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Lipid Peroxidation and Cyclooxygenase Enzyme Inhibitory Activities of Acidic Aqueous Extracts of Some Dietary Supplements

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The botanical supplement market is growing at a fast pace with more and more people resorting to them for maintaining good health. Echinacea, garlic, ginkgo, ginseng, Siberian ginseng, grape seed extract, kava kava, saw palmetto and St John's wort are some of the popular supplements used for a variety of health benefits. These supplements are associated with various product claims, which suggest that they possess cyclooxygenase (COX) enzyme and lipid peroxidation inhibitory activities. COX enzymes are found to be at elevated levels in inflamed and cancerous cells. To test some of the product claims, selected supplements were analysed for their ability to inhibit COX-1 and -2 enzymes and lipid peroxidation *in vitro*. The supplements were extracted with acidified water (pH 2) at 37 °C to simulate the gastric environment. The supplements tested demonstrated varying degrees of COX enzyme inhibition (5–85% for COX-1 and 13–28% for COX-2). Interestingly, extracts of garlic (Meijer), ginkgo (Solaray), ginseng (Nature's Way), Siberian ginseng (GNC, Nutrilite, Solaray, Natrol), kava kava (GNC, Sundown, Solaray) and St John's wort (Nutrilite) selectively inhibited COX-2 enzyme. These supplements also inhibited lipid peroxidation *in vitro* (5–99%). The results indicated that the consumption of these botanical supplements studied possess health benefits. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: botanicals; dietary supplements; COX; lipid peroxidation.

INTRODUCTION

The sale of botanical supplements has increased significantly over the past years (Eisenberg *et al.*, 1998). People have resorted to botanical supplements to maintain good health and vitality. Many botanical supplements claim to perpetuate good health but very little scientific research has been accomplished to corroborate these statements. Research with regard to the efficacy of these supplements would add further notoriety. Echinacea, garlic, ginkgo, ginseng, grape seed extract, kava kava, saw palmetto and St John's wort supplements are among the popular supplements sold in the USA (Blumenthal, 1999). These supplements are stated to promote immune function, cardiovascular health, mental alertness, endurance, well-being, prostate health, enhance the mood and provide antioxidant protection (Tables 1 and 2). Also, it is suggested that these botanicals might possess antioxidant and antiinflammatory properties, which aid in general good health.

Cyclooxygenase (COX) enzymes play an important role in the inflammatory processes. There are two isoforms of the COX enzyme, COX-1 and COX-2. COX-1 is the constitutive form of the enzyme and is responsible for basic regulatory functions in cells and is involved in the production of prostaglandins (Cryer and Dubois, 1998). Prostaglandins are also responsible for the production of gastric secretions. COX-2 is the inducible form, which is produced in response to inflammation (Lipisky, 1999). It has been demonstrated that COX-2 expression has been induced in atheromatous plaques (Baker *et al.*, 1999; Schonbeck *et al.*, 1999) and neoplasms (Dannenberg and Subbaramaiah, 2003). It has also been demonstrated that free radicals, which are implicated in the development of atherosclerosis, cause the induction of COX-2 expression (Adderley and Fitzgerald, 1999). These studies have paved the way for the hypothesis that COX-2 inhibition might be useful in the treatment of inflammatory conditions such as atherosclerosis, arthritis and in various types of cancer. Therefore, selective inhibition of COX-2 enzyme is desirable to prevent the undesirable side effects of COX-1 inhibition such as gastric ulcerations. But, recently selective COX-2 inhibitors, specifically rofecoxib, have been demonstrated to cause an increase in cardiovascular risk (Mukherjee *et al.*, 2001; Juni *et al.*, 2004; Zarraga and Schwarz, 2007). Therefore, judicious discrimination has to be employed in understanding the results of COX-2 inhibition as well as the use of supplements that cause it.

Antioxidant properties can be attributed either to inhibition of the production of reactive oxygen species or to scavenging of free radicals (Arora *et al.*, 1998). Lipid peroxidation is one of the major causes of free radical generation *in vivo*. Oxidative stress has been implicated in many chronic diseases. Therefore, the antioxidant activity of these botanicals can play a major role in imparting good health.

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In a randomized, double-blind, placebo-controlled trial (Weber *et al.*, 2005), a non-alcohol *Echinacea purpurea* liquid (reconstituted dried pressed juice of the above-ground plant parts harvested at flowering) or a similar placebo liquid was administered to 524 children of ages 2–11, for acute upper respiratory tract infections (URIs) that occurred during the study period. The study indicated that *Echinacea purpurea* may be effective in reducing the occurrence of subsequent URIs in children. *Ginkgo biloba* extract (EGb 761) has been demonstrated to have preventive effects on ischemia-reperfusion injury in rat urinary bladder (Yenilmez *et al.*, 2007). In a 24-week, multi-center, double-blind, placebo-controlled, randomized trial (Kanowski and Hoerr, 2003) using *Gingko biloba* extract EGb 761, it was confirmed that EGb 761 improves cognitive function in a clinically relevant manner in patients suffering from dementia. A randomized, double-blind, placebo-controlled, multi-center trial (Kasper *et al.*, 2006) involving 332 patients, using St John's wort extract WS 5570, demonstrated that WS 5570 was consistently more effective than placebo in patients with either less severe or more severe baseline impairment. It has been shown that kava extract WS 1490 can be used effectively and safely to treat sleep disturbances associated with non-psychotic anxiety disorders, in a multicenter, randomized, placebo-controlled, double-blind clinical trial (Lehrl, 2004). But there has been conflicting evidence about hepatotoxicity caused by the use of kava. Some studies (Humberston *et al.*, 2003; Escher *et al.*, 2001) suggest that kava induces necrotizing hepatic failure, whereas other studies suggest that there is no evidence for liver toxicity (Sorrentino *et al.*, 2006; Singh and Devkota, 2003). These differences might be due to the different species in which they are tested and therefore, careful evaluation of liver function tests might be necessary when taking kava supplements.

In addition, it was reported that these botanicals possessed either antioxidant property or inhibition of COX enzymes activity or both. For example, Echinacea was demonstrated to possess antioxidant property (Hu and Kitts, 2000; Facino *et al.*, 1995) and the polyalkamides from it were shown to inhibit microsomal COX enzyme *in vitro* (Muller-Jakic *et al.*, 1994). Similarly, several garlic compounds have been reported to effectively suppress LDL oxidation *in vitro* (Lau, 2001). *Ginkgo biloba* extract (EGb 761) was demonstrated to possess antioxidant property due to the inhibition of free radical formation as well as by scavenging of free radicals (Pietri *et al.*, 1997; Shen *et al.*, 1996). It was reported that a standardized extract of ginseng reduced lipid peroxidation (Cabral de Oliveira *et al.*, 2001). The proanthocyanidins from grape seed extract have been reported to inhibit lipid peroxidation and to modulate the activity of enzyme systems including COX and lipooxygenase enzymes (Bors and Saran, 1987; Kolodziej *et al.*, 1995). They were found to be potent free radical scavengers (Ricardo da Silva *et al.*, 1991; Bagchi *et al.*, 1998; Yamaguchi *et al.*, 1999). Saw palmetto berry extract (SPBE) was observed to inhibit COX-2 expression, which is associated with an increased incidence of prostate cancer (Goldman *et al.*, 2001). Based on the widespread health attributes associated with these botanicals, their ability to inhibit COX enzymes and lipid peroxidation *in vitro* were investigated.

In this study, the botanical supplements were extracted separately with acidified water to simulate the gastric environment (pH = 2, 37 °C). The gastric environment is acidic in nature when food is not ingested. Fasting gastric pH has been well studied (Malagelada *et al.*, 1976; Malagelada *et al.*, 1977) and the generally accepted value for fasting gastric pH is approximately 2 (Dressman *et al.*, 1990).

MATERIALS AND METHODS

Botanical supplement samples. Echinacea, garlic, *Ginkgo biloba*, ginseng, Siberian ginseng, grape seed extract, kava kava, Saw palmetto and St John's wort, manufactured by Nature's Way, Meijer, GNC, Nutrilite, Sundown, Solaray and Natrol, were purchased in 2002 and 2003 from stores in Michigan, Illinois and Indiana (Table 1). All the supplements studied were exclusive preparations of the particular plant of interest except for Nutrilite

Table 1. Health claims reported on the bottle of each supplement studied with the batch numbers and the expiry dates

Botanical	Manufacturer	Claims	Batch numbers	Expiry date (mm/yy)
Echinacea	Nature's Way	Supports the immune system	235204	07/05
	Meijer	Stimulates the immune system	2NB0648	08/05
	GNC	–	C49483	10/04
			35181C4142	09/05
	Nutrilite	Supports body's natural resistance	23101LLA	10/04
	Sundown	Healthy immune function	497015	08/04
			610683	10/05
	Solaray	–	062805	11/05
	Natrol	Supporting the body's defense system	945598	10/03
Garlic	Meijer	Helps retain normal, healthy cholesterol levels	2HB1011	11/03
	GNC	Garlic provides dietary support for normal	2293GC0366	07/05
		healthy cardiovascular function	3803JC0366	10/05
			32671C0366	09/05
	Nutrilite	Supports overall cardiovascular health	2267YC7A	03/04
	Sundown	Healthy heart function	793963	07/04
	Solaray	–	062609	03/06

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Table 1. (*Continued*)

Botanical	Manufacturer	Claims	Batch numbers	Expiry date (mm/yy)
Ginkgo	Nature's Way	For mental function	236066	08/05
	Meijer	Cerebral circulation	2DB0857	04/05
	GNC	*Ginkgo biloba* supports increased blood flow to the brain	96199	08/04
			0660BC4556	02/05
	Nutrilite	Has been studied to improve blood flow to the brain	22680BUA	03/05
	Sundown	Mental alertness	357162	05/04
			1518	07/05
			357164	05/04
	Solaray	Intended to provide dietary support to help promote brain circulation for enhanced neuro activity	060909	03/06
Ginseng	Nature's Way	Enhances physical endurance and mental vitality	235145	07/04
	Sundown	Energy and endurance	809944	07/04
			354550	11/04
Siberian ginseng	Natrol	Siberian ginseng enhances physical and mental resistance to environmental stress while fortifying general endurance	947806	07/04
	Nutrilite	Siberian ginseng has been studied for its effect on work endurance	2302U4LB	10/04
	Solaray	–	062708	02/06
	Meijer	Physical and mental stress	2FB0275	05/05
	GNC	–	2041FC4363	09/08
			35061C4363	06/06
			96293	08/05
Grape seed	Nature's Way	Powerful antioxidant	226989	06/05
	GNC	–	0115AC4626	08/05
			C62819	09/05
			C64621	01/05
	Sundown	Superior antioxidant protection	355357	07/04
			355473	06/04
	Solaray	–	062604	10/05
Kava kava	GNC	–	92297	01/05
	Sundown	Calm well-being	158052	05/05
	Solaray	–	063008	02/06
	Natrol	Calming benefits of kava kava after a stressful day. Its affects relaxation without hampering memory and reaction time	944283	10/03
Saw palmetto	Nature's way	Prostate health	245899	08/05
	Meijer	Prostate health	1DB0411	09/05
	GNC	–	2605GC4552	07/06
			96065	07/05
			95047	06/05
	Nutrilite	For men, Saw palmetto and pumpkin seed oil support normal prostate function. Nettle root supports normal urinary flow	2263WSMB	08/05
	Sundown	Prostate and urinary health	867899	12/03
			867896	07/05
			372498	09/05
	Solaray	–	060208	02/06
St John's wort	Nature's Way	Positive mood	247066	12/05
	Meijer	Mood enhancer	1HB1220	06/04
	GNC	–	82151	09/03
			96523	09/05
			98387	12/05
	Nutrilite	St John's wort is a clinically proven natural approach that helps support a healthy emotional balance and well being	1305POVA	10/03
	Sundown	Mood enhancement	355700	10/05
			354110	01/04
			355701	10/05
	Solaray	–	062307	01/06
	Natrol	St John's wort plays a role in mood enhancement and maintaining a healthy positive mental outlook	947225	05/04

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Ginkgo with DHA (docosahexaenoic acid), Nutrilite Siberian ginseng with *Ginkgo biloba*, Nutrilite Saw Palmetto with Nettle root and Nutrilite St John's wort with lemon balm.

Preparation of extracts for *in vitro* assays. The supplements tested were in the form of capsules, tablets or soft gels. For capsules and soft gels, the shells were removed before extraction. The tablets were powdered and used for extraction. Three unit (1 unit = 1 tablet/capsule/soft gel) contents of each supplement were weighed and extracted with 25 mL of acidified water (pH = 2) by placing it on a shaker for 6 h at 37 °C, and centrifuged. The resulting extracts were lyophilized and the dry extracts were used to perform *in vitro* bioassays. The extraction was carried out at pH = 2 and 37 °C to simulate the gastric environment.

Cyclooxygenase enzyme inhibitory assay. COX-1 activity was assessed using an enzyme preparation from ram seminal vesicles (Oxford Biomedical Research, Inc., Oxford, MI). COX-2 activity was determined using a preparation of human prostaglandin H synthase isoenzyme 2 (hPGHS-2) cloned in insect cells. COX assays were carried out by monitoring the rate of oxygen uptake in an micro chamber and the oxygen electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH) as reported earlier (Wang *et al.*, 2000; Seeram *et al.*, 2001; Francis *et al.*, 2004). Each assay mixture contained 0.6 mL 0.1 M Tris buffer (pH 7), 1 mM phenol, 17 μg hemoglobin. The test samples (6 μL) and the enzyme (10 μL for COX-1 and 30 μL for COX-2) were incubated for 3 min and then with 10 μL of arachidonic acid solution (0.25 mg/0.25 mL Tris buffer) to initiate the reaction. Data were recorded using Quicklog for Windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA). The samples were tested at 25 and 100 μg/mL. Rofecoxib (Vioxx®) (1 μg/mL), celecoxib (Celebrex®) (1 μg/mL), naproxen (1.5 μg/mL) and aspirin (108 μg/mL) were assayed as positive controls.

Lipid peroxidation inhibitory assay. The lipid peroxidation assay was conducted by using a model liposome and its oxidation using fluorescence spectroscopy. Synthetic 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) (Avanti Polar Lipids, Alabaster, AL) was the lipid substrate used. The lipid and the fluorescent probe, 3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (DPH-PA) (Molecular Probes, Inc., Eugene, OR), were dissolved in DMF and dried under vacuum at room temperature. The resulting lipid film was hydrated with a buffer (500 μL containing 0.15 M NaCl, 0.01 M MOPS (pH 7.0) and 0.1 mM EDTA). Large unilamellar vesicles (LUVs) were prepared by subjecting the resuspended mixture to 10 freeze-thaw cycles using a dry ice/ethanol bath, followed by extrusion (29 times) through a 100 nm pore size membrane in a Lipofast extruder apparatus (Avestin Inc., Ottawa, Canada) (Arora *et al.*, 1997). The fluorescent intensity assay described by Arora and Strasburg (1997) was used to assess the antioxidant efficacy of the samples. In the assay, the peroxidative degradation of the probe DPH-PA is indicated by the decrease in fluorescence and is used to monitor the sensitivity of the membrane towards oxidative stress. The final assay volume was 2 mL, consisting of 100 μL HEPES buffer (50 mM HEPES and 50 mM TRIS), 200 μL 1 M NaCl, 1.645 mL N_2 sparged water, 20 μL of test sample or DMSO (blank) and 15 μL aliquot of liposome suspension. Peroxidation was initiated by the addition of 20 μL $FeCl_2$. 4 H_2O (0.5 mM). Positive controls used were BHA, BHT and TBHQ at 1.80 μg/mL, 2.20 μg/mL and 1.66 μg/mL, respectively, and test samples at 25 or 10 μg/mL. Fluorescence was measured at 384 nm and monitored at 0, 1, 3 and every 3 min thereafter up to 21 min using a Turner Model 450 digital fluorometer (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity over time indicated the rate of peroxidation. Relative fluorescence (F_t/F_0) was calculated by dividing the fluorescence value at a given point (F_t) by that at $t = 0$ min (F_0).

RESULTS

The supplements were extracted separately with acidified water (pH = 2) for 6 h at 37 °C, centrifuged and the resulting extracts were lyophilized. The weight of lyophilized extracts varied among supplements (Table 2). The amount of extracts used in the *in vitro* assay was calculated based on the recommended dose of the specific supplement per day. Therefore, the standardized amount of extract per kg body weight varied between 6 and 49 mg, depending on the supplement. For convenience and ease of conducting the bioassays, the concentration selected for COX and lipid peroxidation bioassays was 25 μg/mL.

Garlic (Meijer, Sundown), ginkgo (Solaray), ginseng (Nature's Way, Sundown), Siberian ginseng (Meijer, GNC, Nutrilite, Solaray, Natrol), kava kava (GNC, Sundown, Solaray, Natrol) and St John's wort (Nutrilite) showed only marginal COX-2 enzyme inhibitory activity at 25 μg/mL. Therefore, the assays were repeated at 100 μg/mL for these extracts. Since, these extracts had COX-2 enzyme inhibitory activity at 100 μg/mL, the COX-1 enzyme inhibitory assay was conducted only at 100 μg/mL. At this concentration, most extracts displayed 5–85% of COX-1 and 13–28% of COX-2 enzyme inhibitory activities. The results of COX enzymes inhibitory activities of the standards and extracts are presented in Fig. 1a-1c. Garlic (Meijer, Sundown), ginkgo (Solaray), ginseng (Nature's Way, Sundown), Siberian ginseng (Meijer, GNC, Nutrilite, Solaray, Natrol), kava kava (GNC, Sundown, Solaray, Natrol) and St John's wort (Nutrilite) exhibited selective COX-2 enzyme inhibition at 100 μg/mL (Fig. 1b). However, extracts of garlic (Sundown), ginseng (Nature's Way, Sundown), Siberian ginseng (Meijer), kava kava (Natrol) and St John's wort (Nutrilite) demonstrated COX-1 enzyme inhibition (Fig. 1b). The Siberian ginseng (Meijer) extract gave a higher COX-1 (43%) enzyme inhibition than COX-2 (28%). Echinacea (Meijer), garlic (GNC, Nutrilite), ginkgo (Meijer, GNC), grape seed (GNC, Nature's Way, Solaray), saw palmetto (GNC, Sundown, Nutrilite) and St John's wort (Natrol, Nature's Way) extracts exhibited selective COX-1 enzyme inhibition (Fig. 3).

The inhibition of lipid peroxidation was tested at 25 μg/mL for all supplement extracts (Fig. 2a–f). Most of the extracts tested at 25 μg/mL inhibited lipid peroxidation, which included extracts from echinacea

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Table 2. The yield of extract obtained from each supplement after extraction with acidic water at pH = 2 and 37 °C. Weight of extract per daily dose is based on the recommended serving size

Botanical	Manufacturer	Wt of extract/ unit (mg)	Wt of extract/daily dose (mg)
Echinacea	Nature's Way	154.70	618.8
	Meijer	110.10	990.9
	GNC (*Echinacea purpurea*)	64.70	388.2
	Nutrilite (Triple Guard Echinacea)	36.77	220.62
	Sundown	90.93	363.72
	Solaray (Echinacea root)	32.60	195.6
	Natrol	27.07	27.07
Garlic	Meijer	180.73	542.19
	GNC (Odorless Garlic)	102.90	102.9
	Nutrilite	46.10	92.2
	Solaray	32.60	97.8
	Sundown	85.23	340.92
Ginkgo	Nature's Way	64.80	259.2
	Meijer	192.60	577.8
	GNC	37.77	75.54
	Nutrilite (*Ginkgo biloba* and Dha)	27.30	81.9
	Sundown	56.93	113.86
	Solaray	15.77	31.54
Ginseng	Nature's Way (Korean ginseng)	146.67	293.34
	Sundown (Korean ginseng)	144.03	576.12
Siberian ginseng	Meijer (Siberian ginseng)	13.37	80.22
	GNC (Siberian ginseng)	27.00	54
	Nutrilite (Siberian ginseng with *Ginkgo biloba*)	342.43	1369.72
	Solaray (Siberian ginseng)	4.83	28.98
	Natrol (Siberian ginseng)	40.37	40.37
Grape seed	Nature's Way	93.10	186.2
	GNC	39.17	78.34
	Sundown	221.20	663.6
	Solaray	49.77	99.54
Kava kava	GNC	65.97	395.82
	Sundown	80.67	242.01
	Solaray	51.33	102.66
	Natrol	29.33	58.66
Saw palmetto	Nature's Way	2.43	4.86
	Meijer	232.20	928.8
	GNC	104.43	626.58
	Nutrilite (Saw palmetto and Nettle root)	43.17	129.51
	Sundown	36.60	146.4
	Solaray	101.40	405.6
St John's wort	Nature's Way	228.97	686.91
	Meijer	184.87	1109.22
	GNC	205.20	1231.2
	Nutrilite (St John's wort with Lemon balm)	311.10	933.3
	Sundown	213.77	641.31
	Solaray	23.97	71.91
	Natrol	137.47	412.41

(27–99%), garlic (14–32%), ginkgo (57–96%), ginseng (11–91%), grape seed (68–120%), kava kava (13–52%), saw palmetto (5–87%) and St John's wort (80–93%) supplements. The extracts, which demonstrated a higher lipid peroxidation inhibitory activity than the standards at 25 μg/mL were assayed again at 10 μg/mL (Fig. 2g).

DISCUSSION

Selective inhibition of COX-2 enzyme is desirable for a supplement. This is because COX-2 enzyme is normally induced in response to inflammation. It is also found at elevated levels in many human cancers, especially colorectal cancers (Hsi *et al.*, 1999). Therefore, COX-2 enzyme inhibitors are not only ideal antiinflammatory agents but also useful in the prevention and progression of several types of cancers. The COX-1 enzyme is expressed constitutively in many tissues (Smith and DeWitt, 1996) and it is also involved in the production of prostaglandins. Inhibition of COX-1 enzyme is also implicated in the prevention of cancer (Hsi *et al.*, 1999). But the only adverse effect of COX-1 inhibitors is the gastric ulcerations, as prostaglandins are also involved in the production of a protective mucus in the stomach.

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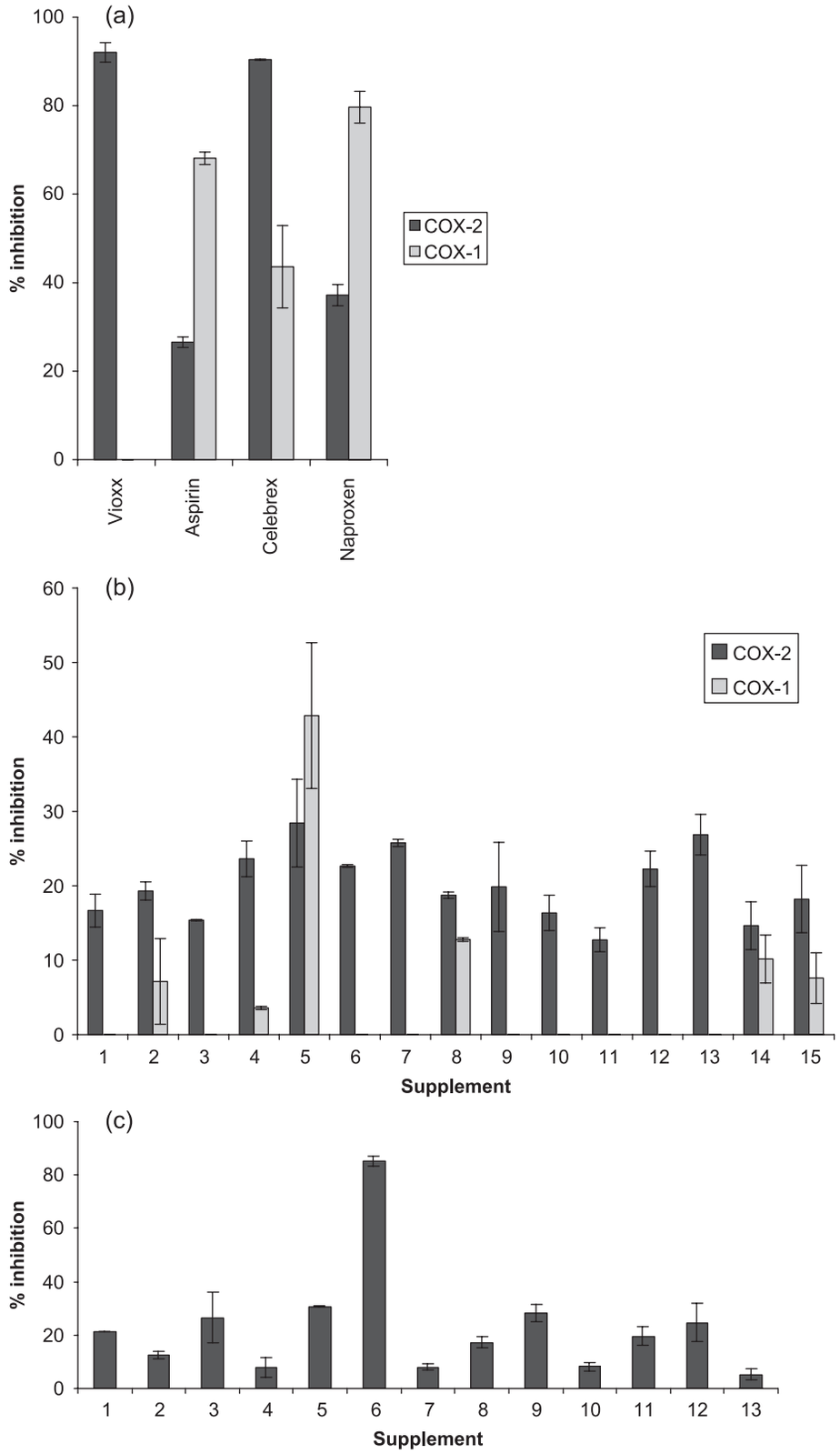

Figure 1. (a) Inhibition of COX enzymes by Vioxx (1.67 μg/mL), aspirin (108 μg/mL), celebrex (1.67 μg/mL), naproxen (2.5 μg/mL). Vertical bars represent the standard deviation of each data point ($n = 2$). (b) Inhibition of COX-1 and 2 enzymes by the acidic aqueous extract prepared from botanical supplements studied at100 μg/mL. The extracts are 1 (Meijer garlic), 2 (Sundown garlic), 3 (Solaray ginkgo), 4 (Nature's Way ginseng), 5 (Meijer ginseng), 6 (GNC ginseng), 7 (Nutrilite ginseng), 8 (Sundown ginseng), 9 (Solaray Siberian ginseng), 10 (Natrol Siberian ginseng), 11 (GNC kava kava), 12 (Sundown kava kava), 13 (Solaray kava kava), 14 (Natrol kava kava) and 15 (Nutrilite St John's wort). Vertical bars represent the standard deviation of each data point ($n = 2$). (c) Inhibition of COX-1 enzyme by the acidic aqueous extract prepared from botanical supplements studies at 100 μg/mL. The extracts are 1 (Meijer echinacea), 2 (GNC garlic), 3 (Nutrilite garlic), 4 (Meijer *Ginkgo biloba*), 5 (GNC *Ginkgo biloba*), 6 (GNC grape seed), 7 (Nature's Way grape seed), 8 (Solaray grapenol), 9 (GNC Saw palmetto), 10 (Sundown Saw palmetto), 11 (Nutrilite Saw palmetto), 12 (Natrol St John's wort) and 13 (Nature's Way St John's wort). Vertical bars represent the standard deviation of each data point ($n = 2$).

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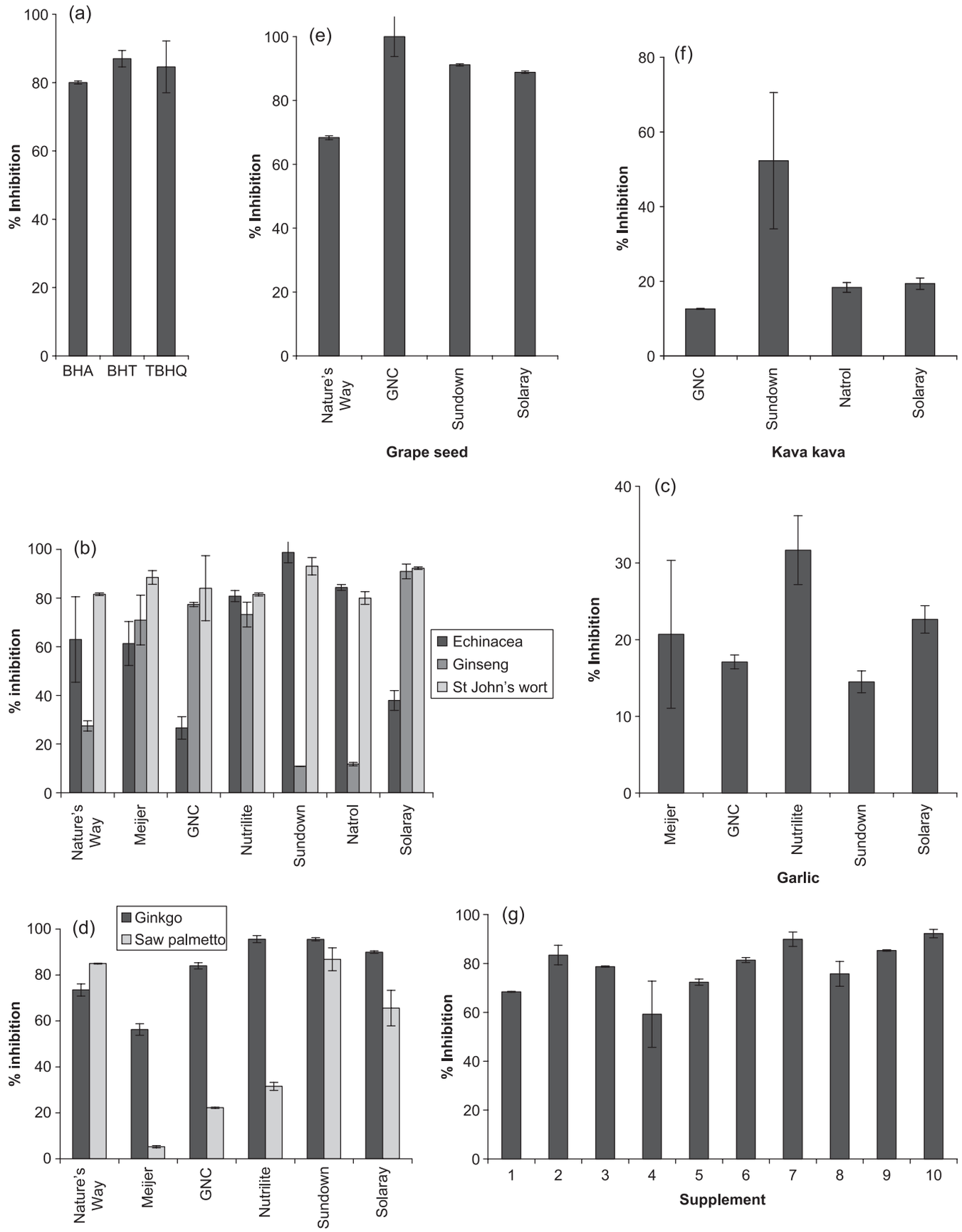

Figure 2. Inhibition of lipid peroxidation at $t = 21$ min. Vertical bars represent the standard deviation of each data point ($n = 2$). (a) Positive controls BHA, BHT and TBHQ tested at 1.80, 2.20 and 1.66 μg/mL, respectively. (b) Echinacea, ginseng and St John's wort supplement extracts at 25 μg/mL. (c) Garlic supplement extract at 25 μg/mL. (d) Gingko and saw palmetto supplements extracts at 25 μg/mL. (e) Grape seed supplement extract at 25 μg/mL. (f) Kava kava supplement extract at 25 μg/mL. (g) The active extracts at 10 μg/mL. The samples are 1 (Solaray *Ginkgo biloba*), 2 (Nutrilite *Ginkgo biloba* and Dha), 3 (Sundown *Ginkgo biloba*), 4 (Solaray Siberian ginseng), 5 (Sundown Grape seed), 6 (GNC Grape seed), 7 (Solaray Grapenol), 8 (Sundown St John's wort), 9 (Solaray St John's wort) and 10 (Meijer St John's wort).

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There is a wealth of evidence that non-steroidal anti-inflammatory drugs (NSAIDs) can prevent colorectal cancers (Luk, 1996; Kate *et al.*, 2002; Herendeen and Lindley, 2003). Most of the NSAIDs inhibit both isoforms of COX and thus, gastric ulcer is usually associated with its use. But it has been suggested that inhibition of both isoforms of COX may have important protective effects against colorectal cancer (Watson, 1998; Slattery *et al.*, 2004).

Lipid peroxidation has been implicated in many of the chronic illnesses. Prevention of free radical generation or its scavenging can be beneficial in maintaining good health. Inhibition of lipid peroxidation *in vivo* can prevent the free radicals involved in oxidative tissue damage. Inhibition of COX enzymes and lipid peroxidation by the extracts produced from the supplements studied indicate that could play a role in maintaining good health. For example, the extracts of garlic (Meijer), ginkgo (Solaray), ginseng (Nature's Way), Siberian ginseng (GNC, Nutrilite, Solaray, Natrol), kava kava (GNC, Sundown, Solaray) and St John's wort (Nutrilite) selectively inhibited the COX-2 enzyme and lipid peroxidation at the recommended dose per day. Hence, these supplements may be beneficial in the prevention and/or treatment of inflammation and cancer. But, it should also be kept in mind that the supplement preparations, which might be available in the market currently, might not produce the same results. It is due to the fact that the samples used in this study were procured at the start of the study and the supplement preparation and its formulation ingredients may have changed since then. A change in the ingredients to formulate the supplement may alter the observed results as this study has been performed on the extracts of the supplement rather than a particular plant extract.

The bioassay results varied among products supplied by the same manufacturer. Also, the variability in biological activity was significant for a given supplement distributed by different manufacturer. One of the primary reasons for this could be the sourcing of plant material used in the product manufacturing since locations and environmental conditions play a significant role in the production of bioactive metabolites. Also, varying extraction procedures and handling techniques contribute to the quality of the product. It is also useful to note that the biological activities varied for the same herbal supplement by different manufacturers as some of the labels state only the genus name of the herb. Species and cultivar differentiation along with plants grown under appropriate environmental and growing conditions are essential elements to yield high quality herbs and its extracts. Manufacturer of the supplement should be aware of these factors before sourcing the herbs. Further research should also be directed to the identification of the active ingredients in the acidic aqueous extracts of these supplements and their effective dosage.

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REFERENCES

Adderley SR, Fitzgerald DJ. 1999. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2. *J Biol Chem* **274**: 5038–5046.

Arora A, Nair MG, Strasburg GM. 1998. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radic Biol Med* **24**: 1355–1363.

Arora A, Strasburg GM. 1997. Development and validation of fluorescence spectroscopic assays to evaluate antioxidant efficacy. Application to metal chelators. *J Am Oil Chem Soc* **74**: 1031–1040.

Bagchi D, Garg A, Krohn RL *et al.* 1998. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol* **30**: 771–776.

Baker CS, Hall RJ, Evans TJ. 1999. Cyclooxygenase-2 is widely expressed in atherosclerotic lesions affecting native and transplanted human coronary arteries and colocalizes with inducible nitric oxide synthase and nitrotyrosine particularly in macrophages. *Arterioscler Thromb Vasc Biol* **19**: 646–655.

Blumenthal M. 1999. Herbal market levels after five years of boom. *HerbalGram* **47**: 64–65.

Bors W, Saran M. 1987. Radical scavenging by flavonoid antioxidants. *Free Radic Res Commun* **2**: 289–294.

Cabral de Oliveira, Antonio C, Perez AC, Merino G, Preito JG, Alvarez Al. 2001. Protective effects of *Panax ginseng* on muscle injury and inflammation after eccentric exercise. *Comp Biochem Physiol* **130C**: 369–377.

Cryer B, Dubois A. 1998. The advent of highly selective inhibitors of cyclooxygenase – a review. *Prostaglandins Other Lipid Mediators* **56**: 341–361.

Dannenberg AJ, Subbaramaiah, K. 2003. Targetting cyclooxygenase-2 in human neoplasia: rationale and promise. *Cancer Cell* **4**: 431–136.

Dressman JB, Berardi RR, Dermentzoglou LC *et al.* 1990. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm Res* **7**: 756–761.

Eisenberg DM, Davis RB, Ettner SL *et al.* 1998. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. *JAMA* **280**: 1569–1575.

Escher M, Desmeulus J, Giostra E, Mentha G. 2001. Hepatitis associated with kava, a herbal remedy for anxiety. *Br Med J* **322**: 139.

Facino RM, Carini M, Aldini G, Saibene L, Pietta P, Mauri P. 1995. Echinosides and caffeoyl conjugates protect collagen from free radical-induced degradation: A potential use of Echinacea extracts in the prevention of skin photodamage. *Planta Med* **61**: 510–514.

Francis JA, Jayaprakasam B, Olson LK, Nair MG. 2004. Insulin secretagogues from *Moringa oleifera* with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. *Helv Chim Acta* **87**: 317–326.

Goldman WH, Sharma AL, Currier SJ, Johnston PD, Ajay R, Pal Sharma C. 2001. Saw palmetto berry extract inhibits cell growth and Cox-2 expression in prostatic cancer cells. *Cell Biol Int* **25**: 1117–1124.

Herendeen JM, Lindley C. 2003. Use of NSAIDs for the chemoprevention of colorectal cancer. *Ann Pharmacother* **37**: 1664–1674.

Hsi LC, Angerman-Stewart J, Eling TE. 1999. Introduction of full-length APC modulates cyclooxygenase-2 expression in HT-29 human colorectal carcinoma cells at the translational level. *Carcinogenesis* **20**: 2045–2049.

Hu C, Kitts DD. 2000. Studies on the antioxidant activity of Echinacea root extract. *J Agric Food Chem* **48**: 1466–1472.

Humberston CL, Akhtar J, Krenzelok EP. 2003. Acute hepatitis induced by kava kava. *J Toxicol Clin Toxicol* **41**: 109–113.

Juni P, Nartey L, Reichenback S, Sterchi R, Dieppe PA, Egger M. 2004. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet* **364**: 2021–2029.

Kanowski S, Hoerr R. 2003. *Ginkgo biloba* extract EGb 761 in dementia: intent-to-treat analyses of a 24-week, multi-center,

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double-blind, placebo-controlled, randomized trial. *Pharmacopsychiatry* **36**: 297–303.
Kasper S, Anghelescu IG, Szegedi A, Dienel A, Kieser M. 2006. Superior efficacy of St. John's Wort extract WS 5570 compared to placebo in patients with major depression: a randomized, double-blind, placebo-controlled, multi-center trial. *BMC Med* **4**: 14.
Kate J, Cheng KK, Langman MJS. 2002. NSAIDs and gastrointestinal cancer prevention. *Drugs* **62**: 945–956.
Kolodziej H, Haberland C, Woerdenbag HJ, Konings AWT. 1995. Moderate cytotoxicity of proanthocyanidins to human tumor cell lines. *Phytother Res* **9**: 410–415.
Lau BHS. 2001. Suppression of LDL oxidation by garlic. *J Nutr* **131**: 985S–988S.
Lehrl S. 2004. Clinical efficacy of kava extract WS1490 in sleep disturbances associated with anxiety disorders. Results of a multicenter, randomized, placebo-controlled, double-blind clinical trial. *J Affect Disord* **78**: 101–110.
Lipisky PE. 1999. The clinical potential of cyclooxygenase-2-specific inhibitors. *Am J Med* **106**: 51S–57S.
Luk GD. 1996. Prevention of gastrointestinal cancer-the potential role of NSAIDs in colorectal cancer. *Schweiz Med Wochenschr* **126**: 801–812.
Malagelada JR, Longstreth GF, Summerskill WH, Go VL. 1976. Measurement of gastric functions during digestion of ordinary solid meals in man. *Gastroenterology* **70**: 203–210.
Malagelada JR, Longstreth GF, Deering TB, Summerskill WH, Go VL. 1977. Gastric secretion and emptying after ordinary meals in duodenal ulcer. *Gastroenterology* **73**: 989–994.
Mukherjee D, Nissen SE, Topol EJ. 2001. Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA* **286**: 954–959.
Muller-Jakic B, Breu W, Probstle A, Redl K, Greger H, Bauer R. 1994. *In vitro* inhibition of cyclooxygenase and 5-lipoxygenase by alkamides from *Echinacea* and *Achillea* species. *Planta Med* **60**: 37–40.
Pietri S, Maurelli E, Drieu K, Culcasi M. 1997. Cardioprotective and anti-oxidant effects of the terpenoid constituents of *Ginkgo biloba* extract (Egb 761). *J Mol Cell Cardiol* **29**: 733–742.
Ricardo da Silva JM, Darmon N, Fernandez Y, Mitjavila S. 1991. Oxygen free radical scavenging capacity in aqueous models of different procyanidins from grape seeds. *J Agric Food Chem* **39**: 1549–1552.
Schonbeck U, Sukhova GK, Graber P, Coulter S, Libby P. 1999. Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions. *Am J Pathol* **155**: 1281–1291.
Seeram NP, Momin RA, Nair MG, Bourquin LD. 2001. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* **8**: 362–369.
Shen J, Zhao B, Li M, Wan Q, Wen-Jian. 1996. Inhibitory effects of *Ginkgo biloba* extract (Egb 761) on oxygen free radicals, nitric oxide and myocardial injury in isolated ischemic-reperfused hearts. In *Proceedings of the International Symposium on Natural Antioxidants*. Molecular mechanisms and Health Effects. AOCS Press, Champaign, Illinois, USA, 453–465.
Singh YN, Devkota AK. 2003. Aqueous kava extracts do not affect liver function tests in rats. *Planta Med* **69**: 496–499.
Slattery ML, Samowitz W, Hoffmann M, Ma KN, Levin TR, Neuhausen S. 2004. Aspirin, NSAIDs, and colorectal cancer: possible involvement in an insulin-related pathway. *Cancer Epidemiol Biomarkers Prev* **13**: 538–545.
Smith WL, DeWitt DL. 1996. Prostaglandin endoperoxide H synthases-1 and -2. *Adv Immunol* **62**: 167–215.
Sorrentino L, Capasso A, Schmidt M. 2006. Safety of ethanolic kava extract: Results of a study of chronic toxicity in rats. *Phytomedicine* **13**: 542–549.
Wang H, Nair MG, Strasburg GM, Booren AM, Gray I, Dewitt DL. 2000. Cyclooxygenase active bioflavonoids from Balaton™ tart cherry and their structure activity relationships. *Phytomedicine* **7**: 15–19.
Watson AJM. 1998. Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2. *Histol Histopathol* **13**: 591–597.
Weber W, Taylor JA, Stoep AV, Weiss NS, Standish LJ, Calabrese C. 2005. *Echinacea purpurea* for prevention of upper respiratory tract infections in children. *J Altern Complement Med* **11**: 1021–1026.
Yamaguchi F, Yoshimura Y, Nakazawa H, Ariga T. 1999. Free radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an H_2O_2/NaOH/DMSO system. *J Agric Food Chem* **47**: 2544–2548.
Yenilmez A, Kilic FS, Sirmagul B, Isikli B, Aral E, Oner S. 2007. Preventive effects of *Ginkgo biloba* extract on ischemia-reperfusion injury in rat bladder. *Urol Int* **78**: 167–172.
Zarraga IG, Schwarz ER. 2007. Coxibs and heart disease: What we have learned and what else we need to know. *J Am Coll Cardiol* **49**: 1–14.

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