Inventory of indigenous plants and minor crops in Thailand based on bioactivities

Kazuhiko Nakahara[1,*], *Molay Kumar Roy*[1], *Najeeb S. Alzoreky*[2], *Vipaporn Na Thalang*[3], *and Gassinee Trakoontivakorn*[3]

[1]Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki 305-8686, Japan
[2]Sana'a University, Sana'a, Yemen
[3]Institute of Food Research and Product Development (IFRPD), Kasetsart University, Chatuchak, Bangkok 10903, Thailand

Abstract

Antimutagenicity, antioxidant activity, cytotoxicity and antimicrobial activity of methanol extracts of some edible plants in Thailand were evaluated. Some plant extracts exhibited definite bioactivities. *Micromelum minutum* (Rutaceae) showed the highest antimutagenicity among 118 samples, and it contained a carbazole alkaloid, mahanine, as an active principle. Mahanine showed a wide variety of bioactivities, particularly antimutagenicity, and toxicity against tumor cell lines such as HL-60. Rhizomes of fingerroot (*Boesenbergia pandurata*), a common Thai spice belonging to the ginger family, showed a remarkable antimutagenicity and six active constituents were isolated and identified. Twigs, fruits and flowers of *Oroxylum indicum* (Bignoniaceae) contained a high concentration of baicalein which is a flavonoid exhibiting antimutagenic and antibacterial activities. Extract of *Azadirachta indica* (neem) inhibited growth of gram-positive bacteria, proliferation of tumor cell lines, and mutagenesis induced by heterocyclic amines. These studies would be a good basis for an inventory of indigenous plants and minor crops based on bioactivities.

Introduction

A WIDE variety of indigenous plants and minor crops have been utilized for daily consumption in Thailand since ancient times. They are not only important ingredients of unique gastronomic dishes but also traditional functional foods to maintain wellness. Some epidemiological studies have suggested that high consumption of fruits and vegetables might prevent several chronic diseases as well as cancer. For example, according to a survey carried out in the middle of the 1980s, the total incidence rate of cancer in Thailand, where many indigenous plants and minor crops were utilized as food and medicine, was much lower than in northern Europe and North America. One of the major reasons for this low incidence of cancer in Thailand was expected to be the foods consumed by Thai people. In order to elucidate such a phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their bioactivities by various methods applying some *in vitro* model systems. In recent years, we have demonstrated that extracts from these plant species exhibit various bioactivities such as antimutagenicity, antioxidant activity, antimicrobial activity and cytotoxicity against human tumor cell lines. To date, approximately 100 species of plants have been examined, and some active constituents have been isolated and identified. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in Thailand and other tropical countries. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural area. Here we present our recent data on bioactivities of some indigenous plants in Thailand, and propose to catalogue previous reports on individual plants and turn that information into a comprehensive inventory of indigenous plants and minor crops based on bioactivities.

* Corresponding author. Email address: <knakahar@affrc.go.jp>

Antimutagenicity of some edible Thai plants, and a bioactive carbazole alkaloid, mahanine, isolated from *Micromelum minutum*

(Nakahara et al. 2002)

THE antimutagenic activity against the heterocyclic amine Trp-P-1 of methanolic extracts of 118 samples (108 species) of edible Thai plants was examined by the Ames test using *Salmonella typhimurium* strain TA98. Tested plants were classified into three groups based on ED_{90}, which is the dose of a plant extract in µL/plate required to inhibit the mutation induced by Trp-P-1 by 90% (Table 1). Five plants, *Micromelum minutum* (Figure 1A), *Oroxylum indicum, Cuscuta chinensis, Azadirachta indica* and *Litsea petiolata*, exhibited significant activity (Table 2) with antimutagenic ED_{90} values lower than 5 µL/plate (0.1 mg of dry plant material equivalent). The activity-guided fractionation of the extract of *M. minutum* which exhibited the highest antimutagenic activity in the screening resulted in the isolation of an active principle, mahanine (Figure 1B), as confirmed by its physicochemical properties. *M. minutum* (Rutaceae) is consumed mainly in the southern part of Thailand as a fresh vegetable served with certain dishes such as *kanom chin nam ya*, a thin rice noodle with spicy sauce, or *kaeng tai pla*, a southern-style fish curry. Various parts of *M. minutum*, including the edible twigs, are used as a folk medicine for fever and giddiness. Mahanine showed a wide variety of biological activity, including antimutagenicity against heterocyclic amines (Trp-P-1, Trp-P-2 and PhIP), cytotoxicity against the tumor cell line HL-60, with a minimal inhibitory concentration (MIC_{100}) of 4.0 µg/mL, and antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus*, with MIC_{100} of 6.25 and 12.5 µg/mL, respectively.

Antimutagenic activity of flavonoids in fingerroot (*Boesenbergia pandurata*)

(Trakoontivakorn et al. 2001)

FINGERROOT (*Boesenbergia pandurata*) is a member of the ginger family (Zingiberaceae), and is consumed as a common vegetable in Thailand (Figure 2). Fresh rhizomes have a characteristic aroma and slightly pungent taste. The rhizomes of fingerroot are used as a food ingredient and a folk medicine for the treatment of colic disorder and as an aphrodisiac in Southeast Asian countries. We found that an methanolic extract of the

Table 1. Classification of edible Thai plants based on antimutagenic activity (ED_{90} = amount of plant extract required to suppress 90% of mutagenesis).

Group A, $ED_{90} < 5$ µL (0.1 mg plant material equivalent): *Azadirachta indica, Cuscuta chinensis, Litsea petiolata, Micromelum minutum, Oroxylum indicum* (5 species)
Group B, 5 µL < ED_{90} < 50 µL: *Anacardium occidentale, Barringtonia acutangula, Boesenbergia pandurata, Bouea oppositifolia, Codiaeum variegatum, Coriandrum sativum, Ficus fistulosa, Glochidion perakense, Limnophila aromatica, Mangifera indica, Piper betle, Syzygium gratum, Toddalia asiatica, Trachyspermum roxburghianum* (14 species)
Group C, $ED_{90} > 50$ µL: *Acacia pennata, Acmella oleracea, Adenanthera pavonina, Amaranthus lividus, Amaranthus tricolor, Amaranthus viridis, Anethum graveolens, Archidendron jiringa, Artocarpus heterophyllus, Basella alba, Bauhinia racemosa, Bombax ceiba, Brassica alboglabra, Brassica juncea, Broussonetia kurzii, Capsicum frutescens, Careya sphaerica, Centella asiatica, Chrysanthemum coronarium, Cissus hastata, Cleome gynandra, Coccinia grandis, Colocasia esculenta, Colocasia gigantea, Cratoxylum formosum, Cucurbita moschata,* Cucurbitaceae sp., *Cymbopogon citratus, Diplazium esculentum, Dregea volubilis, Eleutherococcus trifoliatus, Emilia sonchifolia, Eryngium foetidum, Fernandoa adenophylla, Ficus benjamina, Ficus infectoria, Garcinia cowa, Glinus oppositifolius, Gymnema inodorum, Hibiscus sabdariffa, Houttuynia cordata, Ipomoea aquatica, Ipomoea batatas, Irvingia malayana, Lablab purpureus, Lasia spinosa, Leucaena leucocephala, Limnocharis flava, Marsdenia glabra, Marsilea crenata, Melientha suavis, Momordica charantia, Momordica subangulata, Monochoria vaginalis, Morinda citrifolia, Moringa oleifera, Musa acuminata, Musa sapientum, Neptunia oleracea, Ocimum americanum, Ocimum basilicum, Ocimum gratissimum, Ocimum tenuiflorum, Oenonthe javanica, Parkia speciosa, Parkia timoriana, Parthenocissus vitacea, Piper interruptum, Piper nigrum, Piper retrofractum, Piper sarmentosum, Pisum sativum, Polygonum odoratum, Polyscias fruticosa, Psophocarpus tetragonolobus, Raphanus sativus, Sarcostemma secamone, Sauropus androgynus, Senna siamea, Sesbania grandiflora, Sesbania javanica, Solanum trilobatum, Tamarindus indica, Tiliacora triandra, Tournefortia ovata, Trichosanthes anguina, Vigna unguiculata, Wolffia globosa, Zanthoxylum limonella* (89 species)

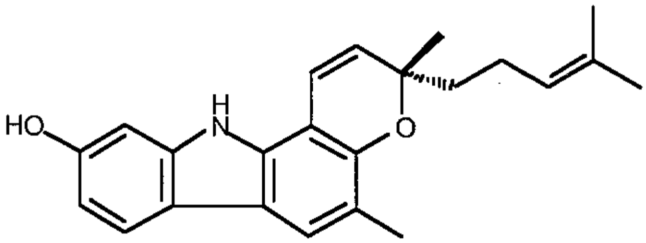

Figure 1. Twigs of *Micromelum minutum* (left) and the structure of mahanine (above).

rhizomes of fingerroot showed a potent antimutagenic effect against Trp-P-1 in the Ames test. Then, six active compounds were isolated from fresh rhizomes of fingerroot as strong antimutagens. These compounds were pinocembrin chalcone, cardamonin, pinocembrin, pinostrobin, 4-hydroxypanduratin A, and panduratin A (Figure 3). 4-hydroxypanduratin A was a novel compound (tentatively termed) and pinocembrin chalcone was not previously reported in this plant. The antimutagenic IC_{50} of those compounds was 5.2 ± 0.4 μM, 5.9 ± 0.7 μM, 6.9 ± 0.8 μM, 5.3 ± 1.0 μM, 12.7 ± 0.7 μM and 12.1± 0.8 μM in the pre-incubation mixtures, respectively. They also similarly inhibited the mutagenesis induced by Trp-P-2 and PhIP. All of them strongly inhibited the *N*-hydroxylation of Trp-P-2. Thus, the antimutagenic effect of all isolated compounds was mainly due to the inhibition of the first step of enzymatic activation of heterocyclic amines.

Figure 2. Rhizomes of fimgerroot (*Boesenbergia pandurata*)

Table 2. Inhibitory effects of methanol extracts from selected edible plants against the mutagenic heterocyclic amines Trp-P-1, Trp-P-2 and PhLP.

Plant extract	Inhibition of mutagenesis (%)		
	Trp-P-1 (50 ng/test)	Trp-P-2 (20 ng/test)	PhLP (250 ng/test)
Micromelum minutum			
(50 μL/plate)	100	99	98
(5 μL/plate)	100	96	94
Oroxylum indicum			
(50 μL/plate)	98	99	98
(5 μL/plate)	91	95	86
Cuscuta chinensis			
(50 μL/plate)	99	99	94
(5 μL/plate)	98	95	88
Azadirachta indica			
(50 μL/plate)	100	99	98
(5 μL/plate)	89	88	86
Litsea petiolata			
(50 μL/plate)	99	99	96
(5 μL/plate)	76	81	80

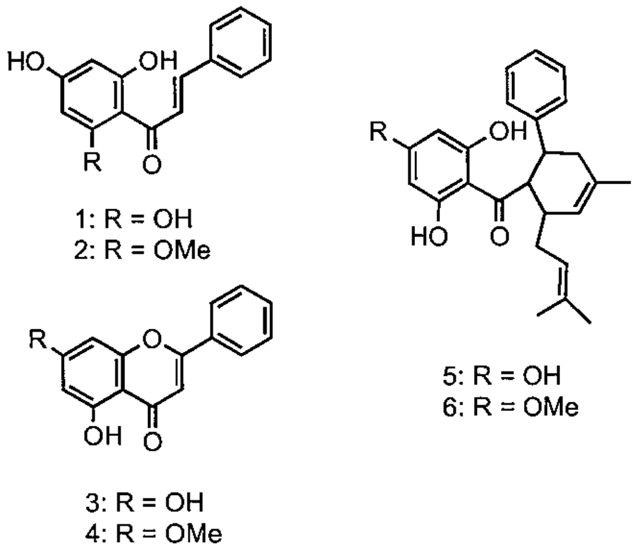

Figure 3. Chemical structures of the antimutagens isolated from fingerroot (1 = pinocembrin chalcone, 2 = cardamonin, 3 = pinocembrin, 4 = pinostrobin, 5 = 4-hydroxypanduratin A, 6 = panduratin A).

Antimutagenic activity of *Oroxylum indicum*

(Nakahara et al. 2001)

OROXYLUM indicum Vent. is a deciduous tree (Figure 4A) belonging to the Bignoniaceae family, which is distributed in South Asia, Southeast Asia and China. It is usually utilized as a crude drug in Indian Ayurvedic medicine and Chinese medicine for curing stomach disorders, diarrhea, dysentery and rheumatic swelling. In Thailand, the fruits (called *peh-gaa*) and flowers of the plant are consumed as a common part of the diet in the north and north-eastern areas. The seeds contain such flavonoids as chrysin (5,7-dihydroxyflavone), oroxylin A (5,7-dihydroxy-6-methoxyflavone), baicalein (5,6,7-trihydroxyflavone) and baicalein glycosides, benzoic acid and fatty acids. Wall et al. (1988) reported significant antimutagenic activity against 2-aminoanthracene in the ethanol-CH_2Cl_2-soluble fraction prepared from twigs and leaves of *O. indicum*. However, there is no information on the antimutagenic constituents in the edible part of this plant. We elucidated the structure and properties of an antimutagenic principle in a methanol extract from the fruit of *O. indicum*. This extract strongly inhibited the mutagenicity of Trp-P-1 in an Ames test. The major antimutagenic constituent was identified as baicalein (Figure 4B), with an IC_{50} value of 2.78 ± 0.15 µM. The potent antimutagenicity of the extract was correlated with the high content (3.95 ± 0.43%, dry weight) of baicalein. Baicalein acted as a desmutagen since it inhibited the *N*-hydroxylation of Trp-P-2.

Antioxidant activity (β-carotene bleaching method) of some leafy vegetables commonly consumed in Thailand

(Na Thalang et al. 2001)

Antioxidant activity of ten leafy vegetables commonly consumed in Thailand was determined using the β-carotene bleaching method. *Neptunia oleracea* (water mimosa) exhibited the highest activity (13.1 mg butylated hydroxyanisole (BHA) equivalent/g dry weight) followed by *Acacia pennata* and *Morinda citrifolia*. Water mimosa is a common vegetable used for *Yam phak-kra-ched*, a spicy and sour salad with seafoods and water mimosa, or *kaeng som*, a sweet and sour soup cooked with tamarind. Nine of ten vegetables showed greater antioxidant activity than 25 mg BHA equivalent/100 g fresh weight, while only one-third of Japanese vegetables exhibited such a high activity. Extract from water mimosa gave five peaks (P-1, P-2, P-3, P-4 and P-5) which showed antioxidant activity, using high-performance liquid chromatography (HPLC) analysis. Based on their ultraviolet (UV) spectra, P-3, P-4 and P-5 are assumed to be derivatives of apigenin.

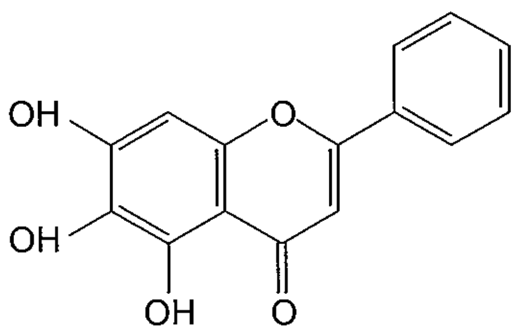

Figure 4. A tree of *Oroxylum indicum* bearing fruits (left) and the structure of baicalein (above).

Cytotoxicity of edible Thai plants against cancer cell lines

(Na Thalang et al. 2000)

SOME 120 samples of edible Thai plants belonging to 51 families were extracted with 80% methanol and determined for cytotoxic effects on two types of cancer cells, blood (U-937 and HL-60) and epithelial (HeLa, and B16 melanoma), and non-cancer cells (CHO-K1). The cell growth was monitored by reduction of an oxidation–reduction dye, Alamar Blue. *Bouea microphylla* (Anacardiaceae) expressed an antiproliferative effect on U-937, HL-60, HeLa and B16 melanoma. Twigs of the Thai neem (*sadao*) tree (*Azadirachta indica*, Meliaceae) showed a promising cytotoxic effect against both U-937 and HL-60 cells, while *Oroxylum indicum* and *Piper nigrum* were toxic against only HL-60 cells. These plant species had a strong effect on the *in vitro* anti-tumor promoting activity according to the Epstein-Barr virus activation assay.

Antibacterial activity of extracts from some edible plants commonly consumed in Asia

(Alzoreky and Nakahara 2002)

Extracts of edible plants (26 species) from China, Japan, Thailand and Yemen were screened for their antibacterial activity against the food-borne pathogens *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella infantis*. Buffered methanol (80% methanol and 20% phosphate-buffered saline—PBS) and acetone extracts from 16 plants showed inhibitory activity in the disk assay. The minimal inhibitory concentrations (MICs) of extracts determined by the agar dilution method ranged from 165 to 2640 mg/L. The most sensitive microorganism to extracts from *Azadirachta indica*, *Cinnamomum cassia*, *Rumex nervosus*, *Ruta graveolens*, *Thymus serpyllum* and *Zingiber officinale* was *B. cereus*, with MIC of 165–660 mg/L. *E. coli* and *S. infantis* were inhibited only by *C. cassia* extracts at the highest MIC (2640 mg/L). *L. monocytogenes* (Tottori) was more resistant than the ATCC 7644 strain to extracts from *Ruta chalepensis*, *Artemisia absinthium* and *Cissus* spp. The chelating agent EDTA (0.85 mM) reduced the MICs of *C. cassia* and *Cissus rotundifolia* by at least 50% when tested against *E. coli*, *S. infantis*, *S. aureus* and *L. monocytogenes*.

References

Alzoreky, N.S. and Nakahara, K. 2002. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology (in press).

Na Thalang, V., Nakahara, K. and Trakoontivakorn, G. 2000. Functional evaluation of edible plants in Thailand by using several animal cell lines. Japan International Research Center for Agricultural Sciences (JIRCAS) Fellowship Program Report.

Na Thalang, V., Trakoontivakorn, G. and Nakahara, K. 2001. Determination of antioxidant activity of some commonly consumed leafy vegetables in Thailand. Japan International Research Center for Agricultural Sciences (JIRCAS) Journal, 9, 39–46.

Nakahara, K., Onishi-Kameyama, M., Ono, H., Yoshida, M. and Trakoontivakorn, G. 2001. Antimutagenic activity against Trp-P-1 of the edible Thai plant, *Oroxylum indicum* vent. Bioscience, Biotechnology, and Biochemistry, 65, 2358–2560.

Nakahara, K., Trakoontivakorn, G., Alzoreky, N.S., Ono, H., Onishi-Kameyama, M. and Yoshida, M. 2002. Antimutagenicity of some edible Thai plants, and a bioactive carbazole alkaloid, mahanine isolated from *Micromelum minutum*. Journal of Agricultural and Food Chemistry, 50, 4796–4802.

Trakoontivakorn, G., Nakahara, K., Shinmoto, H., Takenaka, M., Onishi-Kameyama, M., Ono, H., Yoshida, M., Nagata, T. and Tsushida, T. 2001. Structural analysis of a novel antimutagenic compound, 4-hydroxypanduratin A, and the antimutagenic activity of flavonoids in a Thai spice, fingerroot (*Boesenbergia pandurata* Schult.) against mutagenic heterocyclic amines. Journal of Agricultural and Food Chemistry, 49, 3046–3050.

Wall, M.E., Wani, M.C., Hughes, T.J. and Taylor, H. 1988. Plant antimutagenic agents. 1. General bioassay and isolation procedures. Journal of Natural Products, 51, 866–873.