

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
ENGLISH ABSTRACT	iv
THAI ABSTRACT	vi
LIST OF TABLES	xi
LIST OF ILLUSTRATIONS	xii
ABBREVIATIONS	xiv
CHAPTER I INTRODUCTION	1
1.1. Statement of Problems	1
1.2. Literature reviews	2
1.2.1. The oral environment	2
1.2.1.1. Saliva	4
1.2.2. Periodontium	8
1.2.3. Gingivitis	10
1.2.4. Periodontitis	12
1.2.5. Role of bacteria	13
1.2.6. Proteinases and Matrix Degradation	16
1.2.7. Extracellular matrix - degrading proteinase	17
1.2.8. Role of inflammation in periodontal disease	20
1.2.9. Proteolytic enzymes within inflammatory cell	21
1.2.10. Cysteine Proteinase	23
1.2.11. Serine Proteinases	24

1.2.12.	Endogenous proteinase inhibitors	25
1.2.13.	Cysteine proteinase inhibitors	26
1.2.14.	Enzyme mechanism	26
1.2.15.	Plant proteinase inhibitors	27
1.3.	Objectives	29
CHAPTER II MATERIALS AND METHODS		30
2.1.	Materials	30
2.2.	Group of subjects	32
2.3.	Collection of whole saliva	33
2.4.	Determination of protein concentration	33
2.5.	Determination of total proteinase activity	36
2.6.	Determination of cysteine protease activity	39
2.7.	Determination of elastase activity	41
2.8.	Determination of plant inhibitory activity	44
2.9.	Determination of enzyme inhibition of periodontitis saliva adding with the most inhibitory effect of Thai herbs	48
2.10.	Statistic Analysis	52
CHAPTER III RESULTS		53
3.1.	Clinical measurements	53
3.2.	Biochemical analysis of saliva	53
3.3.	Quantitation of Total proteinase and its inhibitor	55
3.4.	Quantitation of cysteine proteinase and cystatin	58
3.5.	Quantitation of elastase and its inhibitor	63
3.6.	Comparison of HPLC and Spectrophotometric determination of elastase activity	68

3.7.	The electrophoresis of whole saliva	70
3.8.	Determination of total proteinase inhibitory activity in Thai-herbs extracts	72
3.9.	Trypsin inhibitory activity in various species of Thai-herbs	76
3.10.	Trypsin inhibitor with synthetic substrate	77
3.11.	Determination of enzyme inhibitors in periodontitis saliva with the highest inhibitor of Thai herbs	83
CHAPTER IV DISCUSSION		87
REFERENCES		91
APPENDIX		98
CURRICULUM VITAE		109

LIST OF TABLES

Table		Page
Table 1.1	The average composition of mixed human saliva and normal value for plasma	5
Table 1.2	The major functions of saliva	6
Table 1.3	Salivary constituents	7
Table 1.4	Extracellular matrix remodeling events involving proteolysis	16
Table 1.5	Proteinases of connective tissues	18
Table 1.6	Proteinase susceptibility of extracellular matrix proteins	19
Table 1.7	Constituents of neutrophil granules	23
Table 1.8	Polypeptide inhibitors of matrix-degrading proteinases	25
Table 1.9	Proteinase inhibitor by plant extracts	28
Table 2.1	The amount of substrate and enzyme	47
Table 3.1	Descriptive data of sample groups	54
Table 3.2	Salivary parameter of sample groups	54
Table 3.3	The median values of HPLC and spectrophotometric determination	68
Table 3.4	Composition of enzyme in saliva presented in median value	69
Table 3.5	Proteinase inhibitor of Thai herb extracts by unprocessed x-ray film	75
Table 3.6	Proteinase inhibitors (mg/g dry weight) of Thai herb extracts	81
Table 3.7	Proteinase inhibitors (mg/ml) of Thai herb extracts	82
Table 3.8	Enzyme Inhibitors of Periodontitis saliva	85

LIST OF ILLUSTRATIONS

Figure		page
Figure 1.1	Three successive stages in the colonization of a pellicle-covered enamel surface	3
Figure 1.2	The picture of healthy, gingivitis, and periodontitis mouth	9
Figure 1.3	Schematic representation of interactive processes of gingival inflammation	11
Figure 1.4	Proposed chemical structure of the lipid A molecule	15
Figure 1.5	Schematic diagram of the enzymes pathways of host response	15
Figure 1.6	Diagram of collagen degradation	22
Figure 1.7	Diagram of proteoglycan degradation	22
Figure 2.1	The standard curve of protein concentration	35
Figure 2.2	Schematic representation of the proteinase	37
Figure 2.3	Diagram of determination of enzymes inhibition of periodontitis saliva with Thai herbs	48
Figure 2.4	Diagram of determination for saliva sample	49
Figure 2.5	Diagram of determination of inhibitor from Thai herbs	50
Figure 3.1	The standard curve of hydrolysis of biotin-gelatin	56
Figure 3.2	Percent inhibition of trypsin	57
Figure 3.3	The optimum incubation time of papain	59
Figure 3.4	Standard curve for papain assay	60
Figure 3.5	The standard curve of papain concentration	61
Figure 3.6	Percent inhibition of papain	62
Figure 3.7	Chromatograms of enzymatic digests of SAAVNA	64
Figure 3.8	Standard curve of elastase activity	65
Figure 3.9	Percent inhibition of elastase activity by HPLC method	66

Figure 3.10	Standard curve of elastase activity at different concentration by spectrophotometric method	67
Figure 3.11	The pattern of saliva from different individual in normal, gingivitis and periodontitis subjects by SDS-PAGE	71
Figure 3.12	The clear zone on the gelatin coating X-ray film	73
Figure 3.13	The gelatin coating X-ray film demonstrated the inhibition effect	73
Figure 3.14	The clear zone indicated the activity of inhibitor of Thai herb extracts.	74
Figure 3.15	The effect of trypsin inhibitor extracted from the selected Thai-herbs on BApNA	78
Figure 3.16	The effect of papain inhibitor extracted from the selected Thai-herbs on BANA	79
Figure 3.17	The effect of elastase inhibitor extracted from the selected Thai-herbs on SAA VNA	80
Figure 3.18	The enzymes inhibitors in periodontitis saliva	84
Figure 3.19	The SDS-PAGE gel of elastase activity	86

ABBREVIATIONS

%	Percent
°C	Degree Celsius
µg	Microgram
µl	Microliter
Abs	Absorbance
APS	Ammonium persulfate
BANA	α-N-Benzoyl-DL-Arginine-β-Naphthylamine
b-gelatin	biotinylated – gelatin
BSA	bovine serum albumin
cm	Centimeter
CV	Coefficient of variation
DMSO	dimethyl sulfoxide
EDTA	ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
g	Gram
HPLC	High performance liquid chromatography
hrs	Hours
L	Liter
M	Molar
mg	Milligram
Min	Minute
ml	Milliliter

mM	Millimolar
MW	molecular weight
NA	p-Nitroaniline
NaCl	sodium chloride
ng	nanogram
OD	optical density
PMN	polymorphonuclear leukocytes
PMSF	Phenylmethyl sulfonyl fluoride
PPD	probing pocket depth
rpm	revolution per minute
SAAVNA	N-Succinyl-Ala-Ala-Val-Nitroaniline
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
STI	Soybean trypsin inhibitor
TEMED	N, N, N, N, -tetramethyl ethylene-diamine
TNC	Tris-NaCl-CaCl ₂
Tris-base	Tris (hydroxymethyl aminomethane)
U	Unit
UV	ultraviolet
W/V	weight by volume
yr.	year