CHAPTER III

Investigation of Anti-platelet Aggrgeation Activity of Isolated Coumarins

3.1 Experimental Section

Platelet aggregation was monitored according to Born's method (Born, 1962) using dual channel Aggro-Meter 430 (Coulter Electrics, UK) with slight modification. Blood samples were collected from healthy volunteers without taking any medicine at least two weeks. Blood was mixed with 3.2% sodium citrate in a ratio of 9:1 and centrifuged at 160*g* for 10 minutes. Supernatant was taken as platelet-rich plasma (PRP), blood residue was further centrifuged at 2000*g* for 10 minutes. The supernatant was taken as platelet-poor plasma (PPP). The native PRP was prewarmed at 37°C for 10 minutes and incubated with 0.2 mM $CaCl_2$ for 1 min (Mani *et al*, 2005). Test compound was added into mixture and incubated for two minutes (Campillo *et al*, 1999). Aliquot of adenine diphosphate (ADP) was added adjusting to 100μM final concentration and the aggregation was observed for 5 minutes. The final concentration of solvent DMSO was fixed at 0.5% which had no effect on aggregation. Percentage of inhibition values were calculated as described in the literature (Campillo *et al*, 1999).

3.2 Results and Discussion

From phytochemical investigation of *F. lucida* roots extract, eleven coumarins were isolated and evaluated for anti-platelet aggregation activity. The primary screening results showed that most of the isolated coumarins exhibited weak or inactive inhibition of platelet aggregation induced by ADP. Feroniellin B was the most effective compound among the isolated coumarins. From the anti-platelet activity evaluation, it was found that feroniellin B (IC_{50} = 0.287 mM) was more potent than ibuprofen (IC_{50} = 11.2 mM) which was used as a positive control. Interestingly, the potency of feroniellin B was thirty-nine times greater than ibuprofen.

Table 3.1 Effect of isolated furanocoumarins on platelet aggregation (n = 3).

Compounds	Concentration (μM)	% Inhibition (Mean ± SEM)
Feroniellin A	144	19.8 ± 2.1
Feroniellin B	358	59.1 ± 8.2
Feroniellin C	535	9.45 ± 5.4
Marmesin	188	11.6 ± 7.9
Oxypeucedanin hydrate	240	20 ± 9.9
Anisolactone	14	N.A.
2″, 3″-Epoxyanisolactone	13	N.A.
2″, 3″-Dihydroxyanisolactone	25	14 ± 7.2
Psoralen	55	N.A.
Bergapten	45	11.1 ± 8
Isopimpinellin	21	N.A.

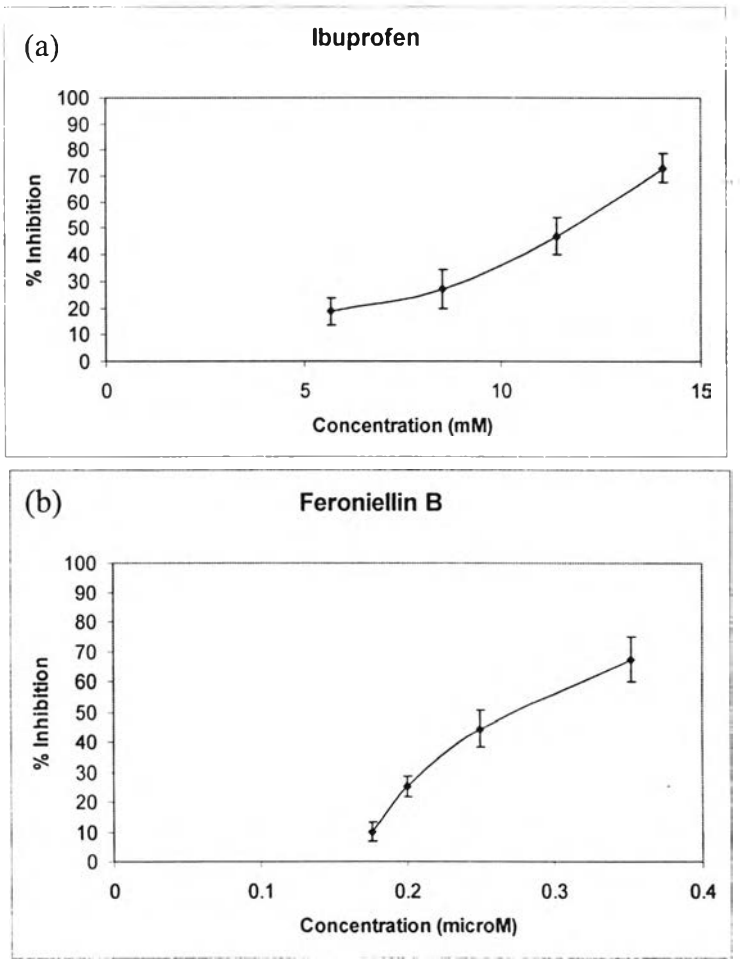

Figure 3.1 Inhibition of ADP-induced platelet aggregation by (a) ibuprofen and (b) feroniellin B (Mean ± SEM, $n = 5$).

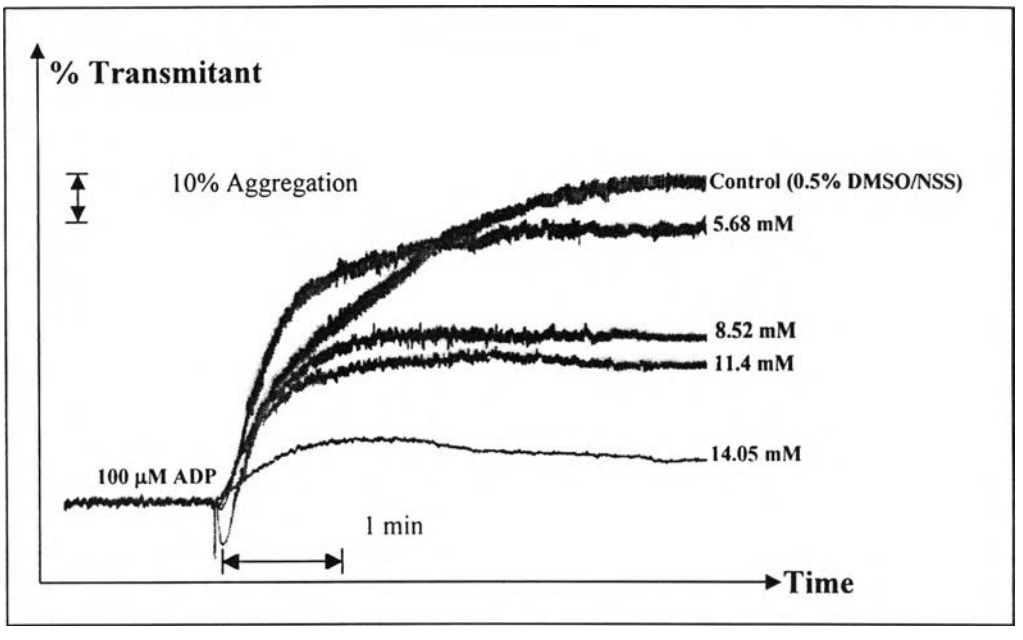

Figure 3.2 Platelet aggregation-induced by ADP in the presence of ibprofen (observed for 5 min.).

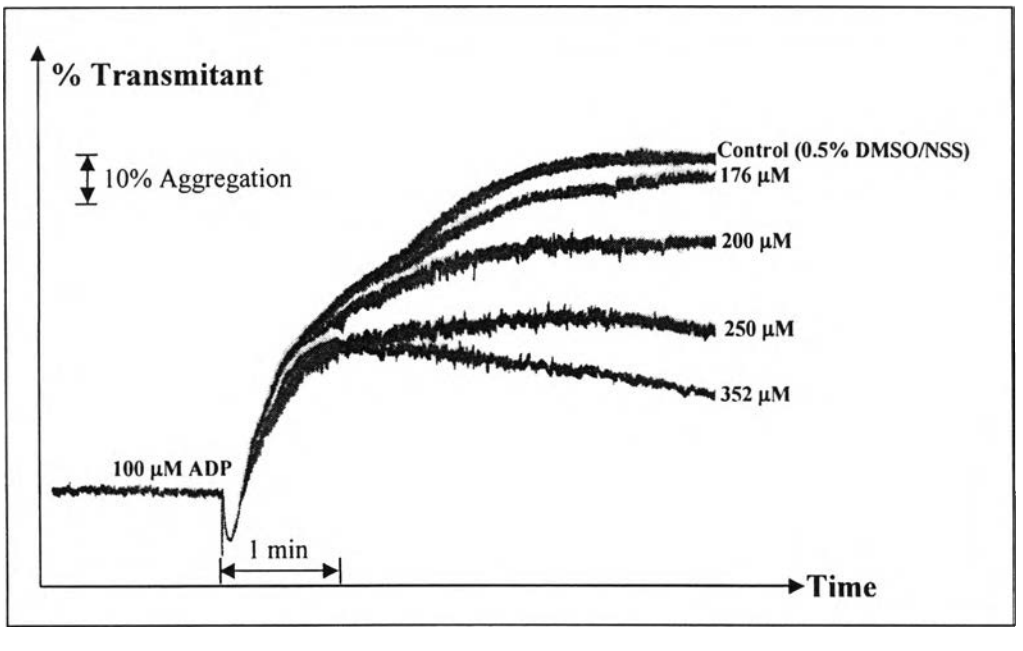

Figure 3.3 Platelet aggregation-induced by ADP in the presence of feroniellin B (observed for 5 min.).

Inhibitory effects of coumarins from plant sources on platelet aggregation have been widely investigated. In addition, several of coumarins exhibit anti-platelet aggregation. Aurapten and its derivatives from *Toddalia asiatica* inhibited rabbit platelet aggregation induced by arachidonic acid and collagen (Chen *et al*, 1995). Some simple

trioxygenated and tetraoxygenated coumarins such as scopoletin, scoperone, esculetin, isopimpinellin and artermicapin B inhibited collagen- and arachidonic acid-induced platelet aggregation (Tsia *et al*, 1998; Wu *et al*, 2001). However modes of action of these coumarins were unclear. Teng and colleague (1992) demonstrated that coumarins from natural sources inhibited platelet aggregation via several agonist inductions. The responses of platelet to various condition such as aggregation, ATP release, thromboxane A2 fromation and phosphoinositide breakdown, were monitored. It was found that the selected coumarins, xanthoxyletin, suberosin, aurapten, poncitrin and xanthyletin, inhibited platelet aggregation in all studied condition. They also found these coumarins interfered thromboxane A2 fromation, ATP release and phosphoinositide breakdown. From these results, the modes of action were proposed as (*i*) inhibition of thromboxane A2 generating enzyme and (*ii*) interference with phosphoinositide breakdown.

This study revealed feroniellin B exhibited strong inhibitory effect of feroniellin B on platelet aggregation induced by ADP. The effect of feroniellin B possibly involved platelet $P2Y_1$ and $P2Y_{12}$ receptor interference (Gachet, 2005). However, the modes of inhibition of feroniellin B must be further clarified by using other type of aggregating agents such as arachidonic acid, collagen, thrombin, etc.