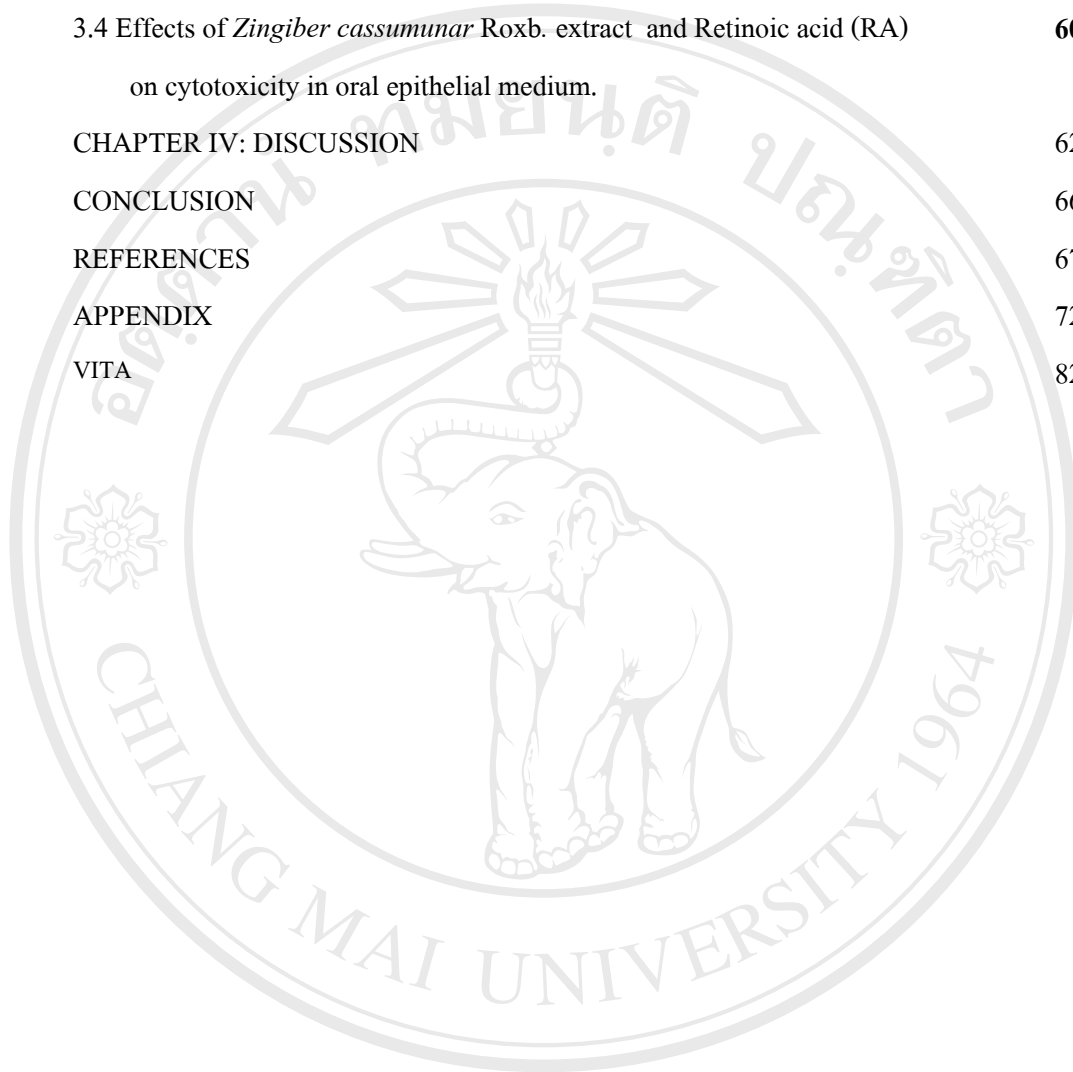


## TABLE OF CONTENTS

	PAGE
<b>ACKNOWLEDGEMENT</b>	iii
<b>ENGLISH ABSTRACT</b>	iv
<b>THAI ABSTRACT</b>	vi
<b>LIST OF TABLES</b>	xi
<b>LIST OF ILLUSTRATIONS</b>	xii
<b>ABBREVIATIONS</b>	xv
<b>CHAPTER I: INTRODUCTION</b>	
1.1 Statement of the problems	1
1.2 Literature reviews	
1.2.1 Wound Healing	4
1.2.2 Hyaluronan	10
1.2.3 Glycosaminoglycan (GAG) and Proteoglycan	12
1.2.4 Matrix metalloproteinase (MMP)	16
1.2.5 <i>Zingiber cassumunar</i> Roxb. Or Plai	20
1.2.6 Objective	25
<b>CHAPTER II: MATERIALS AND METHODS</b>	
2.1 Materials	
2.1.1 Reagents used in this study and their sources	26
2.1.2 Lists of antibodies used in this study	27
2.2 Methods	
2.2.1 Preparation of <i>Zingiber cassumunar</i> Roxb.(Plai) extracts	28
2.2.2 Oral cell culture	28
2.3 Analytical methods	
2.3.1 Dye binding assay	29
2.3.2 Enzyme-linked immunosorbent assay	29
2.3.3 Gelatin Zymography	30
2.3.4 Cytotoxicity detection	30
2.3.5 Protein assay	31

	<b>PAGE</b>
2.3.6 Statistical method	31
<b>CHAPTER III: RESULTS</b>	
3.1 Effect of <i>Zingiber cassumunar</i> Roxb. extract on phenotype of oral fibroblast and epithelial cells	32
3.2 Effects of <i>Zingiber cassumunar</i> Roxb. extract on the level of extracellular matrix (ECM); HA, sulfated-GAG, MMP-2, -9, and the cytotoxicity from oral cells cultured medium.	33
3.2.1 Effects of <i>Zingiber cassumunar</i> Roxb. extract, Retinoic acid (RA), 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the level of hyaluronic acid (HA) in oral fibroblast medium.	33
3.2.2 Effects of <i>Zingiber cassumunar</i> Roxb. extract, Retinoic acid (RA), and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the level of HA from oral epithelial medium.	38
3.2.3 Effect of <i>Zingiber cassumunar</i> Roxb. Extract and Retinoic acid (RA), 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the level of S-GAG from oral fibroblast medium.	43
3.2.4 Effects of <i>Zingiber cassumunar</i> Roxb. extract and Retinoic acid (RA), 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the level of S-GAG from oral epithelial medium.	46
3.2.5 Effect of <i>Zingiber cassumunar</i> Roxb. extract, Retinoic acid (RA), and Interleukin-1 $\beta$ (IL-1 $\beta$ ) on the level of MMP-2, 9 from oral fibroblast medium.	49
3.2.6 Effects of <i>Zingiber cassumunar</i> Roxb. extract, Retinoic acid (RA), Interleukin-1 $\beta$ (IL-1 $\beta$ ), and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the level of MMP-2, -9 from oral epithelial medium.	53
3.3 Effects of <i>Zingiber cassumunar</i> Roxb. extract and Retinoic acid (RA) on cytotoxicity in oral fibroblast medium.	58

	<b>PAGE</b>
3.4 Effects of <i>Zingiber cassumunar</i> Roxb. extract and Retinoic acid (RA) on cytotoxicity in oral epithelial medium.	<b>60</b>
CHAPTER IV: DISCUSSION	62
CONCLUSION	66
REFERENCES	67
APPENDIX	72
VITA	82



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright © by Chiang Mai University  
 All rights reserved

## LIST OF TABLES

TABLE	PAGE
1. Matrix metalloproteinases (MMP) and their substrates.	19
2. All five compounds isolated from the hexane extract of rhizome were found to possess equally or more potent anti-inflammatory activity than the reference drug diclofenac.	23
3. Inhibitory effect of Plai extracts on the release of HA in oral fibroblast medium.	34
4. Inhibitory effects of Plai extracts on the release of HA in oral epithelial medium.	39
5. Effect of ethanol extracted Plai, RA and TPA on the release of S-GAG in oral fibroblast medium.	44
6. Effects of ethanol extracted Plai, RA and TPA on the release of S-GAG in oral epithelial medium.	47

## LIST OF ILLUSTRATIONS

<b>ILLUSTRATIONS</b>	<b>PAGE</b>
1. A cutaneous wound three days after injury.	7
2. A cutaneous wound five days after injury.	8
3. Phases of wound repair.	
4. D-glucuronic acid and N-acetyl glucosamine, the disaccharide backbone of HA.	11
5. The models of typical proteoglycans.	14
6. A schematic diagram demonstrating the molecular structure of glycosaminoglycans.	15
7. The domain structure of the MMPs.	18
8. <i>Zingiber cassumunar</i> Roxb. and its rhizomes.	22
9. Diagram and Main compound of active compounds in <i>Zingiber cassumunar</i> Roxb.	24
10. Inhibitory effect of various concentrations of the extracts of <i>Zingiber cassumunar</i> Roxb. on the releases of HA in oral fibroblast medium.	32
11. Inhibitory effect of various concentrations of the extracts of <i>Zingiber cassumunar</i> Roxb. on the releases of HA in oral fibroblast medium.	35
12. Effect of various concentrations of Retinoic acid (RA) on the releases of HA in oral fibroblast medium.	36
13. Inhibitory effect of various concentrations of the extracts of <i>Zingiber cassumunar</i> Roxb. on the release of HA in oral fibroblast medium.	37
14. Stimulatory effects of various concentrations of the extracts of <i>Zingiber cassumunar</i> Roxb. on the release of HA in oral epithelial medium.	40
15. Effect of various concentrations of Retinoic acid (RA) on the release of HA in oral epithelial medium.	41
16. Stimulatory effects of various concentrations of the extracts of <i>Zingiber cassumunar</i> Roxb. on the releases of HA in oral epithelial medium.	42
<b>ILLUSTRATIONS</b>	<b>PAGE</b>

17. Effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on the releases of S-GAG from oral fibroblast medium.	45
18. Effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on the releases of sulfated-GAG from oral epithelial medium.	48
19. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral fibroblast medium.	50
20. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral fibroblast medium.	51
21. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral fibroblast medium.	52
22. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral epithelial medium.	54
23. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral epithelial medium.	55
24. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral epithelial medium.	56
25. Inhibitory effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on gelatinolytic activity in oral epithelial medium.	57
26. Effects of various concentrations of the ethanol extract of <i>Zingiber cassumunar</i> Roxb. on cytotoxicity in oral fibroblast medium.	59
27. Effects of various concentrations of the ethanol extract of <i>Zingiber</i>	61

*cassumunar* Roxb. on cytotoxicity in oral epithelial medium.

## ABBREVIATIONS

B-HABPs	biotinylated hyaluronan binding proteins
BPE	bovine pituitary extract
BSA	bovine serum albumin
CO <sub>2</sub>	carbon dioxide
CS	chondroitin sulfate
CS-C	chondroitin 6-sulfate
DBA	dye binding assay
DMEM	Dulbecco's modified Eagle's medium
DMMB	1,9-dimethylmethylene blue
DMPBD	(E)-1-(3',4'-dimethoxyphenyl) butadiene
DS	dermatan sulfate
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
GAGs	glycosaminoglycans
GalNAc	N-acetyl-galactosamine
GlcA	D-glucuronic acid
g	gram
HA	hyaluronan
HABPs	hyaluronan binding proteins
hEGF	human Epidermal growth factor
HEPES	N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid
hr.	hour
HS	heparan sulfate
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
IL-1 $\beta$	interleukin-1 $\beta$
KBM	keratinocyte basal medium



kDa	kilodaltons
LDH	lactate dehydrogenase
M	molarity
Min.	minute
MMPs	matrix metalloproteinases
ml.	milliliter
mm.	millimeter
ng.	Nanogram
nm.	Nanometer
P-EtOH	ethanol extracted Plai
P-Hex	hexane extracted Plai
P-H <sub>2</sub> O	water extracted Plai
RA	retinoic acid
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
S-GAG	sulfated glycosaminoglycan
TEMED	N,N,N',N'-tetra-methyl-ethylenediamine
TPA	12-O-tetradecanoyl phorbol-13-acetate
Tween-20	polyoxyethylene sorbitan monolaurate
V	voltage
w/v	weight by volume
w/w	weight by weight
%	percent
°C	degree Celsius
µg	microgram
µM	micromolar
µl	microlitre