

APPENDIX

Reagents and buffers preparation

Articular cartilage explant cell culture

1. DMEM medium

DMEM	1 package (13.4 g)
HEPES	4.8 g
NaHCO ₃	3.7 g
Pen/Strep	20 ml

All chemicals were dissolved in 800 ml of deionized distilled water, adjusted pH to 7.2 and made up to a volume 1,000 ml. Steriled by suction through a filter (membrane pore size 0.2 um). Stored the reagent at 4°C.

Uronic acid assay

1. Papain solution

Cysteine	0.1212 g
EDTA	0.3722 g

All chemicals were dissolved in 0.05 M Phosphate buffer (pH 7.0), made up to a volume 100 ml. Stored the reagent at 4°C.

2. Concentrated sulfuric acid-borate reagent

di-Sodium tetraborate	2.3840 g
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The chemicals was dissolved in conc. Sulfuric acid and made up to a volume 500 ml, kept in a dark bottle. Stored the reagent at 4°C.

3. 0.1% m-phenylphenol

m-phenylphenol	0.1 g
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The chemicals was dissolved in absolute ethanol and made up to a volume 100 ml, kept in a dark bottle. Stored the reagent at 4°C.

Sulfated-GAG assay**1. DMMB Dye**

Glycine	1.52 g
NaCl	1.1850 g

All chemicals were dissolved in 400 ml of deionized distilled water, adjusted to pH 3.0. Add 0.0080 g dimethylmethylene blue and made up to a volume 500 ml. Stored the reagent at room temperature.

Enzyme-linked immunosorbent assay**1. Phosphate buffer saline (PBS)**

NaCl	8.00 g
KCl	0.20 g
Na ₂ HPO ₄	1.44 g
Na ₂ HPO ₄ ·2H ₂ O	0.24 g

All chemicals were dissolved in 900 ml of distilled water, adjusted to pH 7.4 and then added with distilled water to adjust to the volume 1 L. Stored at room temperature. Fore PBS-Tween, Tween-20 was added to 0.05%.

2. Coating buffer (for HA)

NaHCO ₃	8.4 g
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The chemicals was dissolved in 900 ml of distilled water, adjusted to pH 7.4 and then added with distilled water to adjust to the volume 1L.

3. 1% Bovine serum albumin (BSA)

BSA	0.1 g
PBS	10 ml

4. Citrate phosphate buffer

Citric acid monohydrate	10.30 g
Na ₂ HPO ₄ ·3H ₂ O	18.16 g

The chemicals was dissolved in 900 ml of distilled water, adjusted to pH 5.0 and made up to the volume 1 L. Stored the reagent at 4°C

5. Substrate solution

OPD	8 mg
Citrate phosphate buffer	12 ml
35% H ₂ O ₂	7 ul

Prepared the reagent fresh for 1 plate; kept in the dark before used.

Gelatin Zymography**1. Acrylamide/Bis solution**

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

All chemicals were dissolved in deionized distilled water, made up to the volume 100 ml. Filtered through a membrane filter pore size 0.45 um, collected in a dark bottle. Stored the reagent at 4°C.

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

Tris-base	18.15 g
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The chemicals was dissolved in 80 ml of deionized distilled water, adjusted to pH 8.8 and made up to the volume 100 ml. Stored the reagent at 4°C.

3. Stacking gel buffer 0.5 M Tris-HCl, pH 6.8

Tris-base	6.0 g
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The chemicals was dissolved in 80 ml of deionized distilled water, adjusted to pH 6.8 and made up to the volume 100 ml. Stored the reagent at 4°C.

4. 10% SDS solution

SDS	10 g
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The chemicals was dissolved in deionized distilled water, made up to the volume 100 ml.

5. 20% Ammonium persulfate solution

Ammonium persulfate	0.2 g
Deionized distilled water	1.0 ml

6. 0.1% Gelatin solution

Gelatin type B	0.01 g
Deionized distilled water	1.0 ml

7. 2X sample buffer

0.5 M Tris-HCl, pH 6.8	2.5 ml
Glycerol	2.0 ml
10% SDS	4.0 ml
0.1% bromophenol blue	0.5 ml
Deionized distilled water	1.0 ml

8. 10X Running Buffer, pH 8.3

Tris-base	30.3 g
Glycine	144 g
SDS	10 g

All chemicals were dissolved in deionized distilled water, made up volume to 1,000 ml.

Stored reagent at 4°C.

9. Running buffer

10X Running Buffer	100 ml
Deionize distilled water	900 ml

10. 2.5 % Triton X-100

Triton X-100	25 ml
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The chemicals was dissolved in deionized distilled water, made up to the volume 1 L.

Filtered through a membrane filter pore size 0.45 μ m. Stored the reagent at 4°C.

11. Activating buffer

Tris-HCl	6.06 g
CaCl ₂	1.47 g
NaCl	2.92 g

All chemicals were dissolved in 800 ml of deionized distilled water, adjusted to pH 7.6.

Added with 500 μ l of Brij35, and made up to the volume 1 L. Filtered through a membrane filter pore size 0.45 μ m. Stored the reagent at 4°C.

12. Coomassie Brilliant blue G250

Coomassie Brilliant blue G250	1 g
Methanol	50 ml
Acetic acid	10 ml
Deionized distilled water	40 ml

13. Coomassie Brilliant blue destaining solution

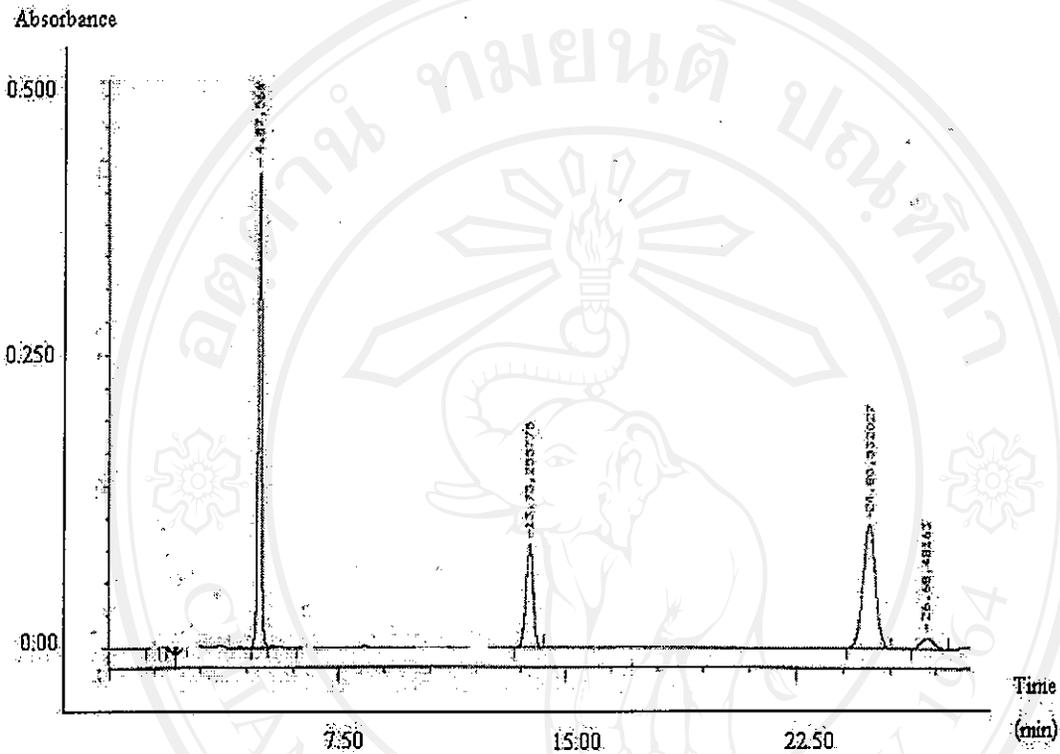
Methanol	50 ml
Acetic acid	10 ml
Deionized distilled water	40 ml

14. 10% Separating gelatin gel

Deionized distilled water	1.95 ml
Acrylamide/Bis solution	3.3 ml
Separating gel buffer	3.75 ml
10%SDS	100 ul
20%APS	100 ul
Gelatin solution	1 ml
TEMED	20 ul

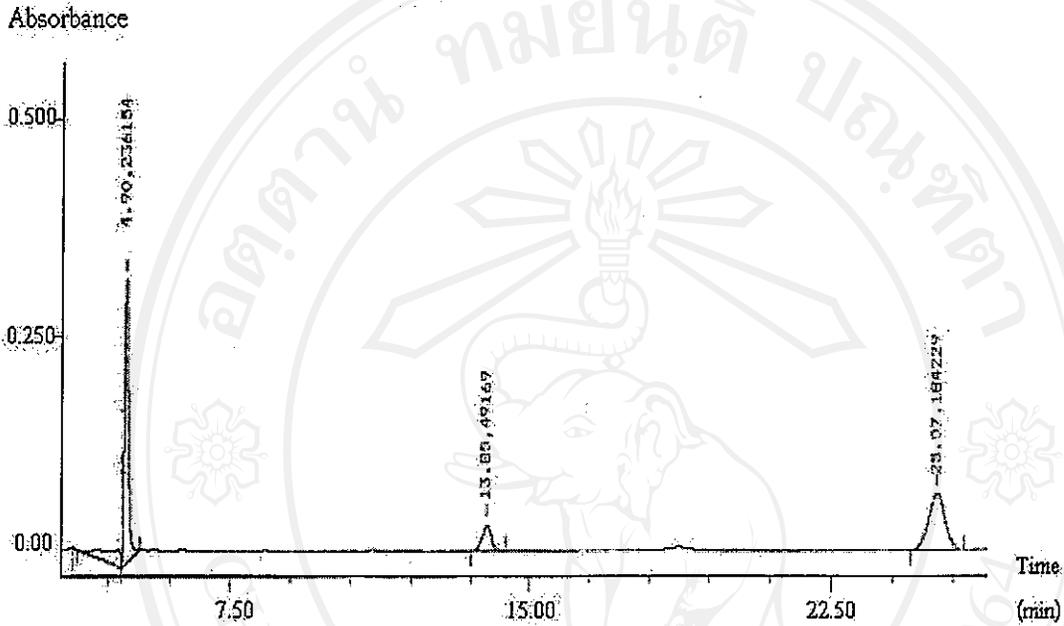
15. 4% Stacking gel

Deionized distilled water	6.10 ml
Acrylamide/Bis solution	1.3 ml
Separating gel buffer	2.5 ml
10%SDS	100 ul
20%APS	100 ul
TEMED	20 ul

HPLC chromatogram of hexane extracted Plai.

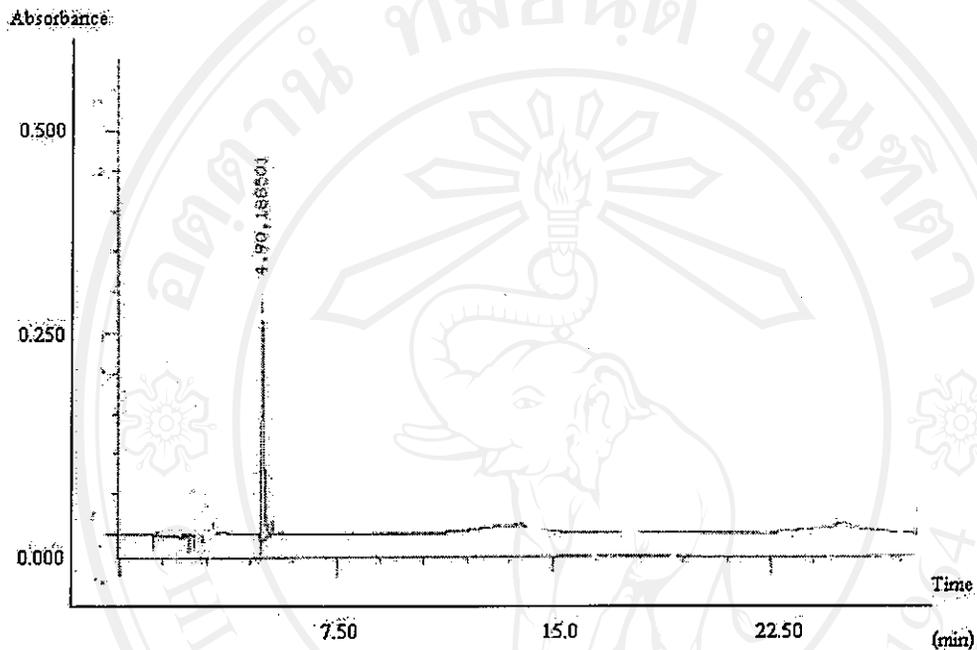
HPLC fingerprint of Hexane extract analysed by the HPLC system for isocratic elution, with 50% acetonitrile mobile phase, Apollo C18 5 u, 250 X 4.6 mm column and 1.0 ml/min flow rate.

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HPLC chromatogram of 70% ethanol extracted Plai.

HPLC fingerprint of 70%Ethanol extract analysed by the HPLC system for isocratic elution, with 50% acetonitrile mobile phase, Apollo C18 5 μ , 250 X 4.6 mm column and 1.0 ml/min flow rate.

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HPLC chromatogram of water extracted Plai.

HPLC fingerprint of water extract analysed by the HPLC system for isocratic elution, with 50% acetonitrile mobile phase, Apollo C18 5 μ , 250 X 4.6 mm column and 1.0 ml/min flow rate.

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Percentage of composition from Plai extracts analysed by HPLC analysis.

	Peak 1 (RT= 4.9)	Peak 2 (RT= 13.7)	Peak 3 (RT= 24.8)	Peak 4 (RT= 26.7)
Hexane	40.41%	18.23%	37.92%	3.433%
70% Ethanol	50.29%	10.47%	39.23%	-
Water	100%	-	-	-

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