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Mycochemical Investigation of the Turkey Tail Medicinal Mushroom *Trametes versicolor* (Higher Basidiomycetes): A Potential Application of the Isolated Compounds in Documented Pharmacological Studies

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ABSTRACT: The purpose of this study was to elucidate the chemical properties of the n-hexane, chloroform, and ethyl acetate extracts of the fruiting body of the medicinal mushroom *Trametes versicolor*. The study led to the isolation of 5 sterols, 2 triterpene derivatives, 1 hydroquinone-derived aromatic compound, and, finally, 1 cerebroside and 1 triglyceride derivative. These compounds were identified for first time in *T. versicolor* and were named as follows: 4-isobutoxyphenyl palmitate (5), N-D-2′-hydroxyheptanoic-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphinga-dienine(cerebroside) (6), 3β-linoleyloxyergosta-7,22-diene (7), 3β-linoleyloxyergosta-7-ene (8), and betulinic acid (9). Other compounds elucidated in our study were ergosterol (1), ergosterol peroxide (2), trilinolein (3), ergosta-7, 22-dien-3β-ol (4), and betuline (10). These compounds were obtained via column or thin-layer chromatography before being identified by nuclear magnetic resonance spectroscopic analyses and infrared data. In addition, the beneficial pharmacological effects of the compounds are described here.

KEYWORDS: medicinal mushrooms, mycochemical analysis, *Trametes versicolor*, sterols, triterpene derivatives, hydroquinone-derived aromatic compound, cerebroside, triglyceride derivative

ABBREVIATIONS: **br m,** broad multiplet; **br s,** broad singlet; **$CHCl_3$,** chloroform; **d,** doublet; **dd,** double doublets; **DEPT,** distortionless enhancement by polarization transfer; **DMSO,** dimethyl sulfoxide; **EtOAc,** ethyl acetate; **H_2SO_4,** sulfuric acid; **J,** coupling constant; **m,** multiplet; **MeOH,** methanol; **NMR,** nuclear magnetic resonance; **ppm,** parts per million; **PSK,** protein-bound polysaccharides; **PSP,** polysaccharopeptide; **s,** singlet; **t,** triplet; **TLC,** thin-layer chromatography; **UV,** ultraviolet.

I. INTRODUCTION

Mushrooms have been used in traditional foods and medicines in Asia for thousands of years. Currently, modern medicine in Eastern countries, such as China, Japan, Korea, and several other Asian countries, still uses mushrooms to treat major diseases.[1] Higher Basidiomycetes mushrooms are a dominant branch of biologically active compounds in the fungal kingdom, and historical traditions, when combined with the extensive research performed in the East Asian countries, validate the preventive and therapeutic properties of many mushroom species.[2] The most important bioactive metabolites belong mainly to 2 groups: polysaccharides, specifically β-D-glucans, and different low-molecular-weight compounds.

One of the most popular medicinal mushrooms used in both traditional medicine and modern clinical practice is turkey tail mushroom *Trametes versicolor* (L.:Fr.) Lloyd (Polyporales, higher Basidiomycetes).[3] Currently, the potential therapeutic applications of *T. versicolor* have been gaining acceptance among patients worldwide. That

the aqueous extracts obtained from *T. versicolor* have a wide array of biological activities, including modulatory and stimulatory effects on different immune cells, as well as the inhibition of cancer cell growth, has recently been demonstrated.[3] Among the different bioactive components that are derived from hot water and standardized ethanol–water extracts of *T. versicolor*, protein-bound polysaccharides (PSK, also known as Krestin) and polysaccharopeptides (PSPs) had vigorous biological activities.[4] The immunological activities of PSPs and PSK have been extensively examined both *in vitro* and *in vivo*. Fortification of immunological function by PSP was studied. Unlike most anticancer drugs currently in use, the antitumor effect of PSP may be produced through immunomodulatory regulation rather than by direct cytotoxicity. Many reports have demonstrated that PSK and PSP activate cellular and humoral components of the host immune system.[5] However, *T. versicolor* seems to trigger enzymatic systems that are involved in the prevention of oxidative damage, such as glutathione peroxidase.[6] Notably, mushrooms not only exhibit direct antioxidant abilities but stimulate antiradical host defenses.[7] Therefore this investigation elucidates the identity of some of the major chemical constituents of *T. versicolor* and reports their documented pharmacological applications.

II. MATERIALS AND METHODS

A. Origin of *T. versicolor*, Extraction, Isolation, and Structure Elucidation

The fruiting bodies of *T. versicolor* were collected from the forests located in northern Iran, Mazandaran. The *T. versicolor* was authenticated by Saeed Ali Mousazadeh (a mycology expert). An herbarium voucher specimen (No-IRAN,MZ.290 F) was deposited at the museum for the Agriculture and Resource Research Center, Mazandaran, Iran. After autoclaving for 48 hours, *T. versicolor* was cut into small pieces and smashed. A percolation extraction method with hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc) as extractive solvents was used at room temperature. After filtration, the extracts were separately concentrated under reduced pressure with a rotatory evaporator. The concentrated extracts obtained from hexane, $CHCl_3$, and EtOAc yielded 13, 18, and 2 g, respectively. The hexane extract was subjected to silica gel column chromatography with mobile phases consisting of hexane:EtOAc (19:1, 9:1, 7:3, 5:5, and 0:1), EtOAc:MeOH (1:1), and methanol (MeOH) to give 9 fractions. Subsequently, after being run through a Sephadex LH-20 Column with $CHCl_3$:MeOH (2:8) as the mobile phase, pure compounds **1** (400 mg), **2** (33 mg), **9** (10 mg) and **10** (12 mg) were obtained. The chloroform extract was subjected to silica gel column chromatography with hexane:EtOAc (49:1, 19:1, 9:1, 7:3, 5:5, and 0:1), EtOAc:MeOH (8:2, 6:4), and MeOH as the eluents to give 21 (CA–CU) fractions. The CA fraction (1050 mg) was subjected to another silica gel column and eluted with hexane:EtOAc (9:1) to obtain 17 fractions (CA_1–CA_{17}). CA fraction 11 contained pure compound **3** (450 mg). The CG fraction (365 mg) was submitted to a silica gel column and washed with hexane:EtOAc (8:2) to isolate compound **4** (17 mg). Subsequently, the column chromatography fraction (98 mg) was loaded onto another silica gel column and washed with hexane:EtOAc (19:1) to obtain pure compound **5** (12 mg). The EtOAc extract was subjected to silica gel column chromatography with hexane:EtOAc (19:1, 9:1, 7:3, 5:5, and 0:1) and EtOAc:MeOH (9:1, 7:3, 5:5, 3:7, and 0:1) as the eluents, to give 17 (EA-EQ) fractions. The EC fraction (165 mg) was submitted to another silica gel column and rinsed with hexane:$CHCl_3$ (19:1, 9:1, 7:3, 6:4, 4:6, 2:8, and 0:1) to furnish compounds **7** (16 mg) and **8** (16 mg). Finally, the EN fraction (695 mg) was loaded onto a Sephadex LH-20 column and eluted with 2 rounds of $CHCl_3$:MeOH (4:6, 3:7) to obtain pure compound **6** (9 mg).

B. General Methods

The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Brucker Avance TM

500 DRX (Karlsruhe, Germany) spectrometer, with tetramethylsilane as an internal standard. The chemical shifts are reported in δ parts per million (ppm) and the coupling constants are revealed in Hertz. The Fourier transform infrared spectra were recorded on a Nicolet 550 instrument on potassium bromide pellets.

C. Chemicals

Column chromatography was carried out using silica gel (70–230 mesh; Merck Co., Darmstadt, Germany) and Sephadex LH-20 (Fluka Co., St. Gallen, Switzerland) as the stationary phase. Precoated silica gel 60 F254 plates (Merck Co.) were used for thin-layer chromatography (TLC). Spots on TLC plates were detected under ultraviolet (UV) light at 254 and 366 nm (UV/visual spectrophotometer; CAMAG Co., Muttenz, Switzerland) and visualized by spraying the developed plates with anisaldehyde, followed by heating for 5 minutes.

III. RESULTS AND DISCUSSION

The chromatography of the hexane, $CHCL_3$, and EtOAc extracts from *T. versicolor* yielded ergosterol (compound 1), ergosterol peroxide (compound 2), trilinolein (compound 3), ergosta-7,22-dien-3β-ol (compound 4), 4-isobutoxyphenyl palmitate (compound 5), N-D-2′-hydroxyheptanoic-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphinga-dienine (compound 6), 3β-linoleyloxyergosta-7,22-diene (compound 7), 3β-linoleyloxyergosta-7-ene (compound 8), betulinic acid (compound 9), and betulin (compound 10).

A. Compound 1, Ergosterol

Ergesterol is a white amorphous powder; it is not visible on TLC plates under UV light (λ = 254 and 366 nm), and it is easily detected following exposure to H_2SO_4anisaldehyde–sulfuric acid (H_2SO_4) spray reagent (dark purple spot) ^{1}H NMR (500 Hz, $CDCl_3$, in parts per million [ppm]): δ 5.6 (1H, double doublets [dd], H7), 5.4 (1H, doublet [d], H5), 5.17 (1H, dd, H22), 5.25 (1H, dd, H23), 3.6 (1H, broad multiplet [br m], H3), 1.03 (3H, d, coupling constant [J] = 6.5, H21), 0.92 (3H, d, J = 6.5, H28), 0.87 (3H, d, J = 7, H26), 0.84 (3H, d, J = 7.3, H27), 0.81 (3H, singlet [s], H19), 0.64 (3H, s, H18); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): see Table 1.

B. Compound 2, Ergosterol Peroxide

Ergesterol peroxide appears as white crystals. It is not visible on TLC plates under UV light (λ = 254 and 366 nm) and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark spot) ^{1}H NMR (500 Hz, $CDCl_3$, in ppm): δ 6.5 (1H, d, J = 8.4, H-7), 6.24 (1H, d, J 0 =8.4, H-6), 5.23 (1H, dd, J = 15.3, J = 7, H-22), 5.14 (1H, dd, J = 15.3, J = 7, H-23), 3.98 (1H, multiplet [m], H-3), 1.00 (3H, d, H-21), 0.91 (3H, d, J = 6.8, H-28), 0.88 (3H, s, H-19), 0.83 (3H, d, J = 6.5, H-27), 0.82 (3H, s, H-18), 0.81 (3H, d, J = 6.8, H-26); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): see Table 1.

C. Compound 3, Trilinolein

Yellow and oily, trilinolein is visible on TLC plates under UV light (λ = 254 nm) as a purple spot and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark purple spot) ^{1}H NMR (500 Hz, $CDCl_3$,in ppm): δ 5.3 (12H, m, H9, H10, H12, and H13), 5.27 (1H, m, H2′ glyceryl), 4.3 and 4.1 (4H, m, H1′, 3′ glyceryl), 2.77 (6H , m , 3× H11), 2.32 (6H, m, 3× H2), 2.05 (12H, m, 3× H8 and H14), 1.6 (6H, m, 3× H3), 1.2 (42H, 21× CH2), 0.91 (9H, t, 3×H18); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): 62.06 (C1, C3 glyceryl), 68.83 (C2 glyceryl), 173.25 (C1′, C3′), 172.83 (C2), 33.98 (C2α), 34.15 (C2β), 24.8 (C3α), 24.8 (C3β), 29.08 (C4α), 29.05 (C4β), 29.2 (C5α), 29.27 (C5β), 29.11 (C6α), 29.18 (C6β), 29.62 (C7α), 29.66 (C7β), 27.16 (both α and β C8), 129.96 (both α and β C9), 128.02 (both α and β C10), 25.58 (both α and β C11), 127.84 (both α and β C12), 130.18 (both α and β C13), 27.16 (both α and β C14), 29 (both α and β C15), 31.48 (C16α),

TABLE 1: ^{13}**C Shifts (ppm) for Identified Compounds of *Trametes versicolor***

	Compound				
Position of C	1 ($CDCL_3$)	2 ($CDCL_3$)	4 ($CDCL_3$)	9 (DMSO)	10 (DMSO)
1	38.35	34.63	37.11	38.24	38.24
2	31.97	30.04	31.45	27.15	26.64
3	70.41	66.4	71.06	76.77	76.77
4	40.77	36.85	37.96	38.49	38.49
5	139.8	82.13	40.22	54.85	54.85
6	119.56	135.38	29.61	17.7	17.68
7	116.27	130.7	117.44	36.71	36.71
8	141.34	79.4	139.54	40.91	40.9
9	46.22	51.63	49.4	49.83	49.91
10	37.1	35.6	34.18	36.32	36.3
11	21.09	20.84	21.52	20.36	20.35
12	39.05	39.28	39.42	24.81	25.06
13	42.79	44.5	43.27	37.57	38.24
14	54.53	51.02	55.91	42.2	41.98
15	22.9	23.35	22.91	29.02	29.22
16	28.28	28.63	28.1	33.81	33.81
17	55.69	56.13	55.8	55.4	47.31
18	12.06	12.83	12.07	47.31	48.52
19	16.26	18.14	13.03	48.15	46.6
20	40.42	39.72	40.49	150.36	150.36
21	21.09	19.61	19.64	29.81	29.8
22	135.55	135.16	131.85	31.7	33.81
23	131.95	132.25	135.66	28.09	28.09
24	42.79	42.73	42.78	15.68	15.68
25	33.06	33.02	33.06	15.91	15.91
26	21.52	19.92	19.93	15.81	15.81
27	19.62	20.59	21.09	14.51	14.38
28	17.58	17.53	17.58	177.22	57.9
29				109.63	109.63
30				18.75	18.92

31.39 (C16β), 22.54 (C17α), 22.65 (C17β), and 14.04 (both α and β C18).

D. Compound 4, Ergosta-7,22-dien-3β-ol

A colorless crystal, ergosta-7,22-dien-3β-ol is not visible on TLC plates under UV light (λ = 254 and 366 nm) and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark purple spot) ^{1}H NMR (500 Hz, $CDCl_3$, in ppm): δ 5.2 (3H, m, H7, H22, and H23), 3.61 (1H, m, H3), 1.02 (3H, d , J = 6.5, H21), 0.91 (3H, d, J = 7, H28), 0.84 (3H, d, J = 7, H26), 0.82 (3H, d, J = 7, H27), 0.80 (3H, s, H19), 0.55 (3H, s, H18); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): see Table 1.

E. Compound 5, 4-Isobutoxyphenyl Palmitate

4-Isobutoxyphenyl palmitate is a white amorphous powder that is visible on TLC plates under UV light (λ = 254 and366 nm; shiny blue spot) and is not detected following exposure to anisaldehyde–H_2SO_4 spray reagent. ^{1}H NMR (500

Hz, CDCl3, in ppm): δ 7.96 (2H, d, J = 8.6, H-2 and H-6 phenyl), 7.89 (2H, d, J = 8.6, H-3 and H-5 phenyl), 4.2 (2H, d, J = 8.4, CH2-isobutyl), 2.3 (2H,t, H-2′ palmitate), 2.05 (2H, t, H-3′ palmitate), 1.77 (1H, m, CH-isobutyl), 1.26 (24H, 12× [CH2] palmitate), 0.89 (6H, d, J = 7, 2× CH3 isobutyl), 0.88 (3H, t, H-16 palmitate); ^{13}C NMR (125 Hz, CDCl3, in ppm): δ 179.66 (C-1′), 166.87 (C-1), 160.25 (C-4), 131.8 (C-2 and C-6), 115.21 (C-3 and C-5), 67.6 (CH2 isobutyl), 37.4 (CH-isobutyl), 34.4 (C-2′), 31.8 (C-3′), 30.2 (C-4′), 29–29.9 (C-5′ to C-12′), 26.7 (C-13′), 25.03 (C-14′), 22.65 (C-15′), 14.08 (C-16′ and 2× CH3 isobutyl).

F. Compound 6, N-D-2′-Hydroxyheptanoic-1-O-β-D-Glucopyranosyl-9-Methyl-4,8-Sphinga-Dienine

A yellow amorphous powder, N-D-2′-hydroxyheptanoic-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphinga-dienine is not visible on TLC plates under UV light (λ = 254 and 366 nm) and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark spot) infrared v_{max}^{KBr} Cm^{-1}: 3436 (OH, NH), 2923, 2853, 1647 (C=O amidic), 1539, 1463, 1078, 1037, 897, 634; ^{1}H NMR (500 Hz, $CDCl_3$, in ppm): δ 10–10.5 (2H, broad singlet [br s], 2× OH), 8.12 (1H, d, J = 6, CONH), 5.3–5.8 (3H, olefinic proton), 4.94 (1H, d, J = 7, anomeric H), 3.84–4.36 (7H, m, H2, 3, 2−, H glycosyl except anomeric H and H6), 3.50 (2H, br s, H1), 3.35 (2H, br s, H6 glycosyl), 1.95 (2H, br t, H10), 1.58 (3H, s, CH3), 1.29 (40H, 20× CH2), 0.89 (6H, t, J = 6.7 Hz, 2× CH3); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): δ 14.09 (CH3 ×2); 15.99 (CH3), 22.68 (CH2 ×2), 25.3 (CH2), 27.69 (CH2), 28.15 (CH2), 29.5 (CH2 ×3), 29.8 (CH2 ×10), 31.4 (CH2), 31.93 (CH2 ×2), 32.7 (CH2), 34.64 (CH2), 39.79 (CH2), 53.39 (C2), 61.06 (C6-OH glycosyl), 62.08 (C1), 69.43 (C3), 72.36 and 73.24 (CH glycosyl ×3), 75.92 (C2′), 76.11 (CH glycosyl), 102.95 (CH anomericglycosyl), 123.09 (C5), 128.55 (C4), 134.48 (C8), 135.99 (C9), and 176.36 (C=O Amide).

G. Compound 7, 3β-Linoleyloxyergosta-7,22-Diene

3β-Linoleyloxyergosta-7,22-diene, a white amorphous powder, is visible on TLC plates under UV light (λ = 254 nm) and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark purple spot). ^{1}H NMR (500 Hz, $CDCl_3$, in ppm): δ 5.4 (4H, olefinic proton oflinoleyl chain), 5.1–5.2 (3H, m, H7, H22, and H23), 4.7 (1H, m, H3), 2.8 (2H, m, H11′), 2.26–2.37 (2H, m, H2′), 2.04 (4H, m, H8′ and 14′), 1.2 (16 H, 8× CH2 of the linoleyl chain), 1.6 (3H, t, H18′), 1.02 (3H, d, J = 5, H21), 0.91 (3H, d, J = 5, H28), 0.83 (3H, s, H19), 0.81 (3H, d, J = 5, H26), 0.79 (3H, d, J = 5, H27), 0.55 (3H, s, H18); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): δ 36.84 (C1), 34.74 (C2), 73.18 (C3), 31.92 (C4), 40.05 (C5), 29.68 (C6), 117.29 (C7), 138.5 (C8), 49.27 (C9), 34.21 (C10), 21.46 (C11), 39.39 (C12), 43.34 (C13), 54.97 (C14), 22.69 (C15), 27.91 (C16), 55.93 (C17), 12.09 (C18), 12.95 (C19), 40.5 (C20), 21.11 (C21), 135.66 (C22), 131.86 (C23), 42.81 (C24), 33.08 (C25), 17.59 (C26), 19.64 (C27), 19.95 (C28), 173.46 (C1′), 33.84 (C2′), 25.07 (C3′), 29.1 (C4′, C5′, C6′, and C7′), 27.19 (C8′), 130.05 (C9′), 128.02 (C10′), 25.63 (C11′), 127.91 (C12′), 130.2 (C13′), 27.54 (C14′), 29.2 (C15′), 31.92 (C16′), 22.68 (C17′), and 14.11 (C18′).

H. Compound 8, 3β-Linoleyloxyergosta-7-ene

3β-Linoleyloxyergosta-7-ene is a white amorphous powder that is visible on TLC plates under UV light (λ = 254 nm) and is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent (dark purple spot). ^{1}H NMR (500 Hz, $CDCl_3$, in ppm): δ 5.4 (4H, olefinic proton of the linoleyl chain), 5.2 (1H, m, H7), 4.7 (1H, m, H3), 2.8 (2H, m, H11′), 2.26–2.37 (2H, m, H2′), 2.04 (4H, m, H8′ and 14′), 1.2 (16H, 8× CH2 of the linoleyl chain), 1.6 (3H, H18′), 1.02 (3H, d, J = 5, H21), 0.91 (3H, d, H28), 0.83 (3H, s, H19), 0.83 (6H, d, H26, H27), 0.56 (3H, s, H18); ^{13}C NMR (125 Hz, $CDCl_3$, in ppm): δ 36.84 (C1), 34.85 (C2), 73.18 (C3), 31.92

(C4), 40.06 (C5), 29.68 (C6), 117.29 (C7), 138.5 (C8), 49.27 (C9), 34.51 (C10), 21.48 (C11), 39.49 (C12), 43.34 (C13), 54.98 (C14), 22.92 (C15), 28.09 (C16), 56.00 (C17), 11.84 (C18), 12.95 (C19), 36.64 (C20), 19.01 (C21), 33.66 (C22), 30.71 (C23), 39.06 (C24), 31.44 (C25), 17.59 (C26), 20.52 (C27), 15.43 (C28), 173.46 (C1′), 33.84 (C2′), 25.07 (C3′), 29.1 (C4′, C5′, C6′ and C7′), 27.19 (C8′), 130.05 (C9′), 128.02 (C10′), 25.63 (C11′), 127.91 (C12′), 130.2 (C13′), 27.54 (C14′), 29.2 (C15′), 31.92 (C16′), 22.68 (C17′), and 14.11 (C18′).

I. Compound 9, Betulinic Acid

A white crystalline solid, betulinic acid is not visible on TLC plates under UV light (λ = 254 and 365 nm) but is easily detected following exposure to anisaldehyde–H_2SO_4 spray reagent. ^{1}H NMR (500 Hz, DMSO, in ppm): δ 12.05 (1H, br s, acidic proton), 4.66 (1H, s, H29b), 4.54 (1H, s, H29a), 4.29 (1H, m, H3), 2.97 (1H, m, H19), 1.63 (3H, s, H30), 0.98 (3H, s, H27), 0.94 (3H, s, H23), 0.88 (3H, s, H26), 0.76 (3H, s, H24), 0.66 (3H, s, H25); ^{13}C NMR (125 Hz, dimethyl sulfoxide [DMSO], in ppm): see Table 1.

J. Compound 10, Betulin

Betulin is a white crystalline solid that is not visible on TLC plates under UV light (λ = 254 and 365 nm) but is easily detected following exposure to an anisaldehyde–H_2SO_4 spray reagent.

^{1}H NMR (500 Hz, DMSO, in ppm): δ 4.69 (1H, s, H29b), 4.56 (1H, s, H29a), 4.24 (1H, dd, J = 4.2 and 11Hz, H3), 3.5 (1H, d, J = 10.1, H28b), 3.09 (1H, d, J = 10.1, H28a), 2.39 (1H, m, H19), 1.63 (3H, s, H30), 0.98 (3H, s, H27), 0.94 (3H, s, H23), 0.88 (3H, s, H26), 0.76 (3H, s, H24), 0.66 (3H, s, H25); ^{13}C NMR (125 Hz, DMSO, in ppm): see Table 1.

Ergosterol is used as an important pharmaceutical intermediate and as the precursor of vitamin D_2 and cortisone. Relatively high ergosterol content could make *T. versicolor* a dietary source for individuals with a limited intake of ergocalciferol from foods of animal origin, for example, vegetarians and vegans.[8] Ergosterol also was identified as one of the most active constituents isolated from the lipid fraction of *Grifola frondosa*; this compound exhibited antioxidant activity and inhibited the cyclooxygenase enzymes COX-1 and COX-2.[9] Takaku et al.[10] in 2001 found that the ergosterol isolated from *Agaricus blazei* inhibited tumor-induced neovascularization and obtained evidence that the mechanism of its antitumor actions might involve the inhibition of tumor-induced angiogenesis. A study by Mizushina et al.[11] in Japan indicated that ergosterol peroxide purified from *Ganoderma lucidum* selectively enhanced the inhibitory effect of linoleic acid on DNA polymerase β. Ergosterol peroxide also inhibits allergic reactions (immunosuppressive)[12] and potentiates adenosine diphosphate–induced platelet aggregation,[13] in addition to having antiviral[14] and antitumor activities.[15] Ergosterol peroxide strongly inhibits the growth of some cancer cells and induces apoptosis in HL60 human leukemia cells, although neonatal normal human dermal fibroblasts and Chinese hamster lung cells remain unaffected.[16] Yasukawa et al.[17] investigated ergosterol-mediated inhibition of phorbol-12-myristate 13-acetate–induced inflammatory ear edema in mice to demonstrate the significant action of this compound. Ergosterol peroxide decreases lipid peroxidation in rat liver microsomes and suppresses the proliferation of rat and human lymphocytes that have been excited with mitogens.[18] In addition, this compound suppresses the lipopolysaccharide-induced proinflammatory gene expression in macrophages and the growth of human colon adenocarcinoma cells.[19] Trilinolein is a triacylglycerol. The olefinic protons (CH=CH) of the polyunsaturated fatty acid chain resonate at 5.3 ppm (m); this m signal was not a baseline artifact because of the comparison with the proton signal at 5.27 ppm (m), which was assigned to H2 of the glycerol backbone. The H1 and H3 protons of glycerol resonate at 4.1 and 4.3 ppm (J = 6 Hz, dd). The protons of bisallylic methylene from the polyunsaturated acyl chains (H11) appear at 2.72 (m), and the protons attached to the allylic carbons resonate at 2.05 ppm. The H2 and H3 protons of the acyl moieties in the triacylglycerol resonate at 2.32 and

1.6 ppm, respectively, and the protons of methylene envelope appear at 1.2 ppm (the integrated area of this region indicated the presence of 42 protons, which is equivalent to 21 methylene groups). The methyl protons of the polyunsaturated fatty acid are shifted to a higher frequency (0.91 ppm, t) relative to the terminal methyl protons of the saturated and unsaturated chains (0.88 ppm, t).[20] In the ^{13}C NMR spectrum, the carbonyl of the triglyceride is revealed as 2 series of resonances: the higher-frequency series denotes the chains esterified at their 1,3-glycerol positions, whereas the low-frequency series encompasses the chains with 2-glycerol substitution. In particular, the signals from the carbonyl carbons on the 1,3-chains are shifted by approximately 0.42 ppm toward higher frequencies than the carbonyls of the 2-chains. This shift difference, which was previously discovered, occurs because the C=O groups of the 2-position chains endure 2 γ-gauche interactions versus the single interaction experienced by the carbonyls of the 1,3-chains.[21] The resonances of the unsaturated carbons, which were assigned according to the chemical shifts of standard triacylglycerols in the region containing δ 127–130 ppm, were observed in a distortionless enhancement by polarization transfer (DEPT) 90 spectrum.[22] The glycerol carbons from the triacylglycerols resonate were observed in the region from 62 to 69 ppm. The chemical shifts were assigned based on the assumption that acylglycerol symmetry, or lack thereof, dictates the number of resonances and their approximate intensities. Therefore, triacylglycerol exhibits 2 signals for the glycerol moiety that have an intensity ratio of 1:2. The use of the DEPT pulse sequence, which presents a method for choosing the carbon-1,3 multiplicity, demonstrated that the resonances of the glycerol C2 shifted to higher frequencies (δ 68.83 ppm) than the signals for the glycerol 1,3-carbons (δ 62.06 ppm). The resonances of the methyl and other methylene carbons in the DEPT experiment and the reported ^{13}C NMR data are validated by previous reports.[23] Trilinolein is a tyrosinase inhibitor[24] and an antibacterial and antifungal compound. It also exhibits myocardial protective effects as a result of its antioxidant ability and inhibits endothelin-1-induced hypertension. Trilinolein also has demonstrated anticancer, thrombogenicity-reducing, erythrocyte-deforming, and anti-ischemic activities.[25] In a study concerning the free radical damage linked with atherogenesis, the myocardial damage caused by ischemia and reperfusion was decreased by trilinolein.[26]

Compound 4 was received as powdery white needles. The ^{1}H NMR spectrum exhibited 3 protons at δ 5.2 ppm and one proton at δ 3.61 ppm as multiplets; in addition, 2 methyl singlets at δ 0.55 and 0.80 ppm and 4 methyl doublets at δ 0.82, 0.84, 0.91, and 1.02 ppm (J = 7) were observed. The ^{13}C NMR spectrum also revealed 24 carbon signals in the high field region (δ 12–71 ppm) and 4 carbon signals in the low field region (δ 117–139.5 ppm). Finally, compound 4 was identified as ergosta-7,22-dien-3β-oland and matched with previously reported data.[27,28] A study that Smania and his coworkers[29] performed on ergosta-7,22-dien-3β-ol, which was isolated from *Ganoderma applanatum*, indicated that ergosta-7,22-dien-3β-ol was weakly active against a number of Gram-positive and Gram-negative microorganisms.

During TLC, compound 5 fluoresced blue when subjected to UV light at 366 nm and could not be stained with the anisaldehyde reagent. This compound is reported here for first time in *T. versicolor*; the ^{1}H NMR, ^{13}C NMR, and DEPT spectra exhibited 2 doublets at δ 7.96 ppm (2H, d, J = 8.6, H2 and H6 phenyl) and δ 7.89 ppm (2H, d, J = 8.6, H3 and H5 phenyl); 2 methine signals at δ 131.8 ppm (C2 and C6) and δ 115.21 ppm (C3 and C5); and 2 quaternary carbons at δ 166.87 ppm (C1) and δ 160.25 ppm (C4). These data indicate the presence of a parasubstituted phenyl moiety with oxygen substituents on both sides. A doublet signal at δ 4.2 ppm (2H, d, J = 8.4, CH2 isobutyl) and a low field signal at δ 67.6 ppm (CH2 isobutyl) indicated that compound 5 has a methylene group bound to oxygen and one adjacent hydrogen. The other quaternary carbon, which was observed at δ 179.66 ppm (C1′), was identified as an ester carbonyl bound to a phenyl moiety. The signal at δ 37.4 (CH isobutyl) in the DEPT spectra and the multiplet observed in the ^{1}H NMR spectra at δ 1.77 ppm (1H, m, CH isobutyl) indicated that

compound 5 has an aliphatic methine (CH) group. The signals in the ^{13}C NMR data at δ 14.08 ppm (CH3 ×3) and in the ^{1}H NMR data at δ 0.89 ppm (6H, d, J = 7, 2× CH3 isobutyl) and δ 0.88 ppm (3H, t, H16 palmitate chain) were assigned to be the 2 terminal methyl groups from the long chains. Finally, the other data that were obtained from the ^{1}H NMR, ^{13}C NMR, and DEPT spectra indicated the following assignments: δ 2.3 ppm (2H, t, H2′ palmitate chain) indicates CH2 protons adjacent to the carboxylic group, δ 2.05 ppm (2H, t, H3′ palmitate chain), δ 1.26 ppm (24H, methylenes of the palmitate chain), and δ 34.4 ppm (C2′), δ 31.8 ppm (C3′), δ 30.2 ppm (C4′), δ 29–29.9 ppm (C5′, C6′, C7′, C8′, C9′, C10′, C11′, and C12′), δ 26.7 ppm (C13′), δ 25.03 ppm (C14′), and δ 22.65 ppm (C15′). Comparing these data with the data in the literature helped us to identify compound 5 as 4-isobutoxyphenyl palmitate.[30,31]

In our study, compound 6 was reported for first time as a component of *T. versicolor*. The infrared spectrum displayed signals that were assigned as follows: jagged hydroxyl absorption bands at 3435 cm^{-1} and bands at 1647, 1539, 1463, 1078, and 1037 cm^{-1} that were assigned to an amide group.[32] The ^{13}C NMR, DEPT 90, and DEPT 135 spectra exhibited 4 signals: δ 123.09 ppm (C5 olefinic), δ 128.55 ppm (C4 olefinic), δ 134.48 ppm (C8 olefinic), and δ 135.99 ppm (C9 olefinic). These data indicated that 2 double bonds were present and that C9 is a quaternary carbon atom bonded to a methyl group; C9 was assigned as such because, in the ^{13}C NMR spectrum, one methyl group was revealed down field (δ 15.99 ppm). In addition, in the ^{1}H NMR (δ 1.58, 3H, s) spectra, the signal was also further down field than the other methyl groups, which demonstrates that it is bound to an olefinic carbon; this assertion was validated by the signals at δ 5.3–5.8 ppm (3H, m) in the ^{1}H NMR spectrum. The signals in the ^{13}C NMR at δ 14.09 ppm (CH3 ×2) and in the ^{1}H NMR at δ 0.89 ppm (6H, t, J = 6.7 Hz) were assigned to be the 2 terminal methyl groups on the continuous chains.[33] At δ 1.29 ppm (40H, 20× CH2) in the ^{1}H NMR and δ 22.68–39.79 ppm in the ^{13}C NMR spectra, the overlapped signals of the methylene groups suggested the presence of 2 long aliphatic chains. In addition, the ^{1}H NMR spectrum also displayed a characteristic a secondary amide NH doublet at δ 8.12 ppm (1H, d, J = 6 Hz, exchangeable with D_2O). The ^{13}C NMR and DEPT spectra of compound 6 indicated the presence of an amide functionality at δ 175.3 ppm (C1′) and δ 53.39 ppm (C2).[34] Eight methine signals were observed at δ 69.43 ppm (C3-OH), δ 72.36 ppm (CH glycosyl ×2), δ 73.24 ppm (CH glycosyl), δ 75.92 ppm (C2′-OH), δ 76.11 ppm (CH glycosyl), and δ 102.95 ppm (CH anomericglycosyl), in addition to the 2 methylene signals at δ 61.06 ppm (C6-OH glycosyl) and δ 62.08 ppm (C1-OH). In the ^{1}H NMR spectrum, compound 6 displayed 7 distinctive signals attributed to protons bound to hydroxyl groups at δ 3.84–4.36 ppm (8H, m, H1, H2, glycosyl protons except the anomeric proton), δ 3.50 ppm (2H, br s, H2′), δ 3.35 ppm (1H, br s, H3), δ 4.94 ppm (1H, d, J = 7, anomericglycosyl proton), and 2 broad singlets at δ 10–10.5 ppm that belong to 2 hydroxyl groups near amidic carbonyl (2H, br s, 2× OH). These findings, combined with the NMR data described above, led to the final assignation of compound 6 as a cerebroside. The cleavage products of cerebrosides (sphingolipids) are ceramides that are associated with various signal transduction pathways.[35]

Extracellular stresses, such as tumor necrosis factor-α and human immunodeficiency virus, activate sphingomyelinases that discharge ceramides, which inhibit cell growth and cause apoptosis.[36] α-Hydroxy fatty acid and ceramide containing C18 phytosphingosine is commonly observed in the bonding form in mushrooms.[37] Studies concerning the chemistry and biology of ceramides have been recently undertaken most importantly because of their most important actions. Compounds 7 and 8 were isolated together and were a combination of 2 linoleic acid stearyl esters, which were identified by comparing their ^{1}H NMR, ^{13}CNMR, and DEPT spectral data with the known data from ergosta-7,22-dien-3β-ol(36,41),ergost-7-en-3β-ol[38] and linoleic acid as 3β-linoleyloxyergosta-7,22-diene and 3β-linoleyloxyergost-7-ene.[28] These compounds were separated from the other species of basidiomycetes, therefore validating the role of the 2 free

sterols as being for fungal growth.[28]

Compound 9 was purified to obtain a white amorphous powder; in the ^{1}H NMR spectrum this substance exhibited signals for methyl groups at δ 1.63 (3H, s, H30), 0.98 (3H, s, H27), 0.94 (3H, s, H23), 0.88 (3H, s, H26), 0.76 (3H, s, H24) and 0.66 ppm (3H, s, H25). The ^{1}H NMR spectrum also revealed signals attributed to olefinic hydrogens at δ 4.66 and 4.54 ppm (2H, s) for H29, hydrogen joined to carbon-bearing OH (H3) at δ 4.29 ppm (1H, m), and an acidic proton at δ 12.05 ppm (1H, br s, acidic proton). In addition, 30 carbon atoms were identified by investigating the signals in the ^{13}C NMR spectrum for compound 9. Doublets for the geminal protons at δ4.66 and 4.54 ppm, in addition to the methyl group at δ 1.63 ppm, suggest that compound 9 is a lupeol-type triterpene derivative. The peak at δ 177.22 ppm indicates the presence of a carboxylic group. The signals at δ 150.36 and δ 109.63 ppm were ascribed to the existence of terminal olefinic carbons in compound 9. The isopropylene groups at C20 and C29 were assigned based on these signals. Furthermore, we identified 23 signals in the DEPT 135 spectrum, of which 11 were negative signals that indicated the presence of 11 methylene carbons in compound 9. Based on the ^{13}C NMR, DEPT 135, and DEPT 90 spectral data, 6 separate methyl group signals were recognized. Finally, compound 9 was proposed to have 30 carbons comprising 6 methyl, 11 methylene, 6 methine, and 7 quaternary carbons. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra data suggest that compound 9 is a pentacyclic lupane-type triterpenoid, and the comparison of its spectral data with published values validated the assignment of compound 9 as betulinic acid.[40] Fulda and coworkers[41] identified that betulinic acid induced apoptosis through direct action on mitochondria, independent of the accumulation of the wild-type p53 protein. Death-inducing ligand/receptor systems, such as CD95, are absent.

Compound 10 was obtained as a white amorphous powder, and its ^{1}H NMR spectrum exhibited the signals for an isopropylene group at δ 4.69 (1H, br s) and 4.56 ppm (1H, br s) for H29. Six signals for methyl groups at 1.63 (3H, s), 0.98 (3H, s), 0.94 (3H, s), 0.88 (3H, s), 0.76 (3H, s), and 0.66 ppm (3H, s) indicate that these groups are attached to a triterpenoidal core.[42] The doublets for the geminal protons at δ 4.69 and 4.56 ppm, in addition to the methyl group at δ 1.63 ppm, as well as the geminal protons at 3.35 and 3.09 ppm (2H each, d, J = 10.1 Hz, H-28) and the oxy-methine group at 4.24 ppm (1H, dd, J = 4.2 and 11 Hz, H3), suggest that compound 10 is a lupeol-type triterpene derivative.[43] The distinctive pair of sp2 carbons for the double bond that is characteristic of lupeol in the ^{13}C NMR and DEPT spectra was observed as resonance at δ 150.36 and 109.63 ppm.[44] Oxygenated carbon signals for C3 and C28 were exhibited at δ 76.77 and 57.9 ppm, respectively. The 30 carbon atoms and 6 methyl groups (the total of which are equivalent to the number of carbon atoms in a triterpenoid) revealed in the spectra indicate that this molecule is a compound with a lupene-type triterpenoidal core and 2 hydroxyl groups at C3 and C28 (a lupeol-type triterpene). Full structural elucidation of compound 10 via comparison with the spectral data from the literature identified it as betulin.[45] Betulin "lup-20(29)-ene-3,28-diol," which is also known as betulinol, betuline, and betulinic alcohol, is a pentacyclic triterpene alcohol with a lupane skeleton. Betulin and betulinic acid both have anti–human immunodeficiency virus, anticancer, anti-inflammatory, antimalarial, antiviral, and other antimicrobial applications.[46] In addition, betulin has antiseptic, antirachitic, cholesterol-lowering, chloritic, and liver-protecting activities.[47]

IV. CONCLUSION

The investigations by the other researchers mentioned above showed compounds of medicinal mushrooms potentially had effective biological actions *in vivo* and *in vitro*. In our study we purified these compounds in *T. versicolor*, indicating that *T. versicolor* would be a valuable source of these compounds. However, intensive and extensive investigations are needed to exploit its valuable therapeutic use.

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