APPENDIX

Preperation of Plai (*Zingiber cassumunar* Roxb.) extracts (57)

Fresh rhizome of Plai were cut into pieces, dried at 50-60° C and ground. Dried powder of Plai samples were extracted with hexane, 70% ethanol and distilled water. Dried ethanolic and water extracts were obtained after removing the solvent by evaporation under reduced pressure in evaporater, then lyophilized. Dried hexane extract was obtained after removing the solvent by evaporation and dry at 37°C. Dried residue was weight and stored at -20°C (22). The extracts were used in all experiments were from the same plant materials. However the HPLC fingerprint of each extract was recorded for further reference.

The HPLC system for isocratic elution

Column : Apollo C18 5 μ, 250 X 4.6 mm

Guard column : Apollo C18 5 μ, 7.5 X 4.6 mm

Mobile phase : 50% acetonitrile

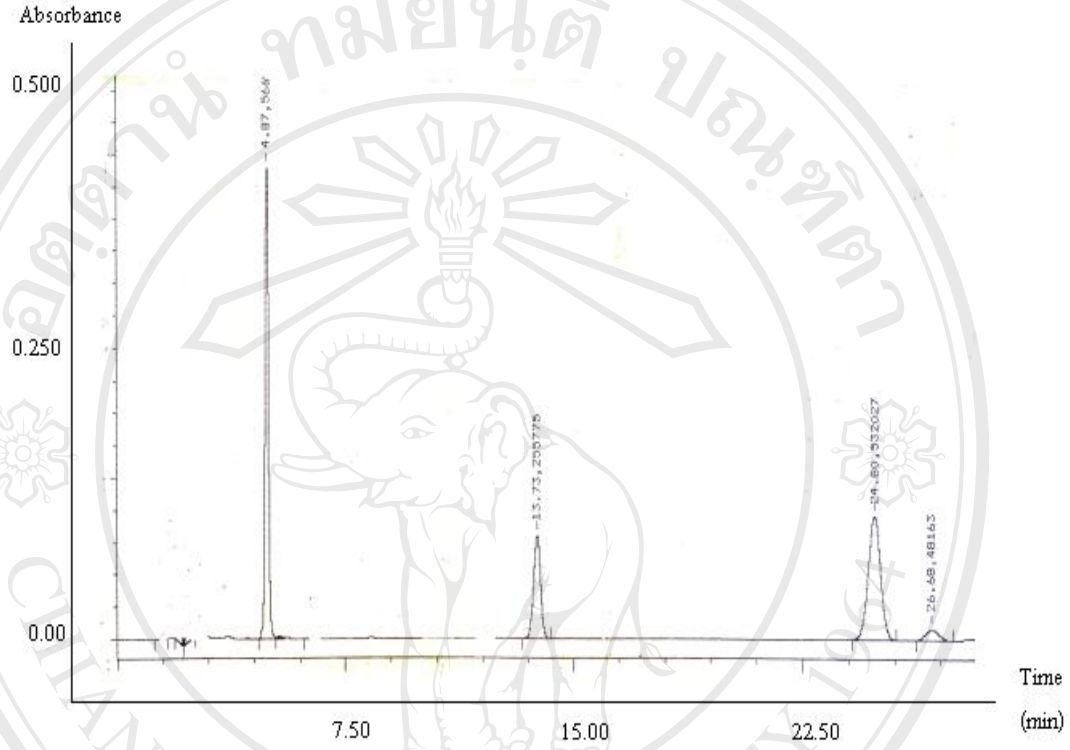
Flow rate: 1.0 ml/min

Injection volume: 10 μl

Run time : 30 min

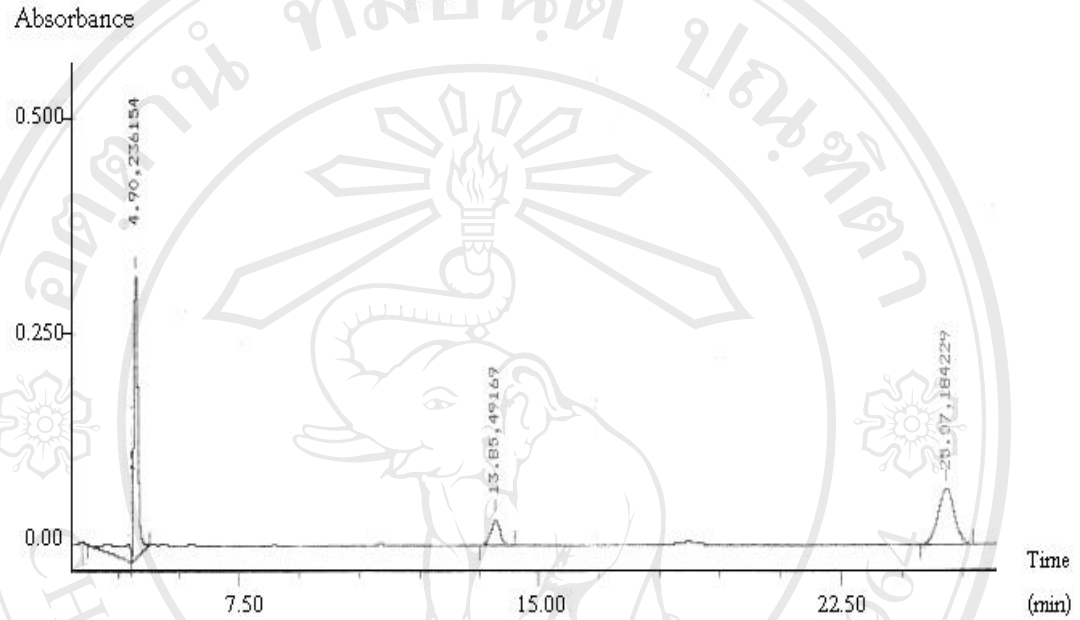
Detection : 267 nm

HPLC chromatogram of hexane extracted Plai. (57)



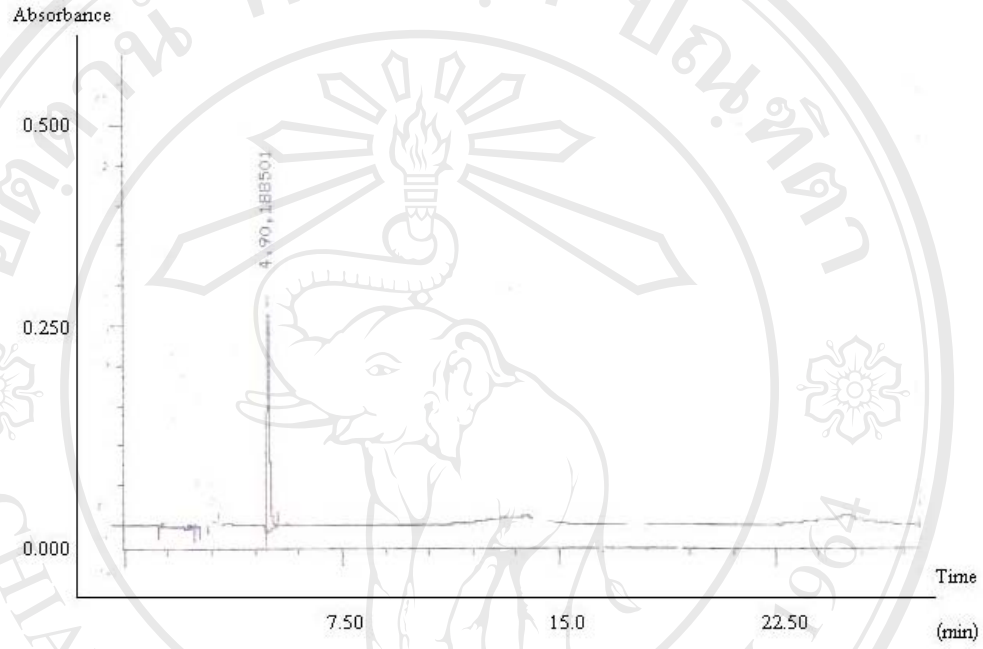
HPLC fingerprint of Hexane extrct analysed by the HPLC system for isocretic elution, with 50% acetonitrile mobile phase, Apollo C18 5 u, 250 X 4.6 mm column and 1.0 ml/min flow rate.

HPLC chromatogram of 70% ethanol extracted Plai. (57)



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

HPLC chromatogram of water extracted Plai. (57)



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

Percentage of composition from Plai extracts analysed by HPLC analysis. (57)

	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%	-	-	-

Reagents and buffers preparation

Enzyme-linked immunosorbent assay

1. Phosphate buffer saline (PBS)

NaCl	8.00	g
KCl	0.20	g
Na_2HPO_4	1.44	g
$Na_2HPO_4.2H_2O$	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. For PBS-Tween, Tween-20 was added to 0.05%.

2. Tris-Incubating buffer

Tris-HCl	1.21	g
NaCl	8.77	g

All chemicals were dissolved in 900 ml of distilled water, adjusted to pH 7.4 and then added distilled water to adjust to the volume 1 L. Added 0.5 g BSA and 1,000 µl Tween-20. Stored the reagent at 4 ^{0}C.

3. Coating buffer (for HA)

$NaHCO_3$	8.4	g

The chemical 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.

4. Coating buffer (foe WF6)

$NaHCO_3$	1.0559	g

The chemical was dissolved in 400 ml of distilled water, adjusted to pH 9.6 and then added with distilled water to adjust to the volume 500 ml.

5. 1% Bovine serum albumin (BSA)

BSA	0.1	g
PBS	10	ml

6. Citrate phosphate buffer

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

The chemicals were dissolved in 900 ml of distilled water, adjusted to pH 5.0 and made up to the volume 1 L. Stored the reagent at 4^0C.

7. Substrate solution

OPD	8	mg
Citrate phosphate buffer	12	ml
35% H_2O_2	7	µl

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

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. Store the reagent at room temperature.

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 µm, collected in a dark bottle. Stored the reagent at 4^0C.

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

Tris-base	18.15	g

The chemical 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 4^0C.

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

Tris-base	6.0	g

The chemical 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 4^0C.

4. 10% SDS solution

SDS	10	g

The chemical 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 ^{0}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

The chemical was dissolved in deionized distilled water, made up to the volume 1 L. Filtered through a membrane filter pore size 0.45 μm. Stored the reagent at 4 ^{0}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 μl of Brij35, and made up to the volume 1 L. Filtered through a membrane filter pore size 0.45 μm. Stored the reagent at 4 ^{0}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% Seperating gelatin gel

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

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	µl
20%APS	100	µl
TEMED	20	µl

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