Chapter 5

Conclusion and Recommendations

The investigation of Prasaprohyai preparation and its ingredient was based on their use by Thai traditional doctors for the treatment of fever, cold and allergy. The objectives of this research were investigated on anti-allergic, antioxidant and anti-inflammatory activities of Thai traditional medicine preparation called Prasaprohyai and its ingredients. The ethanol extracts (Et) and water extracts (EW, HW) of these plants, i.e. *Amomum testaceum* (AmT), *Anethum graveolens* (AnG), *Angelica dahurica* (AnD), *Angelica sinensis* (AnS), *Artemisia annua* (ArA), *Atractylodes lancea* (AtL), *Cuminum cyminum* (CuC), *Dracaena loureiri* (DrL), *Foeniculum vulgare* (FoV), *Kaempferia galanga* (KaG), *Lepidium sativum* (LeS), *Ligusticum sinense* (LiS), *Mammea siamensis* (MaS), *Mesua ferrea* (MeF), *Mimusops elengi* (MiE), *Myristica fragrans* (Chan thet or MyF, Mace or MyF(A), Nutmeg or MyF(S)), *Nelumbo nucifera* (NeN), *Nigella sativa* (NiS), *Syzygium aromaticum* (SyA) and Prasaprohyai formula (PSPY) were prepared. Effects of the ethanolic and water extracts of these plants on antioxidant activity, inhibition of NO production and TNF-α release from RAW 264.7 cells, inhibitory effects on the release of β-hexosaminidase in RBL-2H3 cells were determined.

The free radical-scavenging activity of ethanolic and water extracts of these plants were compared with reference standard, BHT. The present study showed that the water extracts of *Syzygium aromaticum* (EW) showed the highest antioxidant activity in this test (EC_{50} = 4.73 μg/ml) and it also registered stronger activity than BHT (EC_{50} = 11.66 μg/ml). In addition, the seven ethanolic extracts (*Dracaena loureiri*, *Mammea siamensis*, *Mesua ferrea*, *Mimusops elengi*, *Myristica fragrans* (Mace), *Myristica fragrans* (Nutmeg) and *Syzygium aromaticum*) and six water extracts (*Dracaena loureiri* (HW), *Mammea siamensis* (HW), *Mesua ferrea* (EW, HW), *Nelumbo nucifera* (HW) and *Syzygium aromaticum* (HW) showed strong antioxidant activity (EC_{50} < 30 μg/ml).

Anti-inflammatory activities of the ethanolic and water extracts of these plants were tested by measuring their effects on the pro-inflammatory mediators NO in activated macrophages RAW 264.7 cells. The results on this test revealed the water extracts of all plants exhibited inactivity. The ethanolic extract of *Atractylodes lancea* exhibited the most potent inhibitory activity in this test (IC_{50} = 9.70 μg/ml) and it exhibited NO production inhibitory effect higher than Indomethacin (IC_{50} = 20.32 μg/ml). Furthermore, the ethanolic extracts of *Angelica sinensis*, *Artemisia annua*, *Cuminum cyminum*, *Ligusticum sinense*, *Mesua ferrea* and Prasaprohyai formula showed potent inhibition activity (IC_{50} < 30 μg/ml). The anti-inflammatory properties of the ethanolic extracts of *Atractylodes lancea*, *Cuminum cyminum* and Prasaprohyai formula were also supported by the inhibitory effects on LPS-induced TNF-α release in RAW 264.7 cells.

Anti-allergic activities of the ethanolic and water extracts of these plants were tested by measuring their effects on the β-hexosaminidase in stimulated rat basophilic leukemia RBL-2H3 cells. The results on this test revealed that the water extracts of all plants exhibited inactivity. The ethanolic extract of *Mammea siamensis* showed the highest anti-allergic activity (IC_{50} = 7.90 μg/ml). In addition, the ethanolic extracts of *Angelica dahurica*, *Artemisia annua*, *Atractylodes lancea*, *Dracaena loureiri*, *Kaempferia galanga*, *Mesua ferrea*, *Mimusops elengi*, *Myristica fragrans* (Mace, Nutmeg), *Syzygium aromaticum* and Prasaprohyai formula showed the potent inhibitory activity (IC_{50} < 30 μg/ml). The results also indicated that the anti-allergic effects of these plants were higher than that of Ketotifen fumarate (IC_{50} = 40.41 μg/ml).

In summary, the results obtained in this study indicated that Thai medicinal plants, which were the ingredients of Thai folk medicine to treat fever and cold, possessed strong antioxidant activity, active against LPS induced NO production and TNF-α release in RAW 264.7 cell lines, and active against LPS induced β-hexosaminidase in RBL-2H3 cell lines. *Syzygium aromaticum* and *Mimusops elengi* displayed strong antioxidant activity in both ethanolic and water extracts. The ethanolic extracts of *Atractylodes lancea*, *Angelica sinensis* and *Cuminum cyminum* exhibited strong NO-inhibitory activity while *Atractylodes lancea*, *Cuminum*

cyminum and Prasaprohyai formula exhibited strong TNF-α inhibitory activity. Therefore, the ethanolic extracts of *Atractylodes lancea*, *Cuminum cyminum* and Prasaprohyai formula showed strong inhibition on both NO and TNF-α releases. The ethanolic extract of *Mammea siamensis* showed the highest β-hexosaminidase inhibitory activity and the other plants showed inhibitory activity in previous results.

In conclusion, the ethanolic extract of Prasaprohyai preparation showed antioxidant, anti-allergic and anti-inflammatory effect by reducing nitric oxide and TNF-α. The active plant extracts of Prasaprohyai are the ethanolic extract of *Atractylodes lancea* and *Mesua ferrea* because they are active ingredients for anti-allergy, antioxidant and anti-inflammation. The active plant extract of anti-allergic activity is the ethanolic extract of *Mammea siamensis* because it is active ingredient for anti-allergy, whereas the ethanolic extract of *Atractylodes lancea* is active ingredient for anti-inflammatory because it showed the highest NO production inhibitory effect. The active plant extract of antioxidant activity is the water extract (residue) of *Syzygium aromaticum* because it is active ingredient for antioxidant. These results can support using these Thai medicinal plants for treatment of fever, cold, allergic-related diseases and inflammatory-related diseases through the inhibition of NO release by Thai folk medicine. The information from this study may be useful for further studies and the development of this traditional medicine as modern products for treatment of fever, cold, allergic-related diseases and inflammatory-related diseases in the future.

Thus, the future study should be continued to isolate active compounds from the ethanolic extract of *Atractylodes lancea*, *Mesua ferrea* and Prasaprohyai by bioassay guide isolation method. Their active ingredients should be markers for analysis and determine stability of Prasaprohyai preparation in development as modern medicine.

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