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Sterols and Lipids from *Pleurotus florida*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the fruiting bodies of Pleurotus floridaled to the isolation of ergosterol (**1**), ergosterol peroxide (**2**), cerevisterol(**3**), a mixture of palmitic acid and stearic acid, linoleic acid, and oleic acid in about 1.5:1:0.5 ratio, and dilinoleoyloleoylglycerol. The structures of these compounds were identified by comparison of their NMR data with literature data.*

Keywords: *Pleurotus florida,* Pleurotaceae,ergosterol, ergosterol peroxide, cerevisterol, palmitic acid, linoleic acid, oleic acid, dilinoleoyloleoylglycerol

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INTRODUCTION

Pleurotus species belong to phylum Basidiomycota that produce oyster shaped fruiting bodies (basidiocarps) and are called oyster mushroom [1]. Fruiting bodies of *Pleurotus* species were reported to possess a number of medicinal properties, such as anti-inflammatory, immunostimulatory and immunomodulatory[2], anticancer [3], hypoglycemic [4], and anti-HIV [5]. A review on the medicinal properties of *Pleurotus* species (oyster mushroom) has been provided [1].

Pleurotus florida, a species of the genus *Pleurotus*is widely cultivated in the Philippines as commercial oyster mushroom for food. A number of studies have been conducted on the biological activities, nutritional values and chemical constituents of this oyster mushroom. The methanol extract of *P. florida* Eger showed significant activity in ameliorating acute and chronic inflammation at 500 and 1000 mg/kg body weight. The extract also showed significant platelet aggregation inhibiting activity by 88% to 95% at a concentration of 500 µg/mL after a preintubation time of 5, 10 and 20 min [6]. The ethanol extract of *P. florida* was reported to possess appreciable antioxidant activity, as indicated by the polyphenol contents, DPPH scavenging activity, reducing power and anti-lipid peroxide effect. Furthermore, the extract showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi-A* and *Serretiamarcescens*[7]. The aqueous extract of *P. florida* (100, 200, 400mg/kg) revealed an antihepatotoxic action on thioacetamide induced hepatotoxicity in rats [8].The methanol extract of *P. florida* has inhibited the growth of

solid tumour induced by EAC cell line in a dose-dependent manner in experimental animals[9].Another study reported that *P. florida* yielded 113 µg ergosterol per gram of the mushroom[10].

We report herein the isolation of ergosterol (**1**), ergosterol peroxide (**2**), cerevisterol(**3**) (Fig. 1), a mixture of palmitic acid and stearic acid, linoleic acid, and oleic acid in about 1.5:1:0.5 ratio, and dilinoleoyl oleoylglycerolfrom the fruiting bodies of *Pleurotus florida.*

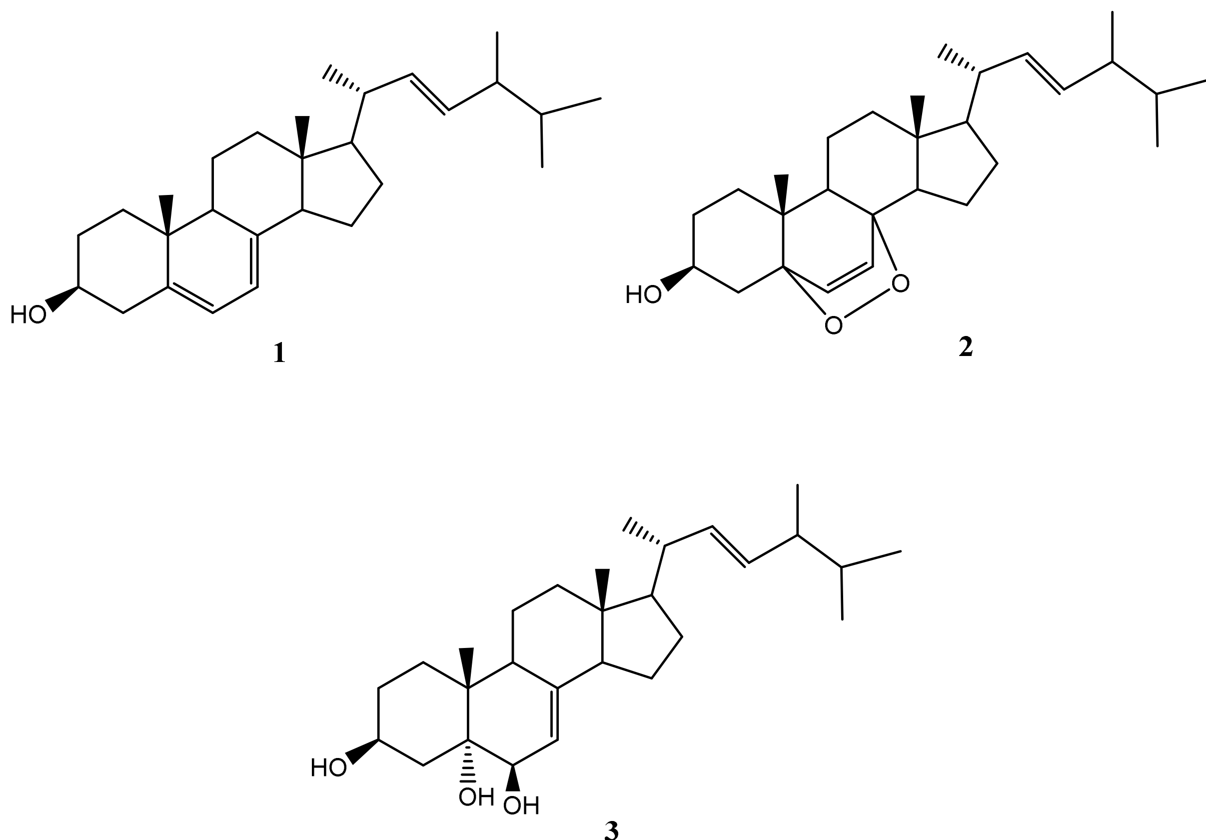

Fig. 1. Chemical structures of ergosterol (1), ergosterol peroxide (2), and cerevisterol (3) from *Pleurotus florida*

MATERIALS AND METHODS

General Experimental Procedure

^{1}H (500 MHz) and^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Propagation and Harvesting

Grain spawn of *P. florida* (commercial strain) was aseptically inoculated into previously pasteurized rice straw – based formulation consisting of 7 parts of composted rice straw and 3 parts of sawdust (v/v) contained in heat resistant polypropylene bags. The inoculated bags were incubated at room temperature (28-30°C) to allow the full ramification of mycelia and the bags were subsequently transferred to the growing room for fruiting in May 2014. Fruiting bodies were harvested at marketable stage.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2(10%increment) as

eluents. Fifty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLCpure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliterfractions were collected.

Isolation of the chemical constituents of the fruiting bodies of P. florida

The freeze-dried fruiting bodies of*P. florida* (200 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (2.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to yield dilinoleoyl oleoyl glycerol(35 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using $CH_3CN:Et_2O:CH_2Cl_2$ (0.5:0.5:9, v/v) to yield **1** (22 mg)after washing with petroleum ether.The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 20% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford a mixture of palmitic acid, stearic acid, linoleic acidand oleic acid(12 mg). The more polar fractions were combined and rechromatographed (3 ×) using $CH_3CN:Et_2O:CH_2Cl_2$ (1:1:8, v/v) to yield**2** (9 mg) after trituration with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using $CH_3CN:Et_2O:CH_2Cl_2$ (2.5:2.5:5, v/v) to yield **3** (2 mg) after trituration with petroleum ether.

Ergosterol (**1**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.57 (dd, *J* = 2.5, 5.5 Hz, H-6), 5.38 (dd, *J* = 2.5, 5.5 Hz, H-8),5.22 (dd, *J* = 7, 15.5 Hz, H-23), 5.17 (dd, *J* = 7.5, 15.5 Hz, H-22), 3.63 (m, H-3), 1.03(d, *J* = 7 Hz, H-21), 0.94 (s, H-19), 0.92 (d, *J* = 7.0 Hz, H-28), 0.84 (d,*J* = 7 Hz, H-26), 0.82 (d, *J* = 7 Hz, H-27), 0.63 (s, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$): δ38.4 (C-1), 32.0 (C-2), 70.4 (C-3), 40.8 (C-4), 139.8 (C-5), 119.6 (C-6), 116.3 (C-7), 141.3 (C-8), 46.2 (C-9), 37.0 (C-10), 21.10, 21.09C-11, C-21), 39.1 (C-12), 42.82, 42.81 (C-13, C-24), 54.5 (C-14), 23.0 (C-15), 28.3 (C-16), 55.7 (C-17), 12.0 (C-18), 17.6 (C-19), 40.4 (C-20), 135.6 (C-22), 132.0 (C-23), 33.1 (C-25), 19.6 (C-26), 19.9 (C-27), 16.3 (C-28).

Ergosterol peroxide (**2**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.51 (d, *J*=8.5, H-6), 6.24 (d, J= 8.5, H-7),5.15 (dd, *J* = 8, 15 Hz, H-22), 5.22 (dd, *J* = 7.5, 15 Hz, H-23), 3.97 (m, H-3), 0.81 (s, Me-18), 0.88 (s, Me-19), 1.00 (d, *J*=7 Hz, Me-21*)*, 0.81 (3H, d,*J* = 7 Hz, H-26), 0.83 (3H, d, *J* = 7 Hz, H-27), 0.90 (3H, d, *J* = 7 Hz, H-28);^{13}C-NMR (125 MHz, $CDCl_3$): δ34.7 (C-1), 30.1 (C-2), 66.4 (C-3), 36.93, 36.95 (C-4, C-10), 82.1 (C-5), 135.4 (C-6), 130.7 (C-7), 79.4 (C-8), 51.7 (C-9), 20.9 (C-11), 39.3 (C-12), 44.5 (C-13), 51.1 (C-14), 23.4 (C-1 5), 28.6 (C-16), 56.2 (C-17), 12.9 (C-18), 18.2 (C-19), 39.7 (C-20), 19.6 (C-21), 135.2 (C-22), 132.3 (C-23), 42.8 (C-24), 33.0 (C-25), 19.9 (C-26), 20.6 (C-27), 17.5 (C-28).

Cerevisterol **(3):** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.35 (d, J = 5.3 Hz, H-7), 5.19 (dd, H-22), 5.19 (dd, H-23), 4.08 (m, H-3), 3.63 (m, H-6), 1.08 (s, H-19), 1.03 (d, J = 6.6 Hz, H-21), 0.92 (d, J = 6.8 Hz, H-28), 0.84 (d, J = 7.4 Hz, H-27), 0.82 (d, J = 7.4 Hz, H-26), 0.60 (s, H-18).

Linoleic acid: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.37 (m, =CH), 2.78 (m), 2.33 (t, *J* = 7.5 Hz, α-CH_2),1.97-2.01 (m, allylic CH_2), 1.60 (m, β-CH_2), 1.24-1.32 (CH_2), 0.86 (t, *J*= 7.0 Hz).

Oleic acid:^{1}H NMR (500 MHz, $CDCl_3$): δ 5.33 (m, =CH), 2.33 (t, *J* = 7.5 Hz, α-CH_2), 1.97-2.01 (m, allylic CH_2), 1.60 (m, β-CH_2), 1.24-1.32 (CH_2), 0.86 (t, *J*= 7.0 Hz).

Palmitic acid **and** ***Stearic acid***:δ 5.37 (m, =CH), 2.78 (m), 2.33 (t, *J* = 7.5 Hz, α-CH_2), 1.60 (m, β-CH_2), 1.24-1.32 (CH_2), 0.86 (t, *J*= 7.0 Hz).

Dilinoleoyl oleoylglycerol:^{1}H NMR (500 MHz, $CDCl_3$): δ 4.29 (dd, *J* = 4.5, 12.0 Hz, glyceryl CH_2O), 4.13 (dd, *J* = 6.0, 12.0 Hz, glyceryl CH_2O), 5.26 (m, glyceryl CHO), 2.30 (t, *J* = 7.5 Hz, α-CH_2), 5.34 (m, olefinic H), 2.75 (t, *J* = 6.5 Hz, double allylic CH_2), 1.97-2.06 (allylic, CH_2), 1.59-1.61 (β-CH_2), 1.25-1.36 (CH_2), 0.87 (t, *J* = 6.0Hz, CH_3), 0.88 (t, *J* = 6.5 Hz, CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *P. florida* yielded ergosterol (**1**) [11], ergosterol peroxide (**2**) [12], cerevisterol(**3**) [13], palmitic acid [14], linoleic acid [15], oleic acid [16]and dilinoleoyloleoylglycerol. The structures of these compounds were identified by comparison of their NMR data with literature data. The fatty acids in the mixture were deduced from the ^{1}H NMR spectrum integrations of the double allylic methylene at δ 2.75 for the linoleic acid, the allylic methylenes for the linoleic acid and oleic acid at δ 1.97-2.04, and the methylene protons at δ1.23-1.35. The presence of saturated fatty acids, palmitic acid and stearic acid in the mixture were suggested by the integrations of the methylene protons at δ 1.23-1.35, methyl protons at δ 0.86, α-methylene protons at δ 2.33, and β-methylene protons at δ 1.60. These fatty acids were reported as major constituents of *Pleurotus* species (Ergonul et al., 2013; Woldegiorgis et al., 2015). The structure of dilinoleoyl oleoyl glycerol was deduced from the integrations of the ^{1}H NMR spectrum of the double allylic methylene at δ 2.75 for the linoleic acid and the allylic methylenes for the linoleic acid and oleic acid at δ 1.97-2.06.

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities.

A study reported that ergosterol (**1**)provides significant protection against the promotion of bladder tumor induced by many types of promoters in the environment [17].

The ergosterol peroxide (**2**)isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm. f. sp. Florida showed strong trypanocidal activity on the intracellular form of *T. cruzi* with an IC_{50} of 6.74 µg/mL[18]. Sterol **2** from an edible mushroom suppresses inflammatory response in RAW 264.7 macrophages and growth of HT29 colon adenocarcinoma cells [19]. Compound **2** was shown to exhibit anti-tumor activity in multiple myeloma U266 cells, Walker carcinosarcoma, human mammary adenocarcinoma, human gastric tumor (SNU-1), human hepatoma (SUN-354), human colorectal tumor (SUN-C4), and murine sarcoma-180 cell lines [20].The IC_{50} value of **2** based on the cell viability of Hep3B was 16.7 µg/mL [21]. It exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU- 145) human prostate cancer cells at µM concentrations [22]and suppressed cell growth and STAT1 mediated inflammatory responses in HT29 cells [23].It inhibited the growth and induced apoptosis of HL60 human leukaemia cells at a concentration of 25 µM, inhibited TPA induced inflammation and tumor promotion in mice and suppressed proliferation of mouse and human lymphocytes stimulated with mitogens [24]. It displayed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295 [25] and induced death of miR-378 cell [26]. It exhibited significant inhibitory activities against leishmaniasis, tuberculosis, *Mycobacterium tuberculosis* H37Rv and *M. avium*[27], and inhibited the hemolytic activity of human serum against erythrocytes[28]. Sterol **2** significantly blocked MyD88 and VCAM-1 expression, and cytokine (IL-1β, IL-6 and TNF-α) production in LPS-stimulated cells and effectively inhibited NF-kB activation. which indicated that it may play an important role in the immunomodulatory activity of GF [29]. It possessed marked activity against PGE2 release with an IC50 value of 28.7 µM. The mechanism in transcriptional level of **2** was found to down-regulate mRNA expressions of iNOS and COX-2 in dose-dependent manners [30].Furthermore, **2** suppressed LPS-induced DNA binding activity of NF-kB and C/EBPβ and inhibited the phosphorylation of p38, JNK and ERK MAPKs. It down-regulated the expression of low-density lipoprotein receptor (LDLR) regulated by C/EBP, and HMG-CoA reductase (HMGCR) in RAW264.7 cells. Moreover, **2** induced the expression of oxidative stress-inducible genes, and the cyclin-dependent kinase inhibitor CDKN1A, and suppressed STAT1 and interferon-inducible genes [31].

Cerevisterol and ergosta-4,6,8(14),22-tetraen-3-one which were isolated from *P. tuber-regium*were tested for anti-inflammatory effects on RAW 264.7 macrophages [32]. Both sterols inhibited the production of NO, TNF-α, and PGE2in LPS-treated RAW 264.7 cells. These compounds also inhibited the expression of the iNOS and COX2 proteins in a dose-dependent manner and repressed the expression of iNOS, COX2, TNF-α, andSOCS3 mRNAs [32].

Linoleic acid belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces risk of colon and breast cancer [33] and lowers cardiovascular disease risk and inflammations[34].Linolenic and linoleic acids inhibited parasites growth by 70% and 64% respectively, against *P.berghei* using the 4-day suppressive test. The two compounds when used in combination inhibited the parasites by 96%on day 4 of treatment[35].

A recent study reported that oleic acid inhibited cancer cell growth and survival in gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines [36]. Another study demonstrated that oleic acid promotes apoptotic cell death of breast cancer cells [37]. It was also shown to be effective at depressing lipogenesis and cholesterologenesis [38]. Furthermore, oleic acid may contribute to the prevention of atherogenesis [39].

Palmitic acid at 12.5 to 50 µg/mL was reported to exhibit selective cytotoxicity to human leukemic cells, induced apoptosis in the human leukemic cell line MOLT-4 at 50 µg/mL, and showed *in vivo* antitumor activity in mice[40]. Another study reported that palmitic acid stimulates incorporation of glucose in the adipocyte by a mechanism dependent upon intracellular Ca^{2+}[41].

CONCLUSION

The dichloromethane extract of the Central Luzon State University commercial strain of *Pleurotus florida* yielded the sterols (**1-2**) and fatty acids which were reported to exhibit anticancer properties. Furthermore, **2** and **3** were reported to show anti-inflammatory effect. Among the isolated compounds, **3** was reported to exhibit more diverse biological activities. The synergistic effect of these anticancer compounds could be partly responsible for the reported anticancer activity of *P. florida.*

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