Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(19):222-225
(http://derpharmachemica.com/archive.html)

Chemical Constituents of *Phellinus gilvus* (Schwein.) Pat.

Consolacion Y. Ragasa[1,2*], Maria Carmen S. Tan[1], Ma. Ellenita G. De Castro[3] and Ian A. van Altena[4]

[1]Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines,
[2]Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines
[3]Biology Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines,
[4]School of Environmental and Life Sciences, Faculty of Science and Information Technology, Chemistry, The University of Newcastle-Australia, Callaghan, NSW, 2308, Australia

---

ABSTRACT

*Chemical investigation of the dichloromethane extract of the fruiting bodies of Phellinus gilvus (Schwein.) Pat. has led to the isolation of ergosterol peroxide (**1**) and triacylglycerols (**2**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its ^{13}C NMR data with literature data.*

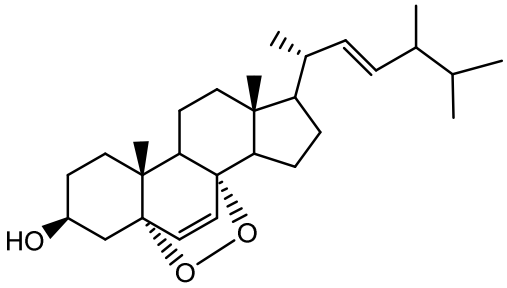
Keywords: *Phellinus gilvus* (Schwein.) Pat., Hymenochaetaceae, ergosterol peroxide, triacylglycerols

---

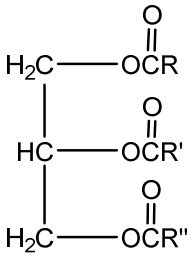
INTRODUCTION

Phellinus gilvus (Schwein.) Pat.is a plant pathogenic fungus having a wide host range worldwide [1].Several studies have been conducted on the chemical constituents and biological activities of *P. gilvus*. An earlier study reported the isolation of the steroidsgilvsins A–D, 24-methylenelanost-8-ene-3b,22-diol and 5α-ergosta-7,22-diene-3-one from *P. gilvus*[2]. The organic extract of the fruiting bodies of*P.gilvus*TMC-1117 exhibited biphasic vasodilator activity on rat aorta with endothelium. The major constituents of this extract were trametenolic acid B and eburicoic acid whichexhibited a moderate vasorelaxant effect on rat aorta [3]. The polysaccharides isolated from *P. gilvus* significantly inhibited melanoma growth in mice by significantly increasing the melanoma apoptosis rate [4]; enhanced wound repair in diabetic impaired healing [5];and inhibited BaP-induced forestomach carcinogenesis in mice by down-regulating mutant p53 expression [6]. Extracts of *P. gilvus* and *P. baumii* may be useful in preventing acute pulmonary inflammation in human diseases [7].

We report herein the isolation of ergosterol peroxide (**1**) and triacylglycerols (**2**)from the fruiting bodies of *P. gilvus*, The structures of **1**-**2**are presented in Fig. 1.



1



2 R, R', R" = long chain fatty acid alkyls

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Bruker Ascend400 spectrometer in $CDCl_3$ at 400 MHz for ^1H NMR and 100 MHzfor^{13}C NMR spectra. Spectra were referenced to the residual proton in $CDCl_3$ at δ 7.24 (^1H) and $CDCl_3$ at δ 77.0 (^{13}C). 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}(0.2 mm layer thickness) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

Fruiting bodies of *Phellinus gilvus* (Schwein.) were collected at various stages of maturity as exemplified by the difference in their colors which range from yellow to brown. Samples were manually detached from rotten stumps and logs found inside the campus of the College of Forestry and Natural Resources, University of the Philippines Los Baños. The collection was done between the months of May to June, 2016, which marks the start of the rainy season in this climatic zone. The matured *Phellinus gilvus* fruiting bodies were collected and identified by one of the authors (MEGDC) based on available published literature and pictorial guides.

General Isolation Procedure

Initial chromatographic steps were performed using a glass column 12 inches in height and 0.5 inch internal; Five 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 TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns; One milliliter fractions were collected.

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

The freeze-dried fruiting bodies of *P. gilvus* (281 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 (1.71 g) which was chromatographed using increasing proportions of EtOAc in petroleum ether in 5% increments. The 5% EtOAc in petroleum ether fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to yield **2** (4 mg). The 15% EtOAc in petroleum ether fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield**1**(2 mg) after trituration with petroleum ether.

Ergosterol peroxide (**1**): ^{13}C-NMR (100 MHz, $CDC1_3$): δ34.68 (C-1), 30.12 (C-2), 66.45 (C-3), 36.89, 36.90 (C-4, C-10), 82.12 (C-5), 135.38 (C-6), 130.71(C-7),79.39 (C-8), 51.65 (C-9), 20.84 (C-11), 39.31 (C-12), 44.53 (C-13), 51.06 (C-14), 23.37 (C-1 5), 28.62 (C-16), 56.17 (C-17),12.84 (C-18), 18.14 (C-19), 39.70 (C-20), 19.61 (C-21), 135.18 (C-22), 132.27 (C-23), 42.75 (C-24), 33.04 (C-25), 19.92 (C-26), 20.60 (C-27), 17.53 (C-28).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the fruiting bodies of *P. gilvus* led to the isolation of compounds **1** and **2**. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its ^{13}C NMR data with literature data [8].The NMR spectra of **2** are in accordance with data reported in the literature for triacylglycerols [9].

A number of studies have been conducted on the biological activities of ergosterol peroxide (**1**). Compound **1** isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm. f. sp. Florida showed strongtry panocidal activity on the intracellular form of *T. cruzi* with an $IC_{50}$of 6.74 μg/mL [10]. Sterol **1**from an edible mushroom suppresses inflammatory response in RAW 264.7 macrophages and growth of HT29 colonadenocarcinoma cells [11]. In addition, **1**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 [12].The IC_{50} value of **1**based on the cell viability of Hep3B was 16.7 μg/mL [13]. It exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU- 145) human prostate cancer cells at μM concentrations [14]and suppressed cell growth and STAT1 mediated inflammatory responses in HT29 cells [15].It inhibited the growth and induced apoptosis ofHL60 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 [16]. It displayed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295 [17] and induced death ofmiR-378 cell [18]. It exhibited significant inhibitory activities against leishmaniasis, tuberculosis, *Mycobacteriumtuberculosis* H37Rv and *M. avium* [19], and inhibited the hemolytic activity of human serum againsterythrocytes [20]. Sterol **1**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 [21]. It possessed marked activity against PGE2 release with an IC_{50} value of 28.7 μM. The mechanism in transcriptional level of **1**was found to down-regulate mRNA expressions of iNOS and COX-2 in dose-dependent manners [22].Furthermore, **1**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 [23].

Triacylglycerols (**2**) have been reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma [24]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*[25]. Another study reported that **2** showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [26].

Acknowledgement
A research grant from the Commission on Higher Education–Philippine Higher Education Research Network (CHED–PHERNet) of the Philippines is gratefully acknowledged.

REFERENCES

[1] A. K. Gautam, A study on mango (*Mangifera indica* L.) trunk decay by *Phellinus gilvus* (Schw.) Pat. from Himachal Pradesh, India. *Int. Lett. Nat. Sci.*,**2014**, 11:9-15.
[2] H.-K., Liu, T.-H. Tsai, T.-T. Chang, C.-J. Chou, L.-C. Lin,*Phytochem*istry,**2009**, 70, 558–563.
[3] T. Hosoe, T. Iizuka, Y. Chiba,T. Itabashi, H. Morita, T. Ishizaki, K- I Kawai, *J. Nat. Med.*,**2006**,60(2), 130–134.
[4] J. S. Bae, K. H. Jang, H. Yim, H. K. Jin,*Cancer Lett*.,**2005**,218(1), 43-52.
[5] J. S.Bae, K. H. Jang, H. K. Jin. *J. Vet. Sci*.,**2005**, 6(2), 161-164.
[6] J. S. Bae, K. H. Jang, H. Yim, S. C. Park, H. K. Jin. *World J. Gastroenterol*. **2005**; 11(4):577-579.
[7] B. S. Jang, J. C. Kim,J. S. Bae, M. H. Rhee, K. H. Jang, J. C. Song, O. D. Kwon, S. C. Park, *Biotechnol. Lett.*,**2004**, 26(1), 31-33.
[8] C. Y. Ragasa, V. D. Ebajo Jr., R. G. Reyes, R. Brkljača, S. Urban, *Der Pharma Chemica*,**2015**, 7(10), 331–336.
[9] N. G. U. Resurreccion, C.-C. Shen, C. Y. Ragasa, *Der Pharmacia Lettre*,**2016**, 8(4), 117–120.
[10] A. Ramos-Ligonio, A. López-Monteon, A. Trigos, *Phytother. Res.*,**2012**, 26(6), 938–43.
[11] M. Kobori, M. Yoshida, M. Ohnishi-Kameyama, H. Shinmoto, *Brit. J. Pharmacol.*,**2007**, 150(2), 209–219.
[12] Y. H. Rhee, S. J. Jeong, H. J. Lee, W. Koh, *BMC Cancer*,**2012**, 12, 28.
[13] T. Takei, M. Yoshida, M. Ohnishi-Kameyama, M. Kobori, *Biosci. Biotechnol. Biochem.***2005**, 69, 212-215.
[14] Y. K. Chen, Y. H. Kuo, Y. Chiang, J. M. Lo, L. Y. Sheen, *J. Agric. Food Chem.*,**2009**, 57, 5713-5719.
[15] A. Russo, V. Cardile, M. Piovano, S. Caggia, C. L. Espinoza, *Chem. Biol. Interact.*,**2010**, 184, 352-358.
[16] F. Leon, L. Brouard, F. Torres, J. Quintana, A. Rivera, *Chem. Biodivers.*,**2008**, 5, 120-125.
[17] X. Liu, C. Y. Wang, C. L. Shao, X. Y. Wei, B. G. Wang, *Biochem.Syst. Ecol.*,**2009**, 37, 127-129.

[18] Q. P. Wu, Y. Z. Xie, Z. Deng, X. M. Li, W. Yang, *PLoS One*,**2012**, 7, e44579.
[19] E. Correa, D. Cardona, W. Quiaones, F. Torres, A. E. Franco, *Phytother. Res*.,**2006**, 20, 497-499.
[20] H. W. Seo, T. M. Hung, M. Na, H. J. Jung, J. C. Kim. Arch. *Pharm. Res*.,**2009**, 32, 1573-1579.
[21] S. J. Wu, T. M. Lu, M. N. Lai, L. T. Ng, *Am. J. Chin. Med*.,**2013**, 41, 131-144.
[22] S. Tewtrakul, P. Tansakul, C. Daengrot, C. Ponglimanont, C. Karalai, *Phytomed*.,**2010**, 17, 851- 855.
[23] J. K. Rugutt, K. J. Rugutt, *Nat. Prod Res*.,**2012**, 26, 1004-1011.
[24] Y. Maeda, M. Sumiyoshi, Y. J. Kimura,*Tradit. Med*.,**2004**, 21(5), 215-220.
[25] Ragasa CY, Lorena GS, Mandia EH, Raga DD, Shen C-C. *Amer. J. Essent. Oils Nat. Prod.*,**2013**, 1(2), 7–10.
[26] M. G. Ferruzzi, J. Blakeslee,*Nutr. Res*.,**2007**, 27, 1–12.