Screening for Inhibitory Activity of Citrus Fruit Extracts against Platelet Cyclooxygenase and Lipoxygenase

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Inhibitory activities of the albedo extracts of 42 species and cultivars of the genus *Citrus* and those of two *Fortunella* and one *Poncirus* species against rat platelet cyclooxygenase and lipoxygenase were screened. Among the species investigated, the extract of Lumie (*Citrus lumia*) was shown to possess the highest inhibitory activity against cyclooxygenase ($IC_{50} = 24$ μg/mL), and that of Shuto (*Citrus aurantium*) was the highest against lipoxygenase ($IC_{50} = 56$ μg/mL). The albedo extracts of citrus from the same taxonomic group appeared to have similar inhibitory tendencies toward these enzymes. The flavedo extract of ripe lumie inhibited cyclooxygenase to the same degree as the albedo, more than the pulp extract. The flavedo, pulp, and juice extracts of ripe Ponkan (*C. reticulata*) also inhibited lipoxygenase in addition to the albedo extract. Both the flavedo and albedo tissues were shown to be effective sources of inhibitory compounds against cyclooxygenase and lipoxygenase.

Keywords: *Citrus; platelet cyclooxygenase; platelet lipoxygenase; arachidonic acid*

INTRODUCTION

There are many papers on the physiological actions of platelet arachidonic acid metabolites. Cyclooxygenase and lipoxygenase catalyze the initial reactions in formation of three major arachidonic acid metabolites, thromboxane B_2 (TXB_2), 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), in platelets (Hamberg and Samuelsson, 1974). Cyclooxygenase-mediated production of thromboxane A_2, an active form of TXB_2, induces platelet aggregation (Hamberg et al., 1975), and lipoxygenase metabolites are involved in atherosclerotic processes (Turner et al., 1975) in addition to platelet aggregation (Lapetina and Cuatrecasas, 1979; Dutilh et al., 1980). In addition, certain metabolic products from both the lipoxygenase and cyclooxygenase pathways appear to play important roles in the process of skin tumor promotion (Fischer et al., 1982; Nakadate et al., 1982). Therefore, a specific inhibitor of cyclooxygenase and lipoxygenase should be useful as a therapeutic drug for the prevention of thrombosis, atherosclerosis, and carcinogenesis. It is well-known that nonsteroid anti-inflammatory drugs, such as aspirin and indomethacin, selectively inhibit cyclooxygenase (Flower, 1974). Sekiya et al. reported that platelet lipoxygenase was specifically inhibited by baicalein (Sekiya and Okuda, 1982) and esculetin (Sekiya et al., 1982), components in Chinese medical plants.

Apart from the utilization of such specific inhibitors as drugs, it is becoming a matter of great significance that these chronic diseases be prevented by daily intake of such inhibitory compounds which occur naturally in food plants (Baughurst et al., 1977; Sekiya et al., 1993). Citrus plants have many kinds of bioactive substances, for example, phenolic compounds including phenylpropanoids and flavonoids (Horowitz and Gentili, 1977), limonoids (Maier et al., 1977), synephrine (Shi et al., 1992), carotenoids (Stewart, 1980), tocopherols (Ting and Newhall, 1965), and so on. In particular, flavonoids have been extensively investigated and shown to act as antioxidants (Chen et al., 1990), to protect against lipid peroxidation (Salvayre et al., 1988; Guengerich and Kim, 1990), to possess anti-inflammatory properties (Busse et al., 1984; Landolfi et al., 1984), and to produce an anticarcinogenic effect (Wei et al., 1990; Deschner et al., 1991). Furthermore, quercetin and certain major flavonoids were reported to be effective inhibitors of 5- and 12-lipoxygenase (Hope et al., 1983; Welton et al., 1986).

The present work attempts to investigate the influence of citrus fruit extracts on platelet arachidonic acid metabolism and to identify the effective citrus species and their tissues for the purpose of generating an increased commercial interest in citrus juices and other food-grade products manufactured from citrus fruits.

MATERIALS AND METHODS

Materials. Five to 15 ripe and unripe fruits were harvested from trees cultivated at the Okitsu Branch, National Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Japan. Samples were dissected into four parts (flavedo, albedo, pulp, and juice) and stored at −20 °C until used. [1-^{14}C]Arachidonic acid and [^{3}H]TXB_2 were obtained from New England Nuclear (Boston, MA). Arachidonic acid was from Sigma Chemical Co. (St. Louis, MO). Precoated silica gel 60 plastic sheets were purchased from E. Merck (Darmstadt, Germany). The Sep-Pak C_{18} cartridge (5 g) was from Millipore Co. (Milford, MA). Other chemicals were of reagent grade.

Preparation of Fruit Extracts. After lyophilization, albedo tissue samples were ground using an Ultra Centrifugal mill (Mitamura Riken Kogyo, Tokyo, Japan) with a 0.5 mm filter. Portions (1.5 g) of these powdered samples were extracted for 12 h with 25 mL of solvent, MeOH−DMSO (1:1

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v/v), in glass-stoppered vessels on a wrist-action shaker at ambient temperature. After centrifugation at 3000*g* for 10 min, the extract of each sample was decanted and the remaining solid residue was extracted three times more with 25 mL of the same solution. To remove ionic compounds, which may cause nonselective inhibition of the enzymes, the combined extract for each sample was diluted 10-fold with water and passed through a Sep-Pak C_{18} cartridge (5 g) that had been preconditioned with 50 mL of methanol followed by 50 mL of 10% methanol. The eluate was discarded, and the cartridge was washed with 50 mL of 10% methanol. The retained compounds were eluted with 50 mL of methanol and evaporated to dryness using an SPE-200 centrifugal evaporator (Shimadzu Rika Kikai, Tokyo, Japan). The dried compounds were suspended in a small amount of water, freeze-dried, and stored at −80 °C until use. Flavedo and pulp extracts of Lumie and Ponkan were prepared in the same way as albedo extracts mentioned above. Juice extracts were prepared by homogenizing the vesicle tissues with a mixer after the other three tissues had been removed from each fruit. The juice extract was clarified by centrifugation at 15000*g* for 20 min, and 45 mL of each extract was passed through a Sep-Pak C_{18} cartridge as described above.

Assay. Rat blood was obtained from normally fed Wister-King rats (200−300 g). Washed platelets were prepared by differential centrifugation as described previously (Sekiya and Okuda, 1982). The experimental conditions of the inhibitory assay were the same as those used by Sekiya et al. (1982). Tris/saline buffer (25 mM Tris, 130 mM NaCl, pH 7.4) containing 1 mM EDTA was used as the solvent in the reaction mixture. The reaction mixture consisted of 130 μL of sonicated platelets (2 mg of protein/mL), 20 μL of sonicated test compounds, and 50 μL of [1-^{14}C]arachidonic acid (0.05 μCi). Platelets were preincubated with test compounds at final concentrations of 0.1−1000 μg/mL for 5 min at 37 °C. Then, [1-^{14}C]arachidonic acid was added (final concentration = 0.9 nmol/0.2 mL per tube), and the mixture was incubated for 5 min at 37 °C. The reaction was terminated by adding 0.2 mL of 0.5 N formic acid. Control in which test compound was omitted was included in every experiment, and each amount of produced TXB_2 and 12-HETE was designated as control value corresponding to 100%. The metabolites were extracted with 3 mL of EtOAc followed by evaporation under N_2 gas. The residue was dissolved in a small volume of EtOAc, applied quantitatively to silica gel 60 plastic sheets, and developed with CH_3Cl−MeOH−AcOH−H_2O (90:8:1:0.8 v/v). Radioactive metabolites were detected and quantified with a Model BAS 1000 laser image analytical system (Fujix, Tokyo, Japan). TXB_2 was identified by comparison with authentic [5,6,8,9,-11,12,14,15-^{3}H]TXB_2 on silica gel TLC. 12-HETE was identified by gas chromatography−mass spectrometry (Sekiya and Okuda, 1982).

RESULTS AND DISCUSSION

Inhibitory Activities of the Albedo Extracts of Citrus Plants against Platelet Arachidonic Acid Metabolism. As shown in Figure 1, both the Lumie (specimen 8) and Ponkan (specimen 30) extracts from albedo tissue inhibited cyclooxygenase and lipoxygenase. Lumie extract inhibited the formation of TXB_2 more than that of 12-HETE at all concentrations (0.1−1000 μg/mL). On the contrary, the extract of Ponkan at concentrations of over 1 μg/mL inhibited the formation of 12-HETE more than that of TXB_2. These results suggested that inhibitory effects of citrus extracts on both of these enzymes differ with citrus species.

Albedo tissue extracts of citrus species inhibited both cyclooxygenase and lipoxygenase as shown in Table 1. To ascertain inhibitory tendency of citrus species, citrus plants were classified according to the guidelines of Tanaka (1969). In general, albedo tissue extracts inhibited cyclooxygenase more than lipoxygenase. Among the species investigated, extracts of Lumie (8), Shikaikan (33), Sweet (7), Citron (5), Kobenimikan (37), and Biroro (4) strongly inhibited cyclooxygenase with IC_{50} values of 24, 50, 66, 67, 71, and 91 μg/mL, respectively. Conversely, Shuto (16), Yatsushiro (27), Tachibana (36), Koji (41), and Ponkan (30) had strong inhibitory activity against lipoxygenase with IC_{50} values of 56, 75, 80, 89, and 93 μg/mL, respectively. Species in *Archicitrus* subgenera generally inhibited cyclooxygenase and had little effect on lipoxygenase. For example, Papeda, Citrophorum, Cephalocitrus, Sinensoides, and Osmocitroides of the *Aurantium* group selectively inhibited cyclooxygenase, while species in *Metacitrus* subgenera had a tendency to inhibit both lipoxygenase and cyclooxygenase.

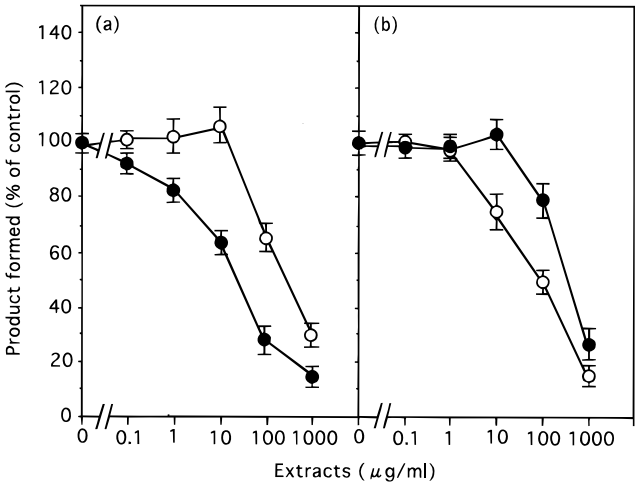

Figure 1. Dose−response curves of the albedo extract of (a) Lumie (*C. lumia*) and (b) Ponkan (*C. reticulata*) for cyclooxygenase (●) and lipoxygenase (○) in platelets. Platelet cyclooxygenase activity was assayed by measuring the formation of TXB_2 from [1-^{14}C]arachidonic acid. Platelet lipoxygenase activity was assayed by measuring the formation of 12-HETE from [1-^{14}C]arachidonic acid. Values were mean ± SE of three replications.

It was reported that extracts of vegetables such as cabbage, cucumber, eggplant, garlic, onion, perilla, and tomato selectively inhibited either cyclooxygenase or lipoxygenase (Sekiya et al., 1993). Although inhibitory compounds in citrus should differ from those in vegetables (Bayer et al., 1988; Morimitsu and Kawakishi, 1991), we have shown the presence of inhibitory activity of citrus groups and species on platelet arachidonic acid metabolism.

Correlation between the Classification of Citrus Plants and the Influences of Their Albedo Extracts on the Formation of TXB_2 and 12-HETE. The inhibitory activity of extracts from tested citrus could be divided into four general groups: extracts that inhibited the formation of both TXB_2 and 12-HETE, selective inhibition of either TXB_2 or 12-HETE formation, and no inhibition (Figure 2). Extracts of Citrophorum (6, 7, 8), Acrumen-Microacrumen-Citroidora-Megacarpa (30, 31, 32, 34, 35), and Acrumen-Anisodora (28, 29) groups and species of Shuto (16), Shikaikan (33), Kobenimikan (37), and Kishu (38) inhibited the formation of both TXB_2 and 12-HETE. Extracts of Aurantium-Osmocitrioides (20, 21), Osmocitrus (22, 24), Acrumen-Euacrumen (25, 26), and Fortunella-Eufortunella (43, 44) groups and Iyo (19) had no effect on either enzyme. Extracts that stimulated the formation of 12-HETE and inhibited formation of TXB_2, such as those of Papeda (1), Limonellus (2, 3, 4), and Aurantium-Sinensoides (17, 18) groups and species of Citron (5) and

Table 1. Inhibitory Activities of Albedo Extracts of Citrus Plants against Platelet Cyclooxygenase and Lipoxygenase Activities[a]

specimen[b]	ref no.	common name	scientific name[b]	IC_{50}[c] (μg/mL) cyclooxygenase	liopxygenase
Citrus-Archicitrus					
Papeda					
	1	Kabuyao	*C. macroptera*	153	>1000
Limonellus					
	2	Mexican	*C. aurentifolia*	124	>1000
	3	Tahiti	*C. latifolia*	134	>1000
	4	Biroro	*C. montana*	91	>1000
Citrophorum					
	5	Citron	*C. medica*	67	>1000
	6	Eureka	*C. limon*	155	500
	7	Sweet	*C. limetta*	66	302
	8	Lumie	*C. lumia*	24	275
Cephalocitrus					
	9	Hirado	*C. grandis* (cv. Hirado)	470	>1000
	10	Shaten yu	*C. grandis* (cv. Shytian you)	305	>1000
	11	Marsh	*C. paradisi*	>1000	>1000
	12	Kinukawa	*C. glaberrima*	>1000	560
	13	Hassaku	*C. hassaku*	890	>1000
Aurantium					
Medioglobosa					
	14	Natsudaidai	*C. natsudaidai*	700	530
	15	Sanbokan	*C. sulcata*	590	230
Aurantioides					
	16	Shuto	*C. aurantium*	360	56
Sinensoides					
	17	Valencia	*C. sinensis* (cv. Valencia)	186	>1000
	18	Morita navel	*C. sinensis* var. *Brasiliensis* (cv. Morita)	470	>1000
	19	Iyo	*C. iyo*	>1000	>1000
Osmocitrioides					
	20	Hyuganatsu	*C. tamurana*	490	>1000
	21	Shunkokan	*C. shunkokan*	980	>1000
Citrus-Metacitrus					
Osmocitrus					
	22	Yuzu	*C. junos*	>1000	>1000
	23	Sudachi	*C. sudachi*	>1000	170
	24	Kabosu	*C. sphaerocarpa*	>1000	>1000
Acrumen					
Euacrumen					
	25	King	*C. nobilis* var. *Knep*	>1000	>1000
	26	Satsuma	*C. unshiu*	>1000	>1000
	27	Yatsushiro	*C. yatsusiro*	720	75
Microacrumen-Anisodora					
	28	Keraji	*C. keraji*	442	>1000
	29	Oto	*C. oto*	580	>1000
Microacrumen-Citroidora-Megacarpa					
	30	Ponkan	*C. reticulata*	298	93
	31	Dancy tangerine	*C. tangerina*	322	302
	32	Jimikan	*C. succosa*	120	158
	33	Shikaikan	*C. suhuiensis*	50	592
	34	Clementine	*C. clementia*	770	>1000
	35	Bergamot	*C. bergamia*	398	275
Microacrumen-Citroidora-Microcarpa-Angstifolia					
	36	Tachibana	*C. tachibana*	810	80
	37	Kobenimikan	*C. erythrosa*	71	420
	38	Kishu	*C. kinokuni*	300	880
	39	Sunki	*C. sunki*	593	930
Microacrumen-Citroidora-Microcarpa-Latifolia					
	40	Shiikuwasha	*C. depressa*	>1000	>1000
	41	Koji	*C. leiocarpa*	>1000	89
Pseudofortunella					
	42	Shikikitsu	*C. madurensis*	>1000	830
Fortunella-Eufortunella					
	43	Oval kumquat	*F. margarita*	643	>1000
	44	Meiwa kumquat	*F. crassifolia*	680	>1000
Poncirus					
	45	Trifoliate orange	*P. trifoliana*	>1000	>1000

[a] Values for lipoxygenase were measured as 12-HETE formation from [1-^{14}C]arachidonic acid; those for cyclooxygenase were measured as thromboxane B_2 formation from [1-^{14}C]arachidonic acid. [b] The classification and the nomenclature of citrus plants were based on Tanaka's classification. [c] Values were average of triplicate five-point titration.

Shaten yu (10), were examples of selective inhibition of cyclooxygenase. Indomethacin was considered to be a specific inhibitor of cyclooxygenase because it inhibited TXB_2 production and enhanced 12-HETE production as

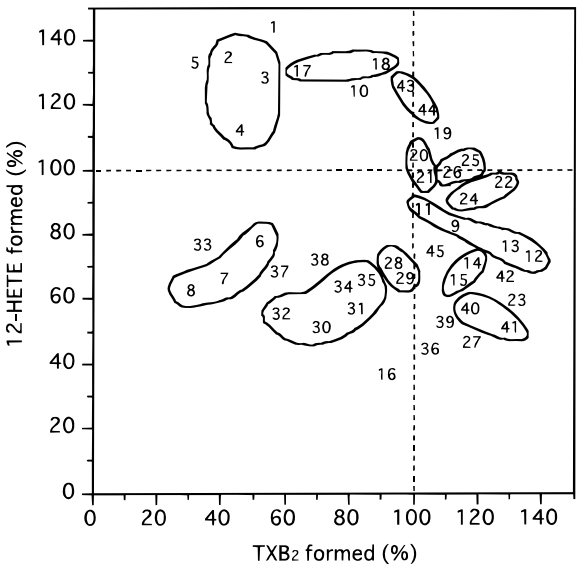

Figure 2. Correlation between the classification of citrus plants and the influences of their albedo extracts on the formation of TXB_2 and 12-HETE. Values were percent formation versus control of TXB_2 and 12-HETE in the presence of each extract at 100 μg/mL (mean of three replications). For the identity of numbers, see Table 1. Encircled numbers showed similar levels of influence on each other among the same group based on Tanaka's classification.

a consequence of elevated levels of arachidonic acid made available to lipoxygenase (Hamberg and Samuelsson, 1974). Extracts from Cepharocitrus (9, 11, 12, 13), Aurantium-Medioglobosa (14, 15), Acrumen-Latifolia (40, 41), Pseudofortunella (42), and Poncirus (45) groups and species of Sudachi (23), Yatsushiro (27), Tachibana (36), and Sunki (39) were considered to selectively inhibit lipoxygenase.

Concerning citrus classification, there was good correlation between the classification of citrus plants and the inhibitory levels of albedo extracts on the formation of the cyclooxygenase and lipoxygenase metabolites, although there were some exceptions. For example, the extract of Citron (5) in the Citrophorum group (5−8) selectively inhibited cyclooxygenase, those of Sudachi (23) in the Osmocitrus group (22−24) and Yatsusiro (27) in the Acrumen-Euacrumen group (25−27) selectively inhibited lipoxygenase, and the inhibitory patterns of the extracts of Acrumen-Microacrumen-Citroidora-Microcarpa-Latifolia group specimens (36−39) separated into two groups. The above findings, however, suggest that inhibitory compounds in citrus plants provide information about the chemotaxonomy.

Effects of Tissue Type and Maturity of Lumie (*C. lumia*) and Ponkan (*C. reticulata*) on the Inhibitory Activity of Extracts on Platelet Cyclooxygenase and Lipoxygenase. Taking into consideration the inhibitory activity of extracts and the marketability of fruit, we have selected Lumie (8) as effective against cyclooxygenase and Ponkan (30) against lipoxygenase. The flavedo, albedo, pulp and juice extracts of unripe and ripe Lumie were investigated for cyclooxygenase inhibition and those of Ponkan for lipoxygenase inhibition (Table 2). In Lumie, the flavedo extracts of both unripe and ripe fruits inhibited enzyme activity to the same degree as those of albedo and were more inhibitory than pulp. There was little difference in extract inhibition between unripe and ripe tissue except for pulp. The inhibitory activity of the pulp extract decreased during ripening, and juice extract had

Table 2. Effects of Tissue Type and Ripeness of Lumie (*C. lumia*) and Ponkan (*C. reticulata*) on the Inhibitory Activity of Extracts on Platelet Cyclooxygenase and Lipoxygenase

	inhibition[a] (%)			
	Lumie[b] (cyclooxygenase)		Ponkan[c] (lipoxygenase)	
tissue	unripe	ripe	unripe	ripe
flavedo	59.5 ± 4.9	58.0 ± 4.1	49.0 ± 6.7	56.5 ± 7.3
albedo	56.8 ± 4.5	56.5 ± 4.3	53.1 ± 6.9	54.6 ± 7.0
pulp	47.8 ± 4.8	29.4 ± 3.8	42.3 ± 6.4	13.9 ± 3.9
juice	0.0	0.0	54.3 ± 6.8	34.5 ± 6.2

[a] Values were percent formation versus control of TXB_2 or 12-HETE in the presence of each extract at 100 μg/mL; mean ± SE of three replications. [b] Mean ± SE weights of unripe and ripe Lumie were 38.4 ± 2.6 and 51.8 ± 4.1 g, respectively. [c] Mean ± SE weights of unripe and ripe Ponkan were 41.7 ± 4.9 and 109.6 ± 6.6 g, respectively.

no effect on the enzyme. The juice extract of unripe or ripe Shikaikan (33) also had no inhibitory activity. The amounts of flavedo, albedo, and pulp extract per ripe fruit (in milligrams) were 70.57 (35.5% of total of the three fractions), 102.35 (51.6%), and 25.63 (12.9%), respectively (triplicate determination). The peel (flavedo and albedo) accounted for about 87% of total dry matter.

On the other hand, all four tissue extracts of both unripe and ripe Ponkan inhibited lipoxygenase. The juice extracts of both unripe and ripe Tachibana (36) inhibited the enzyme by 43.7 and 36.7%, respectively, indicating that some polar compounds in these species are effective against lipoxygenase. The inhibitory activities of flavedo and albedo extracts slightly increased during ripening, while pulp and juice extracts declined in activity. The amounts of flavedo, albedo, pulp, and juice extract per ripe fruit (in milligrams) were 268.91 (36.1%), 265.02 (35.6%), 167.29 (22.5%), and 43.85 (5.9%), respectively (triplicate determination).

The above findings show that both the unripe and ripe peels contain compounds which inhibit cyclooxygenase and lipoxygenase. It is not known, however, which components in fruits contribute to inhibition of cyclooxygenase and lipoxygenase. Further studies on the analysis of inhibitory components in citrus are now in progress.

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LITERATURE CITED

Baughurst, K. I.; Raj, M. J.; Truswell, A. S. Onions and platelet aggregation. *Lancet* **1977**, *8*, 101.

Bayer, T.; Wagner, H.; Wray, V.; Dorsch, W. Inhibitors of cyclooxygenase and lipoxygenase in onions. *Lancet* **1988**, *15*, 906.

Busse, W. W.; Kopp, D. E.; Middleton, E., Jr. Flavonoid modulation of human neutrophil function. *J. Allergy Clin. Immunol.* **1984**, *73*, 801−809.

Chen, Y. T.; Zheng, R. L.; Jia, Z. J.; Ju, Y. Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biol. Med.* **1990**, *9*, 19−21.

Deschner, E. E.; Ruperto, J.; Wong, G.; Newmark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasma. *Carcinogenesis* **1991**, *7*, 1193−1196.

Dutilh, C. F.; Haddeman, E.; Ten Hoor, F. Role of arachidonate lipoxygenase pathway in blood platelet aggregation. *Adv. Prostaglandin Thromboxane Res.* **1980**, *6*, 101−105.

Fischer, S. M.; Mills, G. D.; Slaga, T. J. Inhibition of mouse skin tumor promotion by several inhibitors of arachidonic acid metabolism. *Carcinogenesis* **1982**, *3*, 1243−1245.

Flower, R. J. Drugs which inhibit prostagrandin biosynthesis. *Pharmacol. Rev.* **1974**, *26*, 33−67.

Guengerich, F. P.; Kim, D. M. In vitro inhibition of dihydropyridine oxidation and aflatoxin B1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis* **1990**, *11*, 2275−2279.

Hamberg, M.; Samuelsson, B. Prostaglandin endoperoxides-novel transformations of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3400−3404.

Hamberg, M.; Sevensson, J.; Samuelsson, B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2994−2998.

Hope, W. C.; Welton, A. F.; Fiedler-Nagy, C.; Batula-Bernard, C.; Coffey, J. W. *In vitro* inhibition of the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. *Biochem. Pharm.* **1983**, *32*, 367−371.

Horowitz, R. M.; Gentili, B. Flavonoid constituents of citrus. In *Citrus Science and Technology*; Nagy, S., Shaw, P. E., Veldhuis, M. K., Eds.; AVI: Westport, CT, 1977; pp 397−426.

Landolfi, R.; Mower, R. L.; Steiner, M. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. *Biochem. Pharm.* **1984**, *33*, 1525−1530.

Lapetina, E. G.; Cuatrecasas, P. Rapid inactivation of cyclooxygenase activity after stimulation of intact platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 121−125.

Maier, V. P.; Bennet, R. D.; Hasegawa, S. Limonin and other limonoids. In *Citrus Science and Technology*; Nagy, S., Shaw, P. E., Veldhuis, M. K., Eds.; AVI: Westport, CT, 1977; pp 355−396.

Morimitsu, Y.; Kawakishi, S. Optical resolution of 1-(methylsulfinyl)-propyl alk(en)yl disulfides, inhibitors of platelet aggregation isolated from onion. *Agric. Biol. Chem.* **1991**, *55*, 889−890.

Nakadate, T.; Yamamoto, S.; Ishii, M.; Kato, R. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced epidermal ornithine decarboxylase activity by lipoxygenase inhibitors: possible role of product(s) of lipoxygenase pathway. *Carcinogenesis* **1982**, *3*, 1411−1414.

Salvayre, R.; Nègre, A.; Affany, A.; Lenoble, M.; Douste-Blazy, L. Protective effect of plant flavonoids, analogs, and vitamin E against lipid peroxidation of membranes. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties*; Cody, V., Middleton, E., Jr., Harborne, J. B., Beretz, A., Eds.; Liss: New York, 1988; pp 313−316.

Sekiya, K.; Okuda, H. Selective inhibition of platelet lipoxygenase by baicalein. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1090−1095.

Sekiya, K.; Okuda, H.; Arichi, S. Selective inhibition of platelet lipoxygenase by esculetin. *Biochim. Biophys. Acta* **1982**, *713*, 68−72.

Sekiya, K.; Fushimi, T.; Kanamori, T.; Ishikawa, N.; Itoh, M.; Takita, M.; Nakanishi, T. Regulation of arachidonic acid metabolism in platelets by vegetables. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 670−671.

Shi, L.; Gotou, Y.; Shindo, K.; Ogawa, K.; Shida, Y.; Sashida, Y.; Shimomura, H.; Araki, C.; Yoshida, T. Synephrine contents and their seasonal variation in peels of citrus plants. *Shyoyakugaku Zasshi* **1992**, *46*, 150−155.

Stewart, I. Color as related to quality in citrus. In *Citrus Nutrition and Quality*; Nagy, S., Attaway, A., Eds.; American Chemical Society: Washington, DC, 1980; pp 129−150.

Tanaka, T. Misunderstanding with regards *Citrus* classification and nomenclature. *Bull. Univ. Osaka Pref. Ser. B* **1969**, *21*, 139−145.

Ting, S. V.; Newhall, W. F. Occurrence of a natural antioxydant in citrus fruit. *J. Food Sci.* **1965**, *30*, 57−62.

Turner, S. R.; Tainer, J. A.; Lynn, W. S. Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. *Nature* **1975**, *257*, 680−681.

Wei, H.; Tye, L.; Bresnick, E.; Birt, D. F. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. *Cancer Res.* **1990**, *50*, 499−502.

Welton, A. F.; Tobias, L. D.; Fiedler-Nagy, C.; Anderson, W.; Hope, W.; Meyers, K.; Coffey, J. W. Effect of flavonoids on arachidonic acid metabolism. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*; Cody, V., Middleton, E., Jr., Harborne, J. B., Eds.; Liss: New York, 1986; pp 231−242.

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