CHAPTER V

DISCUSSION

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Farnsworth and Soejarto, 1991).

The use of herbal products as medicinal remedies has increased greatly in the past decade. General consumers always keep in their mind that natural remedies will accomplish cures and imply that the term "natural" means free of side effects. Many herbal products may do what they claim, and some do not. Any product that has a desired effect is also likely to have some undesired side effects. Unfortunately, only a few of the herbal remedies have scientific data to back up their clinical benefits, and a few have been tested for their toxicity.

The antimutagenicity of the extract of red hibiscus (*Hibiscus rosa-sinensis* Linn.) (ชบา), Mexican creeper (*Antigonon leptopus* Hook. & Arn.) (พวงชมพู), ixora (*Ixora coccinea* Linn.) (เข็ม), white frangipani (*Plumeria obtusa* Linn. (ลั่นทม), malay apple (*Syzygium malaccense* (Linn.) Merr.& Perry) (เศรษฐมพู่), kra chiew (*Curcuma sessilis* Gage.) (กระเจียว), sacred lotus (*Nelumbo nucifera* Gaertn.) (บัว), indian cork tree (*Millingtonia hortensis* Linn.) (ปีป), thong pun chang (*Rhinacanthus nasutus* ((Linn.) Kurz.) (ทองพันชั่ง), and pomegranate (*Punica granatum* Linn.) (ทับทิม) has been discovered in the present investigation. Some flowers are being consumed as components of Thai dishes. It was hopeful to search for value-added products in the form of antimutagens for future application.

Extracting with dichloromethane, it was found that red hibiscus, ixora, malay apple, sacred lotus, Indian cork tree, thong pun chang, and pomegranate had color different from methanol or water. The color of methanol or water extract of each flower was supposed to be anthocyanins. Anthocyanins belong to the flavonoid family and, as coloured flavonoids, are prominent in flower petals and fruit peels (Brouillard and Dangles, 1994). Using dichloromethane as an extractant, carotenoids seemed to be the pigments that expressed the yellow color of the extract most flowers while the green color of the extract from thong pun change seemed to be the expression of chlorophyll.

5.1 Safety of the Extracts: Brine Shrimp Bioassay

Bioassays in terms acute toxicity and chemopreventive studies of the crude extracts from various flowers offer special advantages for identification of medicinal botanical extracts. Because desired biological response may not be due to only one component but rather to a mixture of bioactive flower components. Therefore, screening crude fractionated extracts of flowers for biological activity should be directed with bioassays to exploit the bioactive compounds (Jerry and Lingling, 1998.)

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the toxicity of the flower extracts. This assay is a rapid inexpensive and simple method for testing the toxicity of flower extracts. The result in most cases correlates with cytotoxic of the flowers. The LC_{50} (half-inhibition) values obtained after the analysis of the extracts with different polarities demonstrated the negligible toxicity to brine shrimp (most LC_{50} were >1000 μg/ml). The result of this study showed that LC_{50} of the flower extracts were not dependent on polarity of extracting solvents. It suggested the safety of the extracts and required further examination on their antimutagenicity described below.

The high LC_{50} values obtained from the extracts of the studied flower attributed to low amount of cytogenic and toxic compounds present in the flower. High LC_{50} value indicates possibility of using compounds in these flower extracts in food or drug preparation. The knowledge from mutagenicity and antimutagenicity of all extracts described below also confirmed that most extracts were neither toxic to *Salmonella typhimurium* tester strains of the Ames test which are the prokaryote nor

to the higher organism namely, the larvae of *Drosophila melanogaster* tester strain of the SMART.

5.2 Antioxidant Activity and Total Phenolic Content

Interest in the search for new natural antioxidants has grown dramatically over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to ageing related diseases (ARDs) (Finkel and Holbrook, 2000) and a large number of other illnesses. Also the restrictions to the use of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), due to their toxicity (Namiki and Namiki, 1990) has been an important incentive for such research. Minor non-cultivated vegetables as part of a diet with additional health benefits like those discussed in this report have not been investigated systematically for their potential health-benefiting properties; therefore, several of the species discussed here merit further study.

Antioxidant properties of pomegranate and Mexican creeper flowers have been studied relatively well over the past years (Kaur *et al.*, 2006, Vanisree *et al.*, 2008). The antioxidant activity of the samples both in the DPPH and in FRAP assays, are very interesting and both species should be investigated phytochemically and biochemically focusing on these properties. The local processing and cooking procedures should also be taken into consideration. Nothing has so far been reported on the antioxidant properties of other species. The red hibiscus, Mexican creeper, ixora, white frangipani, malay apple, kra chiew, sacred lotus, Indian cork tree, thong pun chang, and pomegranate flowers are also consumed in several areas of Thailand.

Antioxidants, which can quench reactive free radicals, prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases (Shahidi, 1997). The present study has revealed that each extract of flower has antioxidants and certain amount of phenolic compounds. Concerning the practical consumption of some edible flowers in Thailand, Busayaskul (2006) studied the antioxidant activity of raw and conventional processed (boiled, battered and fried) samples of eight edible flowers using scavenging capacity (DPPH) and ferric reducing antioxidant power (FRAP) assays. They were hua-plee (*Musa sapientum* Linn.), dok-khachon (*Telosma minor* Craib.), dok-khem (*Ixora coccinea* Linn.), dok-khae (*Sesbania grandiflora* Desv.), dok-bualuang (*Nelumbo nucifera* Gaertn.), dok-fueangfa (*Bougainvillea glabra* Choisy.), dok-sano (*Sesbania javanica*

Miq.), and dok-anchan (*Clitoria ternatea* Linn.). It was proposed that antioxidant activity and certain amount of phenolic compounds of each edible flower extract might reduce urethane metabolites responsible for inducing wing spots detected in SMART. Therefore, the results of the present investigation that most extracts of the samples contained antioxidants confirmed the benefits of edible Thai flowers. These compounds might, therefore, influence on the mutagenicity of urethane and the product of the reaction mixture of 1-aminopyrene nitrite model discussed below. Moreover, the overall less toxicity of traditionally consumed food flowers offers a unique possibility for developing new health supplements and nutraceuticals from the most interesting non-cultivated Asian flowers. Last, these food supplements may well fulfill the safety requirements for such products much more easily than herbal medicines.

Most extracts of flowers had good reducing power to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. FRAP measures the antioxidant effect of any substance in the reaction medium as reducing ability. They were also good free radicals scavenger of the DPPH model. Reducing ability is considered the ability of natural antioxidant to donate electron and would be considered the mechanism used to describe the antimutagenicity of urethane of the SMART in section 5.4

The antioxidant activity of ethanolic extract of *Punica granatum* flower was not surprised since Kaur *et al* (2006) reported that such extract inhibited hydroxyl radical ($OH^{\cdot}$) induced oxidation of lipids and proteins *in vitro*. In addition, the antioxidant activity of dichloromethane, methanol and water extracts of Mexican creeper had also agreed with the study of Vanisree *et al.* (2008). They reported that the methanol extract of the aerial parts of Mexican creeper inhibited lipid peroxidation (LPO) by 89%. Further purification of the methanolic extract by the same investigators yielded n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside and kaempherol-3-glucoside along with β–sitosterol, β–sitosterol-glucoside and d-mannitol. 4-Hydroxycinnamic acid, quercetin-3-rhamnoside, and kaempherol-3-glucoside inhibited lipid peroxidation by 19.5%, 41.0% and 60.5% respectively, at 5 μg/ml.

The result of total phenolic content, ranged around 3.70-145.13 gallic acid equivs/g of dried extract, might explain the antimutagenicity of the extracts of flowers. Antioxidant activity and certain amount of phenolic compounds of some flowers might reduce the formation of reactive metabolites of the reaction product of

1-aminopyrene-nitrite model in the tester strain of the Ames test discussed below. It might also adjunct to the result of antioxidant activity reduced the formation of ultimate metabolites of urethane responsible for inducing wing spots detected in this investigation. Water extract of ixora showed the highest antioxidant capacity and phenolic content, which was agreed with Saha *et al.* (2008), reported methanol extract of ixora showed strong reducing power and total antioxidant capacity.

5.3 Mutagenicity of Flower Extract in Ames Test

Traditional use of plants with no published toxicological test in daily life frequently provides the basis to select which plant extract worth for studying for the other beneficial role. This study aimed to assess *in vitro*, with the Ames test, the possible risk of using the extracts of flowers. Results obtained with TA98 for frameshift mutations, and TA100 for basepair substituents, show that all extracts were not direct mutagenic, since even at high concentration it did not induce revertants, verified by pre-incubation method. Such information indicated the safety of these flowers if they were consumed in the past and partially convinces at the first step in using the extracts for other propose in food product development.

A good correlation between nitrate intake and gastric cancer mortality in various countries has been documented (Fine *et al.*, 1982). High nitrate intake was associated with gastric cancer in England, Colombia, Chile, Japan, Denmark, Hungary and Italy (Forman and Shuker, 1997). In Thailand, stomach cancer is the eighth most common cancer in males and not in the ten leading sites of cancer in females in 1999. The estimated ASR (aged-standardized incidence rate) was 3.5 for males and 2.8 for females (Khuhaprema and Srivatanakul, 2009). Direct-acting genotoxin can be produced by reaction of their precursors with nitrite under acidic conditions similar to those of gastric juice (Kangsadalampai *et al.*, 1996). Therefore, stomach is most at risk from endogenous mutagenic compound synthesis since stomach acid catalyses nitrosation reactions.

The result that some extracts from flowers showed direct acting genotoxicity in Ames test after being treated with nitrite implied that some extracts from flowers contained certain precursors that could react with nitrite under acidic condition to produce direct mutagenic products causing frame-shift (TA 98) and base-pair substitution (TA 100) was not surprised. Higashimoto *et al.* (1993) reported that methanol and water extract of three kinds of spices (caraway, coriander and black

pepper seeds) treated with nitrite were mutagenic for strain TA 100 without metabolic activation. Several research groups reported the presence of nitrosatable precursors in foods, such as indoles, phenolics and carbolines (Wakabayashi *et al.*, 1989). Ieamworapong (1990) reported that ethanol extracts of *Andrographis paniculata*, *Carthamus tinctorius*, *Cassia alata*, *Cassia angustifolia*, *Cassia fistula*, *Centella asiatica*, *Curcuma domestica*, *Cyperus rotundus* and *Oroxylum indicum* were found to be mutagenic to both strains TA 98 and TA 100 in the absence of activating enzyme after interacted with sodium nitrite in the acid solution (pH 3.0-3.4). Moreover, Pakdee (2001) studied that hot water extract of *Ganoderma lucidum* had mutagen when interacted with nitrite. Therefore, consumers who consume these flowers such as, Mexican creeper, ixora, kra chiew, sacred lotus, Indian cork tree, thong pun chang and pomegranate should avoid nitrite containing food items.

In this experiment, methanol extract of sacred lotus exhibited the highest mutagenicity on both strains. Sacred lotus is the component of Thai folklore medicinal herbs. This is not surprised since nitrosating of extracts from plants e.g. Thai folklore medicinal herbs always generated some direct mutagens towards *S. typhimurium* TA 98 and TA 100 (Kangsadalampai *et al.*, 1995). Therefore, consumers of any folklore medicines should avoid taking extract of sacred lotus after they have a chance to be exposed to nitrite containing food item.

Dichloromethane extracts of red hibiscus, white frangipani, Malay apple, methanol extract of Malay apple and water extract of red hibiscus were an exception because they were not mutagenic after nitrite treatment. It was postulated that they had some compounds that could inhibit the role of nitrite in formation of direct mutagens, possibly be due to the amount of antioxidants in most fractions of these flowers. In a report of a WHO meeting held in Geneva in 1978, Coulston (1980) stated that the development of a systemic approach to the investigation of the potential hazard of nitrosable drugs must be based on two considerations, namely the relative speed at which drugs undergo N-nitrosation under standardized condition *in vitro* and the carcinogenicity of the more strongly-reacting compound in suitable animal models. Suharitdamrong (1996) reported that nitrite treated bromazepam, chlordiazepoxide and isoniazid in acidic condition were mutagenic on both strains (TA 98 and TA 100). Moreover, Limprasertkul (2002) found that nitrite treated menstrual regulatory and haematinic traditional preparations in acidic condition were mutagenic on both strains (TA 98 and TA 100) too. Therefore, dichloromethane

extracts of red hibiscus, white frangipani, Malay apple, methanol extract of Malay apple and water extract of red hibiscus might be reduced mutagenic from nitrite treated drugs.

5.3.1 Modifying Effect of the Extracts of Flowers on the Mutagenicity of the Reaction Product of 1-Aminopyrene-Nitrite Model

The ability of the extracts of flowers to inhibit mutagenic responses induced by the product of the reaction mixture of 1-aminopyrene nitrite model in both strains of *Salmonella typhimurium*, TA 98 and TA 100, indicated that they exerted an inhibitory effect on frame-shift and base-pair type mutation that may, in part, contribute to the induction of tumors.

The first extraction, dichloromethane should contained non-polar compounds. Overall results suggested that all sample with low polarity i.e. dichloromethane extract from each flower inhibited the mutagenicity of the reaction mixture of 1-aminopyrene nitrite model on both tester strains. This agreed with the study of Botting *et al.* (1999) who found that the heptane fraction from petroleum ether of twenty-five food plants eaten by Polynesians were the most antimutagenic against heterocyclic amine 2-amino-3-methylimid-azo[4,5-f]quinoline (IQ).

Methanol and water extracts from flowers in the present experiment should contained polar compounds. The results that water extracts of pomegranate flower and white frangipani flower were high antimutagenic against the reaction product of 1-aminopyrene-nitrite model was not surprised because Kruawan (2001) found that the hot water extracts of bael fruit (*Aegle marmelos*), fragrant screw pine (*Pandanus odorus*), safflower flower (*Carthamus tinctorius*), chrysanthemum flower (*Chrysanthemum indicum*) and roselle (*Hibiscus sabdariffa*)flower had antimutagenic activity against the same mutagen used in *Salmonella typhimurium* TA 100.

In the present study, 1-aminopyrene treated with excess nitrite in acid solution (pH 3.0) for 4 h should give 1-nitropyrene as suggested by Kato *et al.* (1991). 1-Nitropyrene is a potent direct mutagen toward *Salmonella typhimurium* strains TA 98 and TA 100 (Kangsadalampai *et al.*, 1996). The mutagenicity of 1-aminopyrene needs to be activated by nitroreductase (IARC, 1989) and O-acetyltransferase (Mermelstein *et al.*, 1981). These two enzymes are the activating systems of direct-acting mutagens including 1-aminopyrene presented in *Salmonella typhimurium* cell. 1-Nitropyrene binds covalently to the C8 position of 2′-deoxyguanosine upon reductive activation to

generate N-(deoxyguanosin-8-yl)-1-aminopyrene (Howard *et al.*, 1983; Djuric *et al.*, 1988). This adduct induces many types of mutations (Watt *et al.*, 2007).

The ethanol extracts of flowers in this study should contain some flavonoids that are naturally occurring phenolic compounds in plants. However, the information about this matter is scarce. Only the ethanol extract of pomegranate flower are identified to contain polyphenolic compound named pomegranatate; the role of flavonoid in antimutagenicity in Ames test will be discussed below. Together with flavonoid in the extract of pomegranate, some terpenes e.g. ellagic acid, 3,3′,4-tri-O-methylellagic acid, ursolic, and maslinic acids were identified by Wang *et al.* (2006). Loviagina and Shivrina (1962) suggested that triterpene acids and triterpene alcohol components of a mushroom widely used in folkmedicine in Siberia, North America, and North Europe, commonly known as Chaga (*Inonotus obliquus*) had their potential to delay tumorigenicity and inactivate cancer cells. Therefore, it is worth to identify each triterpene found in each fraction of the present study and study whether it has antimutagenicity.

Proposed Mechanism of Antimutagenicity in Ames test

It is hypothesized that the antimutagenicity of the extracts of flowers may be due to their flavonoids since Edenharder and Tang (1997) reported that 1-nitropyrene was in general more effectively antagonized by potent antimutagenic flavonoids. Ramirez-Mares and Gonzalez de Mejia (2003) found that epigallocatechin gallate (flavonoid in green tea) was a suppressor of lipid peroxidation induced by 1-nitropyrene. Kuo, Lee and Lin (1992) proposed that the antimutagenicity of flavonoids may be inhibited the bacterial mutagenesis induced by nitropyrenes. Mechanism of antimutagenic response by flavonoid might result from modulating the nitroreductase in the bacterial strain.

The antimutagenicity of Indian cork tree also supported the hypothesis that its flavonoids content played at least in part in inhibition of the mutagen used in the Ames test of this investigation. Chulasiri *et al.* (1992) reported that hispidulin and hortensin, the flavonoids from *Millingtonia hortensis* (Indian cork tree) flower, inhibited the indirect mutagenicity induced by 2-aminoanthracene, aflatoxin B1 (in TA 98) and dimethylnitrosamine (in TA 100). The information as such suggested that the compounds in indian cork tree might have a general role in inhibiting the action of mutagens.

Overall results suggested that all dichloromethane extract from flowers inhibited the mutagenicity of the reaction mixture of 1-aminopyrene nitrite model on both tester strains. Methanol extract of kra chiew and pomegranate showed the highest antimutagenic activity in TA 98 and TA 100, respectively at high concentration. It might be postulated that components of these flower extracts were antimutagens that worth for research and development of food products.

5.4 Antimutagenicity of Samples in SMART

The finding that co-administration of each flower extract in the present study decreased the mutagenicity of urethane was along with the proposed criteria and supported the work of Busayaskul (2006). This author studied on the antimutagenicity of raw and conventional processed (boiled, battered and fried) eight edible flowers, namely, hua-plee, dok-khachon, dok-khem, dok-khae, dok-bualuang, dok-fueangfa, dok-sano and dok-anchan, against the mutagenicity of urethane in *Drosophila* tester. Since the co-administration study is aimed to elucidate whether each sample can inhibit the mutagenicity of urethane; if number of spots per wing reduces, it postulates that the sample might trap urethane and/or modulate the enzymes of urethane metabolism (e.g. induces glutathione-S-transferase (GST) or inhibites cytochrome P 450 system). Since most of the extracts in the present investigation may be contained polyphenolic compounds; therefore, the mechanism of antimutagenicity of dietary flavonoids and isoflavonoids may be due to the induction of phase II detoxification enzymes in cells (Hoensch and Kirch, 2005).

It was possible that the dichloromethane extract should contain several active compounds. Therefore, the antimutagenicity against urethane might be related at least in part to the chlorophyll content of the extract, originating in the sepals and floral stalk. This assumption agrees with several studies showing a positive correlation between the chlorophyll content of plants and their antimutagenic activity (Grover and Bala, 1993; Lahiri *et al.*, 1993; Salvadori *et al.*, 1993).

Sacred lotus was one of the flowers in the present investigation that had high antimutagenicity. This supported Phoolphithayadhorn (2001) who revealed that hot water extract of flower of sacred lotus was antimutagenic against urethane. The information that conventional processed (boiled, battered and fried) sacred lotus reduced mutagenicity of urethane suggested that antimutagenic substances were at least partially heat stable (Busayaskul, 2006). Sacred lotus flower contains flavonoids

(kaempferol-3-glycoside) and its ether extract of the petals and stamens yields quercetin (ถนอมศรี, 2538). In addition, Yang *et al.* (2008) found five anthocyanins (delphinidin 3-O-glucoside, cyaniding 3-O-glucoside, petunidin 3-O-glucoside, peonidin 3-O-glucoside and malvidin 3-O-glucoside) in sacred lotus. Kraiket (2007) suggested that anthocyanins played a role as antimutagen against urethane which pigments extracted from *Oryza sativa* (black rice), *Clitoria ternatea* (butterfly pea flower), *Hylocereus polyrhizus* (dragon fruit), *and, Hibiscus sabdariffa* (roselle flower) were incorporated to Thai rice noodle (Khanomjeen). It was evident that anthocyanin-rich crude extract from concord grapes and delphinidin (a pure anthocyanin) were associated with a significant increase in activities of the phase 2 detoxification enzymes, namely glutathione-S-transferase and NAD(P)H:quinine reductase (Singletary *et al.*, 2007). Flavonoids which are generally respected as plant anitioxidants and play an important role in scavenging the free radical generated during urethane metabolism are also found in the petal of this flower (Yang *et al.*, 2008). They are quercetin 3-O-rutinoside, quercetin 3-O-galactoside, quercetin 3-O-glucuronide, kaempferol 3-O-robinobioside, kaempferol 3-O-galactoside, isorhamnetin 3-O-rutinoside, kaempferol 3-O-glucoside, kaempferol 3-O-glucuronide, syringetin 3-O-hexose and kaempferol 3-O-pentose) of lotus cultivars.

Busayaskul (2006) revealed that raw and conventional processed ixora flower reduced the mutagenicity of urethane. In this study, water extract of ixora flower showed the highest antimutagenicity. It might possibly be due to flavonoids that Subramanian and Nair (1971) identified as cyanidin-3-rutinoside and leucocyanidin glycoside. Ixora flower also contains ursolic acid (Latha and Panikkar, 1998). Saraswat *et al.* (1996) suggested that ursolic acid inhibits metabolic activation of procarcinogen and the toxic metabolite formation catalyzed by CYPs, which is one of the mechanisms of carcinogenesis inhibition and hepatoprotective effects. This suggestion was confirmed by Kim *et al.*, (2004) who revealed the inhibitory effect of ursolic acid on human liver microsomal CYP2C19. In addition, Resende *et al.* (2006) also revealed a significant reduction in micronucleus frequency in the groups concomitantly treated with the ursolic acid and oleanolic acid and doxorubicin compared to that treated with doxorubicin alone.

The antimutagenicity against urethane of pomegranate flower confirmed the finding of Celik, Temur and Isik (2008). They found that the beverage made from

pomegranate flower impartially protected against the subacute administration of trichloroacetic acid that promoted malondialdehyde concentration fluctuates in the antioxidative systems and elevated tissue damage indicated by serum marker enzymes (AST, ALT, LDH, CK and ALP).

Red hibiscus flower had antimutagenicity on urethane. The constituents present in the extract of red hibiscus flower include quercetin, carotene, niacin, riboflavin, malvalic acid, gentisic acid, margaric acid and lauric acid (Ross, 1999). This study correlates with Sharma, Khan and Sultana (2004) who studied the role of gentisic acid (2,5-dihydroxybenzoic acid) in the chemopreventive activity of *Hibiscus rosa-sinensis* extract on 7,12-dimethyl benz(a)anthracene (DMBA)/croton oil-mediated carcinogenesis in mouse skin via 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced tumour promotion response and oxidative stress. It was noteworthy here that gentisic acid was also known to possess antioxidant, anti-inflammatory and anti-mutagenic properties (Krizkova, Nagy, and Polonyi, 2002; Urig *et al.*, 2000).

Proposed Mechanism of Antimutagenicity of Edible Flowers in SMART

Since most extracts of flowers in this investigation had good reducing power to reduce oxidized compound and scavenge free radical. Reducing ability is considered the ability of natural antioxidant to donate electron and would be considered the mechanism used to describe the antimutagenicity against urethane. The metabolism of urethane is mediated by at least three pathways (Salmon *et al.*, 1991). In rodents, more than 90 % of an administered dose of urethane is hydrolysed to ethanol, ammonia and carbon dioxide by liver microsomal esterase (Skipper *et al.*, 1951; Nomeir *et al.*, 1989). Less than 0.5% of urethane is metabolized to vinyl carbamate by cytochrome P 450. Subsequently, vinyl carbamate oxide is formed, a substance capable of forming DNA adducts *in vivo* (Dahl, Miller and Miller 1978; Miller and Miller, 1983; Guengerich and Kim, 1991). Approximately 0.1% of urethane is reversibly converted by cytochrome P 450 into N-hydroxyurethane (Boyland and Nery, 1965; Nery, 1968). It was documented that N-hydroxyurethane, a urethane metabolite), was hydrolysed by esterase to generate hydroxylamine and exert its carcinogenic effect in multiple organs via generating O_2- and NO· to cause oxidation and depurination of DNA (Sakano *et al.*, 2002). In addition, it was possible that antioxidant activity from flower ingredients may reduce O_2- in urethane metabolism. Thus, it was possible that antioxidant activity of extract of flowers might reduce O_2- and NO· in urethane metabolism since Ferguson, Philpott and Karunasingh

(2004) suggested that antioxidant could scavenge free radicals and prevent their interactions with cellular DNA.

5.5 A Simple Criterion in the Selection of Flower for Further Study

In this study, pomegranate flower showed the antimutagenicity against *Salmonella* in the Ames test. Wang *et al.* (2006) found new polyphenol compound named pomegranatate, together with, ellagic acid, 3,3',4'-tri-O-methylellagic acid, ethylbrevifolincarboxylate, ursolic and maslinic acids and daucosterol, which were isolated from ethanolic extract of the pomegranate flower (*Punica granatum*). Maslinic acid, a natural triterpene from pomegranate flower, exhibited antioxidant activity by decreasing conjugated diene (CD) production of low-density lipoprotein (LDL) susceptibility to oxidation in the rat brain tissue *in vitro*. Moreover, maslinic acid inhibits cell proliferation significantly in a dose-dependent manner and cause apoptotic death in HT-29 colon cancer cells (Reyes-Zurita *et al.*, 2009). In addition, it was found that the extract of white frangipani flower (*Plumeria obtusa*) and kra chiew flower (*Curcuma sessilis*) showed the antimutagenic property in the Ames test. Boonclarm *et al.* (2006) reported that crude ethanol extract of the *Plumeria obtusa* flower contained a large amount of β-glucoside. An iridoid β-glucoside with two glucosyl groups attached, namely plumieride coumarate glucoside, was subsequently isolated from *Plumeria obtusa*. There was no pharmacological study in kra chiew.

Rhinacanthus nasutus (thong pun chang) flower showed the antimutagenic property in the SMART test. Siripong *et al.* (2006) reported that rhinacanthins-C,-N and –Q (three main naphthoquinone esters isolated from the roots of thong pun chang) induced apoptosis of human cervical carcinoma (HeLaS3) cells. Moreover, The ethanol extract of root and aqueous extract of leaves of *Rhinacanthus nasutus* and the active moiety Rhinacanthin C (chemically synthesized from *Rhinacanthus nasutus*) exhibited *in vitro* antiproliferative activity on PC-3 and T 24, which are human prostate and bladder carcinoma cell lines, respectively (Gotoh *et al.*, 2004).

Mutagenicity testing of the selected flower extracts demonstrated that no sample has mutagenic activity. It was found that, results of this study using Ames test (*in vitro*) and somatic mutation and recombination test (*in vivo*) were correlated about no mutagenic activity. Moreover, It showed antimutagenic activity against positive standard mutagen. It was suggest that these flower extracts should be consumed as a health food item to protect the consumer from some mutagens that have similar bio-characteristic to 1-aminopyrene and urethane.

Their intense colours in the visible range of flower extract, such as red have been widely used as colour agents for food additives and dyeing. It was safe for develop to neutraceutical or drug.