## CHAPTER III

## RESULTS

### 3.1 Effects of retinoic acid and interleukin-1β on degradation of extracellular matrix in cartilage explants.

In this study, retinoic acid (RetA) and interleukin-1β (IL-1β) were used as stimulators to induce degradation of extracellular matrix (ECM) of articular cartilage in cartilage explants model. After 3 days of treatment with the stimulators, cartilage tissue was analyzed for uronic acid content using uronic acid assay, and the culture medium was analyzed for sulfated-glycosaminoglycan (sulfated-GAG), hyaluronan(HA), and gelatinolytic activity using three assays which are dye binding assay, a competitive inhibition based ELISA for hyaluronan (HA), and gelatin zymography, respectively.

The results showed that the release of extracellular matrix (ECM) such as sulfated-glycosaminoglycan and HA from cartilage explants into culture medium were increased in dose-dependent manner when stimulated with RetA and IL-1β (Table 5 and Table 6). In addition, the remaining of uronic acid content in cartilage tissue was decreased when stimulated with these stimulators. It was found that 10 μM RetA and 25 ng/ml IL-1β induced degradation of ECM biomolecules in cartilage explants model higher than other concentrations. Therefore, the concentration of 10 μM RetA and 25 ng/ml IL-1β were used in the next experiments.

**Table 5.** Effects of various concentrations of RetA on degradation of extracellular matrix biomolecules in cartilage explants.

| | % change of[a] | | % remaining of uronic acid content in cartilage tissue[b] |
|---|---|---|---|
| Treatment | Sulfated-GAG release | HA-release | |
| The untreated control | 71.08±29.94 | 93.95± 14.46 | 100.00± 0 |
| DMSO | 72.80± 25.28 | 41.14± 3.76 | 108.09± 2.44 |
| 0.1 μM RetA | 169.94± 64.26 | 177.06± 10.85* | 90.24± 1.29 |
| 1.0 μM RetA | 363.31± 2.65** | 455.37± 29.85** | 71.28± 0.16* |
| 10 μM RetA | 834.35± 23.20** | 950.15± 205.62* | 55.56± 1.46** |
| 100 μM RetA | 710.59± 62.44** | 192.061± 19.17* | 80.24± 2.02* |

*, ** Denoted values that were significantly different from the untreated control ($p<0.05$) and ($p<0.01$) respectively.

a= [ ( Day 3 medium – Day 0 medium)/ Day 0 medium] x 100

b= [ uronic acid ( Day 3 of treated) x 100] / uronic ( Day 3 of control)

**Table 6.** Effects of various concentrations of IL-1β on degradation of extracellular matrix biomolecules in cartilage explants.

| Treatment | % change of[a] | | % remaining of uronic acid content in cartilage tissue[b] |
|---|---|---|---|
| | Sulfated-GAG release | HA release | |
| The untreated control | 89.59± 48.87 | 40.12± 2.83 | 100.00± 0 |
| 12.5 ng/ml IL-1 | 189.66± 4.68 | 62.84± 5.75 | 91.78± 0.94 |
| 25 ng/ml IL-1 | 428.03± 28.58* | 139.99± 10.03* | 61.33± 2.31* |
| 50 ng/ml IL-1 | 225.65± 33.87 | 127.08± 27.72 | 76.25± 2.65 |
| 100 ng/ml IL-1 | 315.74± 24.73* | 136.26± 54.22 | 106.02± 5.69 |

* Denoted value that was significantly different from the untreated control ($p<0.05$).

a= [ ( Day 3 medium – Day 0 medium)/ Day 0 medium] x 100

b= [ uronic acid ( Day 3 of treated) x 100] / uronic ( Day 3 of control)

**3.2 Inhibitory effects of Plai extracts on degradation of extracellular matrix biomolecules in cartilage explants stimulated by RetA**

The rhizome of *Zingiber cassumunar* Roxb (Plai). is widely used in Thai traditional medicine for topical treatment of joint inflammations, muscular pain, and similar inflammation-related disorders. Preliminary studies revealed the effect of (E)-1-(3-4-dimethoxyphynyl)butadiene (DMPBD) on anti-inflammatory activity by action on both cyclooxygenase (COX) and lipoxygenase (LOX) in arachidonic acid (AA) metabolic pathways.

In this study, three fractions of Plai extracts i.e. hexane, 70% ethanol and water were used to compare the chondroprotective activity. Various concentrations of Plai extract were co-incubated with RetA (10 μM) in the cartilage explant culture. Then, the releases of ECM biomolecules (sulfated-GAG and HA) were investigated in the culture media. The remaining of uronic acid content in cartilage tissue was also quantified. As show in Figure 13-15, Plai extracts from hexane and ethanol fractions were able to inhibit the release of sulfated-GAG and HA into the culture media in a dose dependent manner but the water fraction was not. In addition, the net loss of uronic acid from the cartilage tissues treated with RetA was prevented when the cartilage explants was co-incubated with Plai extracts from hexane and ethanol fractions as shown in Figureure 13-15, but not in water fraction. When compare between hexane and ethanol extracts, it was found that hexane extract of Plai showed less sulfated –GAG and HA in culture media and more uronic acid content in cartilage tissues than ethanol extract in the same concentration as shown in Figure 13-15.

Co-treatment of RetA (10 μM) and Diacereine, dramatically inhibited the release of sulfated-GAG and HA in culture media and more uronic acid content was found in cartilage tissue. When compare these activity at the same concentration (100 μg/ml) with hexane and ethanol extracts the results showed that Diacereine was able to decrease RetA-induced release of GAG into culture media about 5.3 and 6.9 times respectively, and HA release was about 2.7 times by the ethanol extract. These results agreed with uronic acid content in cartilage which increased for 30% and 32% when compare between hexane and ethanol extracts respectively. Interestingly, water extract showed reverse effects. It showed significantly higher release of ECM biomolecules into culture media than control.

**Table 7.** Inhibitory effect of Plai extracts on the release of ECM biomolecules activated by RetA.

| Treatment | %change[a] | | % remaining of uronic acid content in cartilage tissue[b] |
|---|---|---|---|
| | Sulfated-GAG release | HA release | |
| Untreated control | 92.59 ± 42.19 | 151.49 ± 29.47 | 100.0 ± 0 |
| DMSO | 137.08 ± 45.74 | 143.20 ± 43.49 | 102.6 ± 1.53 |
| 10 μM RetA | 600.06 ± 166.61 | 708.62 ± 174.21 | 66.58 ± 2.35 |
| RetA/+100 μg/ml Diacereine | 60.56 ± 32.97** | 142.94 ± 56.38** | 93.7 ± 3.11* |
| RetA/+50 μg/ml P-Hex | 362.96± 65.72* | 130.18 ± 75.13** | 54.06± 3.70 |
| RetA/+50 μg/ml P-EtOH | 419.97± 195.51 | 469.23±124.14 | 49.75± 2.63 |
| RetA/+50 μg/ml P-$H_2O$ | 773.81± 219.79 | 2020.44± 647.50* | 40.50± 4.97 |
| RetA/+100 μg/ml P-Hex | 319.73± 99.62** | 142.44± 103.64** | 64.76± 4.35 |
| RetA/+100 μg/ml P-EtOH | 415.57± 85.51 | 382.68± 70.99 | 62.89± 3.28 |
| RetA/+100 μg/ml P-$H_2O$ | 862.79± 243.48* | 1366.38± 220.15* | 33.16± 1.61** |
| RetA/+150 μg/ml P-Hex | 207.48± 66.22** | 113.54± 30.18** | 71.12± 2.93 |
| RetA/+150 μg/ml P-EtOH | 411.76± 148.72 | 279.25± 51.66* | 69.26± 3.51 |
| RetA/+150 μg/ml P-$H_2O$ | 1119.85± 394.70* | 1081.98± 455.06 | 38.08± 4.14* |
| RetA/+200 μg/ml P-Hex | 165.40± 83.84** | 100.42± 48.68** | 88.49± 3.11* |
| RetA/+200 μg/ml P-EtOH | 352.15± 71.14 | 234.59± 41.21* | 84.34± 3.61 |
| RetA/+200 μg/ml P-$H_2O$ | 972.32± 157.48** | 765.954± 236.70 | 30.88± 0.98** |

*, ** Denoted values that were significantly different from the untreated control ($p<0.05$) and ($p<0.01$) respectively.

a= [ ( Day 3 medium – Day 0 medium)/ Day 0 medium] x 100

b= [ uronic acid ( Day 3 of treated) x 100] / uronic ( Day 3 of control)

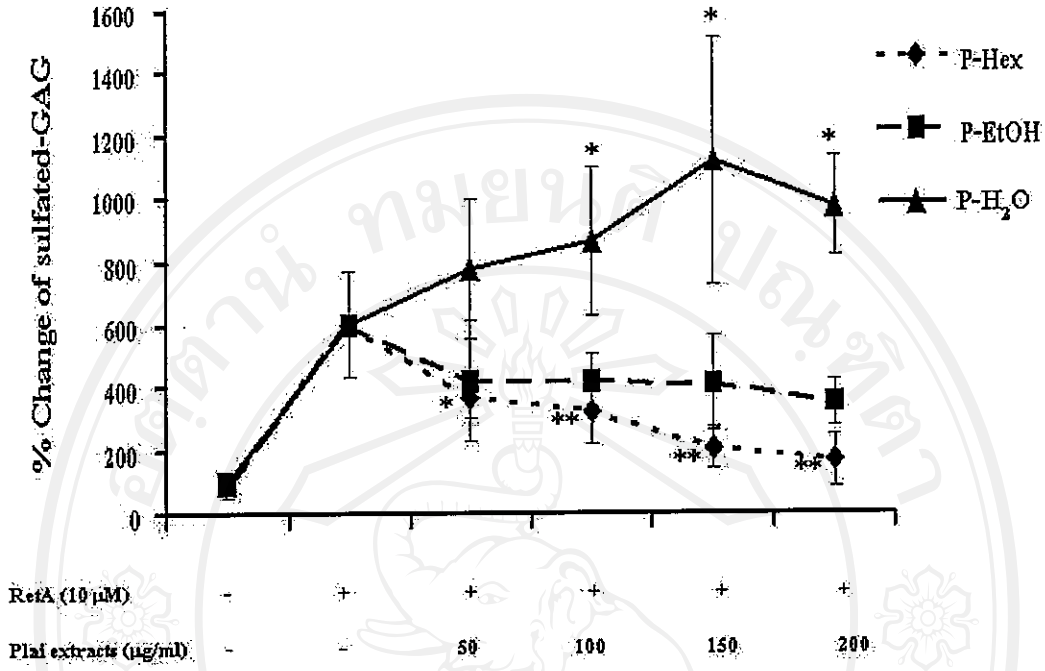


**Figure 13.** Inhibitory effects of Plai extracts on the release of sulfated-GAG. The explants were treated with 10 µM RetA and Plai extracts were added at dose 50, 100, 150, 200 µg/ml.Data are the mean values± standard deviation of triplicate per treatment. *, ** Denoted values that were significantly different from RetA treated control (+RetA/-Plai), ($p<0.05$) and ($p< 0.01$) respectively.

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

P-$H_2O$ = Water extracted Plai

$$\% \text{ Change} = \left[ \frac{(\text{Day 3 medium} - \text{Day 0 medium})}{\text{Day 0 medium}} \right] \times 100$$

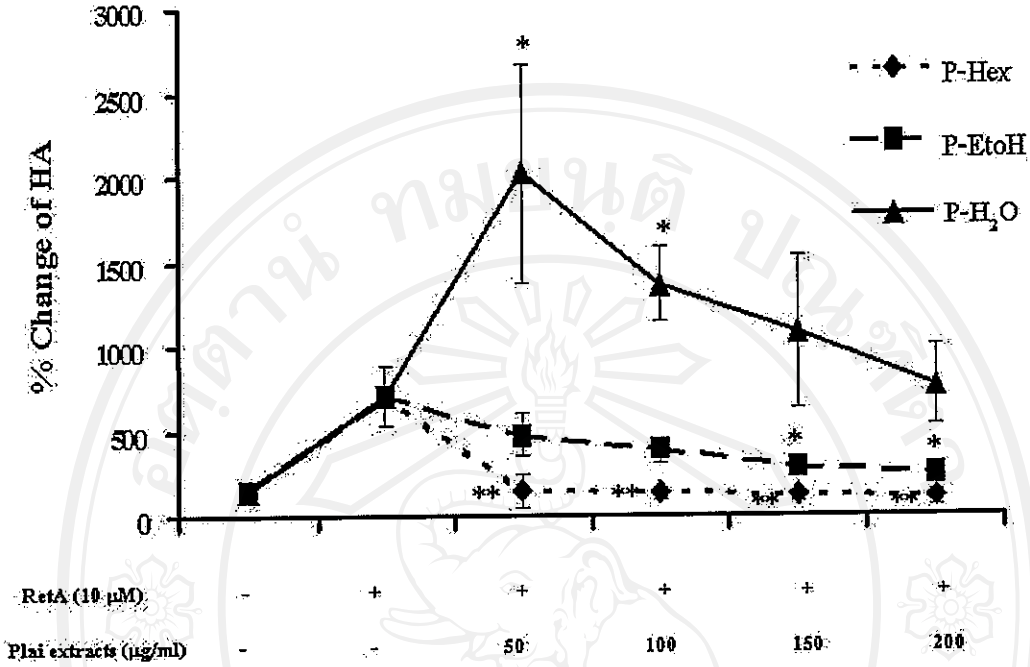


**Figure 14.** Inhibitory effects of Plai extracts on the release of HA. The explants were treated with 10 µM RetA and Plai extracts were added at dose 50, 100, 150, 200 µg/ml. Data are the mean values± standard deviation of triplicate per treatment. *, ** Denoted values that were significantly different from RetA treated control (+RetA/-Plai), ($p<0.05$) and ($p<0.01$) respectively.

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

P-$H_2O$ = Water extracted Plai

$$\% \text{ Change} = \left[ \frac{(\text{Day 3 medium} - \text{Day 0 medium})}{\text{Day 0 medium}} \right] \times 100$$

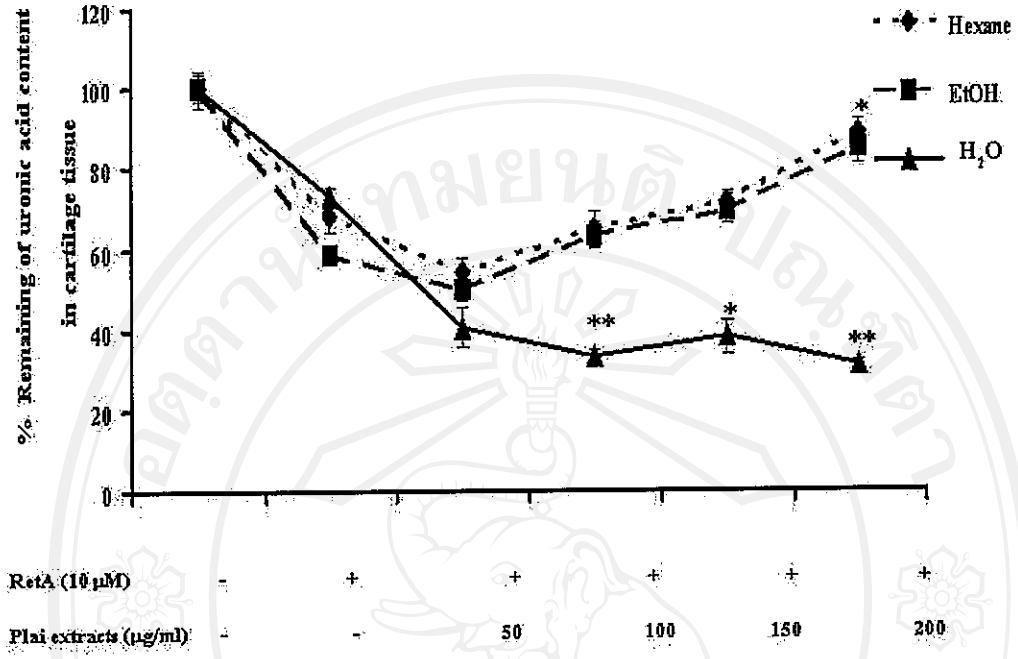


**Figure 15.** Inhibitory effects of Plai extracts on uronic acid content in cartilage tissue treated with RetA. The explants were treated with 10 μM RetA and Plai extracts were added at dose 50, 100, 150, 200 μg/ml. Data are the mean values± standard deviation of triplicate per treatment. *, ** Denote values that were significantly different from RetA treated control (+RetA-Plai), ($p<0.05$) and ($p<0.01$) respectively.

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

P-$H_2O$ = Water extracted Plai

(-RetA/-Plai) = the untreated control

$$\% \text{ Remaining} = \frac{\text{uronic acid (Day 3 of treated)}}{\text{uronic acid (Day 3 of the untreated control}} \times 100$$

**3.3 Effects of Plai extracts on gelatinolytic activity in culture media of cartilage explants activated by RetA.**

In this study, activity of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in the culture media were investigated by gelatin zymography. Culture media were electrophoresed on native, nonreducing, gelatin containing gels, which were subsequently stained with Coomassie blue; resolved gelatinolytic proteins were detected as unstained band. Cartilage explants culture in serum-free medium secreted high levels of gelatinase, which are pro-MMP-2 and pro-MMP-9, when compared to standard enzyme and marker at 72 kDa and 92 kDa respectively.

The results showed that all fractions of Plai extracts (hexane, ethanol and water) significantly down regulate the production of MMP-2 and MMP-9 of the cartilage explant to the culture media in dose- dependent manner, as shown in Figure16 and Figure 17.

According to Diacereine, it showed more dramatic effect to prevent secretion of MMP-2 and MMP-9 into culture medium than hexane and ethanol extracts about 25% and 39%, respectively, at the same concentration (100 μg/ml).

**Table 8.** Effects of Plai extracts on gelatinolytic activity in cartilage explants activated by RetA.

| Treatment | Gelatinolytic activity[a] ( % of Control) |
|---|---|
| The untreated control | 100 |
| 10 μM RetA | 133.42 ± 19.32 |
| RetA/+ 100μ g/ml Diacereine | 35.25 ± 11.18** |
| RetA/+ 50μ g/ml P-Hex | 66.26 ± 30.93** |
| RetA/+ 50 μg/ml P-EtOH | 83.79 ± 26.88** |
| RetA/+ 100 μg/ml P-Hex | 59.84 ± 54.49** |
| RetA/+ 100μ g/ml P-EtOH | 72.40 ± 37.74** |
| RetA/+ 100 μg/ml P-H2O | 160.52 ± 27.96 |
| RetA/ + 200 μg/ml P-Hex | 45.20 ± 20.11** |
| RetA/ + 200 μg/ml P-EtOH | 49.48 ± 8.07** |
| RetA/ + 200 μg/ml P-H2O | 116.88 ± 13.94** |

$$\% \text{ of control} = \frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control)}}$$

** Denoted value that was significantly different from the untreated control ($p<0.01$).

a = total activity of MMP-2 and MMP-9

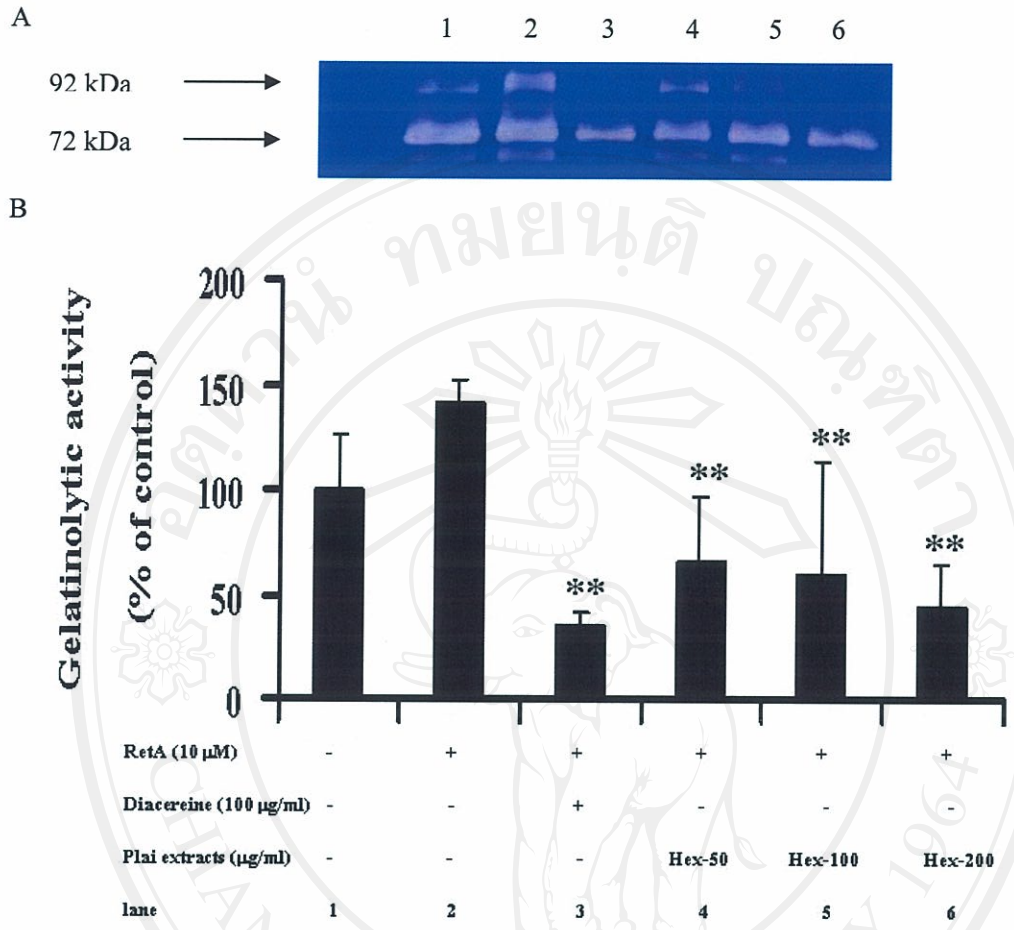


**Figure 16.** Effect of Plai extract (hexane fraction) on gelatinolytic activity in culture media of cartilage explants treated with RetA. Condition medium from day 3 culture was analyzed for genolytic activity. Molecular weights of marker proteins were indicated on the left. Lane 1: the untreated control medium; Lane 2 : the RetA treated control; Lane 3 : the RetA treated with 100 µg/ml Diacereine; Lane 4,5 and 6 : the RetA treated with 50, 100, 200 µg/ml Plai extracts (hexane fraction), respectively (A), they were quantified by densitometry (B) .

(-RetA/-Plai) = the untreated control

% of control = $\frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control}}$

** Denoted value that was significantly different from the untreated control ($p<0.01$).

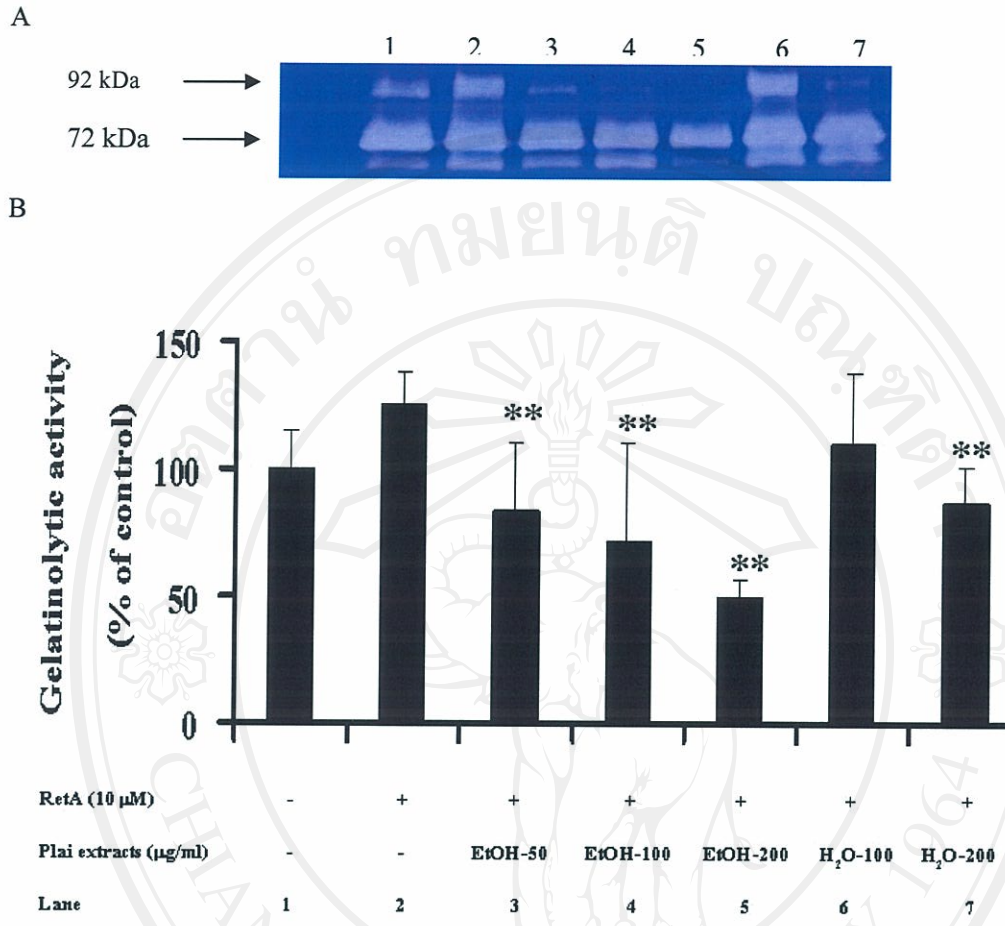


**Figure 17.** Effect of Plai extracts (ethanol and water fractions) on gelatinolytic activity in culture media of cartilage explants treated with RetA. Condition medium from day 3 cultures was analyzed for genolytic activity. Molecular weight of marker proteins are indicate on the left. Lane 1: the untreated control medium; Lane 2 : the RetA treated control; Lane 3, 4 and 5 : the RetA treated with 50, 100, 200 μg/ml Plai-EtOH; Lane 6 and 7 : the RetA treated with 100, 200 μg/ml Plai $-H_2O$, respectively (A), they were quantified by densitometry (B) .

(-RetA/-Plai) = the untreated control

% of control = $\frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control}}$

** Denote values that were significantly different from the untreated control ($p<0.01$).

**3.4 Inhibitory effects of Plai extracts on degradation of extracellular matrix biomolecules in cartilage explants stimulated by IL-1β**

Interleukin-1β (IL-1β), a pro-inflammatory factor, has been shown to accelerate damage of articular tissue *in vitro* and to amplify the inflammatory response. In this study, cartilage explants were co-incubated with 25 ng/ml IL-1β to induce the release of ECM biomolecules such as sulfated-GAG, HA and uronic acid compare with cartilage group which co-incubated with

IL-1β and various concentration of Plai extracts (hexane, ethanol and water).

Then, the release of ECM biomolecules (sulfated-GAG and HA) was investigated in the culture media. The remaining of uronic acid content in cartilage tissue was also quantified. As shown in Figure 18-20, Plai extracts from hexane and ethanol fractions were able to inhibit the release of sulfated-GAG and HA into the culture media in dose- dependent manner. In addition, the net loss of uronic acid from the cartilage tissues treated with IL-1β was prevented when the cartilage explants was co-incubated with Plai extracts from hexane and ethanol fractions as shown in Figure 18-20. In comparison, it was found that hexane extract of Plai showed less sulfated – GAG and HA release into culture media and more remaining of uronic acid content in cartilage tissues than the ethanol extract at the same concentration as shown in Figure18-20.

Co-treatment of IL-1β (25 ng/ml) with Diacereine, dramatically inhibited the release of sulfated-GAG and HA in culture media and more uronic acid content was found in cartilage tissue. When compare the activity at the same concentration (100 μg/ml) with hexane and ethanol extracts the results showed that, Diacereine was able to decrease RetA-induce the release of GAG into culture media about 3 and 2.75 times respectively, and HA release was about 2.4 and 4.2 times respectively. These results agreed with uronic acid content in cartilage which was about 12% and 22% when compare to hexane and ethanol extracted respectively. Interestingly, water extract had reverse effect. It showed significantly higher release of ECM biomolecules into culture media than control.

**Table 9.** Inhibitory effects of Plai extracts on the release of ECM biomolecules activated by IL-1β.

| Treatment | %change | | % remaining of uronic acid content in cartilage tissue[b] |
|---|---|---|---|
| | Sulfated-GAG release | HA release | |
| Untreated control | 184.8 ± 16.70 | 60.98 ± 8.22 | 100.00 ± 0 |
| DMSO | 182.42 ± 40.99 | 71.12 ± 28.57 | 94.01 ± 0.90 |
| 25 ng/ml Il-1β | 513.22 ± 69.4 | 175.73 ± 32.5 | 82.20 ± 1.35 |
| Il-1β/+100 μg/ml Diacereine | 124.36 ± 64.72 | 61.08 ± 21.85 | 98.51 ± 2.34 |
| Il-1β/+50 μg/ml P-Hex | 538.55 ± 55.46 | 175.15 ± 13.77 | 79.42 ± 1.35 |
| Il-1β/+50 μg/ml P-EtOH | 369.25 ± 38.29 | 280.23 ± 132.16 | 72.54 ± 0.63 |
| Il-1β/+50 μg/ml P-$H_2O$ | 1986.66 ± 157.30* | 691.95 ± 238.11 | 80.62 ± 1.20 |
| Il-1β/+100 μg/ml P-Hex | 385.21 ± 86.23 | 147.05 ± 13.28 | 86.12 ± 0.93 |
| Il-1β/+100 μg/ml P-EtOH | 341.79 ± 95.18 | 259.18 ± 39.92 | 76.59 ± 0.36 |
| Il-1β/+100 μg/ml P-$H_2O$ | 1175.67 ± 339.34* | 483.22 ± 40.87* | 43.21 ± 0.22 |
| Il-1β/+150 μg/ml P-Hex | 310.77 ± 20.91** | 82.17 ± 7.66 | 90.72 ± 0.69 |
| Il-1β/+150 μg/ml P-EtOH | 322.37 ± 86.77 | 142.13 ± 10.78 | 88.50 ± 0.57 |
| Il-1β/+150 μg/ml P-$H_2O$ | 2317.45 ± 26.70** | 680.28 ± 112.65* | 47.23 ± 1.27 |
| Il-1β/+200 μg/ml P-Hex | 234.31 ± 15.63** | 66.12 ± 3.77 | 101.24 ± 0.22 |
| Il-1β/+200 μg/ml P-EtOH | 236.39 ± 7.74* | 133.54 ± 32.61 | 92.96 ± 1.74 |
| Il-1β/+200 μg/ml P-$H_2O$ | 1428.74 ± 224.52** | 700.49 ± 185.96 | 48.58 ± 0.73 |

*, ** Denoted values that were significantly different from the untreated control ($p<0.05$) and ($p<0.01$) respectively.

a= [ ( Day 3 medium – Day 0 medium)/ Day 0 medium] x 100

b= [ uronic acid ( Day 3 of treated) x 100] / uronic ( Day 3 of control)

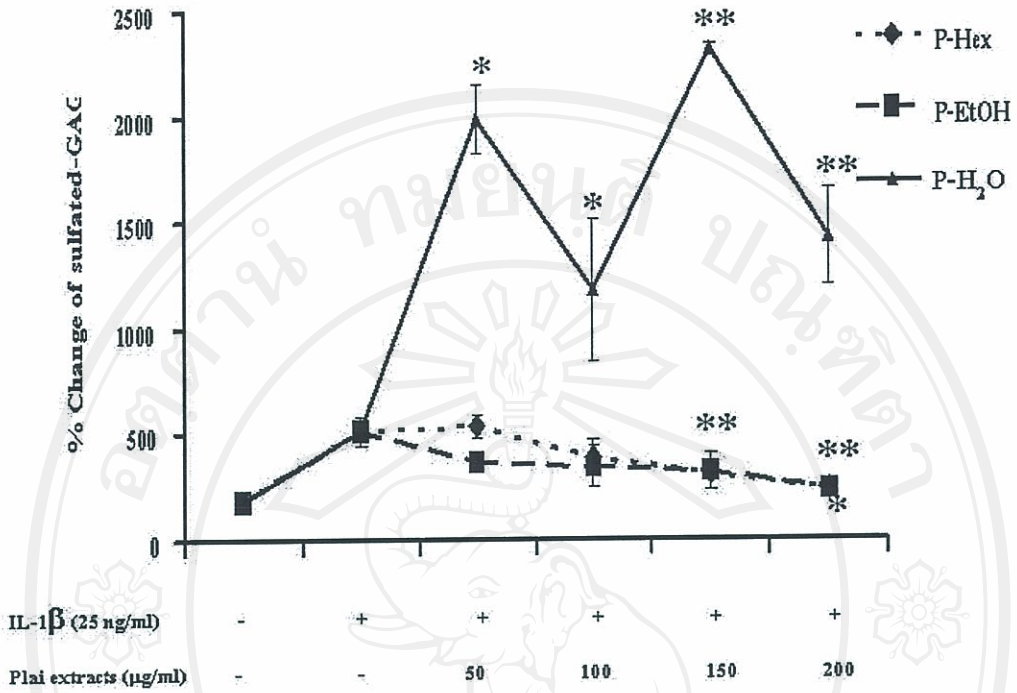


**Figure 18.** Inhibitory effects of Plai extracts on the release of sulfated-GAG. The explants were treated with 25 ng/ml IL-1β and Plai extracts were added at dose 50, 100, 150, 200 µg/ml. Data are the mean values± standard deviation of triplicate per treatment. *, ** Denoted values that were significantly different from RetA treated control (+Il-1β/-Plai), ($p<0.05$) and ($p<0.01$) respectively.

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

$P\text{-}H_2O$ = Water extracted Plai

$$\% \text{ Change} = \frac{(\text{Day 3 medium} - \text{Day 0 medium})}{\text{Day 0 medium}} \times 100$$

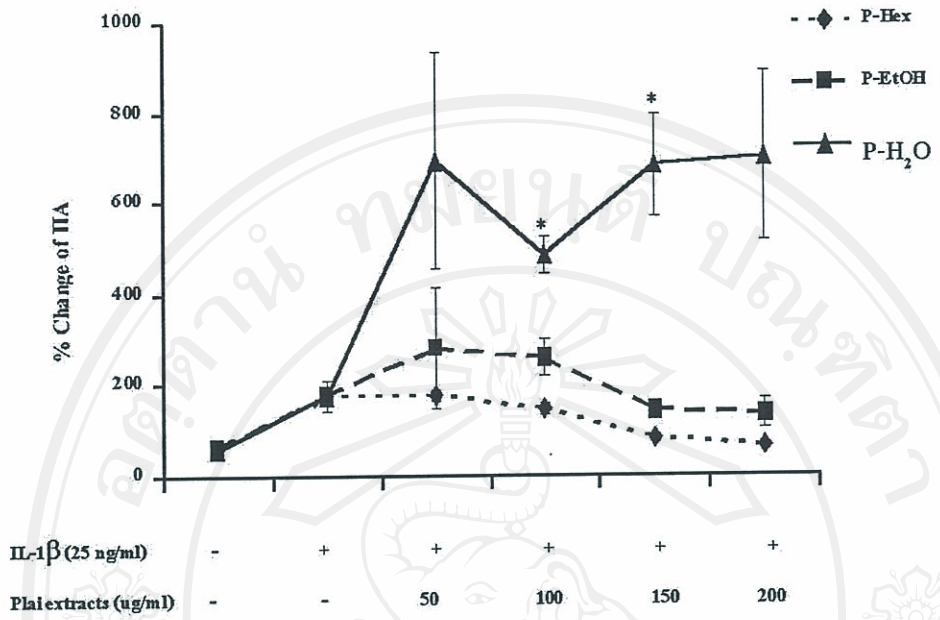


**Figure 19.** Inhibitory effects of Plai extracts on the release of HA. The explants were treated with 25 ng/ml IL-1β and Plai extracts were added at dose 50, 100, 150, 200 μg/ml. Data are the mean values± standard deviation of triplicate per treatment. * Denoted value that was significantly different from IL-1β treated control (+IL-1β/-Plai), ($p<0.05$).

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

P-$H_2O$ = Water extracted Plai

$$\% \text{ Change} = \frac{(\text{Day 3 medium} - \text{Day 0 medium})}{\text{Day 0 medium}} \times 100$$

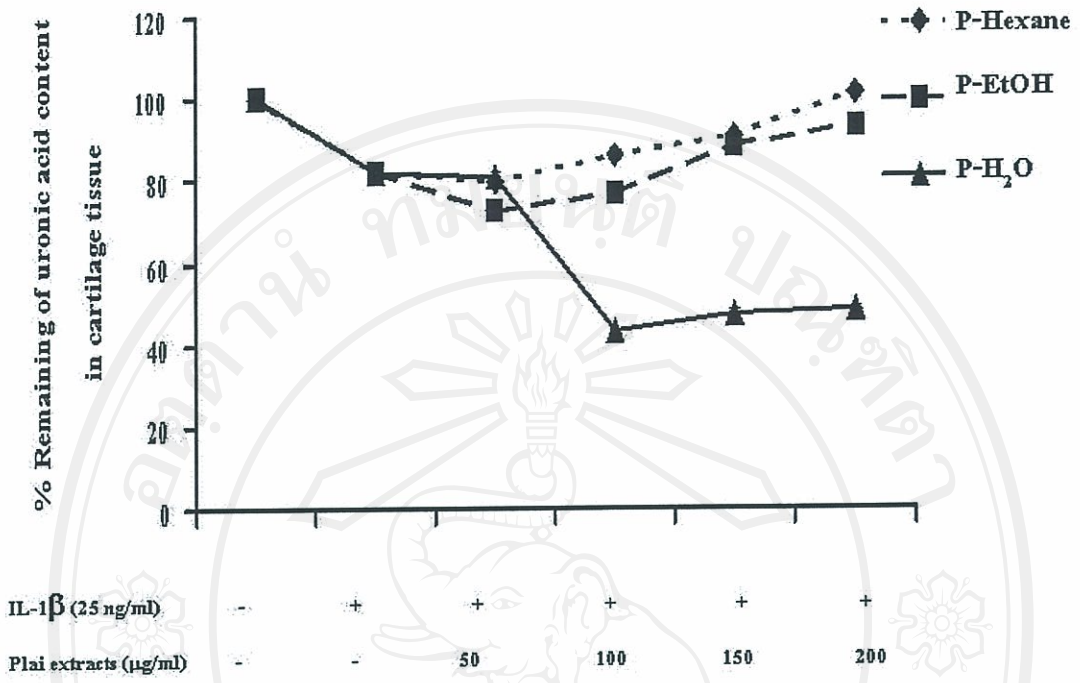


**Figure 20.** Inhibitory effects of Plai extracts on uronic acid content in cartilage tissue treated with IL-1β. The explants were treated with 25 ng/ml IL-1β and Plai extracts were added at dose 50, 100, 150, 200 µg/ml. Data are the mean values± standard deviation of triplicate per treatment.

P-Hex = Hexane extracted Plai

P-EtOH = 70% Ethanol extracted Plai

P-$H_2O$ = Water extracted Plai

(-IL-1β/-Plai) = the untreated control

$$\% \text{ Remaining} = \frac{\text{uronic acid (Day 3 of treated) x 100}}{\text{uronic acid (Day 3 of the untreated control)}}$$

**3.5 Inhibitory effects of Plai extracts on gelatinolytic activity in cartilage explants treated with IL-1β.**

Interleukin-1β (IL-1β), a pro-inflammatory factor has ability to promote tissue degradation appears to be intimately associated with its ability to stimulate the synthesis of matrix metalloproteinases (MMP) such as collagenase, gelatinase and stromelysin. In this study, activity of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) were investigated by gelatin zymography. Culture media were electrophoresed on native, nonreducing, gelatin containing gels, which were subsequently stained with Coomassie blue; resolved gelatinolytic proteins were detected as unstained band. Cartilage explants culture in serum-free medium secreted high levels of gelatinase which should be MMP-2 (72 kDa) and MMP-9 (92 kDa) .

The results showed that hexane extract of Plai significantly decrease MMP-2 and MMP-9 secreted in culture medium in dose dependent manner. Interestingly, MMP-9 was found in the medium from 200 ug/ml hexane extract of Plai, however the total gelatinolytic activity was less than 50 and 100 ug/ml hexane extract of Plai as shown in Figure 21A. While the high dose of the ethanol extract of Plai had ability to significantly decrease the secretion of these enzymes. The reverse effect was apparent in water extract of Plai as shown in Table 10 and Figure 22A.

Diacereine, showed more dramatic effect to prevent secretion of MMP-2 and MMP-9 into culture medium than hexane and ethanol extracs about 27% and 44% respectively at the same concentration (100 μg/ml), as shown in Figure 21A.

**Table 10.** Inhibitory effects of Plai extracts on gelatinolytic activity in cartilage explants treated with IL-1β.

| Treatment | Gelatinolytic activity[a] ( % of Control) |
|---|---|
| The untreated control | 100 |
| 25 ng/ml IL-1β | 124.75 ± 31.88 |
| IL-1β/+ 100 μg/ml Diacereine | 81.74 ± 14.64** |
| IL-1β/+ 50 μg/ml P-Hex | 114.45 ± 18.68 |
| IL-1β/+ 50 μg/ml P-EtOH | 166.34 ± 43.90* |
| IL-1β/+ 100 μg/ml P-Hex | 108.70 ± 33.64* |
| IL-1β/+ 100 μg/ml P-EtOH | 126.01 ± 21.13* |
| IL-1β/+ 100 μg/ml P-$H_2O$ | 208.17 ± 34.18 |
| IL-1β/+ 200 μg/ml P-Hex | 84.95 ± 11.67** |
| IL-1β/+ 200 μg/ml P-EtOH | 89.34 ± 26.35** |
| IL-1β/+ 200 μg/ml P-$H_2O$ | 164.12 ± 25.02** |

$$\% \text{ of control} = \frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control)}}$$

*,** Denoted values that were significantly different from the untreated control ($p<0.05$) and ($p<0.01$) respectively.

a = total activity of MMP-2 and MMP-9

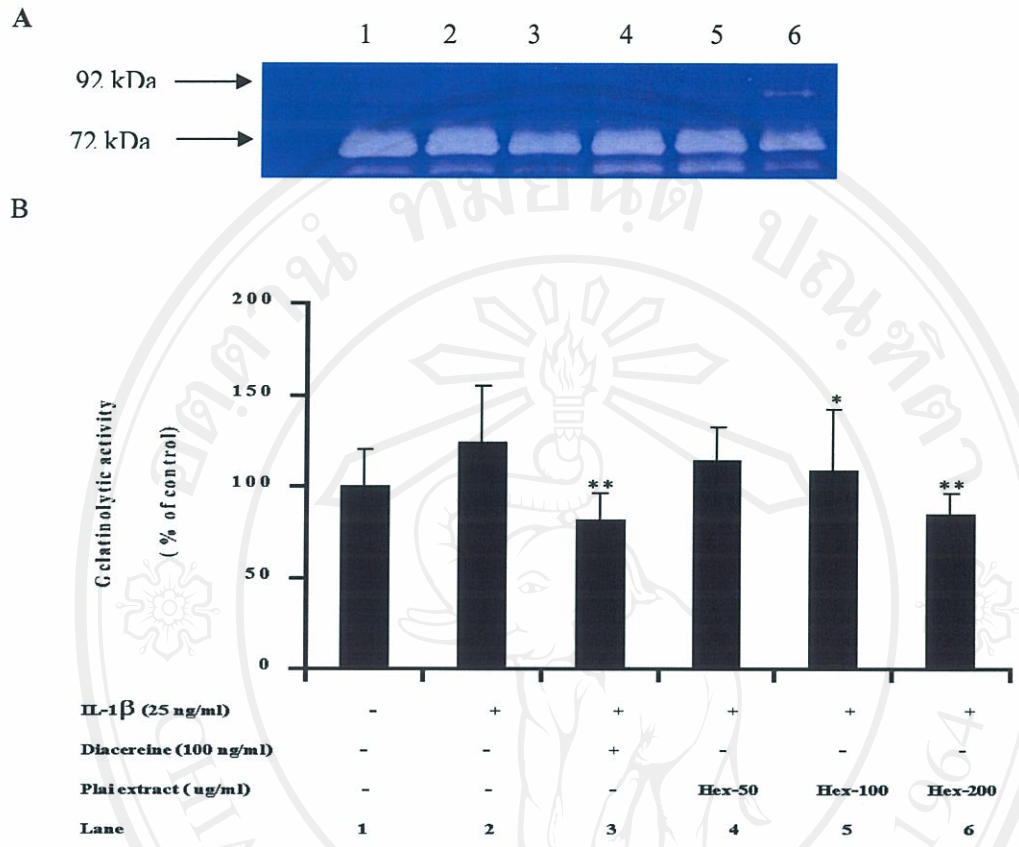


**Figure 21.** Effects of Plai extract (hexane fraction) on gelatinolytic activity in cartilage explants treated with IL-1β.Condition medium from day 3 culture was analyzed for genolytic activity. Molecular weight of marker proteins are indicate on the left. Lane 1: the untreated control medium; Lane 2 : the IL-1β treated control; Lane 3 : the IL-1β treated with 100 ug/ml Diacereine; Lane 4,5 and 6 : the IL-1β treated with 50, 100, 200 μg/ml Plai extracts (hexane fraction), respectively (A), they were quantified by densitometry (B) .

$$\% \text{ of control} = \frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control}}$$

*,** Denote values that were significantly different from the untreated control ($p<0.05$) and ($p<0.01$) respectively.

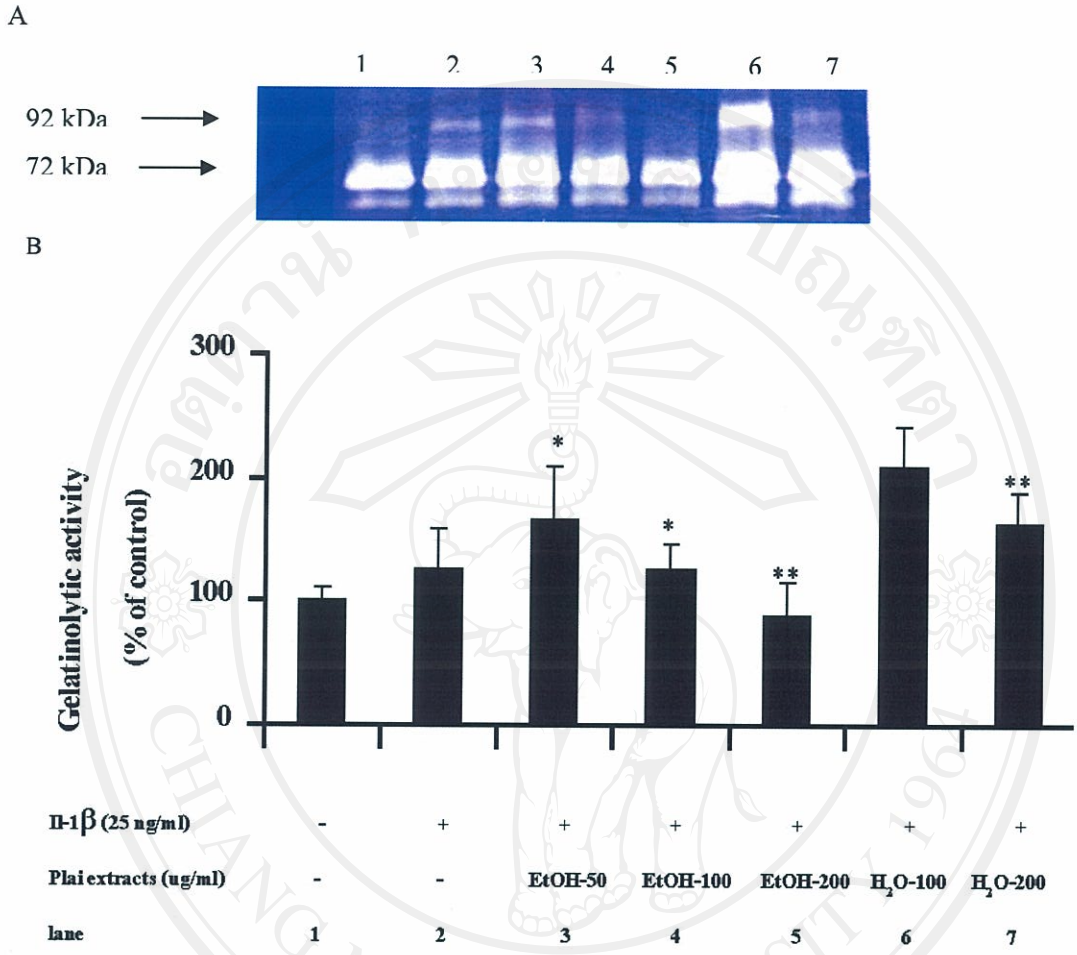


**Figure 22.** Effects of Plai extracts (ethanol and water fraction) on gelatinolytic activity in cartilage explants treated with IL-1β. Condition medium from day 3 culture was analyzed for genolytic activity. Molecular weight of marker proteins are indicate on the left.Lane 1: the untreated control medium; Lane 2 : the IL-1β treated control; Lane 3, 4 and 5 : the IL-1β treated with 50, 100, 200 ug/ml Plai-EtOH; Lane 6 and 7 : the IL-1β treated with 100, 200 ug/ml Plai $-H_2O$, respectively (A), they were quantified by densitometry (B) .

(-IL-1β/-Plai) = the untreated control

% of control = $\frac{\text{density (Day 3 of treated) x 100}}{\text{density (Day 3 of the untreated control}}$