RESEARCH COMMUNICATION

Suppressive Effects of Edible Thai Plants on Superoxide and Nitric Oxide Generation

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Abstract

We screened ethanol extracts from a total of 134 species of edible Thai plants for their suppressive effects on superoxide (O_2^-) generation using a xanthine (XA)-xanthine oxidase (XOD) assay system. When the extracts were tested at a concentration of 500 μg/ml, 28.4% significantly suppressed O_2^- generation. Of these active extracts, it was found that in 17.9% of cases the action was due to XOD inhibition, in 1.5% due to O_2^- scavenging activity, and in 9% due to both XOD inhibition and O_2^- scavenging. In addition, some plant extracts (25 species) which had been known to possibly possess anti-tumor promoting activity were tested for O_2^- and NO generation in cellular systems. In this test, 13 species exhibited strong inhibitory activity toward both O_2^- and NO generation. From the fruit pods of Oroxylum indicum (Bignoniaceae), a traditional vegetable in Thailand, two flavones, oroxylin A and chrysin, and a triterpene carboxylic acid, ursolic acid (UA), were identified as inhibitors of O_2^- generation in XA/XOD system. These compounds also showed marked inhibitory effects on the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced O_2^- generation in dimethylsulfoxide (DMSO)-differentiated HL-60 cells. Our results suggest that, as we have reported earlier, edible Thai plants are promising sources of antioxidants with chemopreventive potential.

Key Words: Anti-oxidation - Thai plants - *Oroxylum indicum* - oroxylin A - chrysin - ursolic acid

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Introduction

Free radicals such as superoxide anion (O_2^-) and nitric oxide (NO) may be involved in a variety of chronic diseases, including cancer (Perchellet et al., 1995; Kensler et al., 1989). Xanthine oxidase (XOD), an enzyme widely distributed in mammal tissues, catalyzes metabolism of hypoxanthine to xanthine (XA), and then xanthine to uric acid in the presence of molecular oxygen, this being accompanied with O_2^- generation. Several cancer preventive phytochemicals such as quercetin, genistein and 1'-acetoxychavicol acetate (ACA) have been reported to be XOD inhibitors (Nakamura et al., 1998 & 2000). Our group has reported that edible plants in the tropical region abundantly contain promising cancer preventives using an inhibition test of Epstein-Barr virus (EBV) activation induced by tumor promoters in Raji cells (Murakami et al., 1995). In the present study we evaluated XOD inhibitory or O_2^- scavenging activity of edible plants in Thailand. In addition, their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced O_2^- generation in differentiated HL-60 human promyelocytic leukemia cells and on combination of lipopolysaccharide (LPS) and interferon (IFN)-γ induced nitric oxide (NO) generation in RAW 264.7 murine macrophage cells, were partly examined. Furthermore, active constituents of *Oroxylum indicum* are also herein reported.

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Materials and Methods

Sample Preparation

Fresh edible plants were collected from the various markets in Thailand in their season through 1995-1999. Each sample was cut into small pieces and extracted with ethanol at room temperature for two weeks. After drying in vacuo, each extract was subjected to bioassay. All of the test samples were prepared as in DMSO solution. The concentration used was 500 μg/ml for testing in XA/XOD system and 200 μg/ml for O_2^- and NO generation inhibitory tests in differentiated HL-60 cells and RAW 264.7 cells, respectively.

Bioassay

XOD inhibitory and O_2^- scavenging assay

The XOD inhibitory and O_2^- scavenging activities were measured by the XA/XOD system using SOD test kit of WAKO, with some modification (Ohnishi et al., 1985). XOD catalyzes the generation of O_2^- through the conversion of XA into uric acid. In this system, the ratio of the O_2^- scavenging activity was obtained by subtracting the XOD inhibitory activity from the total activity (nitroblue tetrazolium (NBT) reduction inhibitory activity). XOD inhibitory and NBT reduction inhibitory activities were measured as previously reported (Murakami et al., 1996). Briefly, the test sample dissolved in 50 ml of DMSO was added to the 1.5 ml microtube contain 0.5 ml of substrate solution (0.40 mM XA and 0.24 mM NBT in 0.1 M phosphate buffer pH 8.0) and 0.5 ml of enzyme solution (0.049 unit/ml, from butter milk) and the mixture was incubated at 37 °C for 20 min. Then 0.5 ml of the reaction solution was separated into another microtube and terminated the reaction by adding 0.5 ml of 69 mM sodium dodecyl sulphate (SDS) and the absorption at 560 nm was measured. The NBT reduction inhibitory activity was expressed by the relative ratio of absorbance of the test sample to that of a control experiment. Heating the remaining solution in the microtube for 5 min in water bath to terminate the reaction. After centrifuged at 6500 rpm, the XOD inhibitory activity was determined by the ratio of XA to uric acid which were detected by HPLC (column: μ-Bondasphere , mobile: 1% acetonitrile in 20 mM phosphate buffer pH 5.0) at UV absorption 290 nm. The control experiment using 0.1 M phosphate buffer solution in the place of enzyme solution, was also performed.

Inhibitory test of TPA-induced O_2^- generation in differentiated HL-60 cells

Inhibitory test of TPA-induced O_2^- generation in DMSO-differentiated HL-60 cells was done as the previously reported (Murakami et al., 1997; Markert et al., 1984). Briefly, the test sample dissolved in 5 ml of DMSO was added to DMSO-induced differentiated HL-60 cell suspension ($1x10^6$/ml) and incubated it at 37 °C for 15 min. The cells were washed with Hank's buffer solution (HBSS) twice for removal of extracellular test compound to omit O_2^- scavenging effect. TPA (100 nM) and cytochrome *c* solution (1 mg/ml) was added to the reaction mixture which was then incubated for another 15 min. Placing the reaction tube on ice to terminate the reaction. After centrifugation at 250 *g*, the absorption at 550 nm was measured. Inhibitory effects were expressed by the relative ratio of absorbance of the test sample to a control experiment. Each experiment was done in duplicate. No significant decrease in cell viability, assessed by the trypan blue-exclusion test, was observed.

Inhibitory test of LPS/IFN-γ-induced NO generation in RAW 264.7 cells

Inhibitory test of LPS/IFN-γ-induced NO generation was done as previously reported (Murakami et al., 1999). Briefly, the test sample dissolved in DMSO at an appropriate concentration was added to 1 ml of medium containing murine macrophage RAW 264.7 cells (2x105 cells/ml) together with LPS (100 ng/ml), tetrahydrobiopterin (BH_4, 10 mg/ml), IFN-γ (100 U/ml) and L-arginine (2 mM). The final DMSO concentration in the medium was 0.5%. The cells were cultivated in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2 atmosphere. After 24 h, the level of both NO_2^- and L-citrulline were measured by Griess method (Green et al., 1982) and diacetyl monoxime (Boyde and Rahmatullah,1980) assays, respectively. Cytotoxicity was measured by a [3-(4,5-dimethylthiazool-2-yl)-2,5-diphenyltetrazolium bromine] (MTT) assay (Sadowski et al., 1992). Each experiment was done in duplicate. No significant decrease in cell viability was observed.

Isolation of Active Compounds

Separation of the active principles was guided in reference to the results from XA/XOD system and also the differentiated HL-60 cells tests. The ethanol extract (6.3 g) from the young fruit pod of *Oroxylum indicum* (100 g) was partitioned between ethyl acetate and water. The active ethyl acetate part (3.3 g) was separated by silica gel column and eluted stepwise with 20% increasing amount of ethyl acetate in toluene, 100% ethyl acetate and then methanol. The active fraction (40-60% ethyl acetate eluate, 1.8 g) was further purified on silica gel and ODS gel to obtain two active fractions, I and II. Preparative high performance liquid chromatography (HPLC) on μ-Bondasphere C_{18} (19 x 150 mm) eluted with 62% methanol in water (7 ml/min) gave active compounds **1** (19 mg) and **2** (20 mg) from fraction I. Also, **3** (2.5 mg) was obtained from fraction II by preparative HPLC on YMC ODS A column (Yamamura Chemical, 19 x 150 mm) eluted with 78 % acetonitrile in water. Compound 1, 2 and 3 were identified as oroxylin A, chrysin and ursolic acid, respectively (Fig 2) by their physicochemical data including data on mass (MS), proton nuclear magnetic resonance (^1H NMR), ultraviolet absorption (UV) and infrared (IR) spectra. Compound **1**: ^1H NMR δ (ppm) (500

MHz, $CDCl_3$): 13.01(5-OH, s), 7.88 (2H, d, *J*=6.8 Hz), 7.54 (2H, d, *J*= 7.55 Hz), 6.66 (1H, s), 6.61(1H, s), 4.05 (3H, s); UV λ_{max}^{MeOH} :250sh., 271, 301sh., 317; UV $\lambda_{max}^{NaOMe/MeOH}$:243sh., 268, 367; UV $\lambda_{max}^{AlCl_3/MeOH}$: 254, 282, 338, 390sh.; UV $\lambda_{max}^{NaOAc/MeOH}$: 268, 366; UV $\lambda_{max}^{NaOAc/H_3BO^3/MeOH}$: 271, 318, IR υ_{max}^{KBr} cm^{-1} : 3034, 1655, 1606; EI-MS at 70 ev $[M]^+$ *m/z*: 284 ($C_{16}H_{12}O_5$). **2**: ^1H NMR δ (ppm) (500 MHz, CD_3OD): 7.97 (2H, dd, *J*=7.9, 1.5 Hz), 7.56 (2H, dt, *J*= 9.0, 8.4 Hz), 6.72 (1H, s), 6.48 (1H, s), 6.23 (1H, s); UV l_{max}^{MeOH}: 248sh, 267, 313; UV $\lambda_{max}^{NaOMe/MeOH}$: 238sh., 276, 362; UV $\lambda_{max}^{AlCl_3/MeOH}$: 251, 280, 327, 382; UV $\lambda_{max}^{NaOAc/MeOH}$:275, 359; UV $\lambda_{max}^{NaOAc/H_3BO_3/MeOH}$: 268, 317; IR υ_{max}^{KBr} cm^{-1} : 3013, 1655, 1612; EI-MS at 70 ev $[M]^+$ *m/z* 254 (C15H10O4). **3**: ^1H NMR δ (ppm) (500 MHz, CD_3OD): 5.23 (1H, t, *J*=3.3 Hz), 3.15 (1H, dd, *J*= 4.5, 11.2 Hz), 2.2 (1H, d, *J*=11.3 Hz), 6.48 (1H, ddd, *J*=3.8 Hz), 1.91 (3H, m) 1.65 (3H, ddd); 1.55(6H, m), 1.35 (6H, m),1.11 (3H, s),1.09 (1H), 0.9-1.0 (11H, m), 0.88 (3H, d), 0.85 (3H, s), 0.77 (3H, s), 0.75 (1H, d); UV λ_{max}^{MeOH} nm: 214; IR υ_{max}^{KBr} cm^{-1} : 2900, 1700, 1450, 1030, 990; methylated of ursolic acid: EI-MS at 70 ev $[M]^+$ *m/z* : 470 ($C_{31}H_{50}O_3$).

Results and Discussion

In the XA/XOD assay, the total inhibition of O_2^- generation determined by the NBT reduction assay consists of both the XOD inhibitory and O_2^- scavenging effects. We tentatively divided the total inhibition activity of the extracts for O2- generation into 4 ranks according to their inhibition rates (IR) at a concentration of 500 mg/ml; strongly active (+++): IR ≥70%, moderately active (++): 70% >IR ≥50%, slightly active (+): 50% >IR ≥30% and inactive (-): 30% >IR.

The screening results are summarized in Table 1. Of the 134 plant species tested, 71 species (53% of the total) reduced the total O_2^- generation at a concentration of 500 µg/ml. Of these active plants 17 species (12.7%) were strongly active (+++), 21 species (15.7%) moderately active (++) and 33 species (24.6%) slightly active (+) as shown in Fig. 1a. Also, the activity of 24 species (17.9%) was mainly due to XOD inhibition, 2 species (1.5%) to O_2^- scavenging, and 12 species (9%) to both XOD inhibition and O_2^- scavenging as shown in Fig. 1b. Interestingly, all of the species in the plant family Nymphaeceae showed significant activities at high rates (+++: 5/8 and ++: 3/8). We found that the major active compounds in the species of Nymphaeceae were polyphenolic compounds, quercetin, gallic acid and its esters (data not shown), whose anti-oxidative properties have already been reported (Salah et al., 1995; Inoue et al., 1994; Chen et al., 1990; Liang et al., 2001).

Murakami et al. (1995) have reported anti-tumor promoting property of Thai vegetables and fruits using EBV activation-inhibition test. They also indicated that inhibition of generation of free radicals (NO and O_2^-) from inflammatory leukocytes may be linked to suppression of carcinogenesis. Therefore, we tested radical generation-inhibitions of the plant extracts (25 species) at 200 µg/ml, which had been found to possess significant EBV activation-inhibition using RAW 264.7 cells and differentiated HL-60 cells. The species selected includes 4 strongly active species in the XA/XOD assay, 2 moderately active species, 5 slightly active species and 14 inactive species as shown in Table 1. Of these selected species, 14 species (56%) showed significant effects (IR≥50%) on both inhibition of NO generation in RAW 264.7 cells and O_2^- generation in HL-60 cells, 6 species (24%) and 2 species (8%) showed only inhibition of NO generation and inhibition of O_2^- generation, respectively. Of the active species in NO generation inhibition, *Glinus oppositifolius* (Aizoaceae) showed strongest activity at an inhibition rate of 81%. In contrast, this species was completely inactive in the total activity test

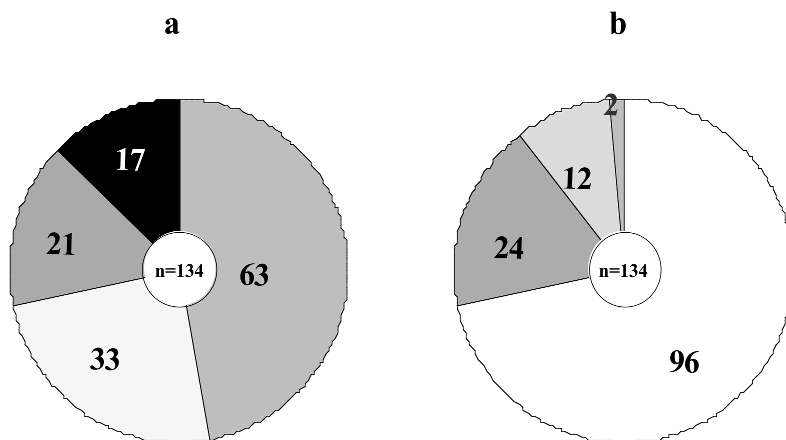

Figure 1. Activity Profiles of O_2^- Generation Inhibition Activity of Edible Plants in Thailand
a proportion of active species sorted as inhibition rate of total activity (IR=inhibition rate, %)
■ +++, (IR ≥70%) ▩ ++, (70%>IR ≥50%) □ +, (50%>IR≥30%) ▩ -, (30%>IR)
b proportion of active species sorted as type of activity
▩ XOD inhibition □ XOD inhibition + O_2^- scavenging ▩ O_2^- scavenging □ IR <50%
(Ethanol extract of sample at concentration = 500 mg/ml)

Table 1. Effect of Thai Plant-extracts on O_2^- generation in a XA/XOD System and on NO and O_2^- generation in Cellular Systems

Family/species	Thai name	Part used [a]	O_2^- generation inhibition[b] (by XA/XOD system)			NO generation inhibiton in RAW 264.7cells[c] (%)	O_2^- generation inhibiton in HL-60 cells[d] (%)
			Total activity	XOD inhibition	O_2^- scavenging		
Acanthaceae							
Andrographis paniculata	Faatalai	W	-	-	-	NT	NT
Asystasiella nusiana	Pakkood	L & St	+	+	-	NT	NT
Rhinacantus nasutus	Thongpunchang	L & St	-	-	-	NT	NT
Agavaceae							
Dracaena conferta Ridl.	Kumlunghanuman	L & St	++	++	-	NT	NT
Aizoaceae							
Glinus oppositifolius	Sadaodin	L & St	-	-	-	81.0	30.4
Alliaceae							
Allium tuberosum	Guichai	l	+	+	-	NT	NT
A. escalonicum	Doakhom	S & Fl	-	-	-	NT	NT
Amaranthaceae							
Amaranthus gracilis	Pak khom	L	+	+	-	76.1	100
Anacadiaceae							
Spondius pinnata	Makok	Fr	-	-	-	NT	NT
Anacardium occidentale	Mamuang himaphan	L	+	+	-	NT	NT
Araceae							
Colocasia esculenta (L) Schott.	Paerk	R	-	-	-	NT	NT
C. esculenta Hasskari	Bonn	St	-	-	-	NT	NT
Lasia spinosa	Paknham	Sh	-	-		65.0	61.8
Araliaceae							
Polyscias fruiticosa	Lebkrud	L	-	-	-	NT	NT
Asclepiadaceae							
Marsedinia glabra	Paksaew	L	+	+	-	NT	NT
Bignoniaceae							
Begonia prolixa Craib.	Bonsom	L	-	-	-	NT	NT
Oroxylum indicum	Pekaa	P (young)	+++	+++	-	75.7	54.1
Bombaceae					-		
Bombax ceiba	Doak-ngew	Fl	+	+	-	NT	NT
Boraginaceae							
Tournefortia ovata Wall. Ex G. Don	Liang	L	-	-	-	NT	NT
Boletaceae							
Thaeogyroporus porentosus (berk. Et Broome) Mc. Nabb	Hedtubtoa	W	++	++	-	NT	NT
Caricaceae							
Carica papaya	Malakor	Fr	-	-	-	NT	NT
Chenopodiaceae							
Basella rubra	Pakplang	L	-	-	-	NT	NT
Spinacia oleracea	Pak puaylaeng	L	-	-	-	NT	NT
Compositae							
Artemisia lactiflora	Doak kaew muang jeen	L	-	-	-	NT	NT
A. vulgaris	Koadchula	Se	+++	+++	-	NT	NT
Cartamus tinctorius	Kumphoi	Fl	++	++	-	NT	NT
Chrysanthemum coronarium	Tang-oh	L	-	-	-	78.0	46.3
Gynura crepidoides	Mohnoi	L	-	-	-	NT	NT
Lactuca sativa Linn.	Pakkardhom	L	+	+	-	NT	NT
Spilanthes iabadicencis	Pakphed	L	-	-	-	NT	NT
S. paniculata	Pakkradhuawaen	L, S &Fl	-	-	-	NT	NT
Cruciferae							
Brassica chinensis	Pak kwangtung	L	+	+	-	NT	NT
B. pekinensis Rupr.	Pakkardkao	L	+	+	-	NT	NT
Rhaphanus sativus cv. Group Rap-tailed radish	Pakkeehud	P (young)	+	+	-	NT	NT
Cucurbitaceae							
Citrullus lanutus Thunb.	Taengmoo	Fr (young)	+	+	+	NT	NT

Family/species	Thai name	Part used [a]	O_2^- generation inhibition[b] (by XA/XOD system)			NO generation inhibiton in RAW 264.7cells[c] (%)	O_2^- generation inhibiton in HL-60 cells[d] (%)
			Total activity	XOD inhibition	O_2^- scavenging		
Coccinia glandis Voigt	Tumlueng	L	-	-	-	NT	NT
Cucumis sativus Linn.	Tang gua	L	+	+	-	NT	NT
Cucurbita moschata	Fugthong	L	+	+	+	NT	NT
C. pepo	Zucchini	Fr	+	+	-	NT	NT
Lagenaria siceraria (Molina) Standl.	Numtao	Fr	-	-	-	NT	NT
Marmodica charantia	Marakeenok	Fr	+	-	+	58.6	94.4
Schium edule Sw.	Fugmaew	Fr	+	-	+	NT	NT
Ebenaceae							
Dioapyros decandra Lour	Lukchan	Fr	-	-	-	NT	NT
Euphobiaceae							
Sauropus androgynus	Pakwanban	L	-	-	-	NT	NT
Phyllanthus amarus Schum & Thomn	Luktaibai	W	+++	-	++	NT	NT
Gramineae							
Cymbopogon winterianus	Takraihom	St	-	-	-	NT	NT
Zea mays Linn.	Kaopoad	Se	-	-	-	NT	NT
Guttiferae							
Garcinia mangostana Linn	Mungkud	Fr	-	-	-	NT	NT
Hydrocharitaceae							
Otellia alesmoides Pers.	Santawaa	L	+	+	-	NT	NT
Labitae							
Mentha cordifolia	Saranae	L	+	+	-	NT	NT
Ocimum basilicum	Horapaa	L	+	+	-	56.7	81.7
O.gratissimum	Yeerha	L	-	-	-	30.9	36.1
O. sanctum	Kraprao	L	+	+	-	31.5	48.1
Lamnaceae							
Walffia globosa Harlog & Plas	Phum	W	++	++	-	NT	NT
Lauraceae						NT	NT
Litsea elliptica Bearl.	Thammung	L	-	-	-	NT	NT
Leguminosae							
Acacia rugata Merr.	Sompoi	L	-	-	-	NT	NT
Cassia siamea Britt	Keeleak	L	-	-	-	NT	NT
Leuceana leucocephala	Krathin	L	-	-	-	NT	NT
Perkia spiciosa	Sa tor	P	-	-	-	NT	NT
Pisum sativum	Tualantao	P	-	-	-	70.5	20.0
Pithecellobium dulce (Roxb) Benth.	Makhamthed	P	+	+	-	NT	NT
Sesbania glandiflora	Kae	Fl	-	-	-	63.0	98.9
Liliaceae							
Aspidistra sutepensis	Doak nang laew	Fl	+	+	-	NT	NT
Malvaceae							
Hibiscus sabdarifla	Krajiebdaeng	Fl	+++	+++	-	NT	NT
Marsiliaceae							
Marsilia crenata Presl.	Pakwaen	L	+	+	-	NT	NT
Meliaceae						NT	NT
Azadirachta indica	Sadao	Sh	-	-	-	78.6	88.1
Minispermaceae							
Tiliacora triandra Diels	Bai ya nang	L	-	-	-	NT	NT
Moraceae							
Artocarpus heterophyllus	Kanun	Fr	-	-	-	NT	NT
Broussonetia kurzii Corner.	Salae	Fl	++	++	-	NT	NT
Streblus asper Lour	Koi	L	+	+	-	NT	NT
Musaceae							
Musa (AA group) 'Kluai Khai'	Kluai Khai	Fr	++	+	+	NT	NT
Musa (ABB group) 'Kluai Nam Wa'	Hoaplee	Fl	+	+	-	NT	NT
Musa (ABB group) 'Kluai Nam Wa'	Kluai Nam Wa	Fr	++	+	+	NT	NT
Myrtaceae							
Eugenia grata	Pakmeg	Sh	-	-	-	NT	NT
E. ridleyi King	Baimug	L	+++	+++	-	NT	NT
Psidium eujavillus	Farang	Fr	+	+	-	NT	NT

Family/species	Thai name	Part used [a]	O_2^- generation inhibition[b] (by XA/XOD system) Total activity	XOD inhibition	O_2^- scavenging	NO generation inhibiton in RAW 264.7cells[c] (%)	O_2^- generation inhibiton in HL-60 cells[d] (%)
Nymphaeacea							
Nelumbo nucifera "Roseum Plenum"	Bualuangdaeng	Sta & Pis	++	+	+	NT	NT
N. nucifera Gaertn	Bualuangdaeng (east indian lotus)	Sta &Pis	++	+	+	NT	NT
N. nucifera Gaertn.	Bualuangkao (Hindu lotus)	Sta &Pis	+++	++	+	NT	NT
N. nucifera "Album Plenum"	Bualuangkao	Sta &Pis	+++	++	+	NT	NT
Nymphaea capensis	Buaphan	St	+++	++	+	NT	NT
N. lotus	Buasaikao	St	+++	++	+	63.0	64.8
N. rubra	Buasaidaeng	St	+++	++	+	NT	NT
N. stellata	Buapheain	St	++	+	+	NT	NT
Palmae							
Caryota urens	Tao rung	Sh	++	++	+	NT	NT
Salacca rumphii Wall.	Ragum	Fr	++	++	-	NT	NT
Trevisia palmata	Doak tang	Fl	-	-	-	62.4	24.3
Peperomiaceae							
Peperomia pellucida	Pakkrasung	L	+	+	-	NT	NT
Piperaceae							
Piper betel	Pluu	L	-	-	-	14.8	81.4
P. nigrum	Prikthaisod	Fr	-	-	-	NT	NT
P. samantosum	Baichapluu	L	-	-	-	NT	NT
Plantaginaceae							
Plantago psyllium Linn.	Tein kred hoi	Se	-	-	-	NT	NT
Polygonaceae							
Polygonum odoratum	Pak pai	L	-	-	-	NT	NT
Rheum officinale Baillon	Koad num tao	Se	+++	+++	-	NT	NT
Ranuculaceae							
Nigella sativa	Teindaeng	Se	-	-	-	NT	NT
Rubiaceae							
Ardisia lenticellata Fletch	Taped tagai	L & St	+++	+	+	NT	NT
Lasianthus virgatus Craib.	Phahoam	L	+	+	-	NT	NT
Rutaceae							
Citrus aurantifolia	Manao	Fr	-	-	-	59.1	78.7
C. hystrix	Makrood	L & Fr	-	-	-	NT	NT
Zanthoxylum limonella	Ma kwaen	Fr	-	-	-	23.4	89.4
Sapotaceae							
Manikara kauki	Lamud	Fr	-	-	-	NT	NT
Scrophulariaceae							
Limnophila aromatica	Pakkayang	L	-	-	-	70.0	100
Nymphoides cristatum	Paktubtao	L	++	++	-	75.2	84.7
Scroparia dulsis Linn.	Pakkrodnum	L	++	-	++	NT	NT
Selaginellaceae							
Selaginella involuta Spreng	Po ka tee mia	Sh	++	++	-	NT	NT
Simaroubaceae							
Eurycoma longifolia	Plalaiphaek	L	+++	+++	-	74.8	99.3
Smilacaceae							
Smilax corburalia Kunth	Kaoyennaey	R	+++	+++	-	NT	NT
Smilax sp.	Kaoyentai	R	++	++	-	NT	NT
Solanaceae							
Capsicum annum Linn	Prikcheefah	Fr	-	-	+	NT	NT
Capsicum frutescens Linn,	Prikkeenu	Fr	-	-	-	NT	NT
Solanum aculeatissima	Makuey leung	Fr	-	-	-	NT	NT
S. mammosum	Makuey nomling	Fr	+	+	-	NT	NT
S. melongena	Makueymuong	Fr	++	++	-	NT	NT
S. melongena	Makuey-yao	Fr	-	-	-	NT	NT
S. sp.	Makuey kunnai	Fr	+	+	-	NT	NT
S. stramonifolium Jacq.	Ma eok	Fr	-	-	-	NT	NT
S. torvum	Makueyphuong	Fr	-	-	-	NT	NT

Family/species	Thai name	Part used [a]	O_2^- generation inhibition[b] (by XA/XOD system) Total activity	XOD inhibition	O_2^- scavenging	NO generation inhibiton in RAW 264.7cells[c] (%)	O_2^- generation inhibiton in HL-60 cells[d] (%)
Sterculiaceae							
Abroma augusta Linn.	Teindum	Se	++	++	-	NT	NT
Stilaginaceae							
Antidesma matabanicum	Sommao	Sh	++	++	-	NT	NT
Thunbergiaceae							
Thunbergia laurifolia Lindl.	Rangjurd	L	++	++	-	NT	NT
Umbelliferae							
Anetheum graveolens	Pakcheelao	W	++	++	-	69.5	75.7
Centella asiatica	Baibuabok	L	-	-	-	64.3	NT
Conioselinum univilatum	Koadhoabua	St	+++	+++	-	NT	NT
Eryngium foetidum	Pakcheefarang	L	+	+	-	78.1	4.9
Foeniculum vulgare Mill	Teinkraeb	Se	+	+	-	NT	NT
Ornanthe stolonifera	Pakcheelom	L	-	-	-	NT	NT
Verbenaceae							
Clerodendrum paniculatum	Nomwan	L	+	+	-	NT	NT
Zingiberaceae							
Amomum utriculosum	Raewhoam	L	-	-	-	NT	NT
Boesenbergia pandurata	Krachai	R	-	-	-	26.2	10.8
Curcuma aromatica Salisb.	Nangdum	L	-	-	-	NT	NT
C. domestica	Khaminchun	R	+++	+++	-	NT	NT
C. zedoaria Rosc.	Khaminkao	R	-	-	-	NT	NT
Languas galanga	Kaa	Fl	+++	+++	-	84.0	100
Nicolaia speiosa	Dalaa	R & Sh	-	-	-	NT	NT
Zingiber zerumbet	Kratue	R & Sh	-	-	-	NT	NT
			-			NT	NT

a Fl = flower, Fr = fruit, L = leaf, P= pods, Pis = pistil, R= root or rhizome, Se = seed, Sh = shoot, St = stem, Sta = stamina, W= whole plant
b rank of % inhibition; – IR < 30%, + IR 30-49 %, ++ IR 50-69 %, +++ IR >70%, IR = inhibition rate, NT = not tested.
c NO_2^- production inhibitory activity was evaluated by the Griess method (9)
d O_2^- inhibitiory activity was expresse by relative decreasing ratio of UV absorbance at 550 nm of test experiment to that of control.

in XA/XOD and slightly active (IR=30.4%) in O_2^- generation inhibition in differentiated HL-60 cells. On the other hand, both of the active species on O_2^- generation inhibition, *Piper betal* (Piperaceae) and *Zanthoxylum limonella* (Rutaceae) showed strong activity (IR > 80%), while both of them were completely inactive in the total activity test in XA/XOD system and IR<30% in NO generation inhibition.

The species that showed significant activity in three bioassay systems are *Languas galanga* (Zingiberaceae), *Eurycoma longifolia* (Simaroubaceae), *Oroxylum indicum* (Bignoniaceae), *Nymphaea lotus* (Nymphaeacea), *Nymphoides cristatum* (Scrophulariaceae) and *Anethum gravelolens* (Umbelliferae).

Both *Azadirachta indica* (Meliaceae) and *Limnophila aromatica* (Scrophulariaceae) were significantly active to suppress both NO and O2- generation in cellular systems but inactive in XA/XOD system.

In chemical analysis of the active compounds in *Oroxylum indicum*, we isolated two known flavonoids, oroxylin A and chrysin and a triterpene carboxylic acid, ursolic acid (Fig 2.) as the XOD inhibitor as well as O_2^- generation inhibitor in TPA-induced O_2^- generation in differentiated HL-60 cells. The XOD and O_2^- generation inhibitory activities of these compounds were shown in Table 2. Chrysin showed more potent XOD inhibition effect (IC_{50} = 7.1 μM) than oroxylin A. This result may indicate that the presence of methoxyl group at C-6 on A-ring is generally a factor to decrease the XOD inhibitory effect of flavonoids. The XOD inhibitory effect of baicalein (IC_{50} =2.79 μM), a flavonoid with hydroxyl groups at C-5, 6 and 7, has been reported to be lower than chrysin (IC_{50} = 0.84 μM) in the comparative method on the examination of xanthine oxidase inhibitors and superoxide scavengers (Cos et al., 1998). In other report, the XOD-inhibitory effect of chrysin, determined by directly measuring uric acid formation by HPLC, was reported to be higher than that of flavone itself (Nagao et al., 1999). In addition, the inhibitory effect of oroxylin A (IC_{50} > 100 μM) on O_2^- generation in HL-60 cells (Table 2) was also lower than that of chrysin (IC_{50} = 69 μM). From the above results, it may be concluded that the hydroxyl groups on A ring of flavonoids are generally a factor to increase the anti-oxidative effect, while the presence of any substitutent at C-6 could reduce the activity, although detailed structure-activity relationship studies are necessary to be performed.

Oroxylin A was reported to be a potent inhibitor of inducible nitric oxide synthase (iNOS) as well as of cyclooxygenase-2 (COX-2) in RAW 264.7 cells stimulated

Table 2. Inhibitory Activities of Compounds Isolated from *Oroxylum Indicum* toward O_2^- Generation

Compound	XOD inhibition IC_{50} (µM)	O_2^- generation inhibition in differentiated HL-60 cells IC_{50} (µM)
Oroxylin A	49	>100
Chrysine	7	69
Ursolic acid	>100	27

with lipopolysaccharide (LPS) through blocking nuclear factor-kB activation (Chen et al., 2000). It should be noted that expressions of iNOS and COX-2 are involved in inflammatory and carcinogenic events.

The activity of triterpene carboxylic acid, ursolic acid, on XOD inhibition was insignificant (IC_{50} > 100 µM), but its activity on TPA-induced O_2^- generation inhibition in HL-60 cells was moderate (IC_{50} = 27.4 µM), as compared with those of natural inhibitors, ACA (IC_{50}= 4.3 µM) and auraptene (IC50 = 1.2 mM) (Murakami et al.,1997). Anti-tumor promoting and anti-inflammatory activities of ursolic and oleanolic acids have been reported in the different *in vitro* assays (Tokuda et al., 1986; Ohigashi et al., 1986), suggesting that O_2^- generation suppressive activity may account for anti-tumor promoting and anti-inflammatory mechanisms of ursolic acid.

Those of results reported previously, together with the results in this study, may indicate that *O. indicum* is a valuable plant source for antioxidative, anti-inflammatory and anti-carcinogenic natural compounds.

Conclusion

The results in this study suggest that edible Thai plant is a promising source to find dietary antioxidants and cancer preventives.

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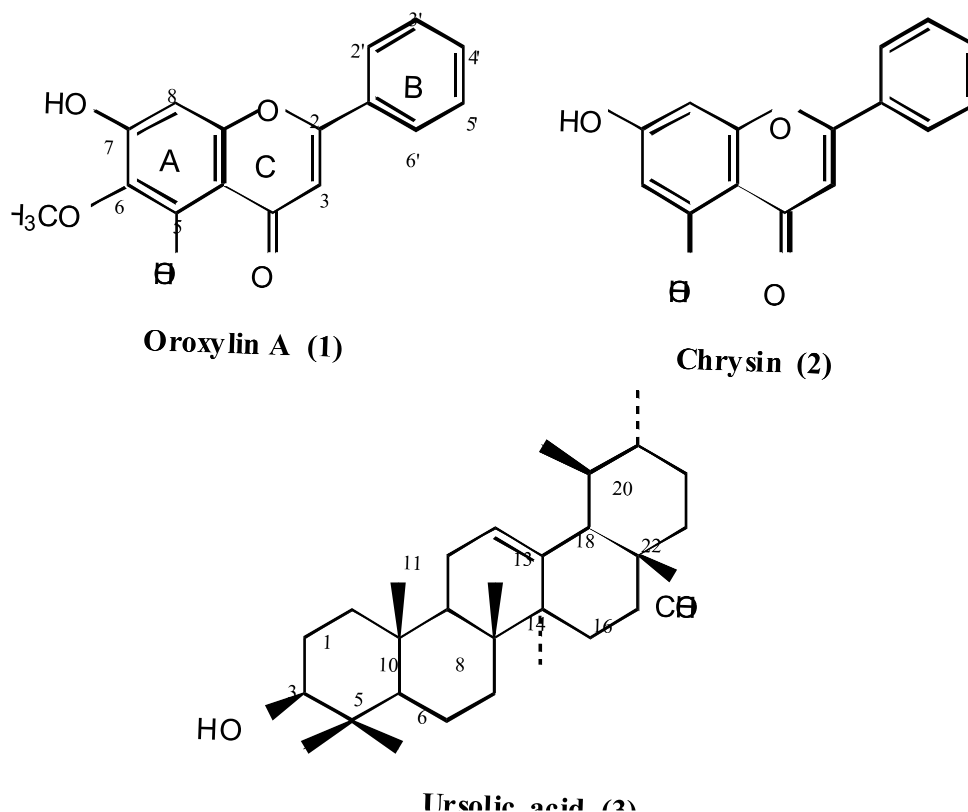

Figure 2. Chemical Structures of the Active Compounds Isolated from *Oroxylum Indicum*

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