Pharmacogn J. 2018; 10(6): 1079-1081
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Sterols from *Lentinus tigrinus*

Consolacion Y. Ragasa[1,2*], Maria Carmen S. Tan[1], Ma. Ellenita De Castro[3], Mariquit M. De Los Reyes[3,4], Glenn G. Oyong[5], Chien-Chang Shen[6]

ABSTRACT

Aim: To investigate the chemical constituents of the dichloromethane extract of the fruiting bodies of the mushroom *Lentinus tigrinus*. **Materials and Methods:** The chemical constituents of *L. tigrinus* were isolated by silica gel chromatography, while the chemical structures of the isolated compounds were identified by NMR spectroscopy. **Results:** The dichloromethane extract of the fruiting bodies of *L. tigrinus* afforded cerevisterol (**1**), and a mixture of stellasterol (**2**) and ergosterol (**3**) in about 4:5 ratio. **Conclusion:** To the best of our knowledge, this is the first report on the isolation of **1**-**3** from the fruiting bodies of *L. tigrinus*.

Key words: *Lentinus tigrinus*, Polyporaceae, Cerevisterol, Stellasterol, Ergosterol.

Consolacion Y. Ragasa[1,2*], Maria Carmen S. Tan[1], Ma. Ellenita De Castro[3], Mariquit M. De Los Reyes[3,4], Glenn G. Oyong[5], Chien-Chang Shen[6]

[1]Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, PHILIPPINES.
[2]Chemistry Department, De La Salle University Science and 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]Biology Department, De La Salle University Science and Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, PHILIPPINES.
[5]Molecular Science Unit Laboratory, Center for Natural Science and Environmental Research, De La Salle University, 2401 Taft Avenue, Manila 1004, PHILIPPINES.
[6]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei 112, TAIWAN.

Correspondence

Prof. Consolacion Y Ragasa

Chemistry Department, De La Salle University Science and Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, PHILIPPINES.

Phone no : +632-5360230

E-mail: consolacion.ragasa@dlsu.edu.ph

History

- Submission Date: 30-03-2018;
- Review completed: 28-06-2018;
- Accepted Date: 18-07-2018

DOI : 10.5530/pj.2018.6.182

Article Available online
http://www.phcogj.com/v10/i6

INTRODUCTION

Lentinus tigrinus, also known as tiger sawgill or *Kabuteng tigre* is an edible saprobic mushroom of the family Polyporaceae which grows on fallen logs in the forest.[1-2] It is considered one of the most common rotting basidiomycetes yet very pleasing to the eye because of its color and appearance.[3] A study reported that the pileus of *L. tigrinus* contained higher amounts of protein (25.9%), fat (2.1%), ash (7.4%) and energetic value (142.1 kcal/100 g) than the stipe, while the stipe contained higher amounts of total carbohydrates (67.7%) than the pileus. The lyophilized hot water extract of the fruiting body of *L. tigrinus* (100 and 250 mg/kg) significantly lowered the glucose level of diabetic mice by 26.9%. The ethanolic extract of the fruiting body and secondary mycelia of *L. tigrinus* exhibited high antibacterial activities against *Staphylococcus aureus*.[1] Another study reported that a 59 kDa laccase which was isolated from the broth of mycelial culture of the mushroom *L. tigrinus* exhibited an inhibitory activity against HIV-1 reverse transcriptase (IC_{50} = 2.4 μM).[4] Furthermore, *L. tigrinus* acetonitrile extract exhibited a 39.2% radical scavenging activity with an EC_{50} value of 637.75 mg/L, contained 451 mg ascorbic acid equivalent/g sample total phenolics, and showed antibacterial activity against *Staphylococcus aureus* with a 9.48 mm diameter zone of inhibition.[5] In addition, *L. tigrinus* also exhibited hyper accumulation potentials with the ability to concentrate varying amounts of lead, cadmium and chromium (9.21, 1.52 and 4.29 ppm, respectively).[3,6] In another study, *L. tigrinus n*-hexane extract was reported to contain flavonoid (3.67 μg/mg) and exhibited antibacterial activity.[7]

We report herein the isolation of cerevisterol (**1**), stellasterol (**2**) and ergosterol (**3**) from the dichloromethane extract of the fruiting bodies of *L. tigrinus* (Figure 1). To the best of our knowledge, this is the first report on the isolation of **1**-**3** from the fruiting bodies of *L. tigrinus*.

MATERIALS AND METHODS

General Experimental Procedure

The NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. 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}. The plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

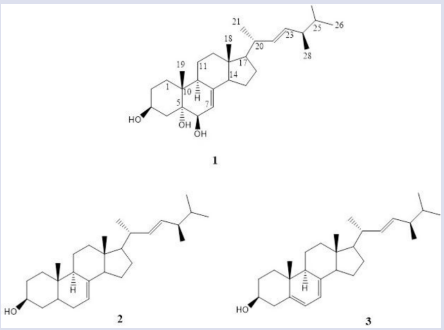


Figure 1: Chemical structures of cerevisterol **(1)**, stellasterol **(2)** and ergosterol **(3)** from the dichloromethane extract of the fruiting bodies of *L. tigrinus*.

Cite this article: Ragasa CY, Tan MCS, Castro MED, Mariquit M. Reyes DL, Oyong GG, Shen C. Sterols from *Lentinus tigrinus*. Pharmacog J. 2018;10(6):1079-81.

Sample Collection

Fruiting bodies of *L. tigrinus* was initially detached from the rotten stumps and logs found within the vicinity of Mt. Makiling, Laguna, Philippines from June to July 2017. The collected specimens were cleaned, placed in a paper bag and was brought to the laboratory for analysis. Authentication of the identity of the collected *L. tigrinus* was done by one of the authors (MEDC) using available published literature and pictorial guides.

Isolation of the Sterols from L. tigrinus

The fruiting bodies of *Lentinus tigrinus* (140 g) were freeze-dried (30.14 g), then ground in a blender, soaked in CH_2Cl_2 for three days, and filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.426 g) which was chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether to afford a mixture of **2** and **3** (77.6 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to provide **1** (3.9 mg) after washing with petroleum ether.

Cerevisterol (**1**): ^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.33 (d, *J* = 5.4 Hz, H-7), 5.20 (dd, *J* = 7.2, 15 Hz, H-23), 5.16 (dd, *J* = 7.2, 15 Hz, H-22), 4.06 (m, H-3), 3.60 (m, H-6), 1.07 (s, H-19), 1.00 (d, *J* = 6.6 Hz, H-21), 0.90 (d, *J* = 7.2 Hz, H-28), 0.82 (d, *J* = 6.6 Hz, H-27), 0.80 (d, *J* = 6.6 Hz, H-26), 0.58 (s, H-18); ^{13}C-NMR (150 MHz, $CDC1_3$): δ 32.96 (C-1), 30.857 (C-2), 67.73 (C-3), 39.22 (C-4), 75.97 (C-5), 73.66 (C-6), 117.53 (C-7), 144.00 (C-8), 43.47 (C-9), 37.14 (C-10), 22.04 (C-11), 39.22 (C-12), 43.76 (C-13), 54.75 (C-14), 22.88 (C-15), 27.90 (C-16), 55.99 (C-17), 12.33 (C-18), 18.82 (C-19), 40.39 (C-28), 19.64 (C-21), 135.36 (C-22), 132.18 (C-23), 42.81 (C-24), 33.07 (C-25), 19.94 (C-26), 21.11 (C-27), 17.58 (C-28).

Stellasterol (**2**): ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.52 (s, H-18), 0.80 (d, *J* = 6.6 Hz, H-26), 0.82 (d, *J* = 7.2 Hz, H-27), 0.77 (s, H-19), 0.90 (d, *J* = 6.6 Hz, H-28), 0.99 (d, *J* = 6.6 Hz, H-21), 3.58 (m, H-3), 5.13 (br t, *J* = 2.4 Hz, H-7), 5.20 (dd, *J* = 7.8, 15.0 Hz, H-23), 5.17 (dd, *J* = 7.8, 15.0 Hz, H-22); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 37.13 (C-1), 31.46 (C-2), 71.06 (C-3), 37.97 (C-4), 40.46 (C-5), 29.63 (C-6), 117.45 (C-7), 139.56 (C-8), 49.45 (C-9), 34.21 (C-10), 21.53 (C-11), 39.44 (C-12), 43.30 (C-13), 55.10 (C-14), 22.92 (C-15), 28.09 (C-16), 55.96 (C-17), 12.08 (C-18), 13.02 (C-19), 40.26 (C-20), 21.10 (C-21), 135.66 (C-22), 131.88 (C-23), 42.81 (C-24), 33.08 (C-25), 19.63 (C-26), 19.94 (C-27), 17.59 (C-28).

Ergosterol (**3**): ^{1}H-NMR (600 MHz, $CDC1_3$): δ 5.55 (dd, *J* = 2.4, 5.4, Hz, H-6), 5.36 (dd, *J* = 2.4, 5.4 Hz, H-7), 5.20 (dd, *J* = 7.8, 15.0 Hz, H-23), 5.17 (dd, *J* = 7.8, 15.0 Hz, H-22), 3.61 (m, H-3), 1.01 (d, *J* = 6.6 Hz, H-21), 0.92 (s, H-19), 0.90 (d, *J* = 6.6 Hz, H-28), 0.82 (d, *J* = 7.2 Hz, H-26), 0.80 (d, *J* = 6.6 Hz, H-27), 0.61 (s, H-18). ^{13}C-NMR (150 MHz, $CDC1_3$): δ 38.36 (C-1), 31.98 (C-2), 70.46 (C-3), 40.78 (C-4), 139.77 (C-5), 119.58 (C-6), 116.28 (C-7), 141.35 (C-8), 46.25 (C-9), 37.02 (C-10), 21.10, 21.09 (C-11, C-21), 39.08 (C-12), 42.82, 42.81 (C-13, C-24), 54.55 (C-14), 22.99 (C-15), 28.27 (C-16), 55.73 (C-17), 12.04 (C-18), 16.27 (C-19), 40.40 (C-20), 135.56 (C-22), 131.97 (C-23), 33.08 (C-25), 19.63 (C-26), 19.94 (C-27), 17.59 (C-28).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the fruiting bodies of *Lentinus tigrinus* yielded cerevisterol (**1**), stellasterol (**2**) and ergosterol (**3**). The NMR data of **1** are in accordance with the data reported in the literature for cerevisterol;[8-9] **2** for stellasterol;[10] and **3** for ergosterol.[8]

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities. Cerevisterol (**1**) and ergosta4,6,8(14),22-tetraen-3-one which were isolated from *P. tuber-regium* were tested for anti-inflammatory effects on RAW 264.7 macrophages. Both sterols inhibited the production of NO, TNF-α, and PGE2 in 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, and SOCS3 mRNAs.[11] Stellasterol (**2**) showed antibiotic activity against Gram positive bacteria.[12] Another study reported that cell cycle arrest against the human cancer cell lines, MCF-7 and SH-SY5Y was exhibited by stellasterol.[13] Furthermore, **2** showed anti-inflammatory activity against iNOS, CHOP and IKB-α expression.[14] A study reported that ergosterol **(3)** provided significant protection against the promotion of bladder tumor induced by many types of promoters in the environment.[15] Moreover, the ergosterol content of brown and white button mushrooms correlated with their antioxidant activities.[16] In another study, **1** was reported to have the capability to inhibit lipid peroxidation.[17]

CONCLUSION

The dichloromethane extract of the fruiting bodies of *L. tigrinus* afforded cerevisterol (**1**), and a mixture of stellasterol (**2**) and ergosterol (**3**). To the best of our knowledge, this is the first report on the isolation of **1**-**3** from the fruiting bodies of *L. tigrinus.* Earlier studies on *L. tigrinus* extracts showed antibacterial properties. This may be partly attributed to stellasterol (**2**) which was reported to exhibit antibacterial properties. Furthermore, an earlier study on *L. tigrinus* extract indicated antioxidant properties. Ergosterol (**3**) which was reported to exhibit antioxidant properties may contribute to this bioactivity. Additionally, **1** and **2** were reported to possess anti-inflammatory activity, while **2** and **3** showed anticancer properties.

ACKNOWLEDGEMENT

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

CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATIONS

CH_2Cl_2: Dichloromethane; **EtOAc:** Ethyl acetate; **CH_3CN:** Acetonitrile; **Et_2O:** Diethyl ether.

SUMMARY

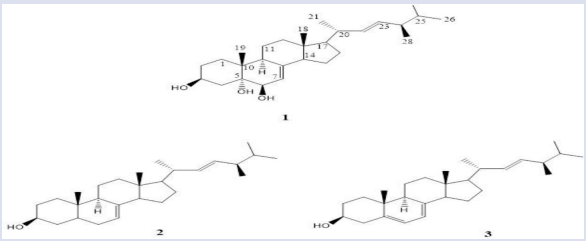
Chemical investigation of the dichloromethane extract of the fruiting bodies of the mushroom *Lentinus tigrinus* afforded cerevisterol (**1**), and a mixture of stellasterol (**2**) and ergosterol (**3**) in about 4:5 ratio. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data with literature data. The structures of **2** and **3** were identified by comparison of their NMR data with those reported in the literature.

REFERENCES

1. *Lentinus tigrinus* (MushroomExpert.Com). Downloaded from www.mushroomexpert.com › Major Groups › Gilled Mushrooms › Pale-Sporedon March. 2018.
2. Dulay RM, Arenas MC, Kalaw SP, Reyes RG, Cabrera EC. Proximate composition and functionality of the culinary-medicinal tiger sawgill mushroom, *Lentinus tigrinus* (higher Basidiomycetes), from the Philippines. Int J Med Mushrooms. 2014;16(1):85-94.

3. Dulay RM, Castro MEGD. Effect of Lead (Pb) mycelia and sporocarp of *Lentinus tigrinus* mushroom. Int J of Biology, Pharmacy and Allied Sciences. 2016;5(6):1323-31.
4. Xu LJ, Wang HX, Ng TB. A laccase with HIV-1 reverse transcriptase inhibitory activity from the broth of mycelial culture of the mushroom *Lentinus tigrinus*. J Biomed Biotechnol. 2012;7. Article ID 536725, doi:10.1155/2012/536725.
5. Dulay RM, Miranda LA, Malasaga JS, Kalaw SP, Reyes RG, Hou CT. Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*. Biocatal Agric Biotechnol. 2017;9:141-4.
6. Dulay RM, Castro MED. Cadmium and chromium tolerance and mycoremediation ability of tiger sawgill mushroom, *Lentinus tigrinus*. Int J of Biology, Pharmacy and Allied Sciences. 2016;5(11):3003-12.
7. Sadi G, Emsen B, Kaya A, Kocabas A, Cinar S, Kartal DI. Cytotoxicity of some edible mushroom extracts over liver hepatocellular carcinoma cells in conjunction with their antioxidant and antibacterial properties. Phcog Mag. 2015;11(Suppl 1):6-18.
8. Ragasa CY, Ebajo VD, Reyes RG, Brkljaca R, Urban S. Sterols and lipids from *Pleurotus florida*. Der Pharma Chemica. 2015;7(10):331-6.
9. Kawagishi H, Katsumi R, Sazawa T, Mizuno T, Hagiwara T, Nakamura T. Cytotoxic steroids from the mushroom *Agaricus blazei*. Phytochemistry. 1988;21(9):2111-9.
10. Borlagdan MS, Castro MEGD, Altena IAV, Ragasa CY. Sterols from *Trametes versicolor*. Res J Pharm Biol Chem Sci. 2017;8(2):740-4.
11. Liu YW, Mei HC, Su YW, Fan HT, Chen CC, Tsai YC. Inhibitory effects of *Pleurotus tuber-regium* mycelia and bioactive constituents on LPS-treated RAW 264.7 cells. J Funct Foods. 2014;7:662-70.
12. Vazirian DM, Faramarzi MA, Ebrahimi SE, Esfahani HR, Samadi N, Hosseini SA, *et al.* Antimicrobial Effect of the Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) and Its Main Compounds. Int J Med Mushrooms. 2014;16(1):77-84.
13. Pereira DM, Correia-da-Silva G, Valentão P, Teixeira N, Andrade PB. Palmitic acid and ergosta-7,22-dien-3-ol contribute to the apoptotic effect and cell cycle arrest of an extract from *Marthasterias glacialis* L. In neuroblastoma cells. Mar Drugs. 2013;12(1):54-68.
14. Pereira DM, Correia-da-Silva G, Valentão P, Teixeira N, Andrade PB. Anti-inflammatory effect of unsaturated fatty acids and ergosta-7,22-dien-3-ol from *Marthasterias glacialis*: Prevention of CHOP-mediated ER-stress and NF-κB activation. PLoS ONE. 2014;9(2):e88341.
15. 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(11):1298-302.
16. Shao SQ, Hernandez M, Kramer JKG, Rinker DL, Tsao R. Ergosterol profiles, fatty acid composition, and antioxidant activities of button mushrooms as affected by tissue part and developmental stage. J Agric Food Chem. 2010;58(22):11616-25.
17. Dissanayake DP, Abeytunga DTU, Vasudewa NS, Ratnasooriya WD. Inhibition of lipid peroxidation by extracts of *Pleurotus ostreatus*. Phcog Mag. 2009; 5(19):266-71

GRAPHICAL ABSTRACT



SUMMARY

Chemical investigation of the dichloromethane extract of the fruiting bodies of the mushroom *Lentinus tigrinus* afforded cerevisterol (1), and a mixture of stellasterol (2) and ergosterol (3) in about 4:5 ratio. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data with literature data. The structures of 2 and 3 were identified by comparison of their NMR data with those reported in the literature.

ABOUT AUTHORS

Consolacion Y. Ragasa: Professor of the Chemistry Department and a University Fellow of De La Salle University - Manila, Philippines.

Maria Carmen S. Tan: Academic Service Faculty of De La Salle University - Manila, Philippines.

Ma. Ellenita De Castro: Assistant Professorial Lecturer of the Biology Department University – Manila - Philippines.

Mariquit M. De Los Reyes: Associate Professor of the Biology Department of De La Salle University – Manila Philippines.

Glenn G. Oyong: Cell and Molecular Biologist and Academic Service Faculty, De La Salle University, Manila, Philippines

Chien-Chang Shen: Associate Research Fellow in Division of Chinese Medicinal Chemistry, National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taiwan, ROC.

Cite this article: Ragasa CY, Tan MCS, Castro MED, Mariquit M. Reyes DL, Oyong GG, Shen C. Sterols from *Lentinus tigrinus*. Pharmacog J. 2018;10(6):1079-81.