ANTIMUTAGENICITY EFFECT OF THE EXTRACTS FROM SELECTED THAI GREEN VEGETABLES

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ABSTRACT: Greater consumption of vegetables has been associated to reduce a risk of cancer. The objective of this research was to investigate the antimutagenicity effect of the extracts from some Thai green vegetables namely *Piper sarmentosum* Roxb. (Cha-plu), *Morinda citrifolia* L. (Yor), *Sauropus androgynus* Merr.(Pak-Waan-Bann), and *Amaranthus lividus* Linn.(Pak-Khom) by Ames mutagenicity assay with *Salmonella typhimurium* strains TA98 and TA100 against mutagen, 1-aminopyrene treated with nitrite. The water-insoluble components of these vegetables were extracted with acetone. The results revealed that the extracts from all vegetable samples showed strong inhibition effect against 1-aminopyrene on both tester strains.

Keywords: Antimutagenicity, Ames, *Piper sarmentosum*, *Morinda citrifolia* L., *Sauropus androgynus* Merr., *Amaranthus lividus* Linn.

INTRODUCTION: Cancer is a leading cause of death in Thailand and is becoming a significant health problem[1]. The main types of cancer leading to overall cancer mortality each year are lung, stomach, colorectal, liver and breast cancer. Cancer is a multi-factorial, multi-stage and multi-mechanistic complex process with multiple risk factors that involve interplay between genetic and environmental components. Diet is also a major risk factor[2].

1-Aminopyrene (1-AP) is a derivative of 1-nitropyrene found in human gastrointestinal tract. Anaerobic bacteria metabolize 1-nitropyrene to 1-aminopyrene[3]. 1-Nitropyrene is generally a product of incomplete combustion and is the predominant nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) emitted in diesel engine exhaust, exhaust of kerosene heaters, petroleum gas burners[4,5]. In food products, 1-Nitropyrene is a result of pyrolysis of fat in meat during barbecuing[6,7]. The most primary route of potential human exposure to 1-nitropyrene is inhalation. 1-Aminopyrene was known to be non-mutagenic when it was tested without metabolic activation (S9 mix)[6]. Kato *et al.*[8] demonstrated that 1-aminopyrene treated with nitrite at pH 3.0 and 37°C showed mutagenicity to *Salmonella typhimurium* strains TA98 and TA100 without S9 mix. The mutation appear to be due to the presence of nitroreductases[9] and *O*-acetyltransferase[10] which are the two activating enzyme systems presented in bacterial cells for nitrite-treated 1-aminopyrene (supposed to be 1-nitropyrene). Such enzymes metabolize 1-nitropyrene to be arylhydroxylamine, which is active to interact with DNA. Evidences had been shown that 1-nitropyrene induced tumors in experimental animals[11, 12]. Thus, the mutagenicity of 1-aminopyrene and nitrite in acid condition has been established as a model for antimutagenicity studies of some chemicals concerning the phenomenon occurred during stomach digestion.

Various kinds of vegetables are commonly consumed in Thailand. Some of them also have diverse medicinal properties. This research selected some green leafy vegetables that usually eaten in the central of Thailand namely *Piper sarmentosum* (Cha-plu), *Morinda citrifolia* L. (Yor), *Sauropus androgynus* Merr. (Pak-Waan-Bann), and *Amaranthus lividus* Linn. (Pak-Khom). However, there is little information available at the present time regarding the antimutagenic and anti-carcinogenic effects of these Thai vegetables.

Piper sarmentosum, a plant in the Piperaceae family, is used as food ingredient in many Southeast Asian cuisines. Subramaniam *et al.*[13] found that methanolic extract of leaves of *Piper sarmentosum* has shown high antioxidant activity by using the Xanthine/ Xanthine Oxidase (X/ XOD) Superoxide Scavenging assay. The cause of

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high antioxidant property of this plant may be an effect of naringenin, a highly potent natural antioxidant compound.

Morinda citrifolia belongs to family Rubiaceae. The leaves are usually eaten blanched and served with chili paste or cooked as curry. Zin *et al.*[14] have evaluated the antioxidative activity of extracts from different parts of *Morinda citrifolia* measured by ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). They found that ethyl acetate extracts from leaves, fruits and roots exhibited significant antioxidative activity. Furthermore, Murakami *et al.*[15] found that methanol extract of *Morinda citrifolia* leaves have shown strongly active anti-tumor promoting activity (*in vitro*).

Sauropus androgynus belongs to family Euphorbiaceae. In Thailand, people cook it with meat, coconut milk and other vegetables to make soup. Leaves are blanched to serve with chili paste. Liu *et al.*[16] found that leaf of this vegetable had high levels of lutein and zeaxanthine. This may be the scientific rationale in support of believes of the local populations, that these leafy vegetables are good for eye health and visions.

Amaranthus lividus is a herb in the Amaranthaceae family**.** Leaves are usually eaten boiled and blanched and served with chili paste as a side dish. Amin *et al.*[17] reported that the extract of *Amaranthus* sp. has shown a high free radical-scavenging activity.

MATERIALS AND METHODS:

Plant material

Fresh samples of *Piper sarmentosum*, *Morinda citrifolia*, *Sauropus androgynus* and *Amaranthus lividus* were purchased from local markets in Bangkok, Thailand. It was identified botanically by Assoc. Prof. Dr. Surattana Amnuoypol of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction

Each sample was cleaned with tap water and dried at room temperature. The leaves (100 g) were chopped and soaked in 400 ml of acetone for 3 days. The acetone extract of each sample was filtered through filter paper No. 541. The extraction process was repeated twice. The combined filtrate was evaporated to dryness under vacuum in a rotary evaporator at 45°C and was sterile by autoclaving at 121°C for 20 minutes. Each sample was diluted with DMSO to obtain appropriate concentrations and stored below 5°C.

Bacterial strains

Two histidine-deficient (His^-) tester strains, TA98 and TA100 of *Salmonella typhimurium* were kindly provided by Assoc. Prof. Dr. Kaew Kangsadalampai, Nutrtion Institute, Mahidol University. Strains TA98 and TA100 are used for the detection of frameshift mutation and base pair substitution mutation, respectively. Prior to use in the assay, each strain was checked for confirm the genotypes as described by Maron and Ames [18]. Overnight cultures of bacteria inoculated from frozen stock culture in Oxoid nutrient broth no.2 at 37°C was used for mutagenicity assay within 24 hours.

1-Aminopyrene treated with nitrite preparation

1-AP was prepared at a concentration of 0.0375 mg/ml in acetonitrile and treated with nitrite in acid condition to provided direct mutagen products[19].

Antimutagenic activity assay

The antimutagenic activity of extracts from study vegetables against 1-AP was determined by a preincubation method[18, 20]. The following materials were added to sterile test tube: 1-AP 0.01 ml (tested on TA98) or 0.02 ml (tested on TA100), 0.74 or 0.73 ml 0.2 N HCl and 0.25 ml of 2 M $NaNO_2$. The entire mixture (pH 3.0-3.5) was shaken in a water bath at 37°C for 4 hours and was stopped by placing the tube in an ice bath for 1 minute. In order to decompose the residual nitrite, 0.25 ml of 2 M ammonium sulfamate was added to the reaction mixture and allowed to stand for 10 minutes in an ice bath. Then 0.1 ml of nitrosated product was mixed with 0.5 ml phosphate buffer (pH 7.4), 0.1 ml of the extract of each vegetable at various concentrations (0.8, 1.6 and 3.2 µg/plate) and 0.1 ml of fresh overnight cultures of the tester strain and incubated at 37°C in a shaking water bath for 20 minutes. After incubation, 2 ml of molten top agar (45°C, containing 0.5 mM histidine / biotin, NaCl and agar) was added. It was mixed well and poured

onto a minimal glucose agar plate (containing magnesium sulfate heptahydrate, citric acid monohydrate, potassium phosphate, dibasic, sodium ammonium phosphate tetrahydrate, 40% glucose and agar). The plate was rotated to achieve uniform colony distribution and incubated at 37°C for 48 hours. After incubation, His^+ revertant colonies were counted. Positive control was consisted of either *S. typhimurium* strains TA98 or TA100 and 1-AP treated with nitrite without the extracts. Spontaneous revertants were determined from bacterial tester strains and DMSO solvent without 1-AP and nitrite. Each assay was performed in hexplicate and antimutagenic activity was shown as percentage of inhibition:

$$Inhibition\ (\%) = [(A-B) / (A-C)] \times 100$$

A = a number of His^+ revertants induced by nitrite treated 1-AP (positive control)

B = a number of His^+ revertants induced by nitrite treated 1-AP in the presence of vegetable extract

C = a number of spontaneous His^+ revertants per plate.

Percentage of inhibition as follows[21]:

> 60%	=	strongly active
41-60%	=	moderately active
21-40%	=	weakly active
0-20%	=	not active

RESULTS: The effect of the extracts from study vegetables on the mutagenicity of nitrite treated 1-aminopyrene on *S. typhimurium* strains TA98 and TA100 are shown in Figure 1 A and B, respectively. The results showed that all of the extracts exhibited the strongly inhibition (> 60% inhibition), especially, the extract from *Piper sarmentosum* at the highest concentration (3.2 mg/plate) showed the strongest inhibition on both tester strains.

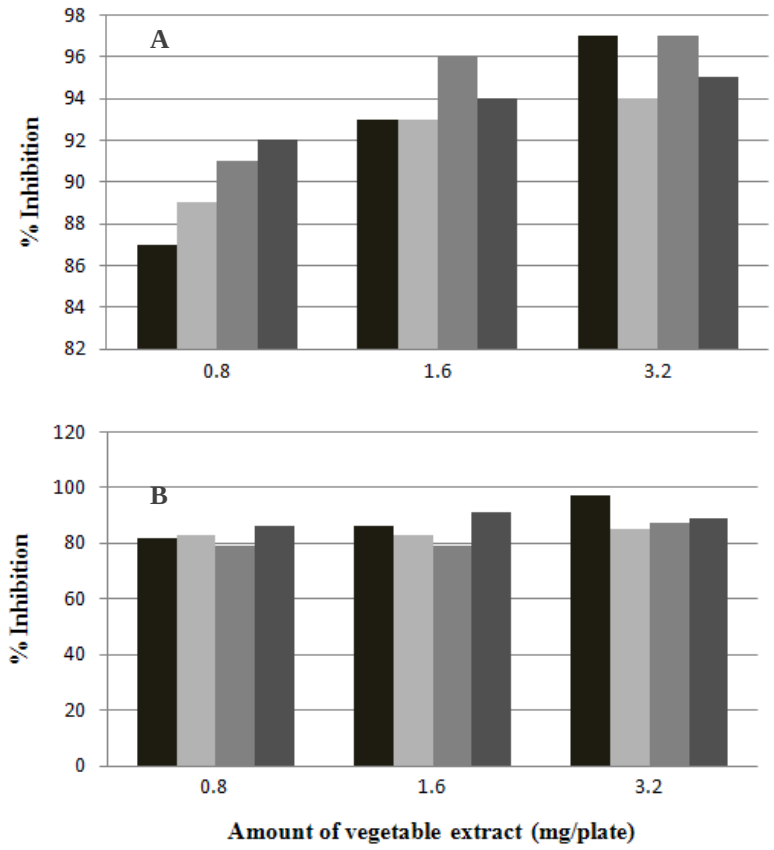


Figure1 The antimutagenicity of the extracts from *Piper sarmentosum* (■), *Morinda citrifolia* (□),*Sauropus androgynus* (▩) and *Amaranthus lividus* (▪) on the mutagenicity of nitrite treated 1-aminopyrene on *S. typhimurium* strains TA98 (0.03 µg/plate) (A) and strains TA100 (0.06 µg/plate) (B). Results are expressed as percentage of inhibition. Spontaneous revertants have been already subtracted from the raw data.

DISCUSSION: The reaction between nitrite and dietary amines and amides under the acidic conditions of the stomach could lead to the formation of nitrosated products. It is possible to the development of gastric cancer in human[22]. On the other hand, it is well known that ingredients in diet including edible plants, fruits and seeds may exert anticarcinogenic and antimutagenic activities[23, 24]. Therefore, the present study is the attempt to elucidate the protective effect of the extracts from *Piper sarmentosum*, *Morinda citrifolia*, *Sauropus androgynus* and *Amaranthus lividus* on the tester strains against mutation induced by nitrite treated 1-AP.

The result indicated that the direct acting mutagens formed from interaction between nitrite treated 1-AP in acid solution could be suppressed by some components in the vegetable extracts. The plant chemicals such as chlorophyll, carotenoids, flavonoid and other phenolic compounds are the contributors to the antioxidant activity of the plants and also found in these selected vegetables[25]. Flavonoids, the active antioxidant compounds, were found commonly in green leafy vegetables[26,27]. Kuo *et al.*[28] found that flavonoids could inhibit nitropyrene reductase and/or O-acetyltransferase which are the activating enzymes in *Salmonella* cell. In the bacterial tester strains (TA98 and TA100) flavonoids inhibited the activity of mutagens identified in foods whether it was direct acting (e.g. 2-aminofluorene) or it required metabolic activation [2-amino-1-methyl-6-pheny limidazo [4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1)][21,29,30]. Furthermore, some constituents found in *Piper sarmentosum* such as naringenin, a highly potent natural antioxidant compound[13], had ability to suppress mutagenesis in *Salmonella typhimurium* strain TA100 NR induced by the direct-acting carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine[31]. Other vegetables such as *Morinda citrifolia, Sauropus androgynus* and *Amaranthus lividus* also have high levels of flavonoids and total phenols[14,16,17].

Acetone extract component in green leafy vegetables, namely chlorophyll[32] has been shown antimutagenic activity. Possible mechanisms of activity of chlorophyll are antioxidative properties[33] and trapping with carcinogens, by which mutagens become unavailable to target cells or organisms[34]. Hayatsu *et al.*[35] reported that chlorophyll and chlorophyllin could inhibit selectively the activity of mutagens having polycyclic structures by forming complexes with them. Therefore, the precursors that could interact with sodium nitrite of this model such as 1-aminopyrene, a polycyclic aromatic hydrocarbons compound, might be trap by chlorophyll to forming complexes. This may describe the mechanism how the extract of selected vegetables in the present study inhibited the mutagenesis of bacterial tester strains.

CONCLUSION: The extracts from *Piper sarmentosum*, *Morinda citrifolia*, *Sauropus androgynus* and *Amaranthus lividus* were good antimutagens in this model. It was proposed that the compounds display the important role as antioxidant and/or antimutagens might be chlorophyll, flavonoids and other phytochemicals. The finding from this experiment suggested that consumption of nitrite-containing foods with these vegetables should prevent the mutagenicity of direct mutagen in gastric like condition.

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