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Antimutagenicity of Some Edible Thai Plants, and a Bioactive Carbazole Alkaloid, Mahanine, Isolated from *Micromelum minutum*

KAZUHIKO NAKAHARA,*,† GASSINEE TRAKOONTIVAKORN,‡ NAJEEB S. ALZOREKY,§ HIROSHI ONO,|| MAYUMI ONISHI-KAMEYAMA,|| AND MITSURU YOSHIDA||

Japan International Research Center for Agricultural Sciences (JIRCAS), 1-1 Owashi, Tsukuba, Ibaraki 305-8686, Japan; Institute of Food Research and Product Development, Kasetsart University, Chatuchak, Bangkok 10903, Thailand; Food Science and Technology Department, Faculty of Agriculture, Sana'a University, Sana'a, Yemen; and National Food Research Institute, 2-1-12 Kan-nondai, Tsukuba, Ibaraki 305-8642, Japan

The antimutagenic activity against Trp-P-1 of methanolic extracts of 118 samples (108 species) of edible Thai plants was examined by the Ames Test. The activity was evaluated by the amount of plant extracts which suppressed 90% of the mutagenesis (ED_{90}). Five plants, *Micromelum minutum, Oroxylum indicum, Cuscuta chinensis, Azadirachta indica,* and *Litsea petiolata,* exhibited significant activity 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 (**1**) as confirmed by its physicochemical properties. Compound **1** showed a wide variety of biological activity, including antimutagenicity against heterocyclic amines such as Trp-P-1 with an IC_{50} of 5.2 μM, cytotoxicity against a tumor cell line HL60 with a MIC_{100} of 4.0 μg/mL, and antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus* with MIC_{100} values of 6.25 and 12.5 μg/mL, respectively.

KEYWORDS: Antimutagen; Trp-P-1; *Micromelum minutum*; mahanine

INTRODUCTION

Food habits are greatly responsible for cancer incidence (*1*). Epidemiological studies revealed that high consumption of fruits and vegetables reduces cancer risks (*1*−*4*). To elucidate this phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their anticancer properties by various methods using bacteria (Ames test), animal cell lines, and rodents (*5*−*7*). To date, numerous antimutagenic substances in edible plants have been isolated and identified (*8*−*10*). Phenolic compounds such as tannic acid (*8*), luteolin (*9*), and myricetin (*8*) in some vegetables and fruits were reported as effective antimutagens. Sulfur compounds (such as 4-(methylthio)-3-butenyl isothiocyanate) in Allium and radish are known to possess antimutagenic activities (*10*).

In Thailand, many indigenous plants belonging to various families are utilized as food and medicine. Previous studies demonstrated that some of those plants exhibited antimutagenic or anticarcinogenic effects, or both. Kusamran et al. investigated antimutagenic and anticarcinogenic activities of some commonly consumed edible plants in Thailand (*11, 12*). Vinitketkumnuen et al. reported a spice in Thailand, lemon grass (*Cymbopogon citratus*), possessed antimutagenic activity toward various mutagens (*13*). In addition, Murakami et al. found that some edible Thai plants had potent antitumor-promoting activity determined by Epstein−Barr virus activation assay (*14, 15*). Recently, we demonstrated that flavonoids isolated from two edible Thai plants, *Boesenbergia pandurata* and *Oroxylum indicum* showed substantial antimutagenic effects against heterocyclic amines such as 3-amino-1,4-dimethyl-5*H*-pyrido[*4,3-b*]indole (Trp-P-1) in the Ames Test (*16*−*18*). These studies suggested that there are some other edible Thai plants containing effective anticarcinogenic or antimutagenic compounds. We now report the result of antimutagenic screening from 118 samples (108 species) of edible Thai plants and isolation of an antimutagenic constituent from *Micromelum minutum*, which showed the highest activity in the screening.

MATERIALS AND METHODS

Chemicals. Mutagens, Trp-P-1, 3-amino-1-methyl-5*H*-pyrido[*4,3-b*]indole (Trp-P-2), and 2-amino-1-methyl-6-phenylimidazo[*4,5-b*]pyridine (PhIP) were products of Wako Pure Chemical (Osaka, Japan). S9 mix from the liver of drug-treated Sprague−Dawley rats (male) for the Ames test was purchased from Kikkoman (Noda, Japan). CD_3OD

* To whom correspondence should be addressed. [telephone and fax: +81-298-386358; e-mail knakahar@jircas.affrc.go.jp].
† JIRCAS.
‡ Kasetsart University.
§ Sana'a University.
|| National Food Research Institute.

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and $CDCl_3$ (NMR grade) were products of Sigma-Aldrich Chemical Co., Ltd. (St. Louis, MO). Unless otherwise stated, all other reagents were of analytical grade.

Plants and Extraction. Plant samples (**Table 1**) were purchased from local markets in Thailand and Japan (*19, 20*). Lyophilized and ground samples (0.5 g) were homogenized in 80% methanol (25 mL), and the supernatant obtained by centrifugation at 10000*g* for 15 min was used for the antimutagenic assay.

Determination of Antimutagenic Activities. The antimutagenic effect of plant extracts against Trp-P-1 in *Salmonella typhimurium* TA98 was assayed by the Ames preincubation method using S9 mix (*21, 22*) with some minor modifications as previously described (*17*). The activity was calculated using the formula defined by Kanazawa et al. (*23*). The bioantimutagenic activity was examined by the method of Kanazawa et al. (*23*) with some minor modifications as follows. Trp-P-1 (50 ng in 50 μL of DMSO) was activated by the incubation with 50 μL of S9 mix and 0.75 mL of 0.1 M potassium phosphate buffer (pH 7.0) for 5 min at 37 °C and then heated for 30 s at 80 °C to inactivate S9 enzymes. After the solution cooled, it was added to bacterial culture (0.1 mL) and incubated for 15 min at 37 °C. The mixture was then incubated with plant extracts (50 μL in methanol) for 20 min at 37 °C and cultured according to the standard protocol of the Ames test. The bioantimutagenic activity was evaluated with a decrease in the number of His^+ revertant by plant extracts.

Analysis of Enzymatic *N*-Hydroxylation of Trp-P-2. The effect of plant extracts on *N*-hydroxylation of Trp-P-2 was determined by HPLC equipped with an electrochemical detector by the procedure of Minamoto et al. (*24*) with slight modifications as previously described (*17*).

Instrumental Analysis. The UV absorption spectra of the compounds in methanol were recorded on a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). The mass spectra were recorded on a JMS-HX/HX-110A spectrometer (JEOL, Tokyo, Japan) by direct electron ionization (EI). The molecular formula was verified by high-resolution mass measurement using an ApexII70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (ESI−FTICRMS) (Bruker Daltonics, Billerica, MA) within an error of 4×10^{-4} (0.4 ppm) in mass assignment on the sample. The ^1H nuclear magnetic resonance (NMR) spectra at 800.03 MHz and the ^{13}C NMR spectra at 201.19 MHz were recorded on an AVANCE 800 spectrometer (Bruker).

Purification and Spectral Analyses of Antimutagenic Constituent of *M. minutum*. *M. minutum* was selected for this experiment because it showed the highest activity in the screening. The plant material used for purification was purchased in Surat Thani, Thailand, in 1999. Lyophilized and ground material (aerial part, 49.0 g) was successively extracted with methanol (2.5 L), hexane (0.5 L), and water (1.0 L). The methanol extract, in which a large part of the activity was recovered, was evaporated at 35 °C, and the viscous residue obtained was partitioned twice with diethyl ether (500 mL) and water (200 mL). Most activity was recovered in the diethyl ether layer. The diethyl ether layer was then concentrated and subjected to chromatography on a column of Wakosil C18 (5.0 × 30 cm). The column was eluted by methanol acidified with formic acid (0.5%). The effluents were analyzed by a PX-8020 HPLC system equipped with a photodiode array detector (Tosoh, Tokyo, Japan) with a 100 mm × 4.6 mm i. d. TSK gel super-ODS column (Tosoh) maintained at 40 °C. The mobile phase system was a linear gradient using 0.5% formic acid and acetonitrile. Successive fractions that showed the activity were combined, and then subjected to chromatography on a column of Wakosil C18 (2.5 × 20 cm). The column was eluted with 90% methanol containing 0.5% formic acid. The active fraction was then subjected to a preparative HPLC on a 250 mm × 20 mm i. d. TSK gel ODS-80Ts (Tosoh). Finally, compound **1** was purified (>95% by HPLC) with a yield of 540 mg (1.1% of the plant material). The physicochemical properties of compound **1** were as follows. EIMS, *m/z*: M^+ 347 (22), 264 (100). High-resolution FT-MS calculated for $C_{23}H_{26}NO_2$ ($[M + H]^+$), 348.1958; found, 348.1964. UV (methanol) λ_{max}, nm: 288(s), 294, 329, 342, 358. ^1H NMR chemical shifts (CD_3OD) were δ 1.410 (3H, s, 3-CH_3), 1.563 (3H, dq, *J* 1.4 and 0.2 Hz, 4′-CH_3), 1.640 (3H, dddq, *J* 1.4, 1.1, 1.1 and 0.2 Hz, 4′-CH_3), 1.694−1.753 (2H, m, 1′-CH_2), 2.128−2.209 (2H, m, 2′-CH_2), 2.271 (3H, d, *J* 0.8 Hz, 5-CH_3), 5.126 (1H, dddqq, *J* 7.3, 7.3, 1.4, 1.4 and 1.4 Hz, 3′-CH), 5.654 (1H, d, *J* 9.8 Hz, 2-CH), 6.602 (1H, dd, *J* 8.3 and 2.2 Hz, 8-CH), 6.794 (1H, dd, *J* 2.2 and 0.5 Hz, 10-CH), 6.815 (1H, d, *J* 9.8 Hz, 1-CH), 7.483 (1H, d, *J* 0.8 Hz, 6-CH), 7.645 (1H, dd, *J* 8.3 and 0.5 Hz, 7-CH). ^1H NMR chemical shifts in $CDCl_3$ were δ 1.430, 1.573, 1.652, 1.676−1.799, 2.086−2.209, 2.311, 5.087−5.120, 5.623, 6.554, 6.677, 6.758, 7.535, 7.704. ^{13}C NMR chemical shifts (CD_3OD) were δ 16.27 (5-CH_3), 17.64 (4′-CH_3), 23.95 (2′-CH_2), 25.87 (4′-CH_3), 26.34 (3-CH_3), 42.01 (1′-CH_2), 79.07 (3-C), 97.64 (10-CH), 105.58 (5-C), 108.85 (8-CH), 117.91 (6a-C), 118.20 (11b−C), 118.31 (6b−C), 119.49 (1-CH), 120.51 (7-CH), 120.92 (6-CH), 125.53 (3′-CH), 129.07 (2-CH), 132.37 (4′-C), 136.73 (11a-C), 143.11 (10a-C), 149.71 (4a-C), 156.28 (9-C). ^{13}C NMR chemical shifts in $CDCl_3$ were δ 16.04, 17.58, 22.73, 25.67, 25.72, 40.66, 78.01, 96.99, 104.34, 108.32, 116.67, 117.47, 118.04, 118.28, 120.00, 120.39, 124.20, 128.72, 131.67, 134.74, 140.73, 148.95, 153.49. Assignments of chemical shifts of ^1H and ^{13}C NMR spectra in CD_3OD were confirmed by 2D-NMR analyses, HMBC, and HSQC.

Cytotoxicity Assay. A tumor cell line HL60 was obtained from the Riken Gene Bank (Tsukuba, Japan). HL60 cells were maintained in RPMI1640 medium supplemented with 10% FCS and antibacteria-antimycotic solution (Sigma, St. Louis, MO). Cells (5×10^3/well) were cultured on a 96-well plate for 24 h at 37 °C in a humidified atmosphere of 5% CO_2, then exposed to various concentrations of compound **1** for 72 h. The cell viability was determined by the colorimetric method using AlamarBlue (Biosource International, Lewisville, TX) (*25*). The MIC_{100} was defined as the lowest concentration of compound **1** in culture media showing equivalent viability as the culture media without cell.

Antibacterial Activity Assay. The antibacterial activity of compound **1** against four foodborn pathogenic bacteria was determined by the agar dilution method (*26*). *Bacillus cereus* 5020024 (MAFF Genebank, Tsukuba, Japan), *Staphylococcus aureus* KR-103 (The Kitasato Institute, Tokyo, Japan), *Escherichia coli* B-1030 (MAFF genebank, Tsukuba, Japan), and *Salmonella infantis* L-0164 (MAFF genebank, Tsukuba, Japan) were used in the study. Bacterial strains were activated in nutrient broth at 36 °C for 24 h prior to use. Ten μL of bacterial cultures (approximately 10^4 cfu) was spotted on Mueller−Hinton agar plates containing various concentrations of compound **1**, and then incubated at 36 °C for 21 h. The MIC_{100} was defined as the lowest concentration of compound **1** in agar plates showing no visible bacterial growth.

RESULTS AND DISCUSSION

Antimutagenic Screening. Tested plants were classified into three groups, A, B, and C, on the basis of 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**). Group A (5 species: *Micromelum minutum*, *Oroxylum indicum*, *Cuscuta chinensis*, *Azadirachta indica*, and *Litsea petiolata*) exhibited ED_{90} values lower than 5 μL/plate (0.1 mg of dry plant material equivalent). Particularly, the extract from *M. minutum* completely suppressed the mutation at a dose of 5 μL/plate (**Table 2**). Group B (17 species) showed ED_{90} values between 5 μL/plate and 50 μL/plate (0.1 to 1.0 mg of dry weight equivalent). The remaining plant samples showed higher ED_{90} values than 50 μL/plate, and were classified into group C.

It is noteworthy that the potent antimutagenic activity of three species (*M. minutum*, *C. chinensis*, and *L. petiolata*) in group A was reported for the first time in this study. The remaining two species, *O. indicum* and *A. indica*, were reported as antimutagenic edible plants (*5*, *11*, *12*, *18*). As we reported previously (*18*), the major active component of *O. indicum* was baicalein (5,6,7-trihydroxyflavone) which was known as a potent antimutagenic flavonoid (*27*). However, the antimutagenic principles in *A. indica* have not yet been reported.

Antimutagenic Properties of Plant Extracts. Extracts from all plants in group A also showed strong inhibitory effect not only against Trp-P-1 but also against the other cooked food mutagens, Trp-P-2 and PhIP (**Table 2**). Thus, it was suggested

Table 1. Antimutagenic Activity of Some Edible Thai Plants

family/species	common name (Thai)	tested part[a]	place of collection	antimutagenic activity[b]
Alismataceae				
Limnocharis flava	Talapat ruesi	S	Ubon Ratchathani	C
Amaranthaceae				
Amaranthus lividus	Phak khom	L	Phayao	C
Amaranthus tricolor	Phak khom suan	L	Ubon Ratchathani	C
Amaranthus viridis	Phak khom hat	L	Ubon Ratchathani	C
Anacardiaceae				
Anacardium occidentale	Ma muang himmapaan	L	Trang	B
Bouea oppositifolia	Ma pring	L	Nakhon Sri Thammarat	B
Mangifera indica	Ma muang	L	Phayao	B
Araceae				
Colocasia esculenta	Bon	S	Chiang Mai	C
Colocasia gigantea	Kun	S	Japan	C
Lasia spinosa	Phak nam	L, S	Chiang Mai	C
Araliaceae				
Eleutherococcus trifoliatus	Phak paem	L	Chiang Mai	C
Polyscias fruticosa	Lep khrut	L	Surat Thani	C
Asclepiadaceae				
Dregea volubilis	Kra thung ma ba	L	Chiang Mai	C
Gymnema inodorum	Phak chiang da	L	Chiang Mai	C
Marsdenia glabra	Phak saeo	L	Phayao	C
Sarcostemma secamone	Chamuk pla lot	L, FL	Udon Thani	C
Basellaceae				
Basella alba	Phak plang	L	Phayao	C
	Dok phak plang	FL	Phayao	C
Bignoniaceae				
Fernandoa adenophylla	Khae hang khang	FL	Phayao	C
Oroxylum indicum	Pheka	FR	Phayao	A
	Dok pheka	FL	Udon Thani	A
Bombacaceae				
Bombax ceiba	Ngio	FL	Lampang	C
Boraginaceae				
Tournefortia ovata	Liang	L	Surat Thani	C
Capparaceae				
Cleome gynandra	Phak sian	L	Phayao	C
Compositae				
Acmella oleracea	Phak khrat	L	Phayao	C
Chrysanthemum coronarium	Phak tang o	L	Chiang Mai	C
Emilia sonchifolia	Hang pla chon	L	Udon Thani	C
	Phak kat nok khao	L	Nakhon Sri Thammarat	C
Convolvulaceae				
Cuscuta chinensis	Foi thong	S	Ubon Ratchathani	A
Ipomoea aquatica	Phak bung	L	Bangkok	C
	Phak bung chin	L	Japan	C
Ipomoea batatas	Man thet	L	Surat Thani	C
Cruciferae				
Brassica alboglabra	Phak khana	L	Bangkok	C
Brassica juncea	Phakkat hin	L	Nong Khai	C
Raphanus sativus	Pak kee huud	FR	Phayao	C
Cucurbitaceae				
Coccinia grandis	Phak tamlueng	L	Bangkok	C
Cucurbita moschata	Fak thong	L, S	Surat Thani	C
Cucurbita moschata	Dok fak thong	FL	Chiang Rai	C
Cucurbitaceae sp.	Buab	FR	Bangkok	C
Momordica charantia	Mara	L	Japan	C
Momordica subangulata	Phak mae	L, S	Nakhon Sri Thammarat	C
Trichosanthes anguina	Boap ngu	FR	Japan	C
Dryopteridaceae				
Diplazium esculentum	Phak kut khao	L, S	Nakhon Sri Thammarat	C
Euphorbiaceae				
Codiaeum variegatum	Koson	L	Chiang Rai	B
Glochidion perakense	Man pu	L	Surat Thani	B
Sauropus androgynus	Phak wan ban	L	Surat Thani	C
Gramineae				
Cymbopogon citratus	Ta khrai	S	Bangkok	C
Guttiferae				
Cratoxylum formosum	Tio khao	L	Ubon Ratchathani	C
Garcinia cowa	Cha muang	L	Surat Thani	C
Ixonanthaceae				
Irvingia malayana	Krabok	FR	Ubon Ratchathani	C
Lamiaceae				
Ocimum basilicum	Horapha	L	Japan	C
Ocimum americanum	Maeng lak	L	Japan	C
Ocimum gratissimum	Yira	FR	Surat Thani	C
Ocimum tenuiflorum	Kaphrao	L	Japan	C
Lauraceae				
Litsea petiolata	Thammang	L	Surat Thani	A
Lecythidaceae				
Careya sphaerica	Kra don	L	Ubon Ratchathani	C
Barringtonia acutangula	Chik na	L	Surat Thani	B

Table 1. Continued

family/species	common name (Thai)	tested part[a]	place of collection	antimutagenic activity[b]
Leguminosae				
Acacia pennata	Cha om	L	Japan	C
Adenanthera pavonina	Ma klam ton	L	Ubon Ratchathani	C
Archidendron jiringa	Cha niang	FR	Nakhon Sri Thammarat	C
Bauhinia racemosa	Chong kho na (siao)	L	Phayao	C
Senna siamea	Khi lek	L	Japan	C
Lablab purpureus	Thua paep	FR	Japan	C
Leucaena leucocephala	Kra thin	FR	Japan	C
	Kra thin	L	Japan	C
	Krathin oon	S	Trang	B
Neptunia oleracea	Phak kra chet	L	Japan	C
Parkia speciosa	Sato	FR	Japan	C
Parkia timoriana	Riang	FR	Surat Thani	C
Pisum sativum	Thua lan tao	L, S	Chiang mai	C
Psophocarpus tetragonolobus	Thua phu	FR	Bangkok	C
Sesbania grandiflora	Khae ban	L, FL	Nong Khai	C
Sesbania javanica	Sano kin dok	FL	Phayao	C
Tamarindus indica	Ma kham	L	Surat Thani	C
Vigna unguiculata	Thua fak yao	FR	Bangkok	C
Lemnaceae				
Wolffia globosa	Khai nae	L, S, FL	Ubon Ratchathani	C
Malvaceae				
Hibiscus sabdariffa	Kra chiap daeng	L	Surat Thani	C
Meliaceae				
Azadirachta indica	Sadao	S, FL	Japan	A
Menispermaceae				
Tiliacora triandra	Thao yanang	L	Ubon Ratchathani	C
Molluginaceae				
Glinus oppositifolius	Phak khuang	L	Ubon Ratchathani	C
Moraceae				
Artocarpus heterophyllus	Khanun	FR	Chiang Mai	C
Broussonetia kurzii	Sa lae	FL	Japan	C
Ficus benjamina	Sai yoi	L	Nong Khai	C
Ficus fistulosa	Ching khao	FR	Surat Thani	B
Ficus infectoria	Liap	L, FL	Chiang Mai	C
Moringaceae				
Moringa oleifera	Ma rum	FR	Chiang Mai	C
Musaceae				
Musa acuminata	Kluai thuean	FR	Trang	C
Musa sapientum	Hua plee	FL	Japan	C
Myrtaceae				
Syzygium gratum	Met chun	L	Surat Thani	B
	Samet chun	L	Ubon Ratchathani	B
Opiliaceae				
Melientha suavis	Phak wan	L	Japan	C
Piperaceae				
Piper nigrum	Phrik thai	FR	Japan	C
Piper retrofractum	Di pli	FR	Ubon Ratchathani	C
Piper betle	Phlu	L	Trang	B
Piper sarmentosum	Cha phlu	L	Japan	C
Piper interruptum	Sakhan	S	Chiang Mai	C
Polygonaceae				
Polygonum odoratum	Phak phai	L, S	Phayao	C
Pontederiaceae				
Monochoria vaginalis	Phak khiat	L, S	Nakhon Sri Thammarat	C
Rubiaceae				
Morinda citrifolia	Yo ban	L	Japan	C
Rutaceae				
Micromelum minutum	Hatsa khun (Mui)	L, S	Surat Thani	A
Toddalia asiatica	Khruea ngu hao (Luk lo)	L, FR	Surat Thani	B
	Khruea ngu hao	L	Surat Thani	B
Zanthoxylum limonella	Kamchat ton	FR	Lampang	C
Saururaceae				
Houttuynia cordata	Phak khao thong	L	Chiang Mai	C
Scrophulariaceae				
Limnophila aromatica	Phak ka yaeng	L	Ubon Ratchathani	B
Solanaceae				
Capsicum frutescens	Phrik khinu	L	Japan	C
Solanum trilobatum	Ma waeng khruea	FR	Surat Thani	C
Umbelliferae				
Anethum graveolens	Phak chi lao	L	Japan	C
Centella asiatica	Bua bok	L	Japan	C
Coriandrum sativum	Phak chi	L	Japan	B
Eryngium foetidum	Phak chi farang	L	Japan	C
	Dok phak chi farang	FL	Chiang Mai	C
Oenonthe javanica	Phak chi lom	L	Surat Thani	C
Trachyspermum roxburghianum	Phak chi lom	L	Phayao	B
	Phak chi lom	L, S	Surat Thani	B
Vitaceae				
Cissus hastata	Som sandan	L	Udon Thani	C
Parthenocissus vitacea	Thao khan	L	Surat Thani	C
Zingiberaceae				
Boesenbergia pandurata	Krachaai ban	Rh	Japan	B

[a] Tested parts: L, leaf; S, stem; FL, flower; FR, fruit; Rh, rhizome. [b] Antimutagenic activity: A, $ED_{90} < 5$ μL/plate (0.1 mg of dry plant material equivalent); B, 50 μL/plate $> ED_{90} > 5$ μL/plate; C, $ED_{90} > 50$ μL/plate.

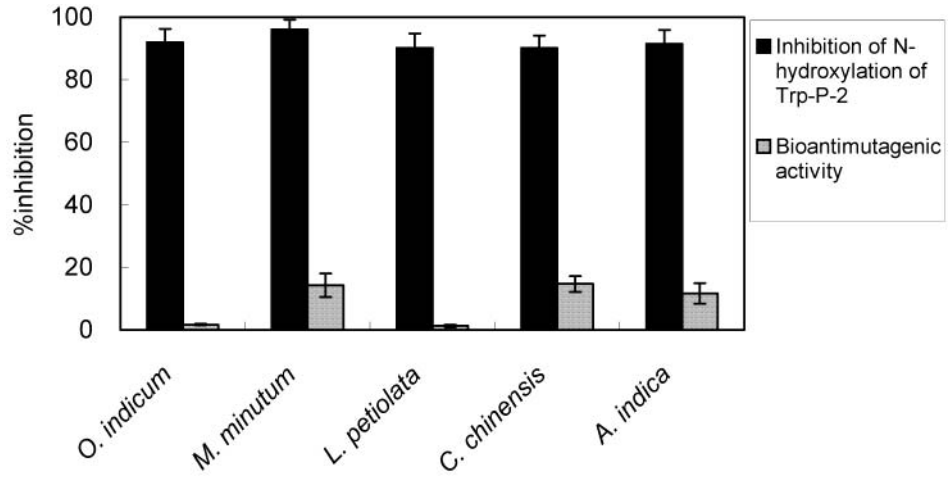

Figure 1. Inhibitory effect on the enzymatic N-hydroxylation of Trp-P-2 and bioantimutagenic activity of the extracts from some edible Thai plants. N-Hydroxy-Trp-P-2 was analyzed by HPLC (TSK gel super-ODS column, 50 mm × 4.6 mm i. d., isocratic elution with 10% acetonitrile in 20 mM KH_2PO_4 and 0.1 mM EDTA-2Na). The reaction mixture (0.5 mL), consisting of 10 μg of Trp-P-2, 25 μL of S9 mix, and 10 μL of the plant extract in 0.1 M potassium phosphate buffer (pH 7.0), was incubated at 37 °C for 15 min. The bioantimutagenic activity was assayed by the method as described in text. Data were average of three independent experiments. Methanol was used as control for both experiments.

Table 2. Inhibitory Effects of Methanol Extracts from Selected Edible Plants toward Mutagenesis Induced by Trp-P-1, Trp-P-2, and PhIP

	% inhibition of mutagenesis		
plant extract	Trp-P-1 (50 ng/test)	Trp-P-2 (20 ng/test)	PhIP (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	9	96
(5 μL/plate)	76	81	80

that plant extracts of group A act on the common process of the mutagenesis induced by heterocyclic amines. In the Ames test, heterocyclic amines such as Trp-P-1 are converted to corresponding N-hydroxyl derivatives by cytochrome P450 (P450) 1A1 or 1A2 monooxygenases in S9 fraction of the rat liver homogenate (*27, 28*). These N-hydroxyl derivatives are then transported into bacterial cells and esterified by phase II enzymes in cytoplasm (*29*). N-Acetoxy or N-sulfonyloxy esters, accounted as the direct-acting mutagen, react with DNA to form a covalent bond inducing mutation of the gene. Antimutagens are classified into two groups, desmutagens and bioantimutagens, based on the mode of action (*30*). Desmutagens neutralize mutagens before or during attack of DNA, whereas bioantimutagens act on DNA repair processes. If the plant extracts were to inhibit phase II enzymes or enhance the DNA repair process, the inhibitory effect would be detected in a bioantimutagenic activity assay. As shown in **Figure 1**, N-hydroxylation of Trp-P-2 was markedly inhibited by extracts from all plant samples in group A by more than 90% at a dose of 10 μL/500 μL assay system, whereas very weak bioantimutagenic activity was detected at a dose of 50 μL/plate. Therefore, the antimutagenic effect of group A plant extracts was mainly due to the inhibition of N-hydroxylation catalyzed by P450 monooxygenases in S9.

Figure 2. Twigs (edible part) of *Micromelum minutum*.

Identification of Antimutagenic Constituent of *M. minutum*. *M. minutum*, which showed the highest activity in the antimutagenic screening, is a shrub belonging to the Rutaceae family. It is consumed mainly in the southern part of Thailand as a fresh vegetable served with some certain dishes such as *kanom chin nam ya* and *ka-nom chin kaeng tai pla*, thin rice noodle with spicy sauce. Various parts, including edible twigs (**Figure 2**), of *M. minutum* are used as a folk medicine for fever and giddiness (*31*). Medicinal principles of *M. minutum* have not yet been elucidated thoroughly, however, many bioactive compounds such as coumarins, a flavanone, a quinolone alkaloid, and carbazole alkaloids have previously been isolated (*31*). In this study, compound **1** was isolated from twigs of *M. minutum* by reversed-phase column chromatography as the major antimutagenic principle. ^1H and ^{13}C NMR chemical shifts of compound **1** in $CDCl_3$ and other physicochemical properties were exactly consistent with those of (+)-mahanine as previously reported by Tachibana et al. (*32*). Thus, compound **1** was identified as (+)-mahanine (**Figure 3**). Mahanine was not previously reported in this plant. It is known that mahanine is a constituent of related species, *Murraya koenigii* (curry leaf), and *Micromelum zeylanicum* and *Murraya euchrestifolia* (*32*−*35*).

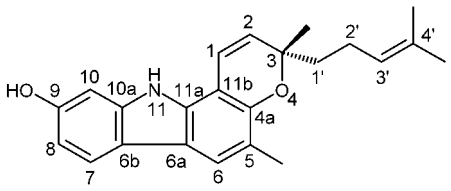

Figure 3. Antimutagenic compound isolated from *Micromelum minutum*.

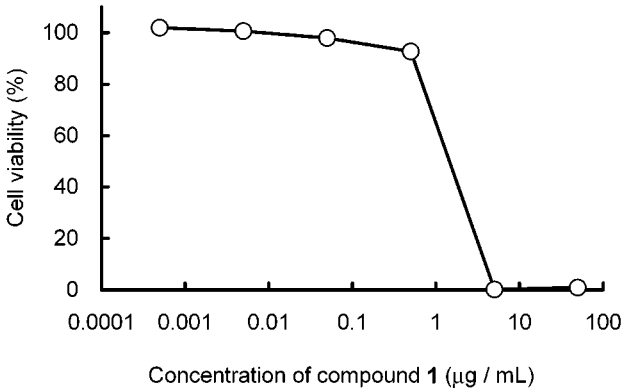

Figure 4. Cytotoxic effect of the compound isolated from *Micromelum minutum*. HL60 human leukemia cells (5×10^3/well) were cultured on a 96-well plate for 24 h, then exposed to various concentrations of compound **1** (dissolved in DMSO) for 72 h. DMSO (0.5%) was used for control. Data were average of four independent experiments.

Bioactivities of Compound 1. In the antimutagenic assay, compound **1** inhibited approximately 99% of the mutation induced by Trp-P-1, Trp-P-2, PhIP, and IQ at the concentration of 20 μM. No toxicity on *S. typhimurium* TA98 was detected at this concentration. The antimutagenic IC_{50} value against Trp-P-1 was 5.2 $\pm$ 0.4 μM (1.8 $\pm$ 0.1 μg/mL). This was closely similar to the antimutagenic IC_{50} values of other flavonoid antimutagens (*27*). Compound **1** was considered as a desmutagen, because it strongly inhibited N-hydroxylation of Trp-P-2 by 95.3 $\pm$ 2.4%, but showed only weak bioantimutagenic activity (7.8%) at concentrations of 50 μM and 20 μM, respectively. In addition, compound **1** did not show inhibition on the NADPH dependent cytochrome *c* reduction catalyzed by NAD(P)H-cytochrome P450 reductase (*36*), which donates electrons to P450 monooxygenases, at a concentration of 20 μM. It is therefore suggested that the antimutagenic effect of compound **1** was mainly due to the inhibition of N-hydroxylation catalyzed by P450 monooxygenases. The inhibitory-type of compound **1** on N-hydroxylation of Trp-P-2 was then determined by a double reciprocal plot analysis of the reaction rate and concentration of Trp-P-2 (6.3 to 50.8 μM) in the presence of compound **1** of the concentration between 0 and 14.4 μM. The result suggested that compound **1** was a competitive inhibitor with a K_i of 3.4 $\pm$ 0.45 μM. The antimutagenic (desmutagenic) activity of compound **1** was found for the first time in this study.

As shown in **Figure 4**, compound **1** showed a potent cytotoxic effect against HL60 cells with a MIC_{100} of 4.0 μg/mL. This value was similar to the cytotoxic LC_{50} against HL60 of genistein (2.9 μg/mL) and matairesinol (6.6 μg/mL) (*37*). Previously, Wu reported the significant cytotoxic effect of (+)-mahanine against KB cells with ED_{50} of 3.0 μg/mL (*35*).

Compound **1** effectively suppressed the growth of gram positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, with the MIC_{100} of 12.5 and 6.25 μg/mL, respectively. However, gram negative bacteria, *Escherichia coli*, and *Salmonella infantis* were not sensitive to compound **1** at the maximum concentration examined (400 μg/mL). These results are in agreement with the previous investigation using the broth microdilution method. Ramsewak et al. (*33*) reported that (+)-mahanine showed lower MIC_{100} against gram positive bacteria (25 μg/mL), *S. aureus*, and *Streptococcus pyogens* than against the gram negative bacteria (100 μg/mL), *E. coli*. The antibacterial activity of compound **1** was comparable to that of a benzylisoquinoline alkaloid, anolobine, against several grampositive bacteria with MIC_{90} values between 12 and 50 μg/mL (*38*).

Table 3. Various Bioactivities of Mahanine

bioactivities
antioxidative activity[a,b]
inhibition on topoisomerase I and II[b]
antimutagenic activity against heterocyclic amines (inhibition on P450 monooxygenase) [c]
antimicrobial activity against gram positive bacteria[b,c]
antimicrobial activity against yeasts[b]
mosquitocidal effect[b]
cytotoxicity against HL60 cell[c]
cytotoxicity against KB cell[d]
antiinflammatory activity[b]

[a] Reported in the literature (*32*). [b] Reported in the literature (*33*). [c] Present study. [d] Reported in the literature (*35*).

Mahanine is an interesting food component exhibiting a wide variety of bioactivities as summarized in **Table 3** (*32*, *33*, *35*). It is expected that edible plants containing mahanine could contribute to reducing cancer risks or to maintaining food safety. To elucidate the modes of action in individual bioactivities, bioavailability, and attainability of mahanine, further biochemical investigations, as well as in vivo studies, are required.

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