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Nitric oxide and ProstaglandinE_2 Synthesis Inhibitory Activities of Flavonoids from the Barks of *Ulmus macrocarpa*

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Abstract – Eight phenolic compounds (**1-8**) which were isolated from the barks of *Ulmus macrocarpa* were evaluated for their inhibitory activities on nitric oxide (NO) and prostagrandin E_2 (COX-2) production in interferon-γ (INF-γ) and lipopolysaccharide (LPS)-activated RAW 264.7 cells *in vitro*. NO and COX-2 levels were moderately reduced by the addition of compounds (**1-8**). Among them, **3, 4, 5, 6, 7** and **8** inhibited NO production in a dose dependent manner with an IC_{50} of 92.2, 97.3, 36.1, 43.5, 32.8, 39.4 and 37.1 μg/ml, respectively (positive control, L-NMMA; 36.4 μg/ml), and **3, 4, 5, 6, 7** and **8** reduced the COX-2 level in a dose dependent manner with an IC_{50} of 43.2, 24.8, 24.8, 33.4, 44.8 and 22.7 μg/ml, respectively (positive control, indomethacin; 23.4 μg/ml). These results suggest that the phenolic compounds may be developed as potential anti-inflammatory and cancer chemopreventive agents.

Keywords – *Ulmus marcroparca*, Ulmaceae, flavanone, flavan 3-ol, Nitric oxide, cyclooxygenase-2, anti-inflammation, cancer chemoprevention

Introduction

The barks of *Ulmus macrocarpa* (Ulmaceae) have been used for the treatment of intestinal parasite and scabies (Bensky D *et al.*, 2004) in oriental traditional medicine. Anti-ulcer (Shibutani *et al.*, 1983) anti-oxidative (Costanitino *et al.*, 1992), anti-fungal (Serit *et al.*, 1991), anti-cancer (Boukharta *et al.*, 1992; Kashiwada *et al.*, 1992; Miyamoto *et al.*, 1993), of this plant were reported. We reported phenolic compounds from the barks of *Ulmus macrocarpa* and its anti-oxidative activities (Kwon *et al.*, 2002).

Nitric oxide (NO) radical play important biological roles in physiological systems like vasodilation, neuro-transmission and platelet aggregation as well as in pathophysiological systems such as acute and chronic inflammation. In addition, the over production of NO is also implicated in the pathogenesis of cancer (Moncada S *et al.*, 1991). Cyclooxygenase-2 (COX-2) is regarded as an inducible enzyme that is responsible for prostaglandin biosynthesis in the inflammatory process as well as in malignant or transformed cells (Subbaramiah *et al.*, 1996). Therefore, compounds that regulate the NO and COX-2 activities might provide a target for the development of new anti-inflammatory (Je *et al.*, 2004; Rie *et al.*, 1999) and cancer chemopreventive (Jang *et al.*, 2003) agents.

As a continuous studies on new anti-inflammatory and anti-tumor promoting agents from natural sources, we investigated the NO and COX-2 inhibitory activities on the phenolic compounds which were isolated from the barks of *U. macrocarpa* (Kwon *et al.*, 2002).

Materials and Method

Materials – Two flavanols, taxifolin 7-O-β-D-glucopyranoside (**1**), taxifolin 3'-O-β-D-glucopyranoside (**2**), two flavanones, eriodictyol 7-O-β-D-glucopyranoside (**3**), nalingenin 7-O-β-D-glucopyranoside (**4**), three flavan 3-ols, (-)-epicatechin (**5**), (+)-catechin (**6**), (+)-catechin 7-O-β-D-glucopyranoside (**7**) and one proanthocyanidin, procyanidin B-1 (**8**) were isolated from the 80% acetone extract of the fresh bark from *U. macroparca* and were used in this experiment (Fig. 1).

Biological Assay

Cell culture – Raw 264.7 cells were purchased from the Korean Cell Line Bank. The cells were grown at 37°C in a humidified atmosphere (5% CO_2) in a DMEM medium containing 10% fetal bovine serum.

MTT assay – The cytotoxicity was measured by the

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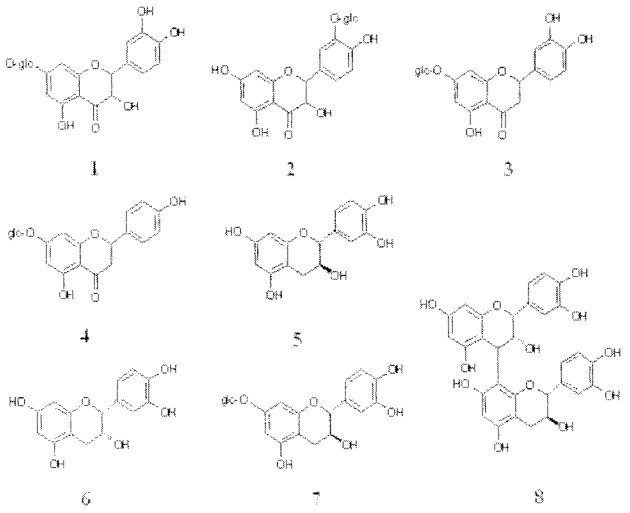

Fig. 1. Structures of compounds **1-8**

motochondrial-dependent reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] to formazan (Mosmann, 1983). The cells were seeded at a density 1×10^6 cells/ml in 96 well-plates. After incubating for 2 h, the cells were treated with the samples. The cells were incubated for an additional 24 h, and the medium was replaced with fresh medium. The medium contained MTT (final concentration: 0.5 mg/ml), and the incubation continued for a further 1 h at 37°C. The medium was then removed and the MTT-formazan produced was dissolved in 200 μl DMSO. The extent of the reduction of MTT to formazan within the cells was quantified by measuring the absorbance at 570 nm using an ELISA reader.

Nitrite assay – Raw 264.7 macrophage cells were cultured in a 24-well plate and preincubated for 2 h at 37°C in a humidified atmosphere (5% CO_2). The cells were then incubated in a medium containing 10 μl LPS, IFN-and the test samples. After incubating for an additional 24 h, the media were removed and analyzed for the level of nitrite accumulation, as an indicator of NO, using the supernatant by a Griess assay. The Griess reagent (0.1% naphthylethylenediamine and 1% sulfanilamide in 5% H_3PO_4 solution, 100 μl) was added to 100 μl of each of the supernatants from the cells treated with the samples. The samples were then read at 540 nm against a standard sodium nitrite curve. The amount of nitrite in the samples was calculated from a sodium nitrite standard curve.

COX-2 enzyme assay – Raw 264.7 macrophages were plated in a 24-well plate and preincubated for 24 h at 37°C in a humidified atmosphere (5% CO_2). The cells were preincubated for 2 h, and 10 μl LPS, IFN-γ was then added. The COX-2 enzyme was then induced for 16 h. After the cells had been washed by the medium, they were added to 170 μl of fresh medium, 20 μl of the test sample and incubated for 15 min. The cells were added to 10 μl arachidonic acid (600 μM) and incubated for 40 min. The inhibitory effects of the test samples upon COX-2 were then determined by a PGE_2 assay.

Table 1. Effects of compounds 1-8 on the LPS, INF--induced NO and COX-2 production in the Raw 264.7 marcrophages.

Compounds	NO	COX-2
	IC_{50} (μl/ml)	IC_{50} (μl/ml)
1	None	None
2	None	None
3	92.2	43.2
4	97.3	24.8
5	36.1	24.8
6	43.5	33.4
7	32.8	44.8
8	37.1	22.7
L-NMMA	**36.4**	-
Indomethacin	-	**23.4**

Results and Discussion

The phenolic compounds were isolated from the bark from *U. macroparca* previously (Kwon *et al.*, 2002) and their inhibitory activities of the isolated phenolic compounds (**1-8**) against NO and COX-2 were evaluated.

The MTT assay showed that- all compounds (**1-8**) did not cause cell cytotoxicity in the treatment ranges of the compounds, 0-100 μg/ml.

The NO levels were moderately reduced as a result of the addition of the compounds (**1-8**) to the RAW 264.7 cell stimulated by- LPS, IFN-γ. Compounds **3, 4, 5, 6, 7** and **8** were found to inhibit NO production in a dose dependent manner with an IC_{50} of 92.2, 97.3, 36.1, 43.5, 32.8, 39.4 and 37.1 μg/ml, respectively. As a positive control, L-NMMA (NO synthesis inhibitory agent) showed significant inhibition with an IC_{50} value of 36.4 μg/ml. The viability of the RAW 264.7 cells were not altered and the NO production level was similar the presence or absence of compounds **3, 4, 5, 6, 7** and **8**, as determined by the MTT assay.

The COX-2 levels were also moderately reduced by the addition of the phenolic compounds (**1-8**). Among them, compounds **3, 4, 5, 6, 7** and **8** were found to inhibit the COX-2 level in a dose dependent manner with an IC_{50} value of 43.2, 24.8, 24.8, 33.4, 44.8 and 22.7 μg/ml

respectively. As a positive control, indomethacin significantly inhibited the COX-2 level with an IC_{50} value of 23.4 μg/ml.

An analysis of the structure and activity relationship among these phenolic compounds, flavanone (**3** and **4**), flavan 3-ol (**5**, **6**, and **7**) and proanthocyanidin (**8**) exhibited potent activities on inhibiting of NO and COX-2 production.

These results suggest that the phenolic compounds isolated from the bark of *U. marcroparca* are potential anti-inflammatory and cancer chemopreventive agents and *U. macrocarpa* is a rich source of these phenolic compounds.

References

Bensky D., Clavey S., Stoger E. and Gamble A., Chinese Herbal Medicine-Materia Medica, Vol.3., *Eastland Press.*, pp. 1004 (2004).

Boukharata, M., Jalbert, G. and Castonguay, A., Biodistribution of ellagic acid dose-related inhibition of lung tumorigenesis in A/J mice., *Nutr. Cancer.*, **18**, 181-189 (1992).

Costantino, L., Albasini, A., Rastelli, G. and Benvenuti, S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med.*, **58**, 342-344 (1992).

Jang D. S., Muriel C., Alison D. P., Leonardus B. S. K., Kazuko K., Norman R. F., Harry H. S. F., John M. P., and A. Douglas K., Potential cancer chemopreventive constituents of the leaves of *Macaranga triloba*. *Phytochemistry*, **65**, 345-350 (2004)

Je K. H., Jan A. R., Lee H. T., Mar W. C. and Seo E. K., The inhibitory Principle of lipopolysaccharide-Induced Nitric Oxide Production from *Inula britannica* var. *chinensis*, *Arch. Pharm. Res.*, **27**, 83-85 (2004).

Kashiwada, Y., Nonaka, G., Nishioka, I., Chang, J. J. and Lee, K. H., Antitumer agents, 129. Tannins and related compounds as elective cytotoxic agents, *J. Nat. Prod.*, **55**, 1033-1043 (1992).

Kwon, Y. M., Lee, J. H. and Lee, M. W., Phenolic compounds from Barks of *Ulmus macrocarpa* and Its Antioxidative Activities., *Kor. J. Pharmacogn.*, **33**, 404-410 (2002).

Miyamoto, L., Nomura, M., Murayama, T., Furukawa, T., Hatano, T., Yoshida, T., Koshiura, R. and Okuda, T., Antitumor activities ellagitannins against sarcoma-180 in mice., *Biol. Pharm. Bull.*, **16**, 279-287 (1993).

Moncada S., Palmer R. M., Higgs E. A., Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacological Reviews.*, **43**, 109-42 (1991).

Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. *J.Iimmunol. Methods.* **65**, 55-63 (1983).

Rie I., Koichi S., Masakazu H., Takeshi S., Susumu K., and Fumio A., Inhibitory Effects of Hydrolyzable Tannins from *Melastoma dodecandrum* Lour. On Nitric Oxide Production by a Murine Macrophage-Like cell Line, RAW 264.7, Activated with Lipopolysaccharide and Interferon-γ. *Biol. Pharm. Bull.*, **22**, 647-653 (1999)

Serit, M., Okubo, T., Hagiwara, N., Kim M., Nonaka, G., Nishioka, I. and Yamamoto, T., Comparative Antibacterial Activity of Quercitol Gallates, *Agric. Biol. Chem.*, **55**, 1893-1894 (1991).

Shibutani, S., Nagasawa, T., Oura H., Nonaka, G. and Nishioka, I., Mechanism of Rhei Rhizoma in Rat. *Chem. Pharm, Bull.*, **31**, 2378-2385 (1983)

Subbaramiah K., Telang N., Ramonetti J. T., Araki T., DeVito B., Weksler B. B. and Dannenberg A. J., Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res.*, **56**, 4424-4429 (1996).

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