Natural Product Sciences
5(4) : 165~171 (1999)

Evaluation of the Antioxidant Potential of Natural Products Mediated by Inhibition of Xanthine Oxidase Activity

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Abstract – Since reactive oxygen species (ROS) play an important role in carcinogenesis and other several human diseases, antioxidants present in consumable fruits, vegetables, and beverages have received considerable attention as cancer chemopreventive agents. Thus, in order to identify antioxidants in plant extracts, potential activity was assessed by determining with inhibition of a xanthine/xanthine oxidase assay system. Approximately 170 plant extracts of Korean herbal medicines were primarily evaluated for the potential of antioxidant activity. As a result, 13 plant extracts were found to be active (IC_{50} < 100 μg/ml). Especially, *Juncus effusus*, *Selaginella tamariscina*, *Pueraria thunbergiana* and *Sedum albroseum* showed strong inhibitory activity in this process. Further studies for the identification of active principles from these active lead plant extracts might be warranted.
Key words – Antioxidants, Natural Products, Xanthine Oxidase

Introduction

Xanthine oxidase (XO) catalyzes the conversion of hypoxanthine to xanthine and then uric acid as a final product in the presence of molecular oxygen to yield superoxide anion (McCord and Fridovich, 1968). It has been reported that xanthine oxidase serum levels are increased in acute liver injury or liver damage and also significantly increased in brain tumor tissues compared to normal brain tissues (Kokoglu *et al.*, 1990). Elevation of xanthine oxidase activity during carcinogenesis including the promotional phase was also reported (Pence and Reiners, 1987). The enzyme also induces gout through the formation of uric acid and causes oxidative damage of tissue in the living body through generation of the superoxide anion radical. Several natural products including phenolic compounds have been reported to inhibit this enzyme, and it is expected that xanthine oxidase inhibitors could prove useful for the treatment of hepatitis, edema, or brain tumor. Allopurinol, first synthesized as a potential antigout agent, is widely used in the treatment of gout which is caused by the deposition of uric acid in the joints. Quercetin, hesperidin, genistein and catechin have been known to act as xanthine oxidase inhibitors and are also reported to have cancer chemopreventive activity (Klaunig, 1992; Tanaka, 1994; Wei *et al.*, 1995). Therefore, the inhibition of superoxide anion production in the xanthine/xanthine oxidase system is due to both scavenger activity and inhibition of the enzymes. Thus, compounds capable of both inhibiting xanthine oxidase and of scavenging superoxide anion (O_2^-) may be useful as antioxidant agents. Based on these considerations, for the procurement of antioxidants from natural products, approximately 170 methanolic extracts of Korean herbal medicines were primarily evaluated using xanthine oxidase inhibition assay.

Experimental

Chemicals – Xanthine, xanthine oxidase, allopurinol, chlorogenic acid and caffeic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from commercial sources and were of the highest purity available.

Plant materials – Medicinal plants were purchased from herbal markets (Han-Yang Yutong) in Seoul, Korea. The botanical identification was performed by Drs. Ihn-Ran Lee and Jung-Ae Do at College of Pharmacy, Ewha Womans Univeristy. The voucher specimen has been deposited in the College of Pharmacy at Ewha Womans Univeristy, Seoul, Korea. Each of the dried herbs was sliced, and

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extracted with 100% methanol. The methanol extracts were concentrated under reduced pressure below 40°C.

Assay for inhibition of xanthine/xanthine oxidase activity – Xanthine solution (100 μM) (980 μl) in sodium phosphate buffer, pH 7.8, with 10 μl of xanthine oxidase solution (0.04 units) and 10 μl of DMSO were incubated for 5 min at room temperature, and the formation of uric acid was measured at 295 nm against a blank sample which did not contain the enzyme, but 10 μl of 0.1 M phosphate buffer solution (pH 7.8) instead. Optical density (O.D.) was recorded for 5 min and the tests were performed in duplicate. Various concentrations of each test sample (10 μl dissolved in DMSO) were added to xanthine buffer solution (980 μl) and phosphate buffer solution (10 μl) as blank tests. Enzyme solution (0.04 units) was added to each 10 μl of various concentrations of test samples in 980 μl of xanthine buffer solution and treated in the same manner as the control. Inhibitory effects on xanthine oxidase activity were measured by a decrease in uric acid formation. The IC_{50} values were calculated from percent inhibition of enzyme activity (Lee *et al.*, 1998).

Results and Discussion

Oxygen free radicals, or reactive oxygen species (ROS), are continuously generated by most cells and may be involved in several disease processes by damaging cellular molecules and structures (Fischer-Nielsen *et al.*, 1994). In addition, ROS are indeed a relevant class of carcinogens and can act at several stages in malignant transformation (Cerutti and Trump, 1991). ROS induce permanent DNA sequence changes in the form of point mutations, deletions, gene amplification and rearrangements which may result in the activation or protooncogenes or the inactivation of tumor suppressor genes (Hsie *et al.*, 1986; Moraes *et al.*, 1990). Further, recent developments have stimulated a growing interest in the possible role of free oxygen radicals in tumor promotion (Crawford *et al.*, 1989; Trush and Kensler, 1991). Some evidence suggests that tumor promoters such as phorbol esters may enhance tumor formation by stimulating superoxide anion production and the degree of stimulation of ROS by various phorbol esters correlates with their promoting activity in mouse skin (Troll and Wiesner, 1985). In recent years, natural product antioxidants present in consumable fruits, vegetables, and beverages have been shown to mediate cancer chemopreventive activity. Thus, as a part of search for novel cancer chemopreventive agents from natural products, we have initiated a program to discover and identify antioxidants from plants. Accordingly, the potential of antioxidant activity of plant extracts has been assessed based on inhibitory activity with a xanthine/xanthine oxidase assay. Using this assay system, superoxide anion (O_2^-)-scavenging capacity and inhibition of xanthine oxidase were studied by measuring the formation of uric acid at 295 nm (Chang *et al.*, 1994; Chan *et al.*, 1995; Cotelle *et al.*, 1996). As shown in Table 1, allopurinol, phenolic compounds such as caffeic acid, chlorogenic acid exhibited potent inhibitory effects on this enzyme-based antioxidant activity assay. Indeed, phenolic compounds such as caffeic acid, chlorogenic acid and ferulic acid, which were previously shown inhibitory effects on xanthine oxidase activity, have been reported to have an inhibitory effect on tumor formation in mouse skin caused by TPA with an increase in the release of ROS (Huang *et al.*, 1988). It has also been reported that ROS and other free radicals play an important role in tumor promotion (Slaga *et al.*, 1983). Thus, xanthine oxidase inhibitors should be considered as potential antioxidants and thus tumor promotion inhibitors. Based on the usefulness of this assay system, active principles have been monitored from plant extracts in this study. As a result, tested with approximately 170 methanolic plant extracts of Korean herbal medicines, thirteen extracts showed potent inhibition activity, judged by $IC_{50} < 100$ μg/ml. Especially, *Juncus effusus*, *Selaginella tamariscina*, *Pueraria thunbergiana* and *Sedum albroseum* showed strong inhibitory activity in this process ($IC_{50} < 50$ μg/ml) (Table 1). Several reports have been shown potential antioxidant activity of plant extracts using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (Choi *et al.*, 1993, Cha *et al.*, 1997). In addition, the methanolic extract of *Salvia miltiorrhiza* and the isolated principles showed antioxidant activity with DPPH free radical scavenging potential. In this study, however, the extract of *Salvia miltiorriza* did not show potent activity (Kang *et al.*, 1997). It might be dependent on action mechanism of antioxidant effect within assay systems. Therefore, the present results will extend the procurement of antioxidants from Korean herbal medicines and further elucidation of active principles from these active leads might be warranted.

Table 1. Inhibitory activity of xanthine oxidase by natural products

Plant name and Authority	Family	Part used[a]	IC_{50} (μg/ml)[b]
Acanthopanax sessiliflorum Seeman.	Araliaceae	BK	> 100
Achyranthes japonica Nakai.	Amaranthaceae	RT	> 100
Aconitum koreanum Raymond	Ranunculaceae	TB	> 100
Aconitum pseudolaeve var. *erectum* Nakai	Ranunculaceae	RT	> 100
Adenophora remotiflora Mig.	Campanulaceae	RT	> 100
Agrimonia pilosa Ledeb var. *japonica* Nakai..	Rosaceae	HR	> 100
Ailanthus altissima (Mill) Swingle.	Simarubeae	SB	> 100
Akebia quinata Dence.	Lardizabalaceae	ST	> 100
Albizzia julibrissin Durazz.	Leguminosae	CR	> 100
Alpinia katsumadai Hayata.	Zingiberaceae	SD	> 100
Amomum cardamomum L.	Zingiberaceae	FR	> 100
Amomum xanthioides Wall.	Zingiberaceae	FR	> 100
Ampelopsis japonica (Thunb.) Makino.	Vitaceae	RT	> 100
Angelica gigas Nakai.	Umbelliferae	RT	> 100
Arctium lappa L.	Compositae	FR	> 100
Areca catechu L.	Palmae	FR	> 100
Aristolochia contorta Bunge.	Aristolochiaceae	FR	> 100
Artemisia apiacea Hance.	Compositae	HR	> 100
Artemisia capillaris Thunb.	Compositae	HR	72.8
Asparagus cochinchinensis (Lour.) Merr.	Liliaceae	TB	> 100
Aster tataricus L. Fil.	Compositae	RT	> 100
Astragalus membranaceus (Fisch.) Bge.	Leguminosae	RT	> 100
Atractylodes japonica Koidz.	Compositae	ST	> 100
Benincasa hispida (Thunb.) Cogn.	Cucurbitaceae	SD	> 100
Betula platyphylla Suk. var. *japonica* Hara.	Betulaceae	CR	> 100
Bletilla striata (Thunb.)Reichb. Fil.	Orchidaceae	TB	> 100
Broussonetia papyrifera (L.)Vent.	Moraceae	FR	> 100
Buddleia officinalis Maxim.	Loganiaceae	FL	> 100
Caesalpinia sappan L.	Leguminosae	LG	> 100
Caragana chamlagu Lam.	Leguminosae	LF	> 100
Carpesium abrotanoides L.	Compositae	FR	> 100
Carthamus tinctorius L.	Compositae	SD	> 100
Chaenomeles sinensis (Thuin) Koehne.	Malaceae	FR	> 100
Chelidonium majus L.	Papaveraceae	HR	> 100
Chrysanthemum zawadskii var *latilobum* Kitamura	Compositae	HR	> 100
Cibotium barometz (L.) J. Sm.	Cyatheaceae	ST	> 100
Cichorium intybus L.	Compositae	LF	> 100
Cinnamomum cassia Blume.	Lauraceae	TW	> 100
Cinnamomum loureirii Nees.	Lauraceae	BK	> 100
Circium japonicum DC	Compositae	RT	> 100
Clematis mandshurica Rupr.	Ranunculaceae	RT	> 100
Codonopsis pilosula (Franch) Nannf.	Campanulaceae	RT	> 100
Corydalis ternata Nakai.	Papaveraceae	TB	> 100
Cremastra variabilis Nakai.	Orchidaceae	TB	> 100
Cucumis melo L. var *makuwa* Makino.	Cucurbitaceae	PF	> 100
Curculigo orchioides Garrtn.	Amaryllidaceae	ST	> 100
Curcuma longa L.	Zingiberaceae	ST	> 100
Curcuma zedoaria Roscoe.	Zingiberaceae	ST	> 100
Cuscuta chinensis Lam.	Convolvulaceae	SD	> 100
Cynanchum atratum Bunge.	Asclepiadaceae	RT	> 100
Cynanchum wilfordii (Max.) Hemsl.	Asclepidaceae	TB	> 100

Table 1. Continued.

Plant name and Authority	Family	Part used [a]	IC_{50} (μg/ml)[b]
Cynomorium songaricum Rupr.	Cynomoriaceae	HR	> 100
Cynthus officinalis	Compositae	RT	> 100
Daphne genkwa Sieb. et Zucc	Thymelaeaceae	FL	> 100
Davallia mariesii Moore.	Davalliaceae	ST	> 100
Dendrobium nobile Lindl.	Orchidaceae	HR	> 100
Dictamnus albus L.	Rutaceae	SB	> 100
Dioscorea japonica Thunb.	Dioscoreaceae	TB	> 100
Dioscorea tokoro Makino.	Dioscoreaceae	TB	> 100
Diospyros kaki Thunb.	Ebenaceae	LF	> 100
Dipsacus japonicus Mig.	Dipsacaceae	RT	> 100
Draba nemorosa L.	Cruciferae	SD	> 100
Eclipta prostrata L.	Compositae	HR	> 100
Ephedra sinica Stapf.	Ephedraceae	RT	> 100
Equisetum hiemale L.	Equisetaceae	HR	> 100
Eriobotrya japonica (Thunb) Lindl.	Malaceae	LF	> 100
Eriocaulon sietoldianun Sieb. et Zucc.	Eriocaulaceae	FT	> 100
Erycibe obtusifolia B.	Convolvulaceae	ST	> 100
Eucommia ulmoides Oliv.	Eucommiaceae	TW	> 100
Eucommia ulmoides Oliv.	Eucommiaceae	SB	> 100
Eugenia caryophyllata Tnunb.	Myrtaceae	SB	93.8
Euphorbia lathyris L.	Euphorbiaceae	SD	> 100
Euphoria longana Steud.	Sapindaceae	FR	> 100
Gallus domesticus Blume.	Phasianidae	ST	> 100
Ganoderma lucidum Karst.	Polyporaceae	WP	> 100
Gardenia jasminoides Ellis.	Rubiaceae	FR	> 100
Gastrodia elata Blume.	Orchidaceae	ST	> 100
Ginkgo biloba L.	Ginkgoaceae	FR	> 100
Glechoma longituba (Nakai) Kupr.	Labiatae	HR	> 100
Gleditsia japonica var. *koraiensis* Nakai.	Leguminosae	TN	> 100
Glycine max (L.) Merr.	Leguminosae	SD	> 100
Glycyrrhiza uralensis Fischer	Leguminosae	RT	> 100
Hemerocallis fluva L.	Liliaceae	LF	> 100
Hordeum vulgale L.	Graminae	FR	> 100
Houttuynia cordata Thunb.	Saururaceae	HR	> 100
Ipomoea hederacea Jacq.	Convolvulaceae	SD	> 100
Juncus effusus L. var. *decipiens* Buchen.	Juncaceae	HR	29.4
Kalopanax pictus (Thunb.) Nakai.	Araliaceae	CR	> 100
Kochia scoparia (L.) Schrad.	Chenopodiaceae	SD	> 100
Leonurus sibiricus L.	Labiatae	SD	> 100
Ligustrum lucidum Ait.	Oleaceae	FR	> 100
Litchi chinensis Sonn.	Sapindaceae	FR	> 100
Lycium chinensis Mill.	Solanaceae	SB	> 100
Lycopus lucidus Turcz.	Labiatae	HR	> 100
Magnolia denudata Desr.	Magnoliaceae	FL	> 100
Magnolia officinalis Rehd. et Wils.	Magnoliaceae	CR	> 100
Malva verticillata L.	Malvaceae	SD	> 100
Melandrium firmum Rohrb.	Caryophyllaceae	SD	> 100
Melia azedarach L. var. *japonica* Makino.	Meliaceae	FR	> 100
Momordica cochinchinensis Spr.	Cucurbitaceae	SD	> 100
Morinda officinalis How.	Rubiaceae	RT	> 100
Morus alba L.	Moraceae	FR	> 100

Table 1. Continued.

Plant name and Authority	Family	Part used [a]	IC_{50} (μg/ml)[b]
Morus alba L.	Moraceae	LF	> 100
Morus alba L.	Moraceae	TW	> 100
Mucuna birdwoodiana Tutcher.	Leguminosae	ST	> 100
Nepeta japonica Max.	Labiatae	HR	> 100
Oldenlandia diffusa (Willd.) Roxb.	Rubiaceae	HR	> 100
Orostachys japonicus A. Berger.	Crassulaceae	HR	> 100
Paeonia moutan Sims.	Paeoniaceae	SB	68.8
Panax notoginseng F. H. Chen.	Araliaceae	RT	> 100
Panax quinquefolium L.	Araliaceae	RT	> 100
Paonia lactiflora Pall.	Paeoniaceae	RT	> 100
Persicaria tinctoria H.Gross.	Polygonaceae	HR	> 100
Phaenosperma globosa M.	Gramineae	HR	> 100
Pharbitis nil Choisy.	Convolvulaceae	SD	86.7
Phragmites communis Trin.	Graminae	RT	78.9
Phyllostachys nigra M. var. *henonis* S.	Bambusaceae	CR	> 100
Phytolacca esculentum Var Houtt.	Phytolaccaceae	RT	> 100
Pinus densiflora Sieb et. Zucc.	Pinaceae	TW	> 100
Piper longum L.	Piperaceae	SD	> 100
Polygala japonica Houtt.	Polygalaceae	HR	> 100
Polygala tenuifolia Willd.	Polygalaceae	RT	> 100
Polygonum aviculare L.	Polygonaceae	HR	> 100
Polygonum multiflorum Thunberg	Polygonaceae	TB	> 100
Poncirus trifloliata Ratin.	Rutaceae	FR	> 100
Poria cocos (S.) Wolf	Polyporaceae	ST	> 100
Poria cocos (S.) Wolf	Polyporaceae	SB	> 100
Poria cocos (S.) Wolf	Polyporaceae	SC	> 100
Portulaca oleracea L.	Portulacaceae	HR	> 100
Prunella vulgaris L. var. *lilacina* Nakai.	Labiatae	HR	> 100
Prunus japonica var. nakaii Rhed.	Amygdalaceae	SD	> 100
Pterocarpus santalinus Lf.	Leguminosae	LG	65.9
Pueraria thunbergiana Bentham	Leguminosae	RT	> 100
Pueraria thunbergiana Bentham	Leguminosae	FL	42.5
Pyrrosia lingua (Thunb) Far W.	Polypodiaceae	LF	> 100
Rehmania glutinosa Liboschitz.	Scrophulariaceae	RT	> 100
Rehmannia glutinosa Liboschitz var. *purpurea* Making	Scrophulariaceae	RT	> 100
Rheum coreanum Nakai	Polygonaceae	ST	50.0
Rhus verniciflua Stokes.	Anacardiaceae	FD	69.2
Rosa laevigata Michx	Rosaceae	FR	> 100
Rosa rugosa Thunb.	Rosaceae	RT	> 100
Rubia akane Nakai	Rubiaceae	RT	> 100
Rubus coreanus Mig.	Rosaceae	FR	> 100
Salvia miltiorrhiza Bunge.	Labiatae	RT	> 100
Sargassum fusiforme (Harv.) Setch.	Sargassaceae	HR	> 100
Saussurea lappa Clarke.	Compositae	RT	> 100
Schizandra chinensis Baill.	Schizandraceae	FR	> 100
Scirpus yagara Ohwi	Cyperaceae	ST	> 100
Scutellaria baicalensis Georgi.	Labiatae	RT	> 100
Sedum albroseum Bak.	Crassulaceae	HR	31.1
Selaginella tamariscina Spring.	Selaginellaceae	HR	33.7
Sesamum indicum DC.	Pedaliaceae	SD	> 100
Siegesbeckia orientalis L. var. *pubescens* Mak.	Compositae	HR	> 100
Sinomenium acutum Rehder et Wilson	Menispermaceae	ST	> 100

Table 1. Continued.

Plant name and Authority	Family	Part used [a]	IC_{50} (μg/ml)[b]
Solanum nigrum L.	Solanaceae	HR	> 100
Sophora japonica L.	Leguminosae	RT	> 100
Sophora subprostrata Chun. et. T. Chen.	Leguminosae	RT	> 100
Spirodela polyrrhiza (L.) Schleid.	Lemnaceae	HR	> 100
Strychnos ignatii Berg.	Loganiaceae	FR	> 100
Thuja orientalis L.	Cupressaceae	FT	> 100
Tribulus terrestris L.	Zygophyllaceae	FR	> 100
Trichosanthes kirilowii Max	Cucurbitaceae	RT	> 100
Trigonella foenum-graecum L.	Leguminosae	SD	> 100
Triticum aestivum L.	Graminae	SD	> 100
Typha orientalis Schum. et Thonn.	Typhaceae	PL	> 100
Ulmus macrocarpa Hance.	Ulmacea	FR	> 100
Ulmus parvifolia Jacq.	Ulmaceae	SB	> 100
Ulmus pumila L.	Ulmaceae	SB	> 100
Uncaria sinensis Havil.	Rubiaceae	TW	> 100
Undaria pinnatifida Sur.	Lamirariaceae	HR	> 100
Xanthium strumarium L.	Compositae	FR	> 100
Zea mays L.	Graminae	CS	> 100
Zingiber officinale Roscoe	Zingiberaceae	ST	> 100
Zizyphus jujuba Mill.	Rhamnaceae	FS	> 100
Zizyphus jujuba Mill. var. *inermis* Rehol.	Rhamnaceae	FR	> 100
Allopurinol			3.5
Caffeic acid			20.6
Chlorogenic acid			83.2
Caffeine			14.6
Butylated hydroxyanisole (BHA)			>100
Gallic acid			>100
Esculetin			20.5

[a]Part used: HR (herb), SB (stem bark), RT (root), ST (stem), LF (leaf), TW (twig), FL (flower), FT (flower + twig), FR (fruit), SD (seed), SC (sclerotium), TB (tuber), PF (penduncle of fruit), PL (pollen), CR (cortex), FD (fluid), CS (corn silk), FS (seed in fruit), WP (whole plant), BK (bark), TN (thorn).
[b]IC_{50}: Inhibition of xanthine oxidase as an IC_{50} (μg/ml).

Acknowledgements

This work was supported by the grant for the National R&D Program for Enhancing R&D Infrastructure of Womans University from the Ministry of Science and Technology.

References

Cerutti, P. A., and Trump, B. F., Inflammation and oxidative stress in carcinogenesis. *Cancer Cells*, **3**, 1-7 (1991).

Cha, B. C., Lee, S. K., Lee, H. W., Lee, E., Choi, M. Y., Rhim, T. J., and Park, H. J., Antioxidant effect of domestic plants. *Kor. J. Pharmacogn.*, **28**, 15-20 (1997).

Chan, W.-S., Wen, P.-C., and Chiang, H.-C., Structure-activity relationship of caffeic acid analogues on xanthine oxidase inhibition. *Anticancer Res.*, **15**, 703-708 (1995).

Chang, W.-S., Chang, Y.-H., Lu, F.-J., and Chiang, H.-C., Inhibitory effects of phenolics on xanthine oxidase. *Anticancer Res.*, **14**, 501-506 (1994).

Choi, J.-S., Lee, J. H., Park, H.-J., Kim, H.-G., Young, H.-S., and Mun, S.-I., Screening for antioxidant activity of plants and marine algae and its active principles from *Prunus davidiana*. *Kor. J. Pharmacogn.*, **24**, 299-303 (1993).

Cotelle, N., Bernier, J.-L., Catteau, J.-P., Pommery, J., Wallet, J.-C., and Gaydon, E.M., Antioxidant properties of hydroxy-flavones. *Free Radic. Biol. Med.*, **20**, 35-43 (1996).

Crawford, D., Amstad, D., Foo, Y., and Cerutti, P. A., Constitutive and phorbol-myristate acetate regulated antioxidant defense of mouse epidermal JB6 cells.

Mol. Carcinogenesis, **2**, 136-143 (1989).

Fischer-Nielsen, A., Jeding, I. B., and Loft, S., Radiation-induced formation of 8-hydroxy-2-deoxyguanosine and its prevention by scavengers. *Carcinogenesis*, **15**, 1609-1612 (1994).

Hsie, A., Recio, I., Katz, D., Lee, C., Wagner, M., and Schenley, R., Evidence for reactive oxygen species inducing mutations in mammalian cells. *Proc. Natl. Acad. Sci. USA*, **83**, 9610-9620 (1986).

Huang, M.-T., Smart, R. C., Wong, C. Q., and Conney, A.H., Inhibitory effects of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol 13-acetate. *Cancer Res.*, **48**, 5941-5946 (1988).

Kang, H. S., Chung, H. Y., Jung, J. H., Kang, S. S., and Choi, J.S., Antioxidant effect of *Salvia miltiorriza*. *Arch. Pharm. Res.*, **20**, 496-500 (1997).

Klaunig, J. E., Chemopreventive effects of green tea components on hepatic carcinogenesis. *Prev. Med.*, **21**, 510-519 (1992).

Kokoglu, E., Belce, A., Ozyurt, E., and Tepeler, Z., Xanthine oxidase levels in human brain tumors. *Cancer Lett.*, **50**, 179-181 (1990).

Lee, S. K., Mbwambo, Z. H., Chung, H., Luyengi, L., Gamez, E. J. C., Mehta, R. G., Kinghorn, A. D., and Pezzuto, J. M., Evaluation of the antioxidant potential of natural products. *Combinatorial Chem. & High Through. Screening*, **1**, 35-46 (1998).

McCord, J. M., and Fridovich, I., The reduction of cytochrome c by milk xanthine oxidase. *J. Biol. Chem.*, **243**, 5753-5760 (1968).

Moraes, E. C., Keyse, S. M., and Tyrrell, R. M., Mutagenesis by hydrogen peroxide treatment of mammalian cells: a molecular basis. *Carcinogenesis*, **11**, 283-293 (1990).

Pence, B. C., and Reiners, Jr, J. J., Murine epidermal xanthine oxidase activity: correlation with degree of hyperplasia induced by tumor promoters. *Cancer Res.*, **47**, 6388-6392 (1987).

Slaga, T. J., Solanki, W., and Logani, M., Studies on the mechanism of action of antitumor promoting agents: Suggestive evidence for the involvement of free radicals in promotion. In: *Radioprotectors and Anticarcinogens*, Nagarrd, O.F., and Simic, M.G. (eds.), Academic Press, New York, 1983, pp. 417-485.

Tanaka, T., Cancer chemoprevention by natural products (Review). *Oncol. Reports*, **1**, 1139-1155 (1994).

Troll, W., and Wiesner, R., The role of oxygen radicals as a possible mechanism of tumor promotion. *Ann. Rev. Pharmacol. Toxicol.*, **25**, 509-528 (1985).

Trush, M. A., and Kensler, T. W., Role of free radicals in carcinogen activation. In: *Oxidative Stress. Oxidants and antioxidants*. Sies. H. (ed.), Academic Press, San Diego, CA, 1991, pp. 277-318.

Wei, H., Bowen, R., Cai, Q., Barnes, S., and Wang, Y., Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.*, **208**, 124-130 (1995).

(Accepted September 16, 1999)