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Chemical constituents of *Pleurotus djamor*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Pleurotus djamor led to the isolation of ergosterol (**1**),triacylglycerols (**2**), and fatty acid methyl esters (**3**). The structures of **1**-**3** were identified by comparison of their NMR data with literature data.*

Keywords: *Pleurotus djamor*, Pleurotaceae, ergosterol, triacylglycerols, fatty acid methyl esters

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INTRODUCTION

The genus *Pleurotus* is composed of a group of edible mushrooms with medicinal properties. Due to the importance of these edible *Pleurotus* species in the food industry, their cultivation has recently expanded. Mushrooms are rich in protein, fiber, carbohydrates, vitamins and minerals. *Pleurotus* species also exhibit medicinal properties, such as hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulating activities [1].

This study was conducted on the dichloromethane soluble chemical constituents of the pink oyster mushroom, *Pleurotus djamor*.An earlier study compared thechemical composition and amino acid profile of *P.djamor*and *P. ostreatus*. No differences were found in crude protein and ash contents of *P. djamor* and *P. ostreatus*. *P. ostreatus* has a higher proportion of valine, isoleucine, phenylalanine, aspartate and alanine, while *P. djamor* had higher proportion of proline and glutamate, but no difference was found in the remaining amino acids. Both species had a high proportion of aspartate and glutamate, while *P. ostreatus* had a lower fiber and fat content [2]. Qualitative analysis of phytochemicals of *P. djamor* extract revealed the presence of anthroquinones, flavonoids, saponins, tannins, and terpenoids; and the absence of cardiac glycosides and steroids. The total phenolic content in the *P. djamor* extract was found to be 32.55 ± 0.21 mg/g gallic acid equivalent of phenols and the flavonoid content was 1.53 ± 0.11 mg/g quercetin equivalent of flavonoids. Antioxidant activity was determined by DPPH method which indicated that 100 µg/ml of mushroom extract and ascorbic acid exhibited 76.4% and 99.3% inhibition with IC50 values of 64.72 and 29.42 µg/ml for mushroom and ascorbic acid, respectively [3]. Another study reported that *P. djamor* contained 28 mg/gm Fr. wt^{-1} protein, 3.4 mg/gm Fr. wt^{-1} glucose, and 9.2 mg/gm Fr. wt^{-1} amino acids. The acetone extract of *P. djamor* at 100 and 150 µg exhibited 12 mm and 11 mm zones of inhibition against *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. On the other hand, the dimethyl sulfoxide extract at

100 and 150 µg exhibited 7 mm and 10 mm zones of inhibition, respectively against *P.aeruginosa* and 5 mm and 8 mm zones of inhibition, respectively against *E. coli* [4].*P. djamor* protein extracts exhibited cytotoxic activities against human hepatoma HepG2 cell, human breast cancer MCF-7 cells, and human lung adenocarcinoma A-549 cell with IC_{50} values of 10, 10, and 14 µg/mL, respectively [5] Another study reported the antioxidant activity of the ethanol extract of *P. djamor* which exhibited IC_{50} values of 115.5 µg/mL and 29,37 µg/mL by DPPH and ABTS methods. Furthermore, 13 sterols from the ethanol extract were identified by GC-MS [6].

This study is part of our research on the chemical constituents of edible mushrooms commercially cultivated in the Philippines. Recently, we reported the isolation of ergosterol, ergosterol peroxide, cerevisterol, palmitic acid, stearic acid, linoleic acid, oleic acid, and dilinoleoyl oleoyl glycerol from *Pleurotus florida*, an edible oyster mushroom cultured at the Central Luzon State University [7]. We report herein the isolation of ergosterol (**1**),triacylglycerols (**2**), and fatty acid methyl esters (**3**) from *Pleurotus djamor*cultured at the Central Luzon State University. The structures of **1**-**3** are presented in Fig. 1.

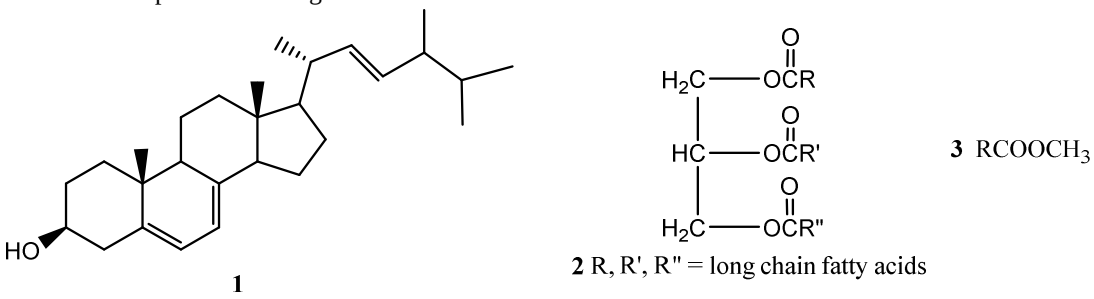

Fig. 1. Chemical structures of ergosterol (1),triacylglycerols (2), and fatty acid methyl esters (3) from *Pleurotus djamor*

MATERIALS AND METHODS

General Experimental Procedure

^1H (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.

Cultivation of Sample

The samples for analysis were prepared as follows:unmilled rice seeds fully permeated with the mycelia of P. djamor were aseptically inoculated into previously pasteurized mixture of 7 parts composted rice straw and 3 parts of sawdust (v/v) contained in heat resistant polypropylene plastic bags. To stimulate the complete colonization of the mycelia into the formulated substrates, the bags were incubated at 28-30oC for 30 days. The fully colonized bags were transferred to the fruiting room and both ends of the bag were opened to allow the emergence of fruiting bodies. Fruiting bodies were harvested 3-5 days after opening the bags.

General Isolation Procedure

A glass column 12 inches in height and 0.5 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. Ten 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. djamor

The freeze-dried fruiting bodies of*P. djamor*(41.52 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 (0.9105 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using 2.5% EtOAc in petroleum ether to afford **2** (8 mg). The 10% acetone in CH_2Cl_2 fraction

was rechromatographed using 2.5% EtOAc in petroleum ether to yield **3**(5 mg). The 20% and 30% acetone in CH_2Cl_2 fractionswere combined and washed with petroleum ether to yield **1** (12 mg).

Ergosterol (**1**): ^{1}H-NMR (500 MHz, CDC1$_3$): 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 Hz, H-23), 5.16 (dd, *J* = 7.5, 15.5 Hz, H-22), 3.63 (m, H-3), 1.03 (d, *J* = 6.5 Hz, H-21), 0.94 (s, H-19), 0.91 (d, *J* = 7.0 Hz, H-28), 0.83 (d, *J* = 7 Hz, H-26), 0.82 (d, *J* = 7.5 Hz, H-27), 0.62 (s, H-18).

Triacylglycerols (**2**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.27 (dd, *J* =4.5, 12 Hz, glyceryl CH_2O), 4.12 (dd, *J* =5.5, 11.5 Hz, glyceryl CH_2O), 5.25 (m, glyceryl CHO), 2.29 (t, *J* = 7.2 Hz, α-CH_2), 5.35 (m, olefinic H), 2.75 (t, *J* = 6.6 Hz, double allylic CH_2), 2.77 (t, *J* = 6.5 Hz, double allylic CH_2), 1.99-2.07 (allylic, CH_2), 1.62 (β-CH_2), 1.25-1.37 (CH_2), 0.88 (t,*J* =6.5 Hz, CH_3), 0.89 (t, *J* =6.5 Hz, 2 ×CH_3).

Fatty acid methyl esters (**3**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.5 Hz), 1.25-1.38 (m), 1.56-1.68 (m), 2.01-2.07 (m), 2.30 (t, *J* =7.5 Hz), 2.77 (t, *J* = 6 Hz), 3.66 (s, CH_3), 5.30-5.41(m).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *P. djamor* yielded ergosterol (**1**) [7, 8],triacylglycerols (**2**) [9], and fatty acid methyl esters (**3**) [10]. The structures of **1**-**3** were identified by comparison of their NMR data with literature data.

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 [11]. Moreover, the ergosterol content of brown and white button mushrooms correlated with their antioxidant activities [12].In another study, ergosterol was reported to have the capability to inhibit lipid peroxidation [13].

Triacylglycerols (**2**) from Tuna (1000 mg/kg) was reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma [14]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes* [15]. Another study reported that triacylglycerols showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [16].

Fatty acid methyl esters (**3**)were reported to exhibit antibacterial and antifungal properties [17]. An invention containing *Annona squamosa*fatty acid effective components or fatty acid methyl ester effective components with antitumor activity has been provided [18]. Another study reported that the antioxidant activity of *Mentha spicata*may be due to the presence of flavonoids and fatty acid methyl esters which have the scavenging potential by reducing the free radicals [19].

CONCLUSION

It is interesting to note that the compounds (**1**-**3**)isolated from *P. djamor* have reported antitumor and antioxidant properties. Thus, the reported antioxidant and anticancer properties of this mushroom may be due in part to these compounds. Furthermore, **2** and **3** were reported to exhibit antimicrobial properties. These compounds may be partially responsible for the reported antimicrobial properties of *P. djamor*.

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REFERENCES

[1] R. Cohen, L. Persky, Y. Hadar,*Appl. Microbiol. Biotechnol.***2002**, 58, 582-594.
[2] C. M. C. Leal, L. A. Mendez, C. A. S. Castro, G. C. Zapata, *Acta Alimentaria*, **2010**, 39(3), 249-255.
[3] R. Sasidhara, T. Thirunalasundari, *J. Chem. Pharm. Res.***2014**,6(4), 950-953.

[4] K.Dharmaraj, T. Kuberan, R. Mahalakshmi,*Int.J.Curr.Microbiol.App.Sci*.,**2014**, 3(6), 518-526.
[5]S. Zhao, Y. Liu, S. Wang, L. Wang, F. Xu, X. Geng, *Faming Zhuanli Shenqing*. **2013**, CN 103272215 A 20130904.
[6] M. Guzman, N. Zuniga, G. G. Santafe, O. Torres, A. Angulo, *Biotecnologia en el Sector Agropecuario y Agroindustrial*, **2009**, 7(2), 63-69.
[7] C. Y. Ragasa, V. D. Ebajo Jr., R. G. Reyes, R. Brkljača, S. Urban, *Der Pharma Chemica*.**2015**,7(10), 331-336.
[8] R. Tao, C.-Z. Wang, Z.-W. Kong, *Molecules*,**2013**, 18, 2166–2182.
[9] C. Y. Ragasa CY, J. Caro, C.-C. Shen C.-C., *DerPharma Chemica*, **2015**, 7(2), 178-182.
[10] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Int, J. Pharm. Clin. Res*., **2015**, 7(5), 356-359.
[11] Y. Yazawa, M. Yokota, K. Sugiyama,Biol. Pharm. Bull.,**2000**, 23(11), 1298–302.
[12]S.-Q. Shao, M. Hernandez, J. K. G. Kramer, D. L. Rinker, R. Tsao, Rong. *J. Agric. Food Chem.*, **2010**, 58(22), 11616-11625**.**
[13] D. P. Dissanayake, D. T. U. Abeytunga, N. S. Vasudewa, W. D. Ratnasooriya, *Phcog. Mag*., **2009**, 5(19), 266-271.
[14] Y. Maeda, M. Sumiyoshi, Y. Kimura, *J. Traditional Med.*, **2004**, 21(5), 215-220.
[15] C. Y. Ragasa, G. S. Lorena, E. H. Mandia, D. D. Raga, C.-C. Shen, *Amer. J. Essent. Oils Nat. Prod.*, **2013**, 1(2), 7–10.
[16] M. G. Ferruzzi, J. Blakeslee, *Nutr. Res*., **2007**, 27, 1–12.
[17] G. Agoramoorthy, M. Chandrasekaran, V. Venkatesalu, M.J. Hsu, *Braz. J. Microbiol*.**2007**, 38:739-742.
[18]X. Li, Y. Chen, J. Chen, Y. Wang, Y. Miao, H. Qiu, F. Yuan, *Faming Zhuanli Shenqing*, **2013**, CN 103239491 A 20130814.
[19]J. R. Naidu, R. B. Ismail, C. Yeng, S. Sasidharan, P. Kumar, *J. Phytol*., **2012**, 4(1), 13-18.