CHAPTER V

DISCUSSION

P. mirifica, *P. lobata, B. superba* and *M. collettii* were investigated for antioxidant activity, mutagenicity and antimutagenicity (Ames' test) and genotoxicity (micronucleus test).

Antioxidant activity

The antioxidant activity test of the 4 plants extracts by DPPH assay revealed that the plants exhibited antioxidant activity in different degree.

M. collettii showed the first order of antioxidant activity (IC_{50} = 55.53 μg/ml). The sample collected from Chiang Rai showed higher antioxidant activity than other samples as well as α-tocopherol, the potent positive control. Flavone (quercetin and kaemperol) is the main chemical constituent of *M. collettii* (Roengsumran *et. al.*, 2000). It has been reported that quercetin could scavenge the stable free radical DPPH stronger than genistein and daidzein (Johnson *et. al.*, 2000). Its antioxidant activity was confirmed in the inhibition of LDL oxidation *in vivo* (Morand *et. al.*, 1998). In general, free radical scavenging and antioxidant activity of phenolics mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolics molecules, and is also affect by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH). Flavonol aglycones such as quercetin and kaempferol, containing multiple hydroxyl groups, had higher antioxidant activity than their glycosides (Cai *et al.*, 2004). Furthermore, quercetin and kaempferol extracts from soybean showed strong antioxidant activity by DPPH assay also (Murota *et. al.*, 2002; Mitchell *et. al.*, 1998). The main reason for the strong antioxidant activity of *M. collettii* might be result from these components. *M. collettii* is thus possible an alternative natural material for commercial antioxidant.

B. superba exhibited the second order of antioxidant activity. The sample collected from Loei showed the highest antioxidant activity. The plant population showed lower antioxidant activity than α-tocopherol. The differences were observed

among tested samples. Flavonoid and flavonoid glycoside was the main chemical constituent of *B. superba* (Roengsumran *et. al.*, 2001). Attempts were made to clarify the antioxidant activity in *B. superba.* Flavonoid and sterol in ethanolic fraction from *B. superba* collected from Lampang were also submitted to DPPH assay to establish a base line for the antioxidant study in this plant. The fraction contains a mixture of flavonoid and flavonoid glycoside at a concentration of 1 mg/ml. The results (Table 4.14) revealed that only flavonoid fraction exhibited weak antioxidant activity (IC_{50} = 1,354.29 μg/ml). Thus flavonoid and flavonoid glycoside could be partially involved in such activity.

P. mirifica showed the third order of antioxidant activity. The sample collected from Uthai Thani showed higher antioxidant activity than other samples. The plants showed significant lower antioxidant activity than α-tocopherol. The differences were observed among tested samples. Isoflavone is the main chemical constituent of *P. mirifica* (Ingham *et. al.*, 1986; Ingham *et. al.*, 1989). The percentage of puerarin, daidzin, genistin, daidzein and genistein (Subtang, 2002) of 6 samples with highest antioxidant activity was compared with the others, 22 samples. It was found that the percentage of daidzein and genistein or isoflavone aglycoside in those samples were significant higher than the others (Table 4.4). The positive control was set with the pure isoflavone DPPH assay. It was found that the antioxidant activity of the pure chemicals was ranked from daidzein (82.58 μg/ml), puerarin (93.26 μg/ml), daidzin (108.26 μg/ml), genistein (155.75μg/ml) to genistin (362.10 μg/ml). The first 3 isoflavone exhibited non-significant different in antioxidant activity. Meanwhile daidzein was found predominantly in the first 6 highest antioxidant activity plants. Thus it could draw a correlation between daidzein content and antioxidant activity of the plants. Genistein and daidzein are the main isoflavone in soybean. The isoflavones were relatively poor hydrogen donors compared with the other estrogenic compounds. However, genistein and daidzein extracts from soy exhibited antioxidant activity *in vivo*, inhibitor among TPA-induced H_2O_2 formation by HL-60 cells (daidzein is the second), inhibit O_2^- generation by xanthine/xanthine oxidase while daidzein showed a moderate inhibitory effect. These results suggest that the antioxidant properties of isoflavones are structurally related and the hydroxyl group at position 4' is crucial in both systems (Wei *et. al.*, 1995). At least the content and

antioxidant activity of daidzein in this positive test was found related to, and could explain the highest antioxidant activity found in the first 6 samples of *P. mirifica* (Table 13). *P. lobata* contained high amount of puerarin and daidzein and the antioxidant was not significantly different from the sample collected from Uthai Thani.

Mutagenicity of plant extracts by Ames' test

Mutagenicity test by Ames test of 3 plant samples with highest antioxidant activity in their population was in comparison with *P. lobata*.

M. collettii exhibited no mutagenicity effect neither in the absence nor in the presence of the activation enzyme both in the TA98 and TA100 strains. *M. collettii* showed dosed-dependent, but no twice fold of spontaneous control, thus classified as non-mutagenic. In the test with TA98 strain, *M. collettii* showed cytotoxicity both in the absence and presence of the activation enzyme. In the test with TA100 strain, *M. collettii* showed toxicity both in the absence and presence of the activation enzyme, but the toxicity was decreased with the activation of enzyme. It could be noticed that *M. collettii* exhibited the high activity for cytotoxicity in TA98; however it was slightly decreased in the presence of activation enzyme in TA100. These results revealed that *M. collettii* exhibited no mutagenicity effect in both frameshift and base pair substitution mutation, neither in the absence nor in the presence of the activation enzyme (2.5-20 mg/plate).

B. superba exhibited no mutagenicity effect neither in the absence nor in the presence of the activation enzyme both in TA98 and TA100 strains. *B. superba* was investigated at the concentration of 0.5-5 mg/plate becaused of its limited disolved. In the test with TA98 strain, *B. superba* showed dose-dependent, but no twice fold of the spontaneous control and no cytotoxic effect both in the absence and presence of the activation enzyme. In test with TA100 strain, activity of *B. superba* was decreased with increasing the concentration in the absence the activation enzyme but no cytotoxicity. However, *B. superba* showed dose-dependent when treated with the activation enzyme in TA100 strain. It could be noticed that *B. superba* might show cytotoxicity if the concentration is increased to 20 mg/plate, however, the cytotoxicity was decreased with the enzyme in the test system. These results revealed that

B. superba exhibited no mutagenicity effect in both framshift and base-pair substitution mutation, neither in the absence nor in the presence of the activation enzyme (0.5-5 mg/plate).

P. mirifica exhibited no mutagenicity effect neither in the absence nor in the presence of the activation enzyme in TA98 and TA100 strains. In the test with TA98, *P. mirifica* showed dose-dependent, but not two fold of the spontaneous control. The cytotoxicity of *P. mirifica* was found; in the absence of the activation enzyme and this effect was decreased with the activation enzyme. In the test with TA100, *P. mirifica* showed dose-dependent, but no twice fold of the spontaneous control. The cytotoxicity effect of *P. mirifica* was found, in the absence and in the presence of the activation enzyme. The cytotoxicity was decreased with the activaiton enzyme as well in the test with TA98. These results revealed that *P. mirifica* induced no mutagenic for frameshift and base-pair substitution mutation (2.5-20 mg/plate). It has been report that daidzein and genistien showed no mutagenic effect to S. Typhimurium strains TA1538, TA98 or TA100 (Bartholomew and Ryan, 1980). The presence of daidzein and genistein in the plant could thus exhibit this activity.

P. lobata, the same as the tested Kwao Krua plants *exhibited* no mutagenicity effect. The samples showed dose-dependent in both in the absence and presence of the activation enzyme in TA98 and TA100 strains. The cytotoxicity of *P. lobata* was found only under in the presence of the activation enzyme in the test with TA100. It could be noticed that the activation enzyme might activate the toxicity of the plant extract. Thus, the consumption of *P. lobata* and Kwao Krua plants should be considered only in the very high amount as well as the effects from the activation via drug metabolizing enzymes.

Antimutagenicity of plant extracts by Ames' test

To clarify that the dead cells during antimutagenic test were the result of antimutagenic or cytotoxic effect of the tested plant extract, the cytotoxicity test of the plant extracts was thus established in both cell types.

Antimutagenicity test of the plant extracts with the highest antioxidant activity, including *P. lobata* extracts by Ames test revealed that the plant extracts exhibited antimutagenicity in different degree.

M. collettii showed the PI value as dose-dependent both in the absence and in the presence of the activation enzyme in TA98 strain. *M. collettii* exhibited strong antimutagenicity at high concentration (10 mg/plate) and the activity was decreased with the acitvation enzyme. Thus *M. collettii* might inhibit the frameshift mutation under the activation enzyme. In the test with TA100, *M. collettii* showed negative antimutagenicity, but the antimutagenicity activity was found strong under the activation enzyme. It could be notice that *M. collettii* might do well inhibit the base pair substitution mutation under the activation of enzyme.

B. superba showed no antimutagenicity. In the test with TA98 strain, *B. superba* showed negative result in both the absence and presence of the activation enzyme. In the test with TA100 strain, *B. superba* showed weak antimutagenicity effect at the lowest concentration and were not found in the presence the activation of enzyme, at high concentration. It could be noticed that *B. superba* showed negative results in the antimutagenicity, may be due to the low amount of test samples (0.5-5 mg/plate) as the crude extract was not easily dissolved in our system.

P. mirifica showed the PI value as dose-dependent. In the test with TA98 strain, the sample showed weak antimutagenicity effect in the absence of the activation enzyme and no antimutagenicity effect in the presence of the activation enzyme. *P. mirifica* could be antimutagenicity for frameshift mutation in the absence of the activation enzyme. In the test with TA100, *P. mirifica* showed negative result in the absence of the activation enzyme, but showed positive antimutagenic effect in the presence of the activation enzyme. *P. mirifica* could be antimutagenicity for base pair substitution mutation under the presence of the activation enzyme.

P. lobata showed the PI value as dose-dependent both in the absence and in the presence of the activation enzyme in TA98 and TA100 strain. In the test with TA98 strian, *P. lobata* exhibited weak antimutagenicity effect under the absence of the activation enzyme (-S9 mix) and showed positive antimutagenic effect under the presence of the activation enzyme (S9 mix). It could be noticed that *P. lobata* could

be antimutagenicity for frameshift mutation both in the absence and presence of the activation enzyme. In the test with TA100 strain, *P. lobata* showed weak antimutagenicity both in the absence and presence of the activation enzyme. It could be noticed that *P. lobata* could inhibit the mutagenicity for base pair substitution mutaion both in the absnce and presence of the activation enzyme.

Antimutagenicity of the plants extracts were the result of the high phytoestrogen content especially flavone and isoflavone in the plants. The chemicals were reported as potent anticancer, including breast cancer (Chen *et. al.*, 2003). The activation of enzymatic was important to activity of phytochemical. This action might involve with the cytochrome P450 enzymes. These enzymes are able to activate harmful chemicals included mutagens and carcinogens, as well as participate in detoxification of these harmful chemicals, and many other beneficial pathways. For the frameshift mutation, these results revealed that *M. collettii* exhibited highest antimutagenicity, *P. lobata* showed antimutagenic activity as second order, *P. mirifica* was third order and *B. superba* exhibited the lowest antimutagenic activity as well as the base pair substitution mutation.

Genotoxicity by micronucleus test

Mutagenic assay by micronucleus test of 3 plant extracts with the highest antioxidant activity and *P. lobata* extracts revealed that the plant extracts exhibited antimutagenicity in different degree.

M. collettii caused significant increase in the frequency of micronucleus in rats after the oral administration for 48 and 72 hr but with a slightly effect when compared with the positive control (Cyclophosphamide). *M. collettii* showed no cytoxicity in rats after treatment. It could be notice that *M. collettii* might show a mild risk in term of mutagenicity within 72 hours of consumption. The preliminary test with *M. collettii* for 30 hours revealed that the plant extract exhibited no change in micronucleus at the doses of 1 and 10 g/kg B.W.., but significant change at 20 g/kg B.W. It implied that micronucleus induction by *M. collettii* happened only at very high not normal consumption dose and could be seen at sooner period.

B. superba caused significant increase in the frequency of micronucleus in rats after the oral administration for 24 hours, with a slightly effect when compared with the positive control (Cyclophosphamide). The frequency of micronucleus formation in rats was decreased after 48 and 72 hours treatment. It could be notied that *B. superba* might be no a risk in term of mutagenicity within 72 hours of consumption. In addition, *B. superba* showed no cytotoxic effect.

P. mirifica as well as *P. lobata* exhibited no significant increase in the frequency of micronucleus and no cytotoxic effect in rats after the oral administration for 24, 48 and 72 hour. It could be noticed that *P. mirifica* and *P. lobata* show no risk in term of mutagenicity.