

Thesis Title Study on Immunomodulating Activity
of Hispidulin from *Millingtonia*
hortensis Linn.f.

Name Alissara Srivattana

Degree Master of Science (Pharmacy)

Thesis Supervisory Committee

Primchanien Moongkarndi, Dr.rer.nat.
Nuntavan Bunyaphratsara, Ph.D.

Date of Graduation 13 August B.E. 2534 (1991)

Abstract

Immunomodulator is defined as agents capable of enhancing or suppressing various immunologic functions. The modulations are often being related to the dose and/or duration of the action. Medicinal plants are believed to promote the restorative and rejuvenating power of the immune system. According to the Thai-traditional practice, dried flowers of *Millingtonia hortensis* Linn.f. is used to relieve bronchial asthma. It is later found the broncho dilator, hispidulin, it is therefore likely that the antiasthmatic activity is due to hispidulin. The purpose of this study was to develop on guideline for quantitative analysis of hispidulin by immunoassay and search for its immunomodulating activity. Quercetin was parallel studied as a control. Experiments were performed by using rabbit anti-hispidulin/anti-quercetin sera which were produced in the laboratory. The results of comparing

sensitivity of every technique which showed from high to low sensitivity for hispidulin were ELISA ($0.5 \mu\text{g/ml}$), Rocket immunoelectrophoresis ($1.2 \times 10^2 \mu\text{g/ml}$), Dot immunoblotting technique ($3.15 \times 10^2 \mu\text{g/ml}$), and countercurrent immunoelectrophoresis ($6.7 \times 10^2 \mu\text{g/ml}$). The results suggested that ELISA is the most reliable, sensitive and appropriate for determining the quantity of hispidulin and quercetin. It could be applied to determine the flavonoid contents in other medicinal plants of interest.

Hispidulin and quercetin showed the immunomodulating activity by enhancing the phagocytosis activity of neutrophils at low concentrations, the best results were obtained at concentrations $10^{-2} \mu\text{g/ml}$ and $10^{-3} \mu\text{g/ml}$ respectively. Both hispidulin and quercetin cannot induce or be synergistic release of lymphokines i.e., leucocyte migration inhibitory factor (LMIF) from the cells. It was shown by the MTT assay that hispidulin was not cytotoxic to the experimental cancer cell lines ($\text{ED}_{50} > 20 \mu\text{g/ml}$) while quercetin showed a potent cytotoxicity at ED_{50} 1.0, 2.75, 7.03, 8.21 $\mu\text{g/ml}$ to the Chago cell line (lung cancer), S102 (hepatoma), SW620 (colon cancer), and BT474 (human breast cancer ATCC HTB20) respectively.

This study demonstrated the application of immunological techniques in the studies of flavonoids. It could be adapted to study for other constituents of plants of interest in the future.