APPENDIX

A. REAGENTS PREPARATION

1. Reagent for Lowry Assay

Reagent A

$CuSO_4.5H_2O$	0.50 g
Na3C6H5O7.2H2O	1.00 g

Reagent B

Na2CO3	20.00 g
NaOH	4.00 g

All reagents were dissolved in 1000 ml. of distilled water and stored at room temperature

Reagent C : Alkaline copper solution

Reagent D : Folin – Ciocalten reagent

Folin – Ciocalteu phenol	10.00 ml
Distilled water	10.00 ml

Solution A and B at ratio 50 : 1 was prepared immediately prior to be used.

2. 1 M Tris. Cl [trischydroxymethyl aminomethame], pH 9.0

Dissolve 121.14 g Tris base in 800 ml of distilled water

Adjust to desired pH with concentrated HCl

Mix and add distilled water to 1 liter

3. TNC – buffer, pH 7.6

1 M Tris-HCl buffer	50.0 ml
NaCl	11.68 g
CaCl2	0.55 g

All reagents were dissolved in 800 ml of distilled water

Adjust to desired pH with concentrated HCl

Mix and add distilled water to 1 liter

4. Phosphate buffer saline (PBS)

NaCl	8.00	g
KCl	0.20	g
$Na_2HPO_4.12H_2O$	2.90	g
K_2HPO_2	0.20	g

All reagents were dissolved in distilled water and made up volume to 1 liter

5. Citrate phosphate buffer

Citric acid monohydrate	10.30	g
$Na_2HPO_4.3H_2O$	18.16	g

All reagents were dissolved in 900 ml of distilled water adjust pH to 5.0 and made up volume to 1 liter Stored reagent at 4°C

6. Carbonate – bicarbonate buffer (coating buffer)

Na_2CO_3	1.59	g
$NaHCO_3$	2.93	g

All reagents were dissolved in 900 ml of distilled water adjust pH to 9.6 and made up volume to 1 liter

7. PBS – Tween 20

PBS	100	ml
Tween-20	0.05	ml

8. Substrate solution

OPD	8.00	mg
Citrate phosphate buffer	12.00	mg
30% H_2O_2	5.00	µl

Prepare reagent fresh for 1 plate ; keep in dark before use.

1. HPLC reagent

1. 0.1 M disodium hydrogen phosphate

Na_2HPO_4	1.42	g
deionized water	100	ml

2. 0.1 M Sodium dihydrogen phosphate

Na_2HPO_4	1.20	g
deionized water	100	ml

3. 0.01 M Sodium phosphate buffer, (pH 3.0)

0.01 M sodium phosphate buffer, (pH 7.4)

Combine	0.1 M Na_2HPO_4	68.4	ml
	0.1 M NaH_2PO_4	31.6	ml

adjusted with 1 M phosphoric acid bring total volume to 1000 ml with deionized water. Filter the solution through a 0.45 µm membrane filter.

4. Buffer solution : 0.50 M Tris-HCl buffer pH 7.5, 0.2 M NaCl

1 M Tris – HCl	50	ml
NaCl	11.68	g
deionized water	800	ml

mixed and adjust to pH 7.5 with 4 N HCl. Bring total volume to 1000 ml with deionized water.

5. Substrate Solution : 0.1% (v/v) dimethyl sulfoxide (DMSO) in buffer solution

Bimethyl sulfoxide	0.1	ml
Buffer solution	99.9	ml

6. Substrate : 1 mM SAAVNA

Stocking 10 mM SAAVNA

SAAVNA	2.5	mg
DMSO	500	µl

diluted to 1 : 10 with substrate solution

2. SDS-PAGE Buffer System

Stock solution and Buffer

1. Solution A : 30% Acrylamide/0.8/bisacrylamide (30% T, 2.67% C)

Acrylamide	29.2	g
N'N'-bis-methylene-acrylamide	0.8	g

Make to 100 ml with deionized water. Filter the solution through a 0.45 µ m filter and store at 4°C in the dark (30 days maximum)

2. Solution B : Separating gel buffer-1.5 M Tris-HCl, pH 8.8

Tris base	18.15	g
Deionized water	80.0	ml

Adjust to pH 8.8 with 4 N HCl. Bring total volume to 100 ml with deionized water. Filter and store at 4°C

3. Solution C : Stacking gel buffer – 0.5 M Tris-HCl, pH 6.8

Tris base	6.05	g
Deionized water	60.0	ml

Adjust to pH 8.8 with 4 N HCl. Bring total volume to 100 ml with deionized water. Filter and store at 4°C

4. 10% (W/V) SDS

Sodium dodecyl sulfate	1	g
Deionized water	10.0	ml

Sterilized by filtration and stored at room temperature

5. 10% APS

ammonium persulfate	0.5	g
Deionized water	5.0	ml

Sterilized by filtration then 200 µl aliquot, store at -20°C

6. Sample Buffer (SDS Reducing Buffer)

Deionized water	3.55	ml
0.5 M Tris-HCl, pH 6.8	1.25	ml
glycerol	2.5	ml
10% (w/v) SDS	2.0	ml
0.5% (w/v) bromophenol blue	0.2	ml
total volume	9.5	ml

Store at room temperature

Use : Add 20 µl β-mercaptoethoanol to 475 µl sample buffer prior to use.

Dilute the sample at least 1 : 1 (v/v) with sample buffer and heat at 95°C for 4 minutes

7. 10 X Electrode (Running) buffer, pH 8.3 (makes 1 L)

Tris base	30.3	g

Glycine	144.0	g
SDS	10.0	g

Dissolve and bring total volume up to 1,000 ml with deionized water

Do not adjust pH with acid or base. Store at 4°C

Use : Dilute 100 ml of 10 X stock with 900 ml deionized water for each electrophoresis run. Mix and filter thoroughly before use.

8. Separating gel preparation (10 ml.) –12% gel, 1.5 M Tris-HCl, pH 8.8

Deionized water	3.4	ml
Acrylamide/bis	4.0	ml
1.5 M Tris-HCl, pH 8.8	2.5	ml
10% w/v SDS	0.1	ml

Prepare the monomer solution by mixing all reagent except the TEMED and 10% APS. Degas the mixture 15 minutes. To initiate polymerization add 100 µl 10% APS and 5 µl TEMED, and swirl gently to mix.

9. Stacking gel preparation (5 ml) – 5% gel, 0.5 M Tris-HCl, pH 6.8

Deionized water	2.85	ml
Acrylamide/bis	0.85	ml
0.5 M Tris-HCl, pH 6.8	1.25	ml

Prepare the monomer solution by mixing all reagent except the TEMES and 10% APS. To initiate polymerization add 50 µl 10% APS and 5 µl TEMED, and swirt gently to mix.

10. 0.1% Commassie blue R-250

Commassie blue R-250	0.1	g
Methanol	40.0	ml
Acetic acid	10.1	ml

Adjust to 100 ml with deionized water

11. Destain solution

Methanol	400.0 ml
Acetic acid	100.0 ml

Adjusted to 1000 ml with deionized water

3. Plant inhibitor analysis

1. stock BApNA solution : 10 mM BApNA

Dissolved 4.4 mg of BApNA in 1 ml of DMSO, and diluted to 10 ml with 0.1 M Tris – HCl buffer pH 7.5

2. Trypsin solution

Dissolved 20 mg trypsin in 20.0 ml of 0.1 M Tris – HCl buffer, pH 7.5

3. Trypsin inhibitor solution

Dissolved 10 mg trypsin inhibitor in 1 ml of 0.1 M Tris – HCl buffer, pH 7.5

4. Cysteine protease determination

1. 0.5 M Na_2HPO_4

Dissolved 3.55 g in 50 ml of distilled water

2. 0.5 M KH_2PO_4

Dissolved 34.0 g in 500 ml of distilled water

3. 0.5 M phosphate buffer, pH 6.0

0.5 M KH2PO4	142.0 ml
0.5 M Na2HPO4	19.5 ml

Adjust volume to 1000 ml with distilled water

4. Incubation buffer : 0.2 M potassiuim phosphate buffer, pH 6.0, 4 mM EDTA,

0.2 mM cysteine

0.5 M phosphate buffer	400	ml
Cysteine	24	mg
Na_2EDTA	1.49	g

Dissolved and adjusted to 1000 ml with distilled water

5. BANA stock solution

Dissolved 40 mg in DMSO 1 ml and store at 4°C

6. 10 mM CMB (4-chloromercuribenzoid acid in 50 mM EDTA)

CMB	3.57	g
0.5 M NaOH	120.0	ml
Na_2EDTA	18.6	g
Distilled water	950	ml

Adjust pH 6.0 with 1 N HCl and made up to 1000 ml with distilled water and stored at room temperature

7. 4% (W/V) Brij 35

30% Brij 35	13.3	ml

Adjust volume to 100 ml with distilled water

8. 0.15 mg/ml Fast Garnet GBC in 4% w/v Brij - 35

4% (w/v) Brij – 35	10	ml
Fast Garnet GBC	1.5	mg

Dissolved, filtered and kept at 4oC for use the same day

9. Papain solution

Dissolved 2.0 mg in 1 ml of 0.1 M Tris – HCl buffer, pH 7.5

No. 17/2001

Documentary Proof of Ethics Clearance
Research Ethics Committee
Faculty of Medicine, Chiang Mai University
Chiang Mai, Thailand

Title of Project: Study of Salivary Proteolytic Enzyme Inhibitors from Thai Herbs in Patients with Oral Inflammatory Diseases

Principal Investigator : Mr. Viboon Rattanapanone

Name of Institution : Department of Biochemistry
Faculty of Medicine, Chiang Mai University

Approved by Research Ethics Committee on : March 7, 2001

Signature of Chairman of the Committee : .....

(Kumpol Klunklin, M.D.)

Signature of Head of the Institution :.....

(Piya Netrawichien, M.D.)

Date of Approval : March 7, 2001

แบบข้อมูลประวัติกลุ่มตัวอย่าง

ลำดับที่_____

ชื่อ _____ นามสกุล_____

อายุ _____ปี เพศ ( ) หญิง ( ) ชาย อาชีพ _____

หลักเกณฑ์การคัดเลือกกลุ่มตัวอย่าง

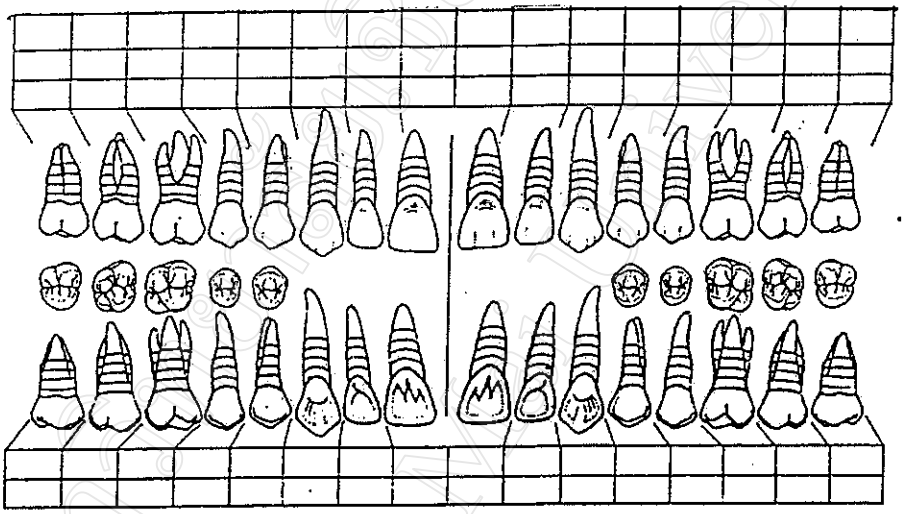
การตรวจสุขภาพ

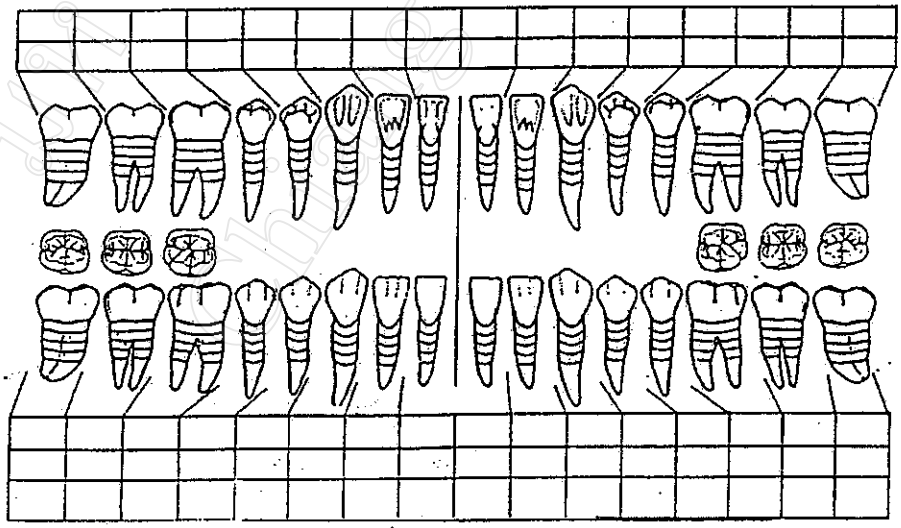
( ) ประวัติสุขภาพร่างกายปกติ

การตรวจสุขภาพช่องปาก

( ) การตรวจมีฟันอยู่ในช่องปากอย่างน้อย 20 ซี่

การตรวจทางปริทันต์(periodontal status)





การจำแนกกลุ่มตัวอย่างจากสุขภาพช่องปาก

( ) ปรกติ ( ) Gingivitis ( ) Periodontitis

ข้อมูลการเก็บน้ำลายตัวอย่างลำดับที่_____

น้ำลาย ปริมาณ_____ ml เวลา _____ นาที Flow rate _____ml/min

pH_____

Total protein

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