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Tumor Cell Proliferation and Cyclooxygenase Inhibitory Constituents in Horseradish (*Armoracia rusticana*) and Wasabi (*Wasabia japonica*)

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Cyclooxygenase and human tumor cell growth inhibitory extracts of horseradish (*Armoracia rusticana*) and wasabi (*Wasabia japonica*) rhizomes upon purification yielded active compounds **1**−**3** from horseradish and **4** and **5** from wasabi rhizomes. Spectroscopic analyses confirmed the identities of these active compounds as plastoquinone-9 (**1**), 6-*O*-acyl-β-D-glucosyl-β-sitosterol (**2**), 1,2-dilinolenoyl-3-galactosylglycerol (**3**), linolenoyloleoyl-3-β-galactosylglycerol (**4**), and 1,2-dipalmitoyl-3-β-galactosylglycerol (**5**). 3-Acyl-sitosterols, sinigrin, gluconasturtiin, and phosphatidylcholines isolated from horseradish and α-tocopherol and ubiquinone-10 from wasabi rhizomes isolated were inactive in our assays. At a concentration of 60 μg/mL, compounds **1** and **2** selectively inhibited COX-1 enzyme by 28 and 32%, respectively. Compounds **3**, **4**, and **5** gave 75, 42, and 47% inhibition of COX-1 enzyme, respectively, at a concentration of 250 μg/mL. In a dose response study, compound **3** inhibited the proliferation of colon cancer cells (HCT-116) by 21.9, 42.9, 51.2, and 68.4% and lung cancer cells (NCI−H460) by 30, 39, 44, and 71% at concentrations of 7.5, 15, 30, and 60 μg/mL, respectively. At a concentration of 60 μg/mL, compound **4** inhibited the growth of colon, lung, and stomach cancer cells by 28, 17, and 44%, respectively. This is the first report of the COX-1 enzyme and cancer cell growth inhibitory monogalactosyl diacylglycerides from wasabi and horseradish rhizomes.

KEYWORDS: Horseradish; wasabi; anticancer; galactosyl diacylglycerides; cyclooxygenase enzyme

INTRODUCTION

Horseradish (*Armoracia rusticana*) is a perennial herb of the Brassicaceae family. Its rhizomes are widely used as a condiment and as a source of horseradish peroxidase. Horseradish peroxidase is a glycoprotein commonly used as a reagent for clinical diagnosis and analytical immunoassays (*1*). Wasabi (*Wasabia japonica*), called Japanese horseradish, is also a member of the Brassicaceae family of vegetables. Either in the fresh form or as a dried powder, wasabi is widely used in the Japanese cuisine to garnish traditional dishes such as sushi and sashimi.

A high consumption of vegetables and fruits has been correlated with a decreased cancer risk. High consumption of Brassica vegetables lowered colon cancer risk in men and women, but the risk was increased in women for rectal cancer (*2*). Consumption of yellow-green vegetables has been correlated with reduced cancer risk from epidemiological studies conducted in Japan (*3*, *4*). Although it was shown that wasabi powder fed to Winstar WKY male rats protected them against *N*-methyl-*N*-nitroso-*N*-nitroguanidine-induced gastrointestinal tumors (*5*), the reports on glucosinolates and isothiocyanates from Brassica spp. and cancer protection are rather ambiguous. For example, isothiocyanates have been shown to inhibit cancer when administered prior to or during treatment with the carcinogen, but they did not perform well when administered after the carcinogen treatment (*6*). In addition, the chemoprotective effects of isothiocyanates seemed to be highly specific to the type of cancer (*6*). Desulfoglucosinolates, the biosynthetic precursors of the glucosinolates, are reported to be compounds with both beneficial and detrimental effects in the Brassica vegetables. We have reported the adverse effects of desulfosinigrin isolated from both wasabi and horseradish as indicated by the in vitro colon cancer cell growth proliferation (*7*). The use of wasabi and horseradish in traditional Japanese dishes and its increased consumption in other countries prompted us to further investigate the presence of potential anticancer and anti-inflammatory compounds in wasabi and horseradish rhizomes.

MATERIALS AND METHODS

Materials. Fresh wasabi rhizomes were purchased from Pacific Farms (Eugene, OR). Fresh horseradish rhizomes were purchased from a supermarket in East Lansing, MI. All solvents were ACS reagent grade and purchased from Spectrum Chemical Co. (Gardena, CA). Ibuprofen, Naproxen, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Celebrex and Vioxx were physician's professional samples supplied by Dr. S. Gupta, Sparrow Hospital, Michigan. COX-1 enzyme was prepared in

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the Bioactive Natural Products and Phytoceuticals Laboratory (BNPP) from ram seminal vesicles and COX-2 enzyme was prepared from prostaglandin endoperoxide H synthase-2 (PGHS-2) cloned insect cell lysate in the Department of Biochemistry and Molecular Biology at Michigan State University. Fetal bovine serum (FBS) and Roswell Park Memorial Institute-1640 (RPMI-1640) medium were purchased from Gibco BRL (Grand Island, NY). Human tumor cell lines, SF-268 (central nervous system, CNS), NCI−H460 (lung), and MCF-7 (breast) were purchased from the National Cancer Institute (NCI, Bethesda, MD) and HCT-116 (colon) and AGS (stomach) from American Type Culture Collection (ATCC, Rockville, MD). All cell lines were maintained at BNPP, Michigan State University.

General Experimental Procedures. ^{1}H NMR spectra were recorded on Varian INOVA (300 MHz) and VRX (500 MHz) instruments. ^{13}C NMR spectra were obtained at 75 and 125 MHz. ^{1}H NMR chemical shifts are reported in ppm relative to $CDCl_3$ at 7.24, DMSO-d_6 at 2.49, D_2O at 4.80, and CD_3OD at 3.30 ppm, respectively. ^{13}C NMR chemical shifts are reported in ppm relative to $CDCl_3$ at 77.0, DMSO at 39.50, and CD_3OD at 49.0, respectively.

A model LC-20 recycling preparative liquid chromatograph, equipped with a model AS-20 fraction collector (Japan Analytical Industry Co., Tokyo) and a 250 × 20 mm, 10 μm, JAIGEL-ODS column (Dychrom, Santa Clara, CA), was used for the purification of extracts. Peaks were detected by UV and refractive index detectors and integrated by using a model D-2500 chromato-integrator (Hitachi, Tokyo). The HPLC system (Waters Corp) used was equipped with a controller, a 717 autosampler, a 2410 RI detector, and a 996-photodiode-array detector and a 250 × 19 mm, 10 μm, XTerra Prep RP-18 preparative column (Waters Corp.). Data were recorded and processed using Empower Pro (Waters Corp., Milford, MA). Merck Silica gel 60 with a particle size of 35−70 μm and C18 with a particle size of 60 μm (Dychrom, Santa Clara, CA) were used for preparative medium-pressure liquid chromatography (MPLC). For the preparative TLC separation, 20 × 20 cm, 1000, 500, and 250 μm silica gel plates (Analtech, Inc., Newark, DE) were used. Gas chromatographic-mass spectrometric (GC-MS) analysis was carried out using an HP 6890 system equipped with an electron capture detector operating at 250 °C, a 30 m × 250 μm × 0.25 μm HP-5MS column, and a 7673-model injector operating at 250 °C in the splitless mode; the injection volume was 1.0 μL. Helium was used as carrier gas at 0.8 mL/min. The temperature started at 50 °C, was held for 2 min, raised to 250 °C at 10 °C/min, and held until the end of the analysis. The quadrupole mass filter was set to scan from *m*/*z* 40 to 550. Samples for GC analyses were dissolved in hexane at 1 mg/mL.

Extraction of Wasabi and Horseradish Rhizomes. Lyophilized horseradish rhizomes (345 g) were sequentially extracted in a glass column with hexane (1 L × 5), ethyl acetate (1 L × 5), and methanol (1 L × 5). The removal of solvents under reduced pressure afforded 1.76, 1.31, and 51 g of hexane, ethyl acetate, and methanol extracts, respectively. Lyophilized wasabi rhizomes (562 g) were sequentially extracted in the same manner and yielded 2.1, 2.2, and 22 g of hexane, ethyl acetate, and methanol extracts, respectively.

Fresh horseradish rhizomes (1025 g) were blended with water and sequentially extracted in a glass column with water, methanol, and ethyl acetate. The water extract was lyophilized and yielded a powder (134 g). The removal of methanol and ethyl acetate under reduced pressure afforded 28 g and 160 mg of extracts, respectively. Fresh wasabi rhizomes (634 g) were blended with water and sequentially extracted in the same manner and yielded 26 g, 7.8 g, and 180 mg of water, methanol, and ethyl acetate extracts, respectively. The methanol and ethyl acetate extracts of fresh and lyophilized wasabi showed identical TLC profiles, respectively. Similarly, the methanol and ethyl acetate extracts of fresh and lyophilized horseradish showed identical TLC profiles, respectively. Therefore, the isolation of compounds was carried out by using the extracts derived from the lyophilized rhizomes of wasabi and horseradish.

Purification of Compounds 1−3 from Horseradish Extract. The hexane extract from horseradish was subjected to preparative TLC purification and yielded compound **1** and 3-acyl-sitosterols (*8*). The acyl moiety of 3-acyl-sitosterols was confirmed to be a 1:1 ratio of palmitic and linolenic acids by GC-MS analysis of the methylated products obtained from its hydrolysis.

The ethyl acetate extract of horseradish rhizomes was stirred with MeOH to afford a MeOH soluble fraction (610 mg). The MeOH soluble fraction (325 mg) was purified by preparative silica (1000 μm) TLC using hexane−acetone (3:1, v/v) as the mobile phase. Two bands collected were at R_f 0.1 (200 mg) and at R_f 0.25 (32 mg). The higher R_f band (15 mg) was further purified by preparative silica TLC (250 μm) using hexane−acetone (3:1, v/v) as the mobile phase to afford compound **2** (R_f 0.125, 10 mg). The lower R_f fraction (180 mg) was further purified by preparative silica TLC (250 μm) using MeOH−$CHCl_3$ (1:6, v/v) as the mobile phase to yield compound **3** (R_f 0.8, 20 mg).

Compound 3 (Colorless Oil). ^{1}H NMR (CD_3OD, δ): 5.25−5.45 (13H, m, H2, 9′, 10′, 12′, 13′, 15′, 16′, 9″, 10″, 12″, 13″, 15″, 16″), 4.43 (1H, dd, J = 12.1, 3.0 Hz, H1a), 4.23 (1H, d, J = 7.5, H1‴), 4.20 (1H, dd, J = 12.1, 6.7 Hz, H1b), 3.82 (1H, dd, J = 11.0, 5.5 Hz, H3b), 3.69−3.80 (3H, overlapping m, H3‴a, 6‴a, 6‴b), 3.45−3.55 (3H, overlapping m, 5‴, 3‴, 2‴), 2.78 (8H, b. t., H11′, 14′, 11″, 14″), 2.30 (4H, overlapping t, H2′, 2″), 1.98−2.10 (8H, m, H17′, 17″, 8′, 8″), 1.60 (4H, m, H3′, 3″), 1.20−1.40 (16H, H4′, 4″, 5′, 5″, 6′, 6″), 0.95 (6H, t, J = 7.6 Hz, 18′, 18″); ^{13}C NMR ($CDCl_3$): 173.67 (C1′), 173.34 (C1″), 131.93 (C16′, C16″), 130.17 (C9′, 9″), 128.31 (C12′, 12″), 128.22 (C13′, 13″), 127.99 (C10′, 10″), 127.12 (C15′, C15″), 104.03 (C1‴), 74.62 (C5‴), 73.51 (C3‴), 71.46 (C2‴), 70.23 (C2), 69.29 (C4‴), 68.22 (C3), 62.78 (C1), 62.25 (C6‴), 34.26 (C2″), 34.11 (C2′), 29.58 (C7′, 7″), 29.04−29.29 (C4′, 4″, 5′, 5″, 6′, 6″), 27.18 (C8′, 8″), 25.61 (C11′, 11″), 25.52 (C14′, 14″), 24.85 (C3′, 3″), 20.50 (C17′, 17″), 14.17 (C18′, 18″).

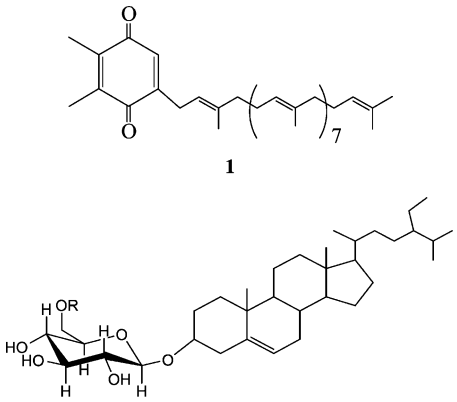
Purification of the methanolic extract of the horseradish rhizomes by various chromatographic methods gave sinigrin, gluconasturtiin, and phosphatidylcholines. Their structures were identified by comparison of NMR data with the previously published literature data (*9*, *10*). These compounds were not biologically active and hence detailed purification methods are not presented in this manuscript.

Purification of Compounds from Wasabi Rhizomes. The hexane extract of wasabi rhizomes was purified by chromatographic methods and afforded α-tocopherol and ubiquinone-10 which were not active in our assays. Their structures were identified by NMR spectroscopic analyses and further confirmed by comparison with published spectral data (*11*, *12*).

The ethyl acetate extract (1.9 g) of wasabi rhizomes was fractionated by preparative silica MPLC with hexane−acetone gradients as mobile phases at 4 mL/min. The bioactive fraction was further purified by preparative silica TLC using $CHCl_3$−MeOH−H_2O (4:1:0.1, v/v) as the mobile phase to afford compound **4** (R_f 0.7, 15 mg).

The methanol extract of the wasabi rhizomes (21.2 g) was stirred with MeOH. The MeOH soluble fraction (17 g) was then stirred with MeOH−H_2O (5:1, v/v) to yield soluble (16 g) and insoluble (900 mg) fractions. The MeOH−H_2O insoluble fraction (880 mg) was fractionated by preparative silica MPLC using $CHCl_3$−MeOH solvent gradients. The active fraction was further purified by preparative C18 MPLC using MeOH−H_2O as the gradient system (started at 20:80 (v/v) and ended with MeOH (100%). Fractions were combined according to their TLC profiles to yield three fractions. Repeated purification of the active fraction 2 (30 mg) by preparative silica TLC using MeOH−$CHCl_3$ (6:1, v/v) as the mobile phase yielded compound **5** (R_f 0.2, 16 mg).

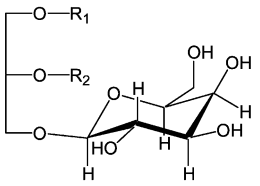
Cancer Cell Growth Inhibitory Assay. The tumor cells were maintained as adherent cell cultures in RPMI-1640 medium supplemented with 10% FBS, 10 units of penicillin, and 10 μg/mL streptomycin at 37 °C in a humidified incubator at 5% CO_2. The MTT cancer cell proliferation method was used in the assay of the extracts and pure compounds from wasabi and horseradish rhizomes. The test compounds were dissolved in DMSO and diluted with supplemented RPMI-1640 medium to the desired concentration, and the final DMSO concentration was 0.1%. The crude extracts of fresh wasabi and horseradish roots were tested at 250 μg/mL, and each sample was tested in triplicate. The pure and active compounds from preliminary screening, **3**, **4**, and **5**, were tested at 7.5, 15, 30, and 60 μg/mL in triplicate. Also, the most active compounds, **3** and **4**, were assayed further in triplicate and repeated three times (*13*).



1

R=Palmitic, oleic, linolenic acids

2



	R_1	R_2
3	linolenic,	linolenic
4	linolenic	oleic * (exchangeable)
5	palmitic	palmitic

Figure 1. The structures of compounds **1**–**5**.

Cyclooxygenase Enzymes Inhibitory Assay. Cyclooxygenase enzymes inhibitory activities of extracts and pure compounds were evaluated using COX-1 and COX-2 enzymes. The rate of oxygen consumption during the initial phase of the enzyme-mediated reaction with arachidonic acid as substrate was measured using a Model 5300 biological oxygen monitor (Yellow Spring Instruments, Inc., Yellow Springs, OH). The reaction mixture, consisting of 0.1 M Tris, 1.0 mM phenol, 17 μg hemoglobin, the enzyme, and 10 μL extract dissolved in DMSO at 1.5% (DMSO alone as solvent control), was held in a 600-μL micro-oxygen chamber (Instech Laboratory, Plymouth Meeting, PA) at 37 °C. After 3 min of incubation, 10 μL of arachidonic acid (1 mg/mL of Tris buffer) was added to initiate the reaction. Data were recorded using Quicklog for Windows (Strawberry Tree Inc., Sunnyvale, CA). The positive controls Ibuprofen, Aspirin, Celebrex, Vioxx, and Naproxen were assayed at 2.06, 180, 1.67, 1.67, and 2.52 μg/mL, respectively (*14*).

Methylation of Samples for GC-MS Analysis. Diazomethane was prepared as follows: KOH (15 g) was dissolved in water (25 mL) cooled in an ice bath. Diethyl ether (100 mL) and then *N*-nitroso-*N*-methylurea (1 g) were slowly added to the KOH solution. Using a separatory funnel, the diazomethane in ether was washed with cold water and the aqueous layer was discarded (*15*). Samples (2 mg) were hydrolyzed with 5% sodium hydroxide in MeOH (1 mL) for 5 min and then acidified with 6 N HCl in MeOH. After evaporation of the solvent, the product was dissolved in anhydrous ether and freshly prepared diazomethane was added until the solution turned yellow. Ether was removed and the resulting residue dissolved in hexane to yield a concentration of 1 mg/mL. Authentic samples of palmitic, oleic, linoleic, and stearic acids were also methylated using diazomethane and used as standards.

RESULTS AND DISCUSSION

The bioassay-guided fractionation and purification of the extracts of fresh wasabi and horseradish rhizomes resulted in the isolation of active compounds **1**−**3** and **4**−**5** (**Figure 1**),

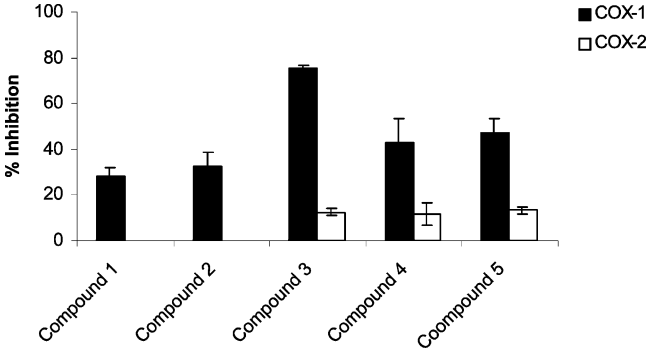


Figure 2. Cyclooxygenase enzyme inhibitory activities of compounds **1** and **2** at 60 μg/mL and **3**–**5** at 250 μg/mL, respectively. Results are expressed as mean value of the percent inhibition of duplicate measurements.

respectively, along with 3-acyl-sitosterols, sinigrin, gluconasturtiin, phosphatidylcholines, α-tocopherol, and ubiquinone-10 using preparative thin-layer (TLC), medium pressure (MPLC), and high-performance (HPLC) liquid chromatographic methods. The structure of compound **1** was established as plastoquinone-9, 2,3-dimethyl-5-[(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl]-2,5-cyclohexadiene-1,4-dione by NMR spectroscopic analyses. The spectral data for compound **1** was in agreement with that reported for plastoquinone-9 (*16*).

The identity of compound **2** as 6-*O*-acyl-β-D-glucosyl-β-sitosterol was determined by NMR spectroscopic analyses. The GC-MS analysis of the hydrolysis products from compound **2** indicated the presence of palmitic, oleic, and linolenic acids at a ratio of 1:1:1. Therefore, compound **2** was characterized as an inseparable mixture of 6-*O*-acyl-β-D-glucosyl-β-sitosterols (*17*) with palmitic, oleic, and linolenic acid moieties as the acyl groups. Mixtures of 6-*O*-acyl-β-D-glucosyl-β-sitosterols have been previously reported as cytotoxic against several cancer cell lines including the MCF-7 breast cancer cells (*18*).

Compounds **3**, **4**, and **5** showed similar NMR spectral data and suggested that they belonged to the monogalactosyl diacylglycerides class. These compounds were hydrolyzed and the resulting products analyzed by GC-MS after methylation with diazomethane. The structure of compound **3** was determined as 1,2-dilinolenoyl-3-β-galactosylglycerol by spectroscopic methods and its data were in agreement with that published for 1,2-dilinolenoyl-3-β-galactosylglycerol (*19*). The two acyl groups in compound **4** were identified as oleic and linolenic acid moieties in a 1:1 ratio. It is possible that compound **4** consisted of a mixture of positional isomers of oleic and linolenic acids in its glycerol backbone. Analysis of compound **5** revealed that palmitic acid was the only acyl moiety in the molecule. The identity of compound **5** was established as 1,2-dipalmitoyl-3-β-galactosylglycerol.

The crude extracts from wasabi and horseradish exhibited both COX-1 and -2 enzyme inhibitory activities. The inhibitions were about 65 and 50% at 250 μg/mL for COX-1 and COX-2 enzymes, respectively. In the COX enzyme inhibitory assays with purified compounds, compounds **1** and **2** inhibited only COX-1 enzyme at 60 μg/mL (**Figure 2**). Similarly, compound **3** inhibited COX-1 enzyme by 75% at 250 μg/mL while showing a marginal COX-2 enzyme inhibition. The COX-1 enzyme inhibition for compounds **4** and **5** was about 45% at 250 μg/mL. The pure compounds did not account for the high COX-1 and -2 enzyme inhibitory activities exhibited by the crude extracts. This was probably due to the high content of fatty acids present in the extracts in addition to components that were not

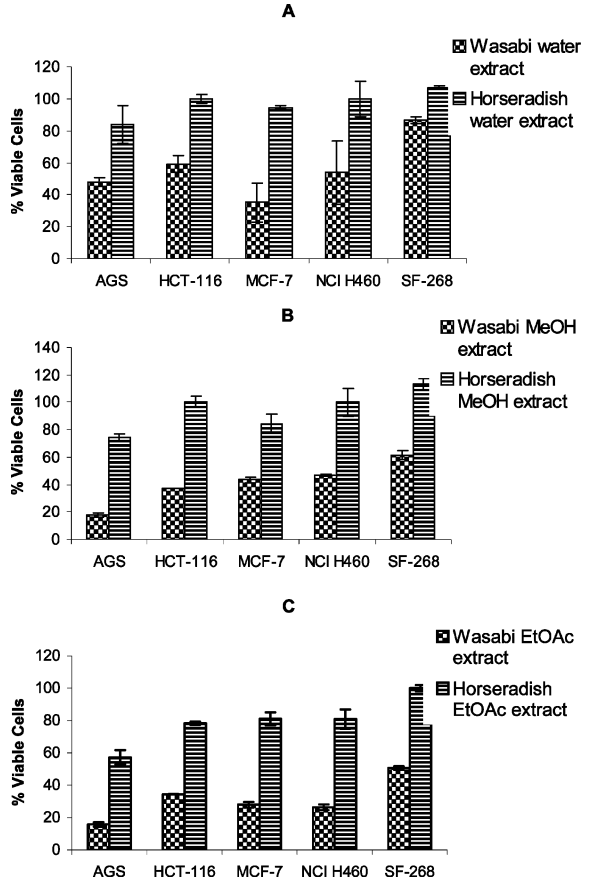


Figure 3. Effect of water (A), methanol (B), and ethyl acetate extracts (C) of fresh wasabi and horseradish roots on the proliferation of human stomach (AGS), colon (HCT-116), and lung (NCI–H460) cancer cell lines as determined by the MTT assay. The extract was tested at 250 μg/mL. The optical density was measured to determine the amount of formazan blue formed by viable cells and compared to the control. The data represents the mean ± SD of one experiment conducted in triplicate.

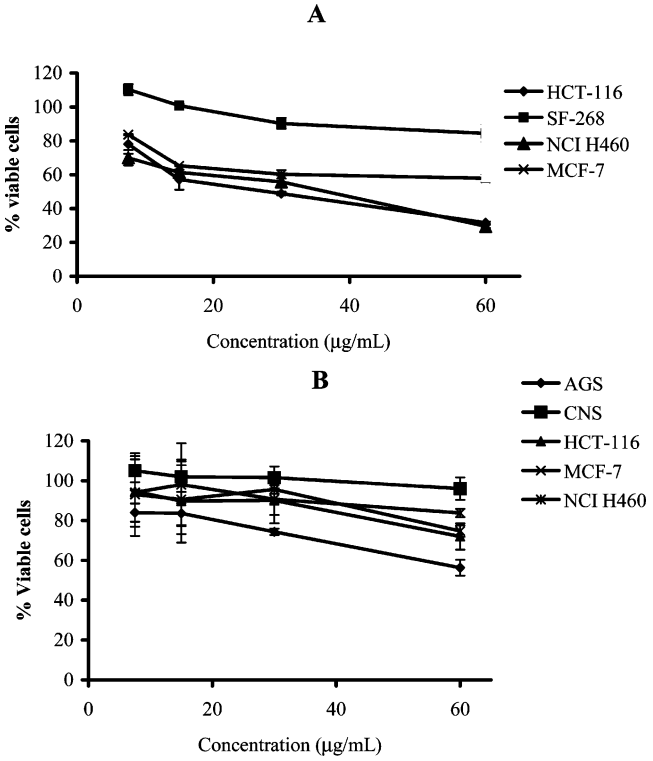


Figure 4. A: Effect of 1,2-dilinolenoyl-3-galactosylglycerol (compound 3) on the proliferation of human colon (HCT-116) and lung (NCI-H460) cancer cell lines as determined by the MTT assay. Growth inhibitory activity was not observed for breast (MCF-7) and CNS (central nervous system, SF-268) cancer cell lines. B: Effect of linolenoyloleoyl-3-β-galactosylglycerol (compound 4) on the proliferation of human stomach (AGS), colon (HCT-116), and lung (NCI-H460) cancer cell lines as determined by the MTT assay. Growth inhibitory activity was not observed for breast (MCF-7) and CNS (central nervous system) (SF-268) cancer cell lines. The optical density was measured to determine the amount of formazan blue formed by viable cells and compared to the control. The data represents the mean ± SD of three parallel experiments conducted in triplicate.

isolated. Fatty acids are known inhibitors of COX enzymes as reported from our previous studies (*20*) and therefore we did not pursue the isolation of fatty acids from these extracts. 1,2-Dilinolenoyl-3-β-galactosylglycerol, isolated from *Euphorbia cyparissias* L., was reported to exert topical anti-inflammatory activity in a mouse edema model (*21*). However, this is the first report of monogalactosyl diacylglycerides isolated from horseradish and wasabi rhizomes with selective COX-1 enzyme inhibitory activity.

Water, methanol, ethyl acetate, and hexane extracts of fresh wasabi and horseradish were tested in cancer cell proliferation inhibitory assay. The water extract of fresh wasabi inhibited 52.3, 40.7, 64.9, 46.2, and 13.2% on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cell growth at 250 μg/mL, respectively (**Figure 3**A). The methanol extract of wasabi showed 82.4, 62.7, 56.6, 53.3, and 38.4% of inhibition on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cell growth at 250 μg/mL, respectively (**Figure 3B**). Similarly, the ethyl acetate extract of wasabi demonstrated inhibitory activity on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cancer cells with 84.3, 65.6, 72.1, 73.7, and 49.4% growth inhibition at 250 μg/mL, respectively (**Figure 3C**). However, the water, methanol, and ethyl acetate extracts of horseradish and the hexane extract of both wasabi and horseradish did not exhibit significant growth inhibitory activity on the cancer cell lines tested at 250 μg/mL.

In tumor cell growth inhibitory assays, compounds **1** and **2** showed little or no growth inhibition of tumor cell lines tested at 30 μg/mL. Compound **3** inhibited the growth of colon (HCT-116) and lung cancer (NCI-H460) cells in a concentration-dependent manner (**Figure 4**A). It inhibited the growth of colon cancer cells by 22, 43, 51, and 68% and lung cancer cells by 30, 39, 44, and 71% at 7.5, 15, 30, and 60 μg/mL, respectively. However, compound **4** inhibited the growth of colon (HCT-116) and lung cancer (NCI-H460) cells only at the highest concentration tested. For example, at 60 μg/mL, it inhibited the growth of colon, lung, and stomach cancer cell lines by 28, 17, and 44%, respectively (**Figure 4B**).

The monogalactosyl diacylglycerides, together with other glycosylated diacylglycerides, belong to the major lipids present in chloroplasts and membranes. The monogalactosyl diacylglycerides have been reported as inhibitors of 12-*O*-tetradecanoylphorbol-13 acetate (TPA)-induced tumor promotion in vitro and in mouse models (*21*−*24*). They are inducers of apoptosis and selective inhibitors of mammalian polymerases (*25*). Upon ingestion, it is expected that monogalactosyl diacylglycerides enzymatically cleave at position 1 by pancreatic lipase enzyme. This may affect their ability to inhibit cancer cell proliferation. In a recent study, monogalactosyl diacylglycerides were hydrolyzed with pancreatic lipase and the resulting monogalactosyl monoacylglycerides (MGMGs) were compared to their monogalactosyl diacylglycerides parents in cancer cell inhibitory assays. The cancer cell growth was inhibited (LD_{50}) by both classes of compounds at 40 μg/mL (*25*).

We had reported the cell proliferating effect of desulfosinigrin in wasabi and horseradish (*7*). Both wasabi and horseradish are rich in glucosinolates and their water extracts primarily contain this class of polar compounds. They are also prone to rapid hydrolysis to release numerous isothiocyanates out of which some are beneficial and others are not. Consumption of compounds such as unsaturated fatty acids, vitamins, anthocyanins, flavonoids, and other antioxidants in herbaceous foods has been implicated to reduce risk of cardiovascular disease and cancer (*26*). Given their abundance in plant foods, monogalactosyl diacylglycerides may play an important role in reducing cancer risk and the occurrence of cardiovascular disease in vegetable and fruit-rich diets. The protective effects of diets rich in fruits and vegetables against oxidative stress, carcinogenesis, and related conditions may not be attributed to a specific class of compounds. It is possible that ubiquitous compounds such as monogalactosyl diacylglycerides, fatty acids, plastoquinone, and others in the food may contribute to the overall protective effects by additive or synergistic effects even if it contains other compounds capable of causing adverse health effects. Wasabi and horseradish are probably examples of such foods with a balance of compounds that could contribute both beneficial and detrimental health effects.

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