Mushroom Science IXX

Science and Cultivation Of Edible Fungi

Edited by J.J.P. Baars & A.S.M. Sonnenberg

PROCEEDINGS OF THE IXXTH INTERNATIONAL CONGRESS ON THE SCIENCE AND CULTIVATION OF EDIBLE AND MEDICINAL FUNGI/AMSTERDAM/THE NETHERLANDS/30 MAY-2 JUNE 2016

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ISBN 978-90-9029771-2

How to cite this publication:

Baars J.J.P. & Sonnenberg A.S.M., ed. 2016. Science and cultivation of edible and medicinal fungi: Mushroom Science IXX. Proceedings of the 19th Congress of the International Society for Mushroom Science, Amsterdam, The Netherlands, 30 May–2 June 2016. International Society for Mushroom Science. Amsterdam

Example of how to cite a paper in this publication:

Semon S. (2016) Development of a Revised UPOV Guideline for New Agaricus Mushroom Varieties. in 'Science and cultivation of edible and medicinal fungi: Mushroom Science IXX', ed. by A.S.M. Sonnenberg & J.J.P. Baars. Proceedings of the 19th Congress of the International Society for Mushroom Science, Amsterdam, the Netherlands, 30 May–2 June 2016. International Society for Mushroom Science. Amsterdam.

Science and Cultivation of Edible Fungi, Baars & Sonnenberg (ed.)

Antiproliferative Activity of Protein Extracts from *Lignosus tigris* (Chon S. Tan) on MCF7 cell line

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ABSTRACT

Mushrooms have received considerable attention for its bioactive compounds with immunomodulatory and anticancer effects. The aim of this study was to investigate the anticancer activity of the fractions and protein components isolated from cold water extract (CWE) of *L. tigris* sclerotium in breast cancer (MCF7) cells. Breast cancer is one of the most common cancers affecting women. Conventional chemotherapy is often limited by the development of drug resistance and various side effects throughout the body. CWE fractionation was carried out on a Sephadex G-50 gel and three fractions of high (HMW), medium (MMW) and low molecular weight (LMW) were collected. High molecular weight (HMW) fraction exhibited greater cytotoxicity compared to other fractions on MCF7 cells. Proteins of the CWE-HMW were isolated by ammonium sulphate precipitation. The CWE-HMW protein was further purified using anion exchange chromatography and protein compounds were identified by LC-MS/MS analysis. Ammonium sulphate precipitated proteins from HMW fraction showed high antiproliferative activity against the MCF7 cells with IC_{50} of 1.17µg/ml. No activity was observed in non-protein fraction of the HMW. Anion exchange chromatographic fractions H1, H2, H3 and H10 showed potent cytotoxicity activity against MCF7 cells with IC_{50} values of 0.79 ± 0.05 µg/mL, 0.72 ± 0.02 µg/mL, 0.78 ± 0.14 µg/mL and 4.17 ± 0.25 µg/mL, respectively and less cytotoxic against breast normal cell lines. LC-MS/MS analysis revealed that lectin was identified as its major component in fraction H10. Mushroom lectins have been shown to possess potential immunomodulatory, antiproliferative and antitumor activities.
Keywords: *L. tigris*, protein, anticancer, MCF7, lectin

INTRODUCTION

Mushrooms have received considerable attention as an edible and medical resource. *Lignosus* spp. also known as the "Tiger Milk mushroom" is a popular medicinal mushroom consumed by

the indigenous people in Southeast Asia and China. The sclerotium is the part of the mushroom with medicinal value and is utilized by the local communities as traditional medicine. In Malaysia, *L. rhinocerotis* has been traditionally utilised by the indigenous communities as general tonic to maintain health and to treat asthma, cough, fever, food poisoning, wounds and breast cancer (Lee et al., 2009).

Studies have reported that the *L. rhinocerotis* sclerotial extracts possess potent antioxidant activity, considerable antiproliferative activity against human breast carcinoma (MCF-7) and human lung carcinoma (A549) cells, immunomodulatory and anti-acute inflammatory properties (Wong et al., 2011; Lee et al., 2012; Yap et al., 2013; Lee et al., 2014). Recently, *L. tigris* (Chon S. Tan), one of the species of the Tiger Milk mushroom has been discovered in the tropical forest located in Pahang, Malaysia (Tan et al., 2013). Recent study has reported that the sclerotium of the *L. tigris* mushroom contains high nutritional value and possesses potent superoxide anion scavenging activity (Kong et al., 2016).

In this study, we aim to investigate the antiproliferative activity of the *L. tigris* sclerotia. Mushroom proteins have been reported to be potential pharmaceutically active components. (Wong et al., 2010). Therefore, in this study, the bioactive proteins that are responsible for anti-proliferative action are isolated and investigated using nano-ESI-LC-MS/MS analysis.

1. Results and Discussion

Cold water extraction of the *L. tigris* sclerotial powder yielded 240 g/kg dry weight of sclerotia with a composition of 40.6% carbohydrate and 7.3% protein. Using the MTT assay, the CWE showed potent antiproliferative activity (IC_{50} = 28.93 ± 7.74 µg/mL) and induced cell death against MCF7 cells in a dose-dependent manner. CWE of the *L. tigris* showed more potent antiproliferative activity against MCF7 cells compared to *L. rhinocerotis* (Lee et al., 2012; Yap et al., 2013).

To further determine the components with antiproliferative properties, CWE was fractionated by Sephadex G-50 gel filtration and three pooled fractions, high (HMW), medium (MMW) and low molecular weight (LMW) were collected. CWE-HMW fraction was the most cytotoxic against MCF7 with IC_{50} values of 4.23 ± 0.08 µg/mL compared to CWE-MMW and CWE-LMW fractions with IC_{50} values of ~35 µg/mL and > 900 µg/mL, respectively (Table 1). Protein in CWE-HMW was precipitated with ammonium sulphate at 100 % saturation. Cytotoxicity of the protein and non-protein parts was evaluated on MCF7 cells. The CWE-HMW protein fraction showed potent cytotoxic activity on MCF7 cells with IC_{50} value of 1.17 ± 0.47 µg/mL, while the non-protein part was not cytotoxic to the tested cell line (IC_{50} = > 1000 µg/mL). HMW proteins were then purified by Resource Q anion exchange chromatography. A total of eleven chromatographic fractions (H1-H11) were collected from the elution with 0.02 M Tris–HCl buffer (pH 8.0) using a linear NaCl gradient. The cytotoxicity activities of the 11 anion exchange chromatographic fractions against MCF7 cells were evaluated using MTT assay. Three eluted fractions, H1, H2 and H3 showed highest cytotoxicity activity against MCF7 cells with IC_{50}

values of 0.79 ± 0.05 μg/mL, 0.72 ± 0.02 μg/mL and 0.78 ± 0.14 μg/mL, respectively, and less cytotoxic against 184B5 cells (breast normal cell line) with selectivity index of 6.14, 3.46 and 2.59, respectively. Fraction H10 showed higher IC_{50} value (4.17 ± 0.25 μg/mL) on MCF7 cells and moderate cytotoxic selectivity to 184B5 cells.

Table 1: Cytotoxic activities (IC_{50}) of *L. tigris* sclerotial extracts and anion exchange chromatographic fractions on breast cancer (MCF7) and normal cell lines (184B5).

Extracts	Cytotoxicity (IC_{50}) (μg/mL)	
	MCF7	184B5
CWE	28.9 ± 7.7	ND
CWE-HMW	4.23 ± 0.08	ND
CWE-MMW	34.75 ± 1.77	ND
CWE-LMW	931 ± 19.8	ND
CWE-HMW Protein	1.17 ± 0.47	ND
CWE-HMW Non-protein	>1000	ND
Anion exchange chromatographic fractions		
H1	0.79 ± 0.05	4.85 ± 0.08
H2	0.72 ± 0.02	2.49 ± 0.40
H3	0.78 ± 0.14	2.02 ± 0.32
H4	4.73 ± 0.22	1.39 ± 0.01
H5	5.66 ± 0.22	6.74 ± 1.15
H6	6.73 ± 0.83	9.56 ± 0.86
H7	4.80 ± 0.24	6.90 ± 0.71
H8	2.21 ± 0.22	2.94 ± 0.09
H9	2.76 ± 0.44	2.48 ± 0.18
H10	4.17 ± 0.25	9.18 ± 0.77
H11	2.44 ± 0.08	2.16 ± 0.25

Abbreviation: CWE, cold water extract; HMW high molecular weight; MMW, medium molecular weight; LMW, low molecular weight; ND, not determined.

Protein composition in the fractions H1, H2, H3 and H10 were further analyzed using LC-MS/MS. LC-MS/MS analysis, using the *L. rhinocerotis* TM02 genome as the matching database. The results revealed that hypothetical protein 876NA and 230NA are the major proteins in fraction H1, H2 and H3. Serine protease was present in the 4 fractions, H1 (11.35%), H2 (9.77%), H3 (7.05%) and H10 (3.80%) and lectin was identified mainly in fraction H10 (44.74%). Yap et al. (2015) has reported that serine proteases isolated from *L. rhinocerotis* sclerotia showed potent antiproliferative activity on MCF7 cells. Lectins from various species of

mushrooms have also been shown to possess antitumor, antiproliferative, and immunomodulatory activities (Xu et al., 2011). This study suggests that serine protease and lectins from the *L. tigris* sclerotia may be the potential bioactive proteins that play an important role in the antiproliferative effect on breast cancer MCF7 cells.

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