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Cyclooxygenase Inhibitory and Antioxidant Compounds from the Mycelia of the Edible Mushroom *Grifola frondosa*

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The bioassay-guided isolation and purification of the hexane extract of the cultured mycelia of *Grifola frondosa* led to the characterization of a fatty acid fraction and three compounds, ergosterol (**1**), ergostra-4,6,8(14),22-tetraen-3-one (**2**), and 1-oleoyl-2-linoleoyl-3-palmitoylglycerol (**3**). The composition of fatty acid fraction was confirmed as palmitic, oleic, and linoleic acids by GC-MS and by comparison with the retention values of authentic samples. The structures of compounds **1**−**3** were established by spectroscopic methods. The fatty acid fraction and compounds **1**−**3** showed cyclooxygenase (COX) enzyme inhibitory and antioxidant activities. The inhibition of COX-1 enzyme by the fatty acid fraction and compounds **1**−**3** at 250 μg/mL were 98, 37, 55, and 67%, respectively. Similarly, COX-2 enzyme activity was reduced by fatty acid fraction and compounds **1**−**3** at 250 μg/mL by 99, 37, 70, and 4%, respectively. The inhibitions of liposome peroxidation by the fatty acid fraction and compounds **1** and **2** at 100 μg/mL were 79, 48, and 42%, respectively. This is the first report of compounds **2** and **3** from the cultured mycelia of *G. frondosa*. The COX inhibitory activities of compounds **1**−**3** are reported here for the first time.

KEYWORDS: ***Grifola frondosa*; mycelia; fatty acid; palmitic acid; oleic acid; linoleic acid; ergosterol; ergostra-4,6,8(14),22-tetraen-3-one; 1-oleoyl-2-linoleoyl-3-palmitoylglycerol**

INTRODUCTION

One of the old Chinese proverbs, "Medicine and foods have a common origin", is quite relevant to those people who consume phytoceuticals as a dietary supplement. Mushrooms are one of such food items that health-conscious people enjoy. They have been used as both food and medicine through recorded history and are one of the natural sources of physiologically active compounds that have been studied for the development of both natural medicine and pharmaceutical products. However, the efficacy of medicinal mushrooms was not clearly established until two decades ago; *Grifola frondosa* is one of such mushrooms belonging to the Basidiomycetes, Aphyllophorales, Polyporaceae. The fruiting body of *G. frondosa* has been sold in the market as an edible mushroom and considered to have diuretic, gonorrhea-therapeutic, and antipyretic activities in Chinese traditional medicine. It has been stated in "Shen Nong Ben Cao Jing" that it has been frequently used for improving spleen and stomach ailments, calming nerves and mind, and treating hemorrhoids (*1*, *2*). It was confirmed that the fruiting body of *G. frondosa* containing substances with antidiabetic activities (*3*). The dried power of the fruiting body of *G. frondosa* has demonstrated a reduction of blood pressure in the spontaneously hypertensive rat (*4*, *5*). The fruiting body of *G. frondosa* has also been recommended as a remedy for palsy, neuralgia, and arthritis (*2*). Many bioactive compounds have been reported from the fruiting bodies of *G. frondosa*. The most important among these were the polysaccharide fractions, which activated immune competent cells and showed anticancer activities (*6*−*11*).

Cyclooxygenase (COX) or prostaglandin endoperoxide H synthase (PGHS) enzymes are widely used to determine the antiinflammatory effects of potential therapeutic products (*12*). COX enzymes are pharmacological targets for the discovery of the nonsteroidal antiinflammatory drugs (NSAIDs) (*13*, *14*). The COX isozymes involved in prostaglandin synthesis are COX-1 and COX-2 (*15*). It is reported that selective COX-2 inhibitors are mainly responsible for antiinflammatory activities (*16*). Epidemiology and experimental studies have demonstrated the effect of NSAIDs in the prevention of human cancers. NSAIDs block endogenous prostaglandin synthesis through inhibition of COX enzymatic activity. COX-2, a key isozyme in the conversion of arachidonic acid to prostaglandin, is inducible by various agents such as growth factors and tumor promoters and is frequently overexpressed in various tumors. Thus, the combination of COX-2 inhibitors with radiation or anticancer or cancer prevention drugs was reported to be beneficial in cancer prevention and treatment (*17*).

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In our investigation of biologically active compounds from *G. frondosa*, the extracts of lyophilized mycelia of *G. frondosa* were evaluated for antioxidant and cyclooxygenase (COX) enzyme inhibitory activities. In this investigation, the bioassay-guided purification and characterization of the compounds from the mycelia of *G. frondosa* with antioxidant and cyclooxygenase inhibitory activities have been studied.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 300 and 500 MHz spectrometers. Compounds were dissolved in $CHCl_3$, and resonances are reported in δ (ppm) based on the residual of $CHCl_3$ at δ 7.24 for ^{1}H NMR and δ 77.0 for ^{13}C NMR. Coupling constants, *J*, are in Hz. Silica gel (30−60 μm particle sizes) used for MPLC was purchased from Merck. TLC plates and Prep-TLC (GF Uniplate, with binder, 250 μm) were the products of Analtech, Inc., Newark, DE. Positive controls *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) used in the antioxidant assay were purchased from Sigma Chemical Co. Vioxx tablets and Celebrex capsules used in the cyclooxygenase inhibitory assay as positive controls were physician's professional samples provided by Dr. Subash Gupta of Sparrow Pain Center, Sparrow Hospital, MI. Yeast extract, malt extract, and dextrose (glucose) used for the liquid media were the products of Difco Laboratories (Detroit, MI). All organic solvents used were ACS regent grade (Aldrich Chemical Co., Inc., Milwaukee, WI).

Materials. The mycelia of *G. frondosa* were grown in YMG liquid media in the Bioactive Natural Product and Phytoceutical Laboratory at Michigan State University. YMG liquid media was prepared by dissolving 2 g of yeast extract, 5 g of malt extract, and 2 g of dextrose (glucose) in 500 mL of distilled water. A 500 mL volume of YMG liquid media was transferred to each incubation flask, capped and autoclaved at 120 °C and 21 psi for 30 min, and cooled to room temperature. Mycelial plugs of *G. frondosa* grown in YMG media for 7 days at 27.5 °C were seeded to the liquid media under aseptic conditions. The flasks were placed on a rotary shaker (130 rpm, 28 °C). After 7 days of incubation, the culture broth was filtered; the mycelial cake was lyophilized and stored at −20 °C in ziplock bags until extraction.

Extraction and Isolation. Lyophilized mycelia of *G. frondosa* (236 g) was ground to a fine powder and extracted sequentially with hexane (3 × 1 L, 24 h), ethyl acetate (3 × 1 L, 24 h), and methanol (3 × 1 L, 24 h) in an extraction column. The solvents were evaporated under vacuum and yielded hexane (**A**, 6.7 g), ethyl acetate (**B**, 4.3 g), and methanol (**C**, 44.5 g) extracts, respectively. All extracts showed activities in our antioxidant and cyclooxygenase (COX) enzyme inhibitory assays at 250 ppm.

Extract **C** (21 g) was dissolved in 200 mL of methanol, the solution was filtered, and the filtrate was extracted with hexane to give hexane-soluble (1.35 g), methanol-soluble (11.42 g), and methanol-insoluble (7.4 g) fractions. The hexane-soluble extract (1.08 g) was subjected to fractionation by silica gel medium-pressure liquid chromatography (MPLC) and eluted with hexane (200 mL), hexane−acetone (8:1, 400 mL), hexane−acetone (4:1, 400 mL), hexane−acetone (2:1, 400 mL), and acetone (400 mL), respectively. The fractions with a similar TLC profile were combined and yielded fractions 1 (361 mg), 2 (268 mg), 3 (65 mg), 4 (61 mg), 5 (78 mg), and 6 (147 mg). Fraction 2 was the most active in the COX inhibitory assays, and 184 mg of it was further purified by prep-TLC (20 × 20 cm, 500 μm, 3 plates) using hexane−ether (2:1) as the developing solvent. This yielded an active band (R_f = 5.5), fatty acid fraction (59 mg). Further TLC analysis revealed that it was pure, as indicated by a single spot on TLC.

Extract **A** (6 g) was redissolved in hexane, and the white precipitate formed was filtered and recrystallized from methanol, resulting in crystalline needles of compound **1** (1.03 g; see **Figure 1**), which melted at 138−140 °C. The mother liquor was combined with the hexane solution and concentrated to yield a residue (4.58 g). This residue (4.3 g) was again dissolved in hexane and subjected to silica gel MPLC and eluted with hexane (300 mL), hexane−ether (8:1, 400 mL), hexane−ether (4:1, 800 mL), hexane−ether (2:1, 400 mL), hexane−acetone (4:1, 400 mL), hexane−acetone (4:1, 400 mL), and acetone (400 mL), respectively. Fractions were collected in aliquots of 15 mL. Fractions with a similar TLC profile were combined and yielded fractions 1 (28.4 mg), 2 (665.0 mg), 3 (1.6474 g), 4 (128.0 mg), 5 (62.4 mg), 6 (121.6 mg), 7 (898 mg), and 8 (522 mg), respectively.

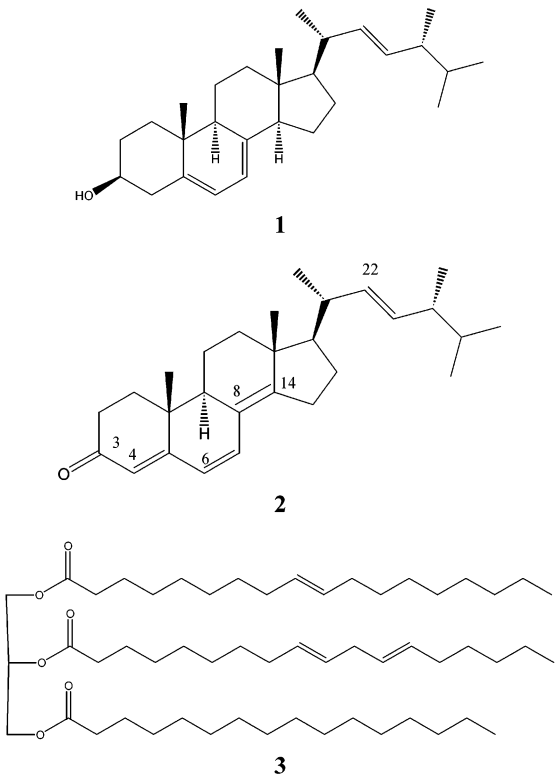

Figure 1. Structures of compounds **1**–**3**.

The bioactive fractions 3 and 6 were further purified. Fraction 6 (121.6 mg) was purified by preparative TLC (20 × 20 cm, 500 μm) using hexane−acetone (4:1) as the developing solvent. A strong UV-active band viewed under 366 nm was collected and eluted with $CHCl_3$ and methanol. Removal of the solvent under reduced pressure gave white needles of compound **2** (2.6 mg), which melted at 113−114 °C. The active fraction 3 (1.64 g) was further purified by silica gel MPLC using hexane−ether (8:1) as the mobile phase. The oily fraction, **3** (230 mg), collected gave one spot on TLC plate using hexane−acetone (10:1) as the developing solvent.

Compound 1: ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, 18-CH_3) 1.01 (d, 3H, J = 6.6 Hz, 21-CH_3), 0.80 (d, 3H, J = 4.8 Hz, 27-CH_3 or 28-CH_3), 0.81 (d, 3H, J = 4.5 Hz, 27-CH_3 or 28-CH_3), 0.89 (d, 3H, J = 6.9 Hz, 26-CH_3), 0.92 (s, 3H, 19-CH_3), 5.17 (2H, m, H-22 and H-23), 5.36 (1H, dd, J = 5.7, 2.7 Hz, H-6), 5.55 (1H, dd, J = 5.7, 2.4 Hz H-7); ^{13}CNMR δ 141.4, 139.8, 135.6, 132.0, 119.6, 116.3, 70.4, 55.7, 54.5, 46.2, 42.8, 40.8, 40.4, 39.1, 38.3, 37.0, 33.1, 31.9, 28.3, 23.0, 21.1, 20.0, 19.6, 17.6, 16.3, and 12.0. The NMR data for compound **1** were found to be in agreement with published data for ergosterol (*18*, *19*).

Compound 2: ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, d, J = 5.4 Hz, 26-CH_3), 0.83 (3H, d, J = 4.8 Hz, 27-CH_3), 0.91 (3H, d, J = 6.6 Hz, 24-CH_3), 0.94 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.04 (3H, d, J = 6.6 Hz, 21-CH_3), 6.60 (1H, d J = 9.3 Hz, H-7), 6.01 (1H, d, J = 9.3 Hz, H-6), 5.72 (1H, s, H-4), 5.21 (2H, m, H-22 and 23). The ^{1}H NMR data confirmed that compound **2** is ergostra-4,6,8(14),22-tetraen-3-one (*20*).

Compound 3: ^{1}H NMR ($CDCl_3$) δ 0.85 (9H, t, J = 6.6 Hz, H-18″, 18″, and 16‴), 1.24 [56H, m, H-(4′−7′, 12′−17′), H-(4″-7″, 15″-17″), and H-(4‴−15‴)], 1.58 (8H, t, J = 2.0 Hz, H-8′, 11′, 8″, and 14″), 2.28 (6H, t, J = 7.5 Hz, H-2′, 2″, and 2‴), 2.76 (2H, t, J = 6.0 Hz, H-11″), 4.11, (2H, dd, J = 12.0, 6.0 Hz, H-1a, 3a), 4.26 (2H, dd, J = 11.7, 4.5 Hz, H-1b, 3b), 5.2−5.4 (7H, m, H-9′, 10′, 9″, 10″, 12″, 13″,

and 2); ^{13}C NMR δ 14.0(C-18′, 18″), 14.1 (C-16‴), 22.6, 22.7, 24.8, 24.9, 25.6, 27.2, 29.0−29.7 [C-(4′−8′, 11′−17′), C-(4″−8″, 14″−17″), C-(4‴−16‴)], 31.5 (C-3′ and C-3‴), 31.9 (C-3″), 33.9 (C-2′ and C-2‴), 34.0 (C-11″), 34.2 (C-2″), 62.1 (C-1 and 3), 68.8 (C-2), 127.8−130.2 (C-9′, 10′, 9″, 10″, 12″, 13″), 172.3, and 173.2 (C-1′, C-1″, and 1‴).

Methylation of Fatty Acids Fraction. Diazomethane was prepared by reacting *N*-nitroso-*N*-methylurea with concentrated KOH solution in ether (*21*). The diazomethane generated was stored in ether and used to methylate the fatty acids. The fatty acid fraction (2 mg) was dissolved in ether and reacted with diazomethane in ether until the solution became pale yellow. The resulting yellow solution was kept at room temperature for 1 h and evaporated; the residue was dissolved in hexane and analyzed by GC-MS.

Saponification and Methylation of Compound 3. Compound **3** (10 mg) was stirred with 5% NaOH in MeOH (2 mL) for 5 min followed by acidification with 6 N HCl in MeOH. This solution was extracted with 5 mL of hexane 3 times. The hexane solution was evaporated under vacuum at 40 °C. The residue containing free fatty acid was dissolved in 2 mL of ether and methylated using diazomethane. Also, the diazomethane in ether was used to methylate linoleic, oleic, and palmitic acids. The methylated products were dissolved in hexane and filtered prior to GC analysis.

GC Analysis of the Hydrolysis Products from 3. GC analysis was performed using a Hewlett-Packard 6890 gas chromatograph (Agilent Technology, Wilmington, DE). Samples were injected into a split injector at 200 °C, and separation was achieved on a 30 × 0.25 mm i.d. HP-5 capillary column with helium carrier gas at a flow rate of 22 mL/min. Compounds were detected using flame ionization at 250 °C. The temperature profile was 150 (2 min) to 200 °C (1 min) at 10 °C/min and then to 250 °C (2 min) at 2 °C/min. Methyl esters of palmitic, linoleic, and olenic acids were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI.

GC-MS Analysis of Fatty Acid Fraction. Analysis of fatty acid methyl esters was carried out on a JEOL AX-505H double-focusing mass spectrometer coupled to a Hewlett-Packard 5890J gas chromatograph via a heated interface. The GC separation of fatty acid methyl ester was accomplished on a 30 m × 6.35 mm i.d, 0.25 μm DBWAX fused-silica capillary column purchased from J & W. Direct (splitless) injection was used, and the carrier gas was helium at 1 mL/min. The GC temperature program consisted of initial temperature 50 °C and then 10 °C/min to reach a final temperature 250 °C. MS conditions were interface temperature 210 °C, ion source temperature ca. 200 °C, electron energy 100 μA, and scan rate 1 scan/s over the range *m*/*z* 45−750.

Cyclooxygenase Inhibitory Assay. COX-1 enzyme inhibitory activity was measured using an enzyme preparation from ram seminal vesicles that was purchased from Oxford Biomedical Research, Inc., Oxford, MI. COX-2 enzyme inhibitory activity was measured using enzyme prepared from HPGHS-2 cloned insect cell lysate (supplied by Dr. Dave DeWitt, Department of Biochemistry, Michigan State University) and diluted with Tris buffer (pH 7) to give an approximate final concentration of 1.5 mg of protein/mL. Assays were conducted at 37 °C and at pH 7 by monitoring the initial rate of O_2 uptake using an Instech micro oxygen chamber and electrode attached to an YSI 5300 biological oxygen monitor. Each assay mixture contained 0.6 mL of 0.1 M Tris buffer (pH 7), 1 mM of phenol, 85 μg of hemoglobin, and 27 μM arachidonic acid. DMSO solutions of test samples or DMSO alone (10 μL) were added to the assay chamber. Reactions were initiated by adding COX enzyme, test sample, and arachidonic acid. The data were recorded using QuickLog for windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA) (*22*, *23*), and each sample was assayed twice.

Antioxidant Assay. This assay was conducted by analysis of model liposome oxidation using fluorescence spectroscopy according to the procedure reported previously (*24*). Peroxidation was initiated by addition of $FeCl_2$ for positive controls of BHA, BHT, TBHQ (1.80, 2.20, and 1.66 μg/mL, respectively), and test samples. 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 fluorimeter (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity with time indicated the rate of peroxidation, and these data are reported for 21 min after the initiation 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) (*25*).

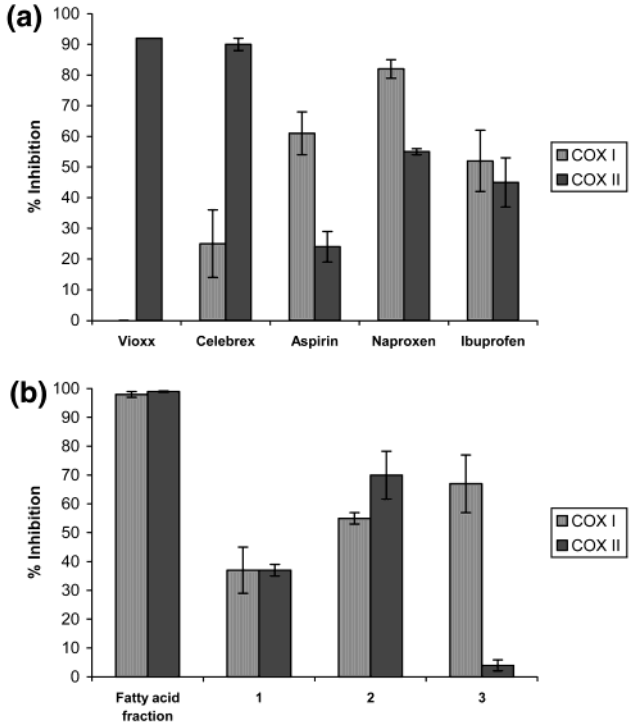

Figure 2. (a) In vitro COX-1 and COX-2 inhibitory activities of Vioxx and Celebrex at 1.67 μg/mL and aspirin, naproxen, and ibuprofen at 180, 2.52, and 2.06 μg/mL, respectively. Vertical bars represent the standard deviation of each data point ($n = 2$). (b) In vitro COX-1 and COX-2 inhibitory activities of fatty acid fraction and compounds **1**–**3** at 250 μg/mL. Vertical bars represent the standard deviation of each data point ($n = 2$).

RESULTS AND DISCUSSION

Compounds isolated from the mycelia of the edible mushroom *G. frondosa* were characterized using ^{1}H and ^{13}C NMR experiments. The fatty acid composition of **3** was confirmed by hydrolysis, methylation of the fatty acids, and GC analysis of the methylated products and by comparison of the retention times of authentic fatty acid methyl esters. The result indicated that the fatty acids of the triglyceride are palmitic, linoleic, and oleic acids. We have reported triglycerides from *Apium graveolens* L. (*26*), *Prunus cerasus* L. (*27*), and *Ocimum sanctum* (*28*). The NMR spectral data for compound **3** were compared to the spectral data of the triglycerides reported (*26*−*28*) and found to be in agreement with the proposed structure of **3** as 1-oleoyl-2-linoleoyl-3-palmitoylglycerol.

The composition of fatty acid fraction was confirmed by GC-MS and by the comparison of retention times of methyl esters of authentic fatty acids. The methyl ester of fatty acids in the fatty acid fraction showed peaks at *m*/*z* 298, 296, and 270 corresponding to retention times of 19, 20.95, and 21.57 min and represented the methyl esters of palmitic, oleic, and linoleic acids, respectively. The height of the GC peaks revealed that linoleic, oleic, and palmitic acids were the main components and the proportion of the fatty acid was about 2:2:1 for linoleic, oleic, and palmitic acid, respectively.

Cyclooxygenase enzyme inhibitory activities for fatty acid fraction and compounds **1**−**3** are shown in **Figure 2b**. The fatty

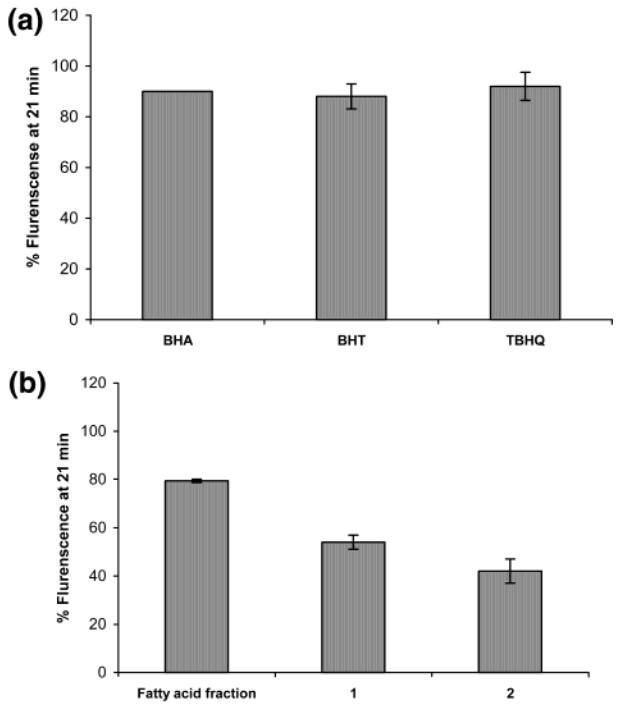

Figure 3. (a) Antioxidant activities of commercial antioxidants in a liposomal model system. TBHQ, BHT, and BHA were evaluated at 1.8, 2.2, and 1.66 μg/mL, respectively. Vertical bars represent the standard deviation of each data point ($n = 2$). (b) Antioxidant activities of the fatty acid fraction and compounds **1** and **2** in a liposomal model system. Samples were tested at 100 μg/mL. Vertical bars represent the standard deviation of each data point ($n = 2$).

acid fraction and compounds **1**−**3** inhibited the activities of both COX-1 and COX-2 enzymes. The inhibition of COX-1 enzyme by fatty acid fraction and compounds **1**−**3** at 250 μg/mL were 98, 37, 55, and 67%, respectively. The positive controls Vioxx (1.67 μg/mL), Celebrex (1.67 μg/mL), aspirin (180 μg/mL), naproxen (2.52 μg/mL), and ibuprofen (2.06 μg/mL) showed 0, 25, 61, 82, and 55% inhibition, respectively (**Figure 2a**). The fatty acid fraction and compounds **1**−**3** inhibited COX-2 enzyme at 250 μg/mL by 99, 37, 70, and 4%, respectively (**Figure 2b**). The positive controls Vioxx (1.67 μg/mL), Celebrex (1.67 μg/mL), aspirin (180 μg/mL), naproxen (2.52 μg/mL), and ibuprofen (2.06 μg/mL) showed 92, 90, 24, 55, and 45% inhibition, respectively (**Figure 2a**).

The results indicated that the fatty acids were the most effective components of the mycelia from *G. frondosa*, which demonstrated both COX-1 and COX-2 enzyme inhibitions. The structure−activity relationship among fatty acids related to COX-1 and COX-2 enzyme inhibitory activities has been reported (*29*, *30*). It was suggested that fatty acids with 20 carbons or more gave higher inhibition to COX enzymes than those with less than 16 carbons. Also, unsaturated fatty acid with two or more double bonds and the chain lengths higher than 20 carbons exhibited COX-2 enzyme inhibitory activities similar to several nonsteroidal antiinflammatory drugs (*30*).

An iron-catalyzed liposome model system (*25*) was employed to evaluate the inhibition of lipid peroxidation by the fatty acid fraction and compounds **1**−**3** as measured by fluorescence spectroscopy. The antioxidant activities of the fatty acid fraction and compounds **1** and **2** at 100 μg/mL were 79, 48, and 42%, respectively (**Figure 3b**). The controls TBHQ (1.66 μg/mL), BHA (1.8 μg/mL), and BHT (2.2 μg/mL) showed peroxidation inhibition at 90, 88, and 92%, respectively (**Figure 3a**). The antioxidant results indicated that fatty acid fraction containing unsaturated fatty acids demonstrated 79% peroxidation inhibition. It is important to note that fatty acids are ubiquitous in several mushrooms, a common ingredient of daily diet for many people. These compounds may be responsible for some of the anecdotal health claims implicated to the consumption of mushrooms.

Although compound **1**, ergosterol, exhibited lower COX enzyme inhibitory activity than fatty acid fraction, the COX-2/COX-1 inhibitory ratio was similar to the fatty acids (**Figure 2b**). It is important to note that ergosterol was produced in large quantities (about 1% of the dry weight) by the mycelia of *G. frondosa* as in the case of the fruiting bodies. Recent studies strongly suggest that ergosterol could provide significant protection against the promotion of bladder tumor induced by many types of cancer promoters in the environment (*31*). Ergosterol showed only a mild antioxidant activity compared to that of fatty acid fraction. The oxidized product of ergosterol showed strong antimicrobial activities (*32*).

We have evaluated the antiinflammatory and antioxidant activities of the extracts of *G. frondosa* fruiting body, grown in Bioactive Natural Product and Phytoceutical green houses at Michigan State University, along with the mycelial extracts. Our results indicated that both fruiting body and mycelial extracts exhibited the same activities at 250 μg/mL. Since the production of the fruiting body of *G. frondosa* takes time, the mycelial production of these active compounds is economical. Therefore, we are interested in the production and characterization of active compounds in the liquid culture of *G. frondosa*.

The antioxidant and COX enzyme activities of the compounds isolated from the mycelia of *G. frondosa* suggest that the consumption of *G. frondosa* either as daily food or as an ingredient in food preparation may contribute to potential health benefits. These benefits may include reducing pain related to inflammation, reducing the incident of cardiovascular disease, and cancer prevention by acting as an antioxidant and/or as antiinflammatory agents.

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LITERATURE CITED

(1) Jiangsu New Medical College. *Dictionary of traditional Chinese medicine*; Shanghai Scientific Edition: Shanghai, P. R. China, 1986; pp 2191−2193.

(2) Mizuno, T.; Zhuang, C. Maitake, *Grifola frondosa*: Pharmacological effects. *Food Rev. Int.* **1995**, *11*, 135−149.

(3) Kubo, K.; Aoki, H.; Naba, N. Anti-diabetic activities present in the fruit body of *Grifola frondosa* (maitake). *Biol. Pharm. Bull.* **1994**, *17*, 1106−1110.

(4) Kabir, Y. Effects of shiitake (*Lentinus edodes*) and maitake (*Grifola frondosa*) mushrooms on blood pressure and plasma lipids of spontaneously hypertensive rats. *J. Nut. Sci. Vitaminol.* **1987**, *33*, 341−346.

(5) Kabir, Y.; Kimura, S. Dietary mushrooms reduce blood pressure in spontaneously hypertensive rats. *J. Nut. Sci. Vitaminol*. **1989**, *35*, 91−94.
(6) Kato, K.; Inagaki, T.; Shibagaki, H.; Yamauchi, R.; Okuda, K.; Sano, T.; Ueno, Y. Structural analysis of the β-D-glucan extracted with aqueous zinc chloride from the fruit body of *Grifola frondosa*. *Carbohydr. Res*. **1983**, *123*, 259.
(7) Kato, K.; Inagaki, T.; Teranishi, T.; Yamauchi, R.; Okuda, K.; Sano, T.; Ueno, Y. Structural analysis of the alkali-soluble β-D-glucan from the fruit body of *Grifola frondosa*. *Carbohydr. Res.* **1983**, *124*, 247.
(8) Mizuno, T.; Ohsawa, K.; Hagiwara, N.; Kuboyama, R. Studies on the host-mediated antitumor polysaccharides. Part IX. Fractionation and characterization of antitumor polysaccharides from Maitake, *Grifola frondosa*. *Agric Biol. Chem*. **1986**, *50*, 1679.
(9) Ohno, N.; Suzuki, I.; Oikawa, S.; Sato, K.; Miyazaki, T.; Yadomae, T. Antitumor activity and structural characterization of glucans extracted from cultured fruit bodies of *Grifola frondosa*. *Chem. Pharm. Bull*. **1984**, *32*, 1142−1151.
(10) Ohno, N.; Suzuki, I.; Sato, K.; Oikawa, S.; Miyazaki, T.; Yadomae, T. Purification and structural characterization of an antitumor β-1,3-glucan isolated from hot water extract of the fruit body of cultured *Grifola frondosa*. *Chem. Pharm. Bull*. **1985**, *33*, 4522−4527.
(11) Matsui, K.; Kodama, N.; Nanba, H. Effects of Maitake (*Grifola frondosa*) D-fraction on the carcinoma angiogenesis. *Cancer Lett.* **2001**, *172*, 193−198.
(12) Bayer, T.; Breu, W.; Seligmann, O.; Wray, V.; Wagner, H. Biologically active thiosulfinates and alpha-sulfinyl disulfides from *Allium cepa*. *Phytochemistry* **1989**, *28*, 2373−2378.
(13) Humes, J.; Winter, C. A.; Sadowski, S. J.; Kuehl, F. A., Jr. Multiple sites on prostaglandin cyclooxygenase are determinants in the action of nonsteroidal anti-inflammatory agents. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2053−2056.
(14) Rome, L. H.; Lands, W. E. Structural requirements for time dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4863−4865.
(15) Hemler, M.; Lands, W. E. Purification of the cyclooxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzymes. *J. Biol. Chem*. **1976**, *251*, 5575−5579.
(16) Masferrer, J. L.; Zweifel, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P. C.; Seibert, K. Selective inhibition of inducible cyclooxygenase 2 in vivo is anti-inflammatory and nonulcerogernic. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3228−3232.
(17) Xu, Xiao-Chun. COX-2 inhibitors in cancer treatment and prevention, a recent development. *Anti-Cancer Drugs* **2002**, *13*, 127−137.
(18) Gan, K.-H.; Kuo, S.-H.; Lin, C.-N. Steroidal constituents of *Ganoderma applanatum* and *Ganoderma neo-japonicum. J. Nat. Prod.* **1998**, *61*, 1421−1422.
(19) Zhou, W.-X.; Nes, W. D. Stereochemistry of hydrogen introduction at C-25 in ergosterol synthesized by the mevalonate-independent pathway. *Tetrahedron Lett.* **2000**, *41*, 2791−2795.
(20) Ishizuka, T.; Yaoita, Y.; Kikuchi, M. Sterol constituents from the fruit bodies of *Grifola frondosa* (Fr.) S. F. Gray. *Chem. Pharm. Bull*. **1997**, *45*, 1756−1760.
(21) Gonzalez, A. G.; Barrera, J. B.; Toledo Marante, F. J. The steroids and fatty acids of the Basidiomycete *Scleroderma polyrhizum*. *Phytochemistry* **1983**, *22*, 1049−50.
(22) Laneuville, O.; Breuer, D. K.; Dewitt, D. L.; Hla, T.; Funk, C. D.; Smith, W. L. Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs. *J. Pharm. Exp. Ther*. **1994**, *271*, 927−34.
(23) Kelm, M. A.; Nair, M. G. Mosquitocidal compounds and a triglyceride, 1,3-dilinoleneoyl-2-palmitin, from *Ocimum sanctum*. *J. Agric. Food Chem*. **1998**, *46*, 3092−3094.
(24) Wang, H.; Nair, M. G.; Strasburg, G. M.; Chang, Y.-C.; Booren, A. M.; Gray, I. J.; Dewitt, D. L. Antioxidant and anti-inflammatory activities of anthocyanins and their aglycone, cyanidin, from tart cherries. *J. Nat. Prod.* **1999**, *62*, 294−296.
(25) Arora, A.; Nair, M. G.; Strasburg, G. M. *A novel fluorescence assay to evaluate antioxidants efficiency: application to flavonoids and isoflavonoids*; Aruoma, I., Cuppett, S. C., Eds.; AOCS Press: Champaign, IL, 1997; pp 205−222.
(26) Momin, R. A.; Ramsewak, R. S.; Nair, M. G. Bioactive compounds and 1,3-di[(*cis*)-9-octadecenoyl]-2-[(*cis*,*cis*)-9,12-octadecadienoyl]glycerol from *Apium graveolens* L. seeds. *J. Agric. Food Chem.* **2000**, *48*, 3785−3788.
(27) Chandra, A.; Nair, M. G. Characterization of pit oil from montmorency cherry (*Prunus cerasus* L.). *J. Agric. Food Chem*. **1993**, *41*, 879−881.
(28) Kelm, M. A.; Nair, M. G. Mosquitocidal compounds and a triglyceride, 1,3-dilinolenoyl-2-palmitin, from *Ocimum sanctum*. *J. Agric. Food Chem*. **1998**, *46*, 3092−3094.
(29) Ringbom, T.; Huss, U.; Stenholm, L.; Flock, S.; Skattebol, L.; Perera, P.; Bohlin, L. COX-2 Inhibitory effects of naturally occurring and modified fatty acids. *J. Nat. Prod.* **2001**, *64*, 745−749.
(30) Henry, G. E.; Momin, R. A.; Nair, M. G.; DeWitt, D. L. Antioxidant and cyclooxygenase activities of fatty acids found in food. *J. Agric. Food Chem*. **2002**, *50*, 2231−2234.
(31) Yazawa, Y.; Yokota, M.; Sugiyama, K. Antitumor promoting effect of an active component of polyporus, ergosterol and related compounds on rat urinary bladder carcinogenesis in a short-term test with concanavalin A. *Biol. Pharm. Bull.* **2000**, *23*, 1298−1302.
(32) Fujii, N.; Yuasa, K. Antimicrobial activity of soy sauce oil. *Nippon Shoyu Kenkyusho Zasshi* **1996**, *22*, 105−109.

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