CHAPTER VI
CONCLUSIONS

1. Pharmacokinetic parameters and oral bioavailability

PMF, TMF, and DMF which were markers in KP crude extract were detected in blood samples after administration of 250 mg/kg of the plant extract. For oral route, PMF, TMF, and DMF levels rapidly reached the maximum concentration (0.55-0.88 μg/ml) within 1-2 h and then slowly eliminated ($t_{1/2}$ = 3-6 h). Vd of PMF, TMF, and DMF ranged from 2.4 to 3.0 L. The oral bioavailability values of the methoxyflavones were very low about 1-4%. In case of intravenous route, Cmax of PMF, TMF, and DMF ranged from 46.4 to 71.6 μg/ml. After that, the methoxyflavones quickly excreted out from the blood ($t_{1/2}$ = 2-5 h). Vd values were 30-70 ml.

2. Organ distribution study

PMF, TMF, and DMF were found in the tested organs including liver, lung, kidneys, brain as well as testes. PMF and DMF were found in liver followed by kidney, lung, testes, and brain, respectively. TMF concentration was highest in liver following by kidney, brain, testes, and lung, respectively. The highest methoxyflavones levels was in liver. It should be noted that PMF, TMF, and DMF can cross blood-brain barrier as detected the compounds in brain.

3. Effect of KP crude extract on CYP450 metabolizing enzymes

In vitro studies using non-induced mouse hepatic microsomes in the presence or absence of KP crude extract showed that the extract altered CYP1A1, CYP1A2, CYP2B, and CYP2E1 activities by non-competitive, mixed-competitive, competitive, and uncompetitive mechanisms, respectively. Among these enzymes, CYP1A2 was affected by KP based on the highest value of Vmax and lowest of Ki value. In addition, the plant extract also modulated CYP2B activity based on the low Km value.

For *in vivo* studies, mice were orally treated with 250 mg/kg of KP crude extract for 7, 14, and 21 days. The results demonstrated that KP crude extract significantly induced CYP1A1, CYP1A2 enzyme activities following short-term treatment. CYP2B enzyme activities were markedly increased in all KP crude extract treatment timepoints, whereas KP crude extract significantly enhanced CYP2E1

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activity only after long-term treatment. However, KP crude extract did not affect on CYP3A enzyme activity. In summary, KP crude extract modulated several CYP450 enzyme activities with weak or medium modulation, thus, its utilization with drugs or other herbs should raise concern for potential drug-herb interactions.

4. Excretion and identification of the metabolites

The parent substances, PMF, TMF, and DMF, were still detected in urine and feces after administration of 750 mg/kg of KP solution. PMF, TMF, and DMF concentration in feces samples were higher than those of urine samples. The highest levels of three compounds were found at 24-30 h in urine and 24 h in feces. Then, they were gradually excreted out or were changed to metabolite forms.

In urine, methoxyflavones were excreted in demethylated, glucuronide, and sulfide forms whereas in feces, they were only in demethylated forms. These metabolites were probably occurred in liver because they were not detected in blood and basolateral side of transport experiment in Caco-2 cells treated with KP crude extract.

5. Development of products to increase oral absorption

According to low oral bioavailability of KP crude extract in pharmacokinetic study, KP-SMEDDS and KP-CD complex were developed to enhance its solubility, dissolution, and absorption. KP-SMEDDS formulation composing of 80% of Cremophor® EL and propylene glycol (at ratio of 2:1) with 20% of triglyceride of coconut oil (S-3-80) and KP-HP-β-CD complex successfully improved solubility, dissolution rate, and oral bioavailability of methoxyflavones in KP crude extract. Both of developed formulations can improve dissolution rate of methoxyflavones both in 0.01 HCl and 0.2 M PBS pH 6.8. These results showed the increase Papp values of methoxyflavones in Caco-2 cells by S-3-80 (about 10-fold comparing with KP crude extract). Papp of KP-HP-β-CD complex was greater than that of KP crude extract at 3.8, 5.0, and 5.1-fold for PMF, TMF, and DMF, respectively. The oral bioavailability values of S-3-80 were higher than those of KP crude extract (25.38, 42.00, and 26.01–fold for PMF, TMF, and DMF, respectively). For KP-HP-β-CD complex, the oral bioavailability values were 21.63, 34.20, and 22.90-fold greater than those of KP crude extract, respectively.

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