Chapter 4
DISCUSSION

Plants in the human diet synthesize chemicals in large amount probably to discourage insects and animals from eating them. The variety of these chemicals is so great that their characterization has contributed to the identification of many natural mutagens, carcinogens as well as antimutagens/anticarcinogens (Hayatsu, 1991., Ivie, et al, 1981; Toth and Erickson, 1986 ; and Sugimura, 1979., and Sugimura, 1982). Many papers have reported that many substances in plants inhibit the mutagenicity of various mutagens (Hayatsu, et al., 1988 ; Namiki and Osawa, 1986 ; Ramel, et al, 1986 ; and Stich et al, 1984). The preirous reports have shown that Thai vegetables also contained antimutagens (Itokawa, et al., 1986., Rojanapo, et al., 1990; Rojanapo and Tepsuwan, 1992 ; Vinitketkumnuen, et al., 1992; and Vinitketkumnuen, et al., 1993).

The antimutagenic effect of plant extracts was mainly caused by adsorption of mutagen. Plant mucilages have a high molecular weight and may be able to adsorb mutagens (Sato, et al., 1990).

Lemon grass has been tested for mutagenic properties in a battery of assays, covering a range of genetic end points and test organisms. The battery of test included in vitro assays for the induction of gene mutations in prokaryotes; Ames test, (this investigation) and the induction of chromosonal damage in vivo in somatic cells micronucleus test (Pinsaeng, 1993).

The Ames test is the most rapid, sensitive and best-validated mutagenicity test currently available. Testing lemon grass extract up to concentrations that induced slight cytotoxicity, as measured by background lawn growth, gave no evidence of mutagenic activity in Salmonella typhimurium strain TA 98 and TA 100 in the absence or presence of S9 metabolic activation, by two independent experiments (Tables 2, and 3).

While this investigation was to determin the mutagenicity properties of lemon grass, other laboratory (Mevatee, et al, 1993) has investigated the in vitro assay for chromosomal aberrations measures chemically induced breakage of chromosomal material resulting in structural chromosomal aberration. Their results indicated that lemon grass did not have clastogenic effect.

Since there is no criteria far separation between gene mutation and chromosomal damage, and chemical mutagens frequently induce both kinds of mutation. The lack of activity in the gene mutation and chromosomal aberration assays are mutually confirmatory which indicating that lemon grass does not induce genetic damage in mammalian cells.

Up to now, there are no published data on the antimutagenicity and mechanism of lemon grass could be obtained (in a search of the CD-ROM database). This investigation indicated that lemon grass extract exert an antimutagenic action against the mutagenicity of various known mutagens, such as AFB_1, B (a) P, Trp-P-1, IQ, MNNG, AF-2 and 4-NQO to S.typhimurium strain TA 98 and TA 100. The extracts did not affect on the mutagenicity of NaN_3.

Cooking, especilly heating during food preparation, sometimes considerably reduced the antimutagenic capacities. The objective of this study was also to examine the effect of heating on the antimutagenicity lemon grass. When the aqueous extract was prapared from lemon grass was made by heated at 100 ^{o}C for 8 hours, the antimutagenicity of lemon grass hot extract was similar to the aqueous cold extract which prepared at room temperature as shown in Tables.6, 7. Therefore, heat or boiling did not destroy the antimutagenic activity of

water extract. The methanol and hexane extracts prepared by both hot and cold extraction conditions were also similar antimutagenicity. It could, at least be assume that heating could not alter the antimutagenicity of lemon grass extract.

The methanol extract of lemon grass decreased mutagenicity of AFB_1, B (a) P, IQ, Trp-P-1 , MNNG , 4-NQO and AF.2 . The inhibitory effects have dose-relationship (Figure 9, 10).

There was no significant change in mutagenesis of AF-2, 4-NQO, B (a) P, DMBA and NaN_3 mutagenesis when they were combind with an aqueous extract of lemon grass (Figures 7, 8).

The hexane extract decreased the mutagenicity on most of mutagens studied, but the extract showed to have toxicity to bacteria as the amount of the extract increased (Figures 11 and 12)

It was interesting that the methanol extract generally depressed most of known mutagens either in the presence or absence metabolic activation. Lemon grass extracts have been shown to have antimutagenic properties either through the inhibition of metabolic activating enzymes (S9 fraction) (Figure 16) or S9 mix (Figure 15) and trapping on known mutagen, AFB_1 (Figure. 13) and MNNG (Figure 14).

Several reports indicated that MNNG can be "activated" in mammalian cells and in bacteria after binding to molecules containing thiol groups such as cysteine and glutathione (Lowley and Thatcher, 1970; Mohn, et al, 1983). With respect to these reports, it is probable that the antimutagenic activity of lemon grass extract found under the present experimental conditions was due to reaction of some constituents with MNNG. It can be tentatively assumed that the one of the antimutagenic properties of lemon grass is mediated via inactivation of active mutagenic compound like MNNG by mutagen scavenging which might be sulfur-containing compounds.

It was interesting that lemon grass extracts depressed the mutagenicity of the amino acid pyrolysates, IQ and Trp-P-1. It was reported that the major metabolic pathways in the presence of S9 mix of the amino acid pyrolysates occurred via N-hydroxylation (Hirayama, et al., 1989 ; Brumborg,et al, 1989). Thus, it might be suggested that the desmutagenic effect of lemon grass on the amino acid pyrolysates may be due to prevention of the N-hydroxylation reaction.

Aflatoxin B_1 is the potent carcinogen and its metabolism has been studied extensively. Aflatoxin B_1 metabolism is mediated by

This study showed that the decrease in His^+ revertant colonies of S.typhimurium TA 98 induced with AFB_1 and B(a)P has occurred in the presence of methanol extract of lemon grass. Therefore, the antimutagenic compounds in lemon grass might have the ability to interact with both cytochrome P-450 dependent enzymes and cytochrome P-448 pathway.

According to Kada and Shimoi, (Kada and Shimoi, 1987) there are two possible mechanism for preventionof inducing cellular mutagenesis. Firstly, mutagens are inactivated by "desmutagens" before they can attack the DNA. Secondly, " bio-antimutagens" interfere cellular fixation processes which had effect on damage in DNA.

This investigation has revealed that desmutagenic activity for AFB_1, amino acid pyrolysates, IQ and Trp-P-1, B(a) P, MNNG as well as 4-NQO does exist in lemon grass (Figures. 7 ,8, 9, 10, 11, and 12). Desmutagenic factors in lemon grass may modify the mutagens' activities The possible mode of inhibiting action of antimutagens in lemon grass on AFB_1 and MNNG were investigated. In the present investigation, it might be able to explain for action of the factor which inhibited the activity of the microsomal activation system (S9 fraction), (Figure. 15, 16).

peak 2. After rechromatographed and further purified by HPLC two antimutagenic peaks were obtained, peak 2a, and peak $2a_2$. (Figure 26). The antimutagenicity of these two peaks against AFB_1 was still demonstrated (Figure 27) but not MNNG (Figure 28). The results obtained for isolation of antimutagenic substance in this investigation was summarized in Table 18.

In the present study, there is evidence that the methanol extract of lemon grass still contain several antimutagenic compounds. In this investigation, two active antimutagens were partially purified. It is expected that a future purification of other antimutagens may yield a good results. Along with future study, the identification and the mechanism of action of these antimutagens may complete by validate the information which can be used to assess whether these minor dietary components will be beneficial to cancer prevention.