

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 $\mu\text{g/ml}$, 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 O₂⁻ and NO generation inhibitory tests in differentiated HL-60 cells and RAW 264.7 cells, respectively.

Bioassay

XOD inhibitory and O₂⁻ scavenging assay

The XOD inhibitory and O₂⁻ 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 O₂⁻ through the conversion of XA into uric acid. In this system, the ratio of the O₂⁻ 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 O₂⁻ generation in differentiated HL-60 cells

Inhibitory test of TPA-induced O₂⁻ 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 (1x10⁶/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 O₂⁻ 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 (BH₄, 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% CO₂ atmosphere. After 24 h, the level of both NO₂⁻ 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-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (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 C₁₈ (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, CDCl₃): 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 λ_{max}^{NaOMe/MeOH}: 243sh., 268, 367; UV λ_{max}^{AlCl₃/MeOH}: 254, 282, 338, 390sh.; UV λ_{max}^{NaOAc/MeOH}: 268, 366; UV λ_{max}^{NaOAc/H₃BO₃/MeOH}: 271, 318, IR ν_{max}^{KBr} cm⁻¹: 3034, 1655, 1606; EI-MS at 70 ev [M]⁺ *m/z*: 284 (C₁₆H₁₂O₅). **2:** ¹H NMR δ (ppm) (500 MHz, CD₃OD): 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 λ_{max}^{MeOH}: 248sh, 267, 313; UV λ_{max}^{NaOMe/MeOH}: 238sh., 276, 362; UV λ_{max}^{AlCl₃/MeOH}: 251, 280, 327, 382; UV λ_{max}^{NaOAc/MeOH}: 275, 359; UV λ_{max}^{NaOAc/H₃BO₃/MeOH}: 268, 317; IR ν_{max}^{KBr} cm⁻¹: 3013, 1655, 1612; EI-MS at 70 ev [M]⁺ *m/z*: 254 (C₁₅H₁₀O₄). **3:** ¹H NMR δ (ppm) (500 MHz, CD₃OD): 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 ν_{max}^{KBr} cm⁻¹: 2900, 1700, 1450, 1030, 990; methylated of ursolic acid: EI-MS at 70 ev [M]⁺ *m/z*: 470 (C₃₁H₅₀O₃).

Results and Discussion

In the XA/XOD assay, the total inhibition of O₂⁻ generation determined by the NBT reduction assay consists of both the XOD inhibitory and O₂⁻ 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 O₂⁻ 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 O₂⁻ scavenging, and 12 species (9%) to both XOD inhibition and O₂⁻ scavenging as shown in Fig. 1b. Interestingly, all of the species in the plant family Nymphaeaceae showed significant activities at high rates (+++: 5/8 and ++: 3/8). We found that the major active compounds in the species of Nymphaeaceae 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 O₂⁻) 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 O₂⁻ generation in HL-60 cells, 6 species (24%) and 2 species (8%) showed only inhibition of NO generation and inhibition of O₂⁻ 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

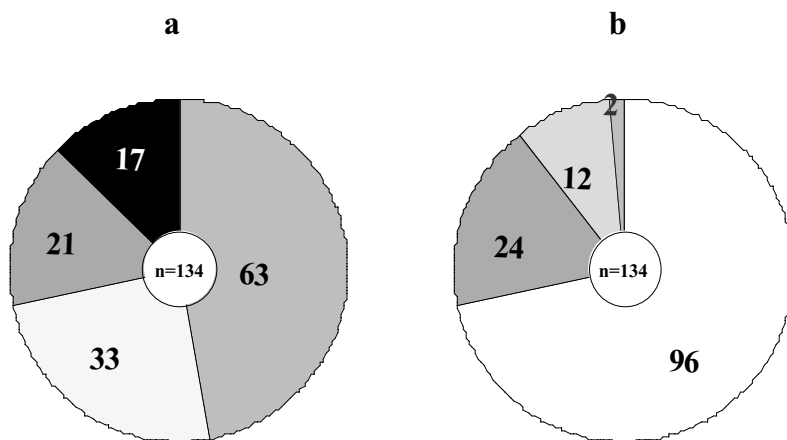


Figure 1. Activity Profiles of O₂⁻ Generation Inhibition Activity of Edible Plants in Thailand

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 + O₂⁻ scavenging ▒ O₂⁻ scavenging □ IR <50%

(Ethanol extract of sample at concentration = 500 mg/ml)

Table 1. Effect of Thai Plant-extracts on O₂⁻ generation in a XA/XOD System and on NO and O₂⁻ generation in Cellular Systems

Family/species	Thai name	Part used ^a	O ₂ ⁻ generation inhibition ^b (by XA/XOD system)			NO generation	O ₂ ⁻ generation
			Total activity	XOD inhibition	O ₂ ⁻ scavenging	RAW 264.7 cells ^c (%)	HL-60 cells ^d (%)
Acanthaceae							
<i>Andrographis paniculata</i>	Faatalai	W	-	-	-	NT	NT
<i>Asystasiella nusiana</i>	Pakkood	L & St	+	+	-	NT	NT
<i>Rhinacantus nasutus</i>	Thongpunchang	L & St	-	-	-	NT	NT
Agavaceae							
<i>Dracaena conferta</i> Ridl.	Kumlungphanuman	L & St	++	++	-	NT	NT
Aizoaceae							
<i>Glinus oppositifolius</i>	Sadaodin	L & St	-	-	-	81.0	30.4
Alliaceae							
<i>Allium tuberosum</i>	Guichai	L	+	+	-	NT	NT
<i>A. esculonicum</i>	Doakhom	S & Fl	-	-	-	NT	NT
Amaranthaceae							
<i>Amaranthus gracilis</i>	Pak khom	L	+	+	-	76.1	100
Anacardiaceae							
<i>Spondius pinnata</i>	Makok	Fr	-	-	-	NT	NT
<i>Anacardium occidentale</i>	Mamuang himaphan	L	+	+	-	NT	NT
Araceae							
<i>Colocasia esculenta</i> (L.) Schott.	Paerk	R	-	-	-	NT	NT
<i>C. esculenta</i> Hasskari	Bonn	St	-	-	-	NT	NT
<i>Lasia spinosa</i>	Paknham	Sh	-	-	-	65.0	61.8
Araliaceae							
<i>Polyscias fruticosa</i>	Lebkrud	L	-	-	-	NT	NT
Asclepiadaceae							
<i>Marsedinia glabra</i>	Paksaew	L	+	+	-	NT	NT
Bignoniaceae							
<i>Begonia prolixa</i> Craib.	Bonsom	L	-	-	-	NT	NT
<i>Oroxylum indicum</i>	Pekaa	P (young)	+++	+++	-	75.7	54.1
Bombaceae							
<i>Bombax ceiba</i>	Doak-ngew	Fl	+	+	-	NT	NT
Boraginaceae							
<i>Tournefortia ovata</i> Wall. Ex G. Don	Liang	L	-	-	-	NT	NT
Boletaceae							
<i>Thaeogyroporus porentosus</i> (berk. Et Broome) Mc. Nabb	Hedtubtoa	W	++	++	-	NT	NT
Caricaceae							
<i>Carica papaya</i>	Malakor	Fr	-	-	-	NT	NT
Chenopodiaceae							
<i>Basella rubra</i>	Pakplang	L	-	-	-	NT	NT
<i>Spinacia oleracea</i>	Pak puaylaeng	L	-	-	-	NT	NT
Compositae							
<i>Artemisia lactiflora</i>	Doak kaew muang jeen	L	-	-	-	NT	NT
<i>A. vulgaris</i>	Koadchula	Se	+++	+++	-	NT	NT
<i>Cartamus tinctorius</i>	Kumphoi	Fl	++	++	-	NT	NT
<i>Chrysanthemum coronarium</i>	Tang-oh	L	-	-	-	78.0	46.3
<i>Gynura crepidoides</i>	Mohnoi	L	-	-	-	NT	NT
<i>Lactuca sativa</i> Linn.	Pakkardhom	L	+	+	-	NT	NT
<i>Spilanthes iabadicencis</i>	Pakphed	L	-	-	-	NT	NT
<i>S. paniculata</i>	Pakkradhuawaen	L, S & Fl	-	-	-	NT	NT
Cruciferae							
<i>Brassica chinensis</i>	Pak kwangtung	L	+	+	-	NT	NT
<i>B. pekinensis</i> Rupr.	Pakkardkao	L	+	+	-	NT	NT
<i>Rhaphanus sativus</i> cv. Group Rap-tailed radish	Pakkeehud	P (young)	+	+	-	NT	NT
Cucurbitaceae							
<i>Citrullus lanatus</i> Thunb.	Taengmoo	Fr (young)	+	+	+	NT	NT

Family/species	Thai name	Part used ^a	O ₂ ⁻ generation inhibition ^b (by XA/XOD system)			NO generation	O ₂ ⁻ generation	
			Total activity	XOD inhibition	O ₂ ⁻ scavenging	inhibitor in RAW 264.7 cells ^c (%)	inhibitor in HL-60 cells ^d (%)	
<i>Coccinia glandis</i> Voigt	Tumlueng	L	-	-	-	NT	NT	
<i>Cucumis sativus</i> Linn.	Tang gua	L	+	+	-	NT	NT	
<i>Cucurbita moschata</i>	Fugthong	L	+	+	+	NT	NT	
<i>C. pepo</i>	Zucchini	Fr	+	+	-	NT	NT	
<i>Lagenaria siceraria</i> (Molina) Standl.	Numtao	Fr	-	-	-	NT	NT	
<i>Marmodica charantia</i>	Marakeenok	Fr	+	-	+	58.6	94.4	
<i>Schium edule</i> Sw.	Fugmaew	Fr	+	-	+	NT	NT	
Ebenaceae								
<i>Dioapyros decandra</i> Lour	Lukchan	Fr	-	-	-	NT	NT	
Euphobiaceae								
<i>Sauropus androgynus</i>	Pakwanban	L	-	-	-	NT	NT	
<i>Phyllanthus amarus</i> Schum & Thonn	Luktaibai	W	+++	-	++	NT	NT	
Gramineae								
<i>Cymbopogon winterianus</i>	Takraihom	St	-	-	-	NT	NT	
<i>Zea mays</i> Linn.	Kaopoad	Se	-	-	-	NT	NT	
Guttiferae								
<i>Garcinia mangostana</i> Linn	Mungkud	Fr	-	-	-	NT	NT	
Hydrocharitaceae								
<i>Otellia alesmoides</i> Pers.	Santawaa	L	+	+	-	NT	NT	
Labiatae								
<i>Mentha cordifolia</i>	Saranae	L	+	+	-	NT	NT	
<i>Ocimum basilicum</i>	Horapaa	L	+	+	-	56.7	81.7	
<i>O.gratissimum</i>	Yeerha	L	-	-	-	30.9	36.1	
<i>O. sanctum</i>	Kraprao	L	+	+	-	31.5	48.1	
Lamnaceae								
<i>Walffia globosa</i> Harlog & Plas	Phum	W	++	++	-	NT	NT	
Lauraceae								
<i>Litsea elliptica</i> Bearn.	Thammung	L	-	-	-	NT	NT	
Leguminosae								
<i>Acacia rugata</i> Merr.	Sompoi	L	-	-	-	NT	NT	
<i>Cassia siamea</i> Britt	Keeleak	L	-	-	-	NT	NT	
<i>Leuceana leucocephala</i>	Krathin	L	-	-	-	NT	NT	
<i>Perkia spiciosa</i>	Sa tor	P	-	-	-	NT	NT	
<i>Pisum sativum</i>	Tualantao	P	-	-	-	70.5	20.0	
<i>Pithecellobium dulce</i> (Roxb) Benth.	Makhamthed	P	+	+	-	NT	NT	
<i>Sesbania glandiflora</i>	Kae	Fl	-	-	-	63.0	98.9	
Liliaceae								
<i>Aspidistra sutepensis</i>	Doak nang laew	Fl	+	+	-	NT	NT	
Malvaceae								
<i>Hibiscus sabdarifla</i>	Krajiebdang	Fl	+++	+++	-	NT	NT	
Marsiliaceae								
<i>Marsilia crenata</i> Presl.	Pakwaen	L	+	+	-	NT	NT	
Meliaceae								
<i>Azadirachta indica</i>	Sadao	Sh	-	-	-	78.6	88.1	
Minispermaceae								
<i>Tiliacora triandra</i> Diels	Bai ya nang	L	-	-	-	NT	NT	
Moraceae								
<i>Artocarpus heterophyllus</i>	Kanun	Fr	-	-	-	NT	NT	
<i>Broussonetia kurzii</i> Corner.	Salae	Fl	++	++	-	NT	NT	
<i>Streblus asper</i> Lour	Koi	L	+	+	-	NT	NT	
Musaceae								
<i>Musa</i> (AA group) 'Kluai Khai'	Kluai Khai	Fr	++	+	+	NT	NT	
<i>Musa</i> (ABB group) 'Kluai Nam Wa'	Hoaplee	Fl	+	+	-	NT	NT	
<i>Musa</i> (ABB group) 'Kluai Nam Wa'	Kluai Nam Wa	Fr	++	+	+	NT	NT	
Myrtaceae								
<i>Eugenia grata</i>	Pakmeg	Sh	-	-	-	NT	NT	
<i>E. ridleyi</i> King	Baimug	L	+++	+++	-	NT	NT	
<i>Psidium eujavillus</i>	Farang	Fr	+	+	-	NT	NT	

Family/species	Thai name	Part used ^a	O ₂ generation inhibition ^b (by XA/XOD system)			NO generation	O ₂ generation
			Total activity	XOD inhibition	O ₂ ⁻ scavenging	inhibitor in RAW 264.7 cells ^c (%)	inhibitor in HL-60 cells ^d (%)
Nymphaeaceae							
<i>Nelumbo nucifera</i> “Roseum Plenum”	Bualuangdaeng	Sta & Pis	++	+	+	NT	NT
<i>N. nucifera</i> Gaertn	Bualuangdaeng (east indian lotus)	Sta & Pis	++	+	+	NT	NT
<i>N. nucifera</i> Gaertn.	Bualuangkao (Hindu lotus)	Sta & Pis	+++	++	+	NT	NT
<i>N. nucifera</i> “Album Plenum”	Bualuangkao	Sta & Pis	+++	++	+	NT	NT
<i>Nymphaea capensis</i>	Buaphan	St	+++	++	+	NT	NT
<i>N. lotus</i>	Buasaikao	St	+++	++	+	63.0	64.8
<i>N. rubra</i>	Buasaidaeng	St	+++	++	+	NT	NT
<i>N. stellata</i>	Buapheain	St	++	+	+	NT	NT
Palmae							
<i>Caryota urens</i>	Tao rung	Sh	++	++	+	NT	NT
<i>Salacca rumphii</i> Wall.	Ragum	Fr	++	++	-	NT	NT
<i>Trevisia palmata</i>	Doak tang	Fl	-	-	-	62.4	24.3
Peperomiaceae							
<i>Peperomia pellucida</i>	Pakkrasung	L	+	+	-	NT	NT
Piperaceae							
<i>Piper betel</i>	Pluu	L	-	-	-	14.8	81.4
<i>P. nigrum</i>	Prikthaisod	Fr	-	-	-	NT	NT
<i>P. samantosum</i>	Baichapluu	L	-	-	-	NT	NT
Plantaginaceae							
<i>Plantago psyllium</i> Linn.	Tein kred hoi	Se	-	-	-	NT	NT
Polygonaceae							
<i>Polygonum odoratum</i>	Pak pai	L	-	-	-	NT	NT
<i>Rheum officinale</i> Baillon	Koad num tao	Se	+++	+++	-	NT	NT
Ranunculaceae							
<i>Nigella sativa</i>	Teindaeng	Se	-	-	-	NT	NT
Rubiaceae							
<i>Ardisia lenticellata</i> Fletch	Taped tagai	L & St	+++	+	+	NT	NT
<i>Lasianthus virgatus</i> Craib.	Phahoam	L	+	+	-	NT	NT
Rutaceae							
<i>Citrus aurantifolia</i>	Manao	Fr	-	-	-	59.1	78.7
<i>C. hystrix</i>	Makrood	L & Fr	-	-	-	NT	NT
<i>Zanthoxylum limonella</i>	Ma kwaen	Fr	-	-	-	23.4	89.4
Sapotaceae							
<i>Manikara kauki</i>	Lamud	Fr	-	-	-	NT	NT
Scrophulariaceae							
<i>Limnophila aromatica</i>	Pakkayang	L	-	-	-	70.0	100
<i>Nymphoides cristatum</i>	Paktubtao	L	++	++	-	75.2	84.7
<i>Scroperia dulcis</i> Linn.	Pakkrodnum	L	++	-	++	NT	NT
Selaginellaceae							
<i>Selaginella involuta</i> Spreng	Po ka tee mia	Sh	++	++	-	NT	NT
Simaroubaceae							
<i>Eurycoma longifolia</i>	Plalaiphaek	L	+++	+++	-	74.8	99.3
Smilacaceae							
<i>Smilax corburalia</i> Kunth	Kaoyennaey	R	+++	+++	-	NT	NT
<i>Smilax sp.</i>	Kaoyentai	R	++	++	-	NT	NT
Solanaceae							
<i>Capsicum annum</i> Linn	Prikcheefah	Fr	-	-	+	NT	NT
<i>Capsicum frutescens</i> Linn.	Prikkeenu	Fr	-	-	-	NT	NT
<i>Solanum aculeatissima</i>	Makuey leung	Fr	-	-	-	NT	NT
<i>S. mammosum</i>	Makuey nomling	Fr	+	+	-	NT	NT
<i>S. melongena</i>	Makuey muong	Fr	++	++	-	NT	NT
<i>S. melongena</i>	Makuey-yao	Fr	-	-	-	NT	NT
<i>S. sp.</i>	Makuey kunnai	Fr	+	+	-	NT	NT
<i>S. stramonifolium</i> Jacq.	Ma eok	Fr	-	-	-	NT	NT
<i>S. torvum</i>	Makuey phuong	Fr	-	-	-	NT	NT

Family/species	Thai name	Part used ^a	O ₂ ⁻ generation inhibition ^b (by XA/XOD system)		NO generation inhibition ^b in RAW 264.7 cells ^c		O ₂ ⁻ generation inhibition ^b in HL-60 cells ^d	
			Total activity	XOD inhibition	O ₂ ⁻ scavenging (%)	NO generation inhibition (%)	O ₂ ⁻ generation inhibition (%)	
Sterculiaceae								
<i>Abroma augusta</i> Linn.	Teindum	Se	++	++	-	NT	NT	
Stilaginaceae								
<i>Antidesma matabanicum</i>	Sommao	Sh	++	++	-	NT	NT	
Thunbergiaceae								
<i>Thunbergia laurifolia</i> Lindl.	Rangjurd	L	++	++	-	NT	NT	
Umbelliferae								
<i>Anethum graveolens</i>	Pakcheelao	W	++	++	-	69.5	75.7	
<i>Centella asiatica</i>	Baibuabok	L	-	-	-	64.3	NT	
<i>Conioselinum univilatum</i>	Koadhoabua	St	+++	+++	-	NT	NT	
<i>Eryngium foetidum</i>	Pakcheefarang	L	+	+	-	78.1	4.9	
<i>Foeniculum vulgare</i> Mill	Teinkraeb	Se	+	+	-	NT	NT	
<i>Ornanthe stolonifera</i>	Pakcheelom	L	-	-	-	NT	NT	
Verbenaceae								
<i>Clerodendrum paniculatum</i>	Nomwan	L	+	+	-	NT	NT	
Zingiberaceae								
<i>Amomum utriculosum</i>	Raewhoam	L	-	-	-	NT	NT	
<i>Boesenbergia pandurata</i>	Krachai	R	-	-	-	26.2	10.8	
<i>Curcuma aromatica</i> Salisb.	Nangdum	L	-	-	-	NT	NT	
<i>C. domestica</i>	Khaminchun	R	+++	+++	-	NT	NT	
<i>C. zedoaria</i> Rosc.	Khaminkao	R	-	-	-	NT	NT	
<i>Languas galanga</i>	Kaa	Fl	+++	+++	-	84.0	100	
<i>Nicolaia speiosa</i>	Dalaa	R & Sh	-	-	-	NT	NT	
<i>Zingiber zerumbet</i>	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 NO₂⁻ production inhibitory activity was evaluated by the Griess method (9)

d O₂⁻ inhibitory activity was expressed 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 O₂⁻ generation inhibition in differentiated HL-60 cells. On the other hand, both of the active species on O₂⁻ generation inhibition, *Piper betel* (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* (Nymphaeaceae), *Nymphoides cristatum* (Scrophulariaceae) and *Anethum graveolens* (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 O₂⁻ generation inhibitor in TPA-induced O₂⁻ generation in differentiated HL-60 cells. The XOD and O₂⁻ generation inhibitory activities of these compounds were shown in Table

2. Chrysin showed more potent XOD inhibition effect (IC₅₀ = 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 (IC₅₀ = 2.79 μM), a flavonoid with hydroxyl groups at C-5, 6 and 7, has been reported to be lower than chrysin (IC₅₀ = 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 (IC₅₀ > 100 μM) on O₂⁻ generation in HL-60 cells (Table 2) was also lower than that of chrysin (IC₅₀ = 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 substituent at C-6 could reduce the activity, although detailed structure-activity relationship studies are necessary to be performed.

Oroxylum 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} (μ M)	O_2^- generation inhibition in differentiated HL-60 cells IC_{50} (μ M)
Oroxylin A	49	>100
Chrysin	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 ($IC_{50} > 100 \mu$ M), but its activity on TPA-induced O_2^- generation inhibition in HL-60 cells was moderate ($IC_{50} = 27.4 \mu$ M), as compared with those of natural inhibitors, ACA ($IC_{50} = 4.3 \mu$ M) and auraptene ($IC_{50} = 1.2 \text{ mM}$) (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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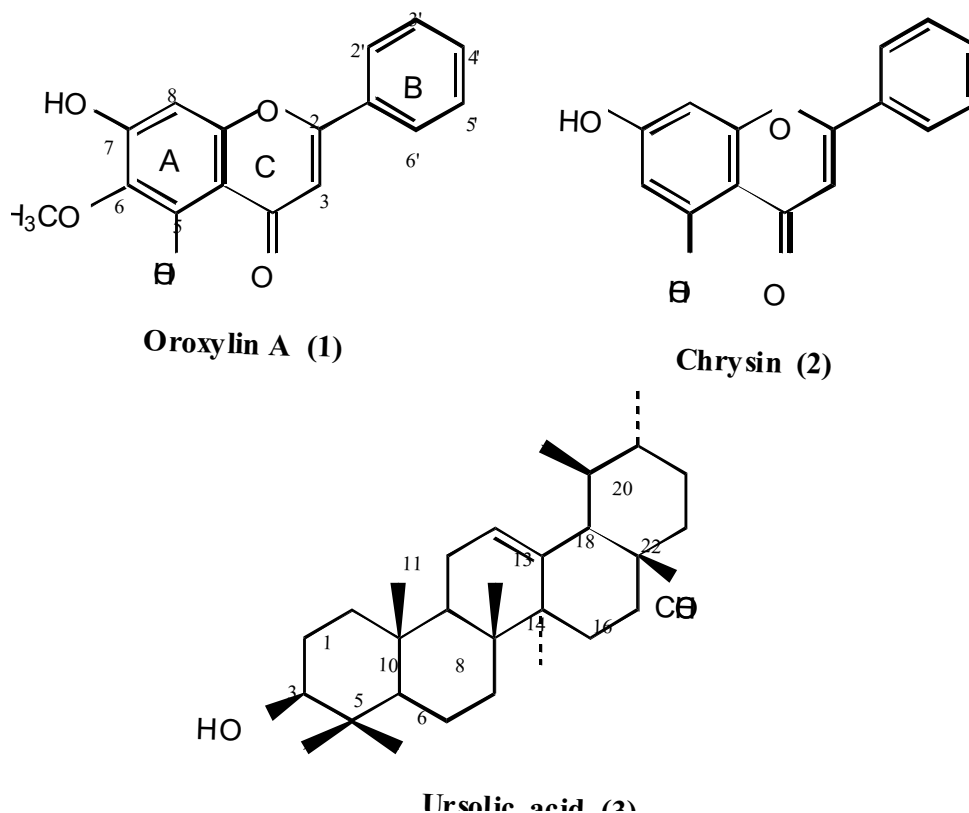


Figure 2. Chemical Structures of the Active Compounds Isolated from *Oroxylum Indicum*

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