MUTAGENICITY STUDY OF WEEDS AND COMMON PLANTS USED IN TRADITIONAL MEDICINE AND FOR ANIMAL FEED

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Abstract. Mutagenicity and antimutagenicity potentials were tested using Ames' test in crude distilled water and absolute ethanol extracts from the stems and leaves of *Peperomia pellucida* (Linn.) Kunth, *Eichhornia crassipes* Solms, *Colocasia esculenta* Schott and *Brachiaria mutica* (Forssk.) Stapf, and the stems of *Musa sapientum* Linn. No mutagenic effect was found in any of the 10 mg/plate crude extracts of these plants for either TA98 or TA100 of *Salmonella typhimurium*, in a direct test and a mutagenic induced test by S-9 mix. Both distilled water and absolute ethanol extract of 0.5–10 mg/plate *B. mutica* showed strong antimutagenicity to AFB1, B(a)P and 4NQO in two tester strains. Ethanol extract of 0.1–0.5 mg/plate *C. esculenta* also showed antimutagenicity to AFB1, B(a)P and 4NQO in two tester strains, but the 0.5–10 mg/plate water extract had an antimutagenic effect only for B(a)P in TA98. The ethanol extracts of 5 mg/plate *B. mutica* and 0.5 mg/plate *C. esculenta* are cytotoxic, as indicated by their partial killing effect.

INTRODUCTION

Common weeds and plants, such as Krasank, Pak-tob-java, Bond, Ya-kon and Yuak-kluey are used as traditional medicine and local animal feeds in rural areas of Thailand. These plants are grown and are widely available all over the country. In traditional medicine, these plants are used as antibacterials, anti-inflammatories, and as a coagulating factor. Farmers used to mix these plants with commercial feeds to reduce costs. Some people consume these plants as a local dish. The nutritional values and protective effects of these plants are carefully considered to sustain human and animal health. The Thai common names and the scientific names of these plants are shown in Table 1.

Krasank is a short-lived weed that is seen all over Thailand, especially in the rainy reason (Fig 1a). The stems and leaves of Krasank are used for their anti-fever, anti-inflammatory and wound-healing properties (Bunyapratsara, 1996c). Pak-tob-java is a water plant (Fig 1b). Sun-dried Pak-tob-java is commonly mixed with broken rice, rice bran and left-over house foods to feed animals, *ie* chickens, geese, rabbits, pigs, and cattle. The dried stems and leaves of Pak-tob-java contain 2% calcium, 0.5% phosphorus, lysine 6.7g/100g protein and carotene 584-667 ppm. Consumption of large amounts of Pak-tob-java is disadvantageous, because of the oxalate it contains. A mixture of 25% Pak-tob-java makes a good animal feed (Lopez *et al*, 1981). Pak-tob-java's medicinal benefit is to release poison from insect bites and as an anti-inflammatory (Bunyapratsara, 1996c). Moreover, the dried stem of Pak-tob-java can serve as a material for making handicrafts, while fermented Pak-tob-java can be used as manure (http://www.ku.ac.th/AgrInfo/thaifish/aqplant). Bond is used for human consumption in some areas (Fig 1c). The Bond extract is used as a coagulating factor, as an antibacterial, and for the relief of diarrhea (Bunyapratsara, 1996b). Ya-kon grows commonly in slushy areas or riversides (Fig 1d). The leaves and stems of Ya-kon are used as animal feeds, especially for cattle (Anonymous, 2005a,b). The banana is not only a nutritious fruit; all parts of the banana palm are valuable (Fig 1e). Local farmers use Yuak-kluey, the banana stem, chopped and mixed with rice bran to feed pigs. The heart of this stem is used as a human food and blended Yuak-kluey is applied to prevent hair loss and encourage the growth of new hair (Bunyapratsara, 1996a).

The Ames Salmonella assay remains the most widely used *in vitro* test for genotoxicity, and using this technique we examined the mutagenicity and anti-mutagenicity of these common plants.

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MATERIALS AND METHODS

Materials

All solvents and chemicals used were of analytical grade and obtained from Merck, Sigma and Difco. *Salmonella typhimurium* strains were obtained from

Fig 1- The common plants in this study; (a) Krasang [*Peperomia pellucida* (Linn.) Kunth], (b) Pak-tob-java (*Eichhornia crassipes* Solms), (c) Bond (*Colocasia esculenta* Schott), (d) Ya-kon [*Brachiaria mutica* (Forssk.) Stapf], (e) Yuak-kluey (*Musa sapientum* Linn].

Table 1
Common name, Thai common name and scientific name of the studied plants (Smitinand, 2001).

Common name	Thai common name	Scientific name
Peperomia	Krasang	*Peperomia pellucida* (Linn.) Kunth
Water hyacinth	Pak-tob-java	*Eichhornia crassipes* Solms
Bond	Bond	*Colocasia esculenta* Schott
Paragrass, Buffalo grass, Panicum grass	Ya-kon	*Brachiaria mutica* (Forssk.) Stapf
Herbaceous stalk of banana plant	Yuak-kluey	*Musa sapientum* Linn.

Professor BN Ames, University of California, through the National Cancer Institute, Thailand. The positive control chemicals were Aflatoxin B_1(AFB_1), 4-Nitroquinoline-N-oxide (4-NQO) and Benso(a)pyrene [B(a)p], purchased from Sigma.

Preparation of medicinal plant extracts

Krasank, Pak-tob-java, Bond, Ya-kon and Yuak-kluey (Smitinand, 2001) were obtained from different gardens all over Bangkok. The plants were carefully washed, air dried, blended and extracted with distilled water and 95% ethanol. Both water and ethyl alcohol extract were centrifuged at 12,000 rpm for 10 minutes to remove crude particles. Alcohol was removed from the ethyl alcohol extract under vacuum evaporation at 50-60˚C. The powder or semisolid extract of the plants from both water and alcohol extract, were lyophilized and kept at -20˚C until used. All plant extracts were resuspended in water, except for the alcohol extract of Ya-kon using DMSO, at a concentration of 100 mg/ml and a dilution series of 50 mg/ml and 5 mg/ml were prepared. These 3 dilutions were included in the investigation. It was found that the Bond alcohol extract at these 3 dilutions showed highly toxicity; therefore, a dilution series of 5, 2.5 and 1 mg/ml was prepared.

Mutagenicity and antimutagenicity assay

The Ames assay was performed with *S. typhimurium* strain TA98 and strain TA 100. The well-known plate incorporation procedure described by Maron and Ames

(1983) was used. Ten microliters of bacterial stock were incubated in 12 ml of Oxoid nutrient broth for 16 hours at 37°C on a rotative shaker. Of this overnight culture, 0.1 ml was added together with 0.1 ml test solution and 0.5 ml phosphate buffer were added for exposure without metabolic activation or 0.5 ml metabolic activation mixture containing an adequate amount of post-mitochondrial fraction (S9 mix), then incubated at 37°C for 20 minutes. The test solutions were mixed with 2.0 ml top agar and poured over the surface of a minimal agar plate, then incubated for 48 hours at 37°C.

After incubation, the number of revertant colonies was counted. All cultures were made in triplicate. Absence of toxicity was examined by observing background bacterial growth, which should normally be present. The positive controls used were 4-NQO at a concentration of 4 µg/ml for TA98 and 2 µg/ml for TA100, AFB_1 at a concentration of 0.3 µg/ml for TA98 and TA100, and B(a)P at a concentration of 100 µg/ml for TA98 and 50 µg/ml for TA100. The S9 mix was prepared from the livers of Napthoflavone-induced rats.

The antimutagenicity of 4-NQO, AFB_1 and B(a)P in the absence of test samples was defined as 100% or 0% inhibition. The calculation of % inhibition was done according to the formula given by Ong *et al* (1986); % inhibition=[1-T/M] 100, where T is

Table 2
Mutagenicity of plant water extracts on *Salmonella typhimurium* TA 98 and TA 100.

Plant extract	Amount (mg/plate)	No. of His+ Revertants/plate[a]			
		TA98		TA100	
		-S9	+S9	+S9	-S9
Krasang	0	24	33	55	94
(stem and leaf)	0.5	20	40	94	91
	5	29	27	68	94
	10	22	41	84	119
Pak-tob-java	0	24	33	55	94
(stem and leaf)	0.5	21	33	66	72
	5	25	33	67	72
	10	24	29	76	82
Bond	0	22	38	198	168
(stem and leaf)	0.5	25	37	120	151
	5	24	55	105	135
	10	31	48	119	152
Ya-kon	0	27	47	112	125
(stem and leaf)	0.5	24	33	158	141
	5	34	36	153	145
	10	27	47	161	194
Yuak-kluey	0	24	28	72	93
(stem)	0.5	18	28	72	58
	5	23	40	66	75
	10	20	42	60	83
Positive control					
4NQO	0.2 µg	ND	ND	748±161	ND
	0.4 µg	183±33	ND	ND	ND
AFB_1	0.03 µg	ND	719±66	ND	1136±156
B(a)P	5 µg	ND	ND	ND	879±139
	10 µg	ND	509±155	ND	ND

[a]Mean of triplication; PK = partial killing effect; ND = not determined

Table 3
Mutagenicity of plant ethanol extracts on *Salmonella typhimurium* TA 98 and TA 100.

Plant extract	Amount (mg/plate)	No. of His+ Revertants/plate[a]			
		TA98		TA100	
		-S9	+S9	+S9	-S9
Krasang	0	22	38	198	168
(stem and leaf)	0.5	25	36	142	159
	5	29	45	171	243
	10	34	72	180	268
Pak-tob-java	0	18	34	84	102
(stem and leaf)	0.5	17	33	77	105
	5	14	29	89	108
	10	21	38	84	168
Bond	0	25	29	84	106
(stem and leaf)	0.5	18	39	PK	129
	5	PK	29	PK	PK
	10	PK	25	PK	PK
Ya-kon	0	25	29	84	106
(stem and leaf)	0.5	28	42	81	139
	5	34	50	PK	135
	10	PK	60	PK	156
Yuak-kluey	0	24	33	55	94
(stem)	0.5	17	26	83	77
	5	10	30	72	90
	10	20	30	72	96
Positive control					
4-NQO	0.2 µg	ND	ND	1,027±92	ND
	0.4 µg	231±85	ND	ND	ND
AFB_1	0.03 µg	ND	719±66	ND	1,246±22
B(a)P	5 µg	ND	ND	ND	1,257±215
	10 µg	ND	667±18	ND	ND

[a] Mean of triplication; PK = partial killing effect; ND = not determined

the number of revertants per plate in the presence of mutagen and the test sample, and M is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and the denominator. The antimutagenic effect was considered moderate when the inhibitory effect was 25-40% and strong when >40%. Inhibitory effects <25% were considered weak and not recognized as positive (Ikken *et al*, 1999).

RESULTS

Our findings in mutagenic activity tests, using *Salmonella typhimurium* strains TA98 and TA100, with or without S9 metabolic activation, indicated no mutagenicity in all plants to both tester strains, either with or without the S9 mixture (Tables 2 and 3). The mutagenicity of the test specimens was assessed by increasing His+ revertant, *ie* to at least 20-50 revertant colonies/plate for TA98, and 120-200 revertant colonies/plate for TA100; a test sample produced a positive dose-response relationship over three concentrations, with the highest increase not less than twice the negative control value. Moreover, the trace of histidine in the top agar allowed all the bacteria on the plate to undergo several divisions and produce a faint background lawn, which could be examined under a dissecting microscope. The background lawn is essential to the test as an indicator

of growth caused by the toxicity of the test sample. If massive cell deaths occur, defined as the killing effect or partial killing effect, the background lawn on the test plate will be sparse compared with the control plate. In this case, more histidine is available to the surviving bacteria, which undergo more cell divisions and may appear as small colonies. These colonies can be mistaken for revertant.

The mutant colonies of *Salmonella typhimurium* indicated an antimutagenicity test to 4NQO without S9 metabolic activation, AFB_1 and B(a)P with S9 are shown in Tables 4 and 5. Both water and ethanol extracts of Krasank, Pak-tob-java, and Yuak-kluey were synergistic to all tested carcinogens in both tester strains. The water extract of Bond (0.5-10 mg/plate) was antimutagenic to B(a)P tested with TA 98, but synergistic to this carcinogen with TA 100, and to AFB_1 and 4NQO with both tester strains, whereas its ethanol extract at the same concentration had an undetectable effect with all three carcinogens. Thus, dilutions of 0.1-0.5 mg/plate were tested and ethanol extract Bond had a high inhibitory effect with all three carcinogens, tested with both strains. Fig 2 shows the 29.4-39.5% antimutagenic effects of water extract Bond on the antimutagenicity of B(a)P at concentrations of 0.5-10 mg/plate (tested with TA 98). Bond absolute ethanol (concentration 0.1-0.5 mg/plate) extract had an inhibitory effect with 4-NQO 25.1-43.2%, tested with TA 98; 74.6-76.1% tested with TA 100, to AFB_1 38.1-38.7% with TA 98; 49.2-56.1% with TA 100 and to B(a)P 30.2-41.4% with TA 98; 40.1-51.3% with TA 100 (Fig 3), when an inhibitory effect of 25-40% was considered moderate and >40% was strong. Bond alcohol extract inhibited effects of 3 carcinogens powerfully, was tested with TA 100, which are capable of detecting base-pair substitute mutagen. The highest concentration of Bond ethanol (0.5 mg/plate) had a partial killing effect in AFB_1 (TA98) and 4-NQO (TA100). Both water and ethanol extract of Ya-kon (0.5-10 mg/plate) had an antimutagenic effect with all 3 carcinogens. Fig 4 shows the inhibitory effect of Ya-kon water extract to 4-NQO 20.9-38.3% tested with TA 98; 10.0-31.2% tested with TA 100, to AFB_1 7.4-75.6% with TA 98; 26.5-75.0% with TA 100 and to B(a)P 39.7-57.4% with TA 98; 28.8-59.2% with TA 100. Fig 5 shows the inhibitory effect of Ya-kon ethanol extract to 4-NQO 87.4-93.7% tested with TA 98; 56.0% tested with TA 100, to AFB_1 23.5-78.0% with TA 98; 42.1-60.0% with TA 100 and to B(a)P 52.0-59.0% with TA 98.

Our results indicated a stronger effect of Ya-kon than Bond, that high concentration of Ya-kon water extract had strong antimutagenic effect on AFB_1 and B(a)P tested with TA98 and AFB_1 with TA100, whereas 5-10 mg/plate of its had a strong effect on B(a)P. Ya-kon ethanol extract had a stronger inhibitory effect with all 3 carcinogens with both tester strains, which are capable of detecting frame shift and base-pair substitute mutagen, respectively, except for B(a)P with *S. typhimurium* TA100. Partial killing effect occurred at a concentration of 10 mg/plate to 4-NQO; TA98 and 5-10 mg/plate 4-NQO; TA100.

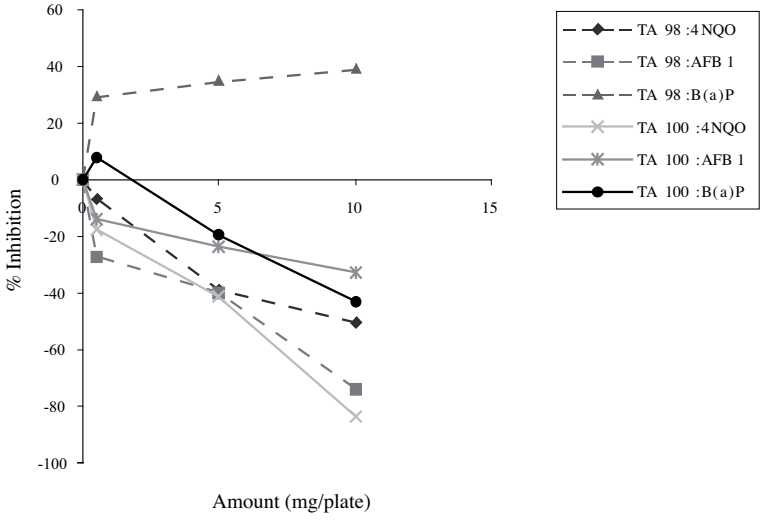

Fig 2- The antimutagenic effect of Bond extracted with distilled water.

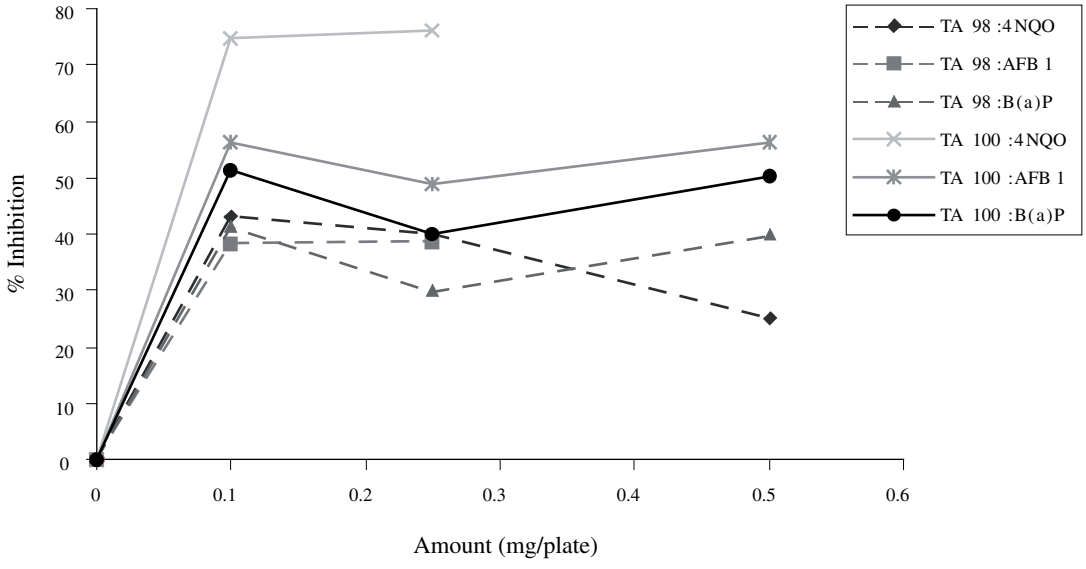

Fig 3- The antimutagenic effect of Bond extracted with 95% ethanol.

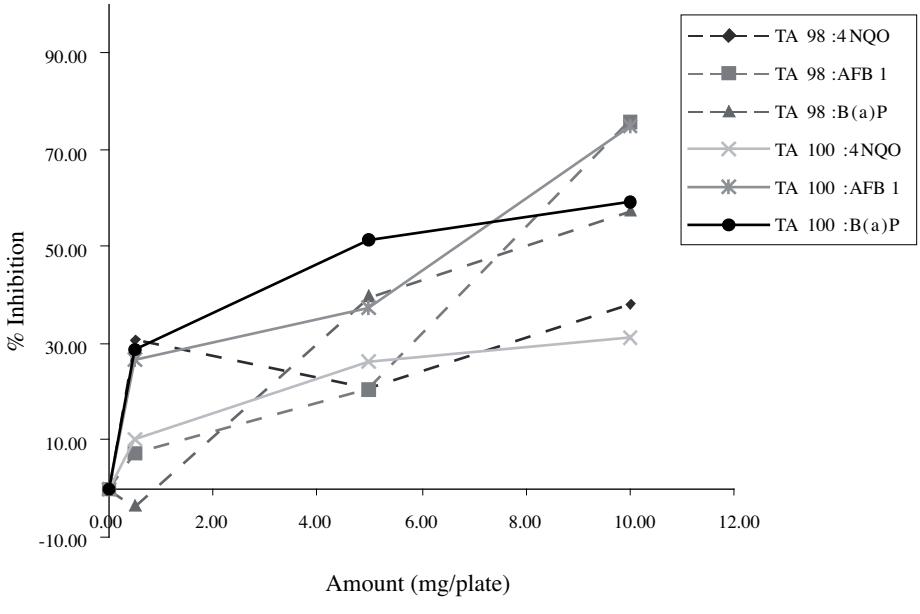

Fig 4- The antimutagenic effect of Ya-kon extracted with distilled water.

DISCUSSION

Studies have demonstrated that many edible plants contain a variety of antimutagenic substances as well as the enzymatic machinery to activate environmental mutagens/carcinogens. Most studies were of plants for human consumption. Cortés-Eslava *et al* (2004) found strong antimutagenicity for coriander juice on mutagenesis produced by 4-nitro-o-phenylenediamine, m-phenylenediamine and 2-aminofluorene. Negi *et al* (2003) found pomegranate peel extracts to have both antioxidant and decreased sodium azide mutagenicity. Although mutagenicity was observed in some orange juice studies, the risk for humans

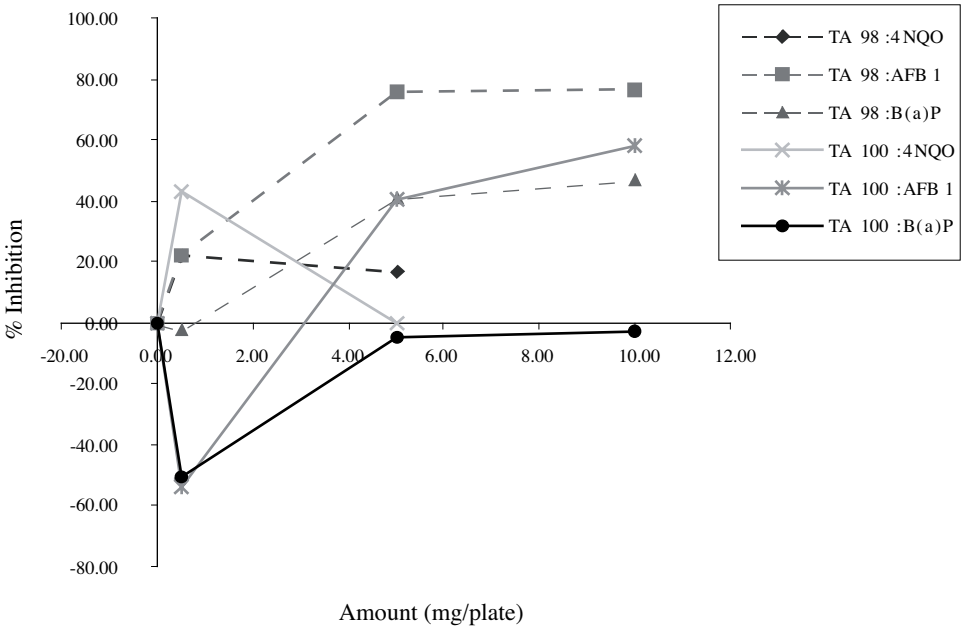

Fig 5- The antimutagenic effect of Ya-kon extracted with 95% ethanol.

consuming orange juice may be low, due to enzyme activity and pH changes in the digestive tract. Also, it contains antimutagenic-acting juice ingredients and detoxificants, *eg* carotenoids and some vitamins (Franke *et al*, 2004). Green tea extract antimutagenicity was demonstrated by Geetha *et al* (2004) against oxidative mutagen tertiary butyl hydroperoxidase using *Salmonella typhimurium* TA 102, which readily responds to reactive oxygen species.

In Thailand, Kusamran *et al* (1998) studied the antimutagenicity and anticarcinogenic potentials of some Thai vegetables. They found the methanol extract of neem leaves contained weak antimutagen inhibiting the mutagenicity of direct-acting mutagens, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) and sodium azide (NaN_3). They also demonstrated that Thai bitter gourd fruits markedly exhibited some inhibitory effects on 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced mammary gland carcinogenesis in animal experiments. The study by Nakahara *et al* (2002) indicated remarkable antimutagenicity for the rhizomes of fingerroot (*Boesenbergia pandurata*), a common Thai spice in the ginger family, and they also found antimutagenicity and antibacterial activity in the twigs, fruits, and flowers of *Oroxylum indicum* (Bignoniaceae).

In our finding, Bond alcohol extract had strong antimutagenic activity on TA100; Ya-kon, both water and alcohol extracts, had strong effects on *S. typhimurium* TA98 and TA100 strains. Their mutagenicity inhibition ranged between 40-90% for TA98, and 40-70% for TA100 in the presence or absence of metabolic activity. Percent inhibition was slightly more in TA98 with frame-shift mutation than in TA100 with base-substituted. This effect was mildly significant in the absence of metabolic activation. The data presented here support their consideration as pharmacologically important in cancer prevention. Bond ethanol extract showed stronger antimutagenic activity than water extract.

In our study, we could not identify the active substances in Bond, which may be due to variations in the quality and quantity of the bioactive compounds present in the different extracts. The dipole moments of water and ethanol are 1.85 D and 1.69 D, respectively (Anonymous, 2005c). Further work is required on isolating and characterizing the individual bioactive compounds present in various extracts, and determining the mechanisms involved in the synergic and antimutagenic effects of Bond and Ya-kon extracts. The synergic effects found in Krasank, Yuak-kluey, and Pak-tob-java are an important precaution in using single or mixed plant extracts.

In condusion, this study evaluated the genotoxic and antigenotoxic efects of the widespread plants, Krasank, Pak-tob-java, Bond, Ya-kon and Yuak-

kluey, which are used in traditional medicine and as local animal feed in rural areas in Thailand by Ames Salmonella/microsomal test. The results indicated significant antimutagenicity of Bond and Ya-kon *in vitro*, suggesting a potential pharmacological importance for health maintenance and the prevention of cancer and other chronic diseases.

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