A novel glycoprotein from mushroom Hypsizygus marmoreus (Peck) Bigelow with growth inhibitory effect against human leukaemic U937 cells

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ABSTRACT

This study was to isolate the anti-leukaemic component from edible mushroom Hypsizygus marmoreus (Peck) Bigelow. Crude protein was extracted from the basidioma, and then purified with DEAE-Sepharose CL-6B ion exchange chromatography followed by Sephacryl S-300 gel filtration. A protein which exerted high growth inhibitory effect on human leukaemic U937 cells and sufficient toxicological safety on normal human white blood cells was isolated and named HM-3A. Electrophoresis showed HM-3A approximately 52 kDa in size. N-terminal analysis found the amino acid sequence ATTQWKTSAA and confirmed HM-3A a novel protein. High-performance anion-exchange column chromatography revealed HM-3A a glycoprotein with galactose as the major monosaccharide. Haemagglutination assay proved it non-lectin. We suggest that HM-3A is worth further investigation for antitumour use.

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1. Introduction

Leukemia is the most common haematological malignancy. It is characterized by the abnormal increase of immature leukocytes. The current treatments for leukemia include pharmaceutical medication, chemotherapy and radiation therapy to reduce and eliminate the immature leukocytes. However, these treatments often have some adverse effects, including the damage to normal human cells, the reduction in patients' immunity, the increase of risk for infection or bleeding, and the occurrence of mouth sores, nausea, vomiting, hair loss, etc. (Wesa & Cassileth, 2009). It has become a highly hoped task to look for natural bioactive components with good functionality and no serious side effects for leukemia prevention or treatment.

Mushrooms production worldwide have increased more than sixfolds in 30 years, from about 1,258,804 metric tons in 1980 to about 7,397,558 metric tons in 2010 (Food, 2012). Mushrooms have become an important source of antitumor bioactive components (Ferreira, Vaz, Vasconcelos, & Martins, 2010; Wasser, 2010). The principal cultivated edible mushrooms belong to the order Agaricales (Rühl, Fischer, & Kües, 2008). Some proteins from the dietary Agaricales mushrooms Agrocybe aegerita, Agaricus blazei and Pleurotus citrinopileatus have been proven to be effective in inhibiting the growth of leukaemic cells (Chen, Ma, Tsai, Wang, & Wu, 2010; Kim, Jiang, Leung, Fung, & Lau, 2009; Wang, Huang, & Chang, 2004).

Another Agaricales species, Hypsizygus marmoreus (HM), is a popular dietary mushroom in East Asian regions, including China, Japan and Taiwan. The annual production of HM in Japan reached 83,832 metric tons in 2000 (Nakamura, 2006). HM has been reported to contain anti-leukaemic components, including an 18 kDa ribonuclease that inhibits mouse leukemia L1210 cells (Guan, Wang, & Ng, 2007), a 20 kDa ribosome-inactivating protein that inhibits human leukemia HL-60 and L1210 cells (Lam & Ng, 2001), and a polyterpene that inhibits HL-60 cells (Mizumoto et al., 2008). These reports showed significant growth inhibitory effects of the bioactive components on leukaemic cells but presented no data regarding to the safety of these components on normal cells.

The present study was aimed to look for an anti-leukaemia bioactive component with toxicological safety on normal human white blood cells from the fresh basidioma of mushroom HM and to elucidate the basic structural characteristics of this novel bioactive component.

2. Materials and methods

2.1. Collection of crude protein

The fresh fruiting bodies (200 g) of the mushroom HM were purchased from a contracted supplier in Taiwan. They were...
blended with 10% NaCl solution at 4 °C, 500 × g for 1 min by using a Waring blender (Cycle blender, Osterizer, Mexico), and then continuously stirred for 24 h to obtain a homogeneous slurry. The slurry was centrifuged at 8500g for 50 min to collect the supernatant. Ammonium sulphate (Sigma, St. Louis, MO, USA) was added to the supernatant to 40% saturation, and then centrifuged. More ammonium sulphate was added into the supernatant to 80% saturation, and then centrifuged again to collect the 40–80% saturation precipitate. The precipitate was dissolved in a small volume of distilled water and then dialysed against distilled water to obtain the crude mushroom extract. The crude extract was then freeze-dried for the following experiments.

The growth inhibitory effect of the crude mushroom component on human myeloid leukemia U937 cells (American Type Culture Collection, Rockville, MD, USA) was evaluated following the procedure described by Zweier, Kuppusamy, and Lutty (1988) with some modifications. Briefly, U937 cells were cultured in RPMI-1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) in a humidified atmosphere containing 5% CO2 at 37 °C. The cells were harvested and re-suspended in the medium at the density of 1.25 × 105 cells/mL. The crude extract was diluted with phosphate buffer saline (PBS) and then added to the cell suspension at 100–500 μg/mL concentrations. The cultures were incubated at 37 °C for 48 h to harvest the cells. The total cell count was performed using a haemocytometer. Counting of viable cells was done by Trypan Blue Dye (Gibco) exclusion test. The growth inhibitory effect was calculated using the following formula:

\[
\text{Growth inhibitory effect (\%)} = \frac{1 - \left( \frac{\text{number of viable cells in the treated group}}{\text{number of viable cells in the control group}} \right)}{100}\%.
\]

2.2. Isolation of bioactive component

The crude extract was dissolved in distilled water and run through a 2.6 × 30 cm DEAE-Sepharose column (GE Healthcare, Uppsala, Sweden), which had been equilibrated with 0.05 M Tris–HCl buffer (pH 8.0), in two steps. In the first step, the column was eluted with five bed volumes of the Tris–HCl buffer to deprive of the unadsorbed fractions. In the second step, protein–rich fractions were collected through the elution with five bed volumes of 0–1 M NaCl gradient Tris–HCl buffer. The growth inhibitory effect of each fraction on U937 cells was evaluated following the above-described procedure with a change in protein concentration to 50 μg/mL. The fraction with the highest inhibitory effect was collected and further fractionated by gel filtration in a 1.6 × 100 cm Sephacryl S-300 column (GE Healthcare) eluted with three bed volumes of the Tris–HCl buffer. The growth inhibitory effect of each sub-fraction on U937 cells was then evaluated to collect the sub-fraction with higher inhibitory effect. The sub-fraction was freeze-dried to powder as the bioactive component for storage before use.

2.3. Analysis of bioactive component

2.3.1. Molecular mass estimation

The native molecular mass of the bioactive component was determined by gel filtration chromatography on Sephacryl S-300 gel referring to Andrews (1964). The molecular mass protein markers (669, 440, 232, 140 and 66 kDa) were purchased from GE Healthcare (Buckinghamshire, UK). The bioactive component was also analyzed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) followed with Sypro Ruby staining (Sigma) to confirm its purity and to estimate the molecular mass of its subunits with the use of low molecular weight protein markers (170, 130, 70, 55, 35, 25 and 15 kDa) (GE Healthcare). Periodic acid (Sigma) Schiff staining was applied to check for the presence of carbohydrate moiety in the sample, with phytohaemagglutinin from Phaseolus vulgaris (referring to PHA (Sigma) as the positive control, referring to the method reported by Segrest and Jackson (1972).)

2.3.2. Protein analysis

The bioactive component was analyzed following the method of Lowry, Rebebrugh, and Farr (1951) for protein content, with bovine serum albumin as the standard.

The amino acid composition of bioactive component was revealed by hydrolysis in 6 M HCl at 110 °C for 24 h followed with the use of a Hitachi Model L-8800 Amino Acid Analyzer (Tokyo, Japan). The N-terminal sequence was determined by automated Edman degradation protocol using a Model 477A Amino Acid Sequencer from Applied Biosystems (Foster City, CA, USA) with the manufacturer’s standard program and chemicals.

2.3.3. Carbohydrate analysis

Carbohydrate content of the bioactive component was determined by the phenol–H2SO4 method using glucose as the standard (Mizuno et al., 1990).

The active component was hydrolyzed following a procedure that involved methanalysis and trifluoroacetic acid (TFA) hydrolysis to prepare the sample for monosaccharide composition analysis. Approximately 1 mg of the bioactive component was mixed with 1 mL of 2 M HCl anhydrous solution in absolute methanol in a sealed hydrolytic tube, and heated at 80 °C for 12 h. The solvent was then removed under a vacuum. The residue was mixed with 1 mL of 2 M TFA (Sigma) and heated at 100 °C for 1 h to complete the hydrolysis process. The monosaccharide composition of the hydrolysate was determined in high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD). The equipment was consisted of a 4 × 250 mm PA1 column (Dionex, Sunnyvale, CA, USA) and a Bioscan 817 IC System (Metrohm, Herisau, Switzerland). A pump, injection valve unit 812 with 20 μL loop, and an electrochemical detector with gold working electrode (E1 = 0.05 V, 0.44 s; E2 = 0.80 V, 0.18 s; E3 = −0.30 V, 0.36 s). Isocratic elution was performed at the rate of 0.5 mL/min with 19 mM NaOH eluent containing 1 mM Ba(OAc)2 (Sigma) (Talaga, Vialle, & Moreau, 2002).

2.3.4. Haemagglutinating assay

The active component was dissolved in pH 7.2 PBS containing 8 g of NaCl/1.15 g of Na2HPO4/0.20 g of KH2PO4/0.20 g of KCl/L, and then diluted in twofold series. Each 50 μL aliquot of the sample solution was pipetted into a well on a microtitre V-plate and then added with 50 μL of a PBS suspension (2%; v/v) of rabbit erythrocytes purchased from Fitzgerald Industries (North Acton, MA, USA) or human erythrocytes obtained from three normal adult volunteers (aged between 22 and 25, in blood type A, B or O) with informed consent. The haemagglutination titer was recorded after these plates were incubated at 37 °C for 2 h when complete precipitation was observed in the control that contained no bioactive component.

2.4. Growth inhibitory effect of bioactive component

The growth inhibitory effect of the bioactive component on human mononuclear cells (MNC) and U937 cells was tested. Human peripheral blood was obtained from healthy adult volunteers. MNC were recovered from the blood of each person by density centrifugation (400g, 30 min) in Ficol–Hypaque solution (1.077 g/mL).
Pharmacia Fine Chemicals, Uppsala, Sweden). U937 cells were cultured and harvested as above-described.

FBS was held at 56 °C in a water bath for 30 min to destroy heat-labile complement proteins before preparing the medium 2 mM glutamine (Gibco)/10% heat-inactivated FBS/RPMI 1640 medium (Gibco). MNC and U937 cells were then suspended in the medium in the density of $1 \times 10^8$ and $1.25 \times 10^5$ cells/mL, respectively. The bioactive component was dissolved in phosphate buffer saline (PBS) and then added to the cell suspension. The cultures were incubated at 37 °C for 24, 48, and 72 h to harvest the cells. The total cell count was performed using a haemocytometer. Counting of viable cells was done by trypan blue dye (Gibco) exclusion test. The growth inhibitory effect was calculated as above-described.

2.5. Statistical analysis

Each experiment was repeated three times using duplicate samples. The results were expressed as means ± standard deviations. Statistical comparisons were made by one-way analysis of variance (ANOVA), followed by Duncan’s multiple-comparison test. Differences were considered significant when the $p$-values were <0.05.

3. Results and discussion

3.1. Isolation of bioactive component.

The crude extract from HM, in a concentration between 100% and 500 µg/mL, exhibited a growth inhibition rates between 28% and 91% on U937 cells in 48 h incubation. Four protein-rich fractions (named HM-1, -2, -3 and -4) were recovered from the crude extract through DEAE-Sepharose ion-exchange column (Fig. 1A). The growth inhibition rates of each fraction, at the concentration of 50 µg/mL, in incubating with U937 cells for 48 h were 7.8%, 5.9%, 63.2% and 13.6%, respectively. Among them, the fraction HM-3, which corresponded to 0.95 M NaCl elution concentration, had the highest growth inhibitory effect. This fraction was further

![Fig. 1. Chromatographic isolation and purification of HM-3A.](image-url)

(A) DEAE-Sepharose ion-exchange chromatograph of 40–80% ammonium sulphate precipitates from 10% NaCl extract of Hypsizygus marmoreus. The column (2.6 cm × 30 cm) was eluted with a gradient of 0–1 M NaCl in 0.05 M Tris–HCl buffer (pH 8.0) at 0.5 mL/min to recover the protein-rich fractions HM-1–4. (B) Sephacryl S-300 gel filtration chromatograph of fraction HM-3. The column (1.6 × 100 cm) was eluted with 0.05 M Tris–HCl buffer (pH 8.0) at 0.5 mL/min to recover protein-rich subfractions HM-3A and -3B.
separated by a Sephacryl S-300 gel filtration column into two sub-
fractions, named HM-3A and HM-3B (Fig. 1B). The growth inhibi-
tion rates of HM-3A and HM-3B, at 50 μg/mL, in incubating with
U937 cells for 48 h were 76.5% and 23.6%, respectively. HM-3A
was significantly stronger than HM-3B in the inhibition on U937
cells, and therefore taken as the bioactive component for subse-
quent analyses.

The recovery of HM-3A was approximately 0.015 mg/g dry
weight of the basidioma. It is close to the recovery of another bio-
glycoprotein which was isolated from Golden oyster mush-
room P. citrinopileatus, 0.023 mg/g dry weight of the basidioma
(Chen, Wang, & Wu, 2009).

There are affirmative reports on the retention of protein activi-
ties through oral administration. For example, the protein-bound
polysaccharide (PSK), a heterogeneous mixture of glycoproteins,
was reported to induce gene expression and production of TNF-α
and IL-8 (Kato et al., 1995; Matsunaga et al., 1998). It is possible
that HM-3A may retain its bioactivity as ingesting as a food
ingredient.

3.2. Identification of HM-3A

3.2.1. Molecular mass estimation

The protein content of HM-3A was found to be 70.2 ± 1.6% by
Lowry's method. The apparent molecular mass of HM-3A, as deter-
mined by gel filtration chromatography, was 525 kDa. SDS–PAGE
followed by Sypro Ruby staining revealed a single band at 52 kDa
molecular mass (Fig. 2A), indicating that each HM-3A molecule is
probably composed of 10 subunits. PAS staining of the gel revealed
an apparent single band, indicating that HM-3A is a glycoprotein
(Fig. 2B).

3.2.2. Protein analysis

The amino acid composition of HM-3A in relative higher con-
tents include aspartic acid (11.46%), glutamic acid (9.13%), arginine
(8.91%), alanine (8.56%), proline (7.79%), methionine (7.64%), gly-
cine (7.31%), threonine (7.21%), phenylalanine (6.23%), and serine
(5.12%), and in lower contents include lysine (4.29%), cysteine
(3.55%), leucine (3.46%), valine (2.59%), isoleucine (2.56%),
tryptophan (2.21%) and histidine (1.98%). The present study also
identified a sequence of 10 amino acids, ATTQWKTSAA, in the
N-terminal of HM-3A. It was found to be different from any re-
ported N-terminal amino acid sequence of mushroom proteins in
GeneBank database (BLAST search, including the antitumor glyco-
proteins from mushrooms HM, F. velutipes (Ko, Hsu, Lin, Kao, & Lin,
1995), G. lucidum (Tanaka, Ko, & Kino, 1989), Volvariella volvacea
(Hsu, Hsu, Lin, Kao, & Lin, 1997) and Xerocomus spadixicus (Liu,
Wang, & Ng, 2004) (Table 1). The above-described biochemical
analyses confirmed that HM-3A is a novel bioactive glycoprotein
never reported before.

3.2.3. Carbohydrate analysis

The carbohydrate content of the glycoprotein HM-3A was found
to be 21.7 ± 1.9% by Phenol–H2SO4 method. After hydrolysis with
2 M TFA for 1 h, the monosaccharide profile was determined by
HPAEC–PAD. TFA hydrolysis followed with HPAEC–PAD analysis
showed that HM-3A contains Gal (galactose) as the most abundant
monosaccharide, at 46.73% (mol/mol) of the total monosaccha-
drides, followed by six other monosaccharides, namely Man
(mannose; at 23.88%), Fuc (fucose; at 13.07%), Glc (glucose; at 11.70%),
Xyl (xylose at 3.47%), Ara (arabinose; at 1.05%), and Rha (rham-
nose; at 0.11%) in diminishing order (Fig. 3). The monosaccharide
profiles in the reported antitumor polysaccharides in mushrooms
often include glucose, galactose, mannose, xylose, arabinose, fu-
cose, ribose and glucuronic acid (Zhang, Cui, Chueng, & Wang,
2007). Among them, ribose and glucuronic acid are absent in
HM-3A.

3.2.4. Haemagglutinating assay

Lectins present in many varieties of mushrooms (Feng et al.,
2006). They are a glycoprotein with haemagglutinating activity on
mammalian erythrocytes (Grahn, Askarieh, & Holmner, 2007;
Pemberton, 1994). They may also be inhibitory to the growth of
leukaemic cells (Abdullaev & de Mejia, 1997). When consumed in
excess by sensitive individuals, lectins may cause severe symp-
toms, including intestinal damage, Gell–Coombs types of food aller-
gies (Roitt, 1989), and anemia (Hamid & Masood, 2009). Before
starting any further investigation, it is preferable to know whether
HM-3A is a lectin or not.

Human blood is usually classified into types A, B, AB and O
based upon the presence or absence of the antigens A and B on
the surface of erythrocytes. All types of blood also contain antigen
H. All these antigens, A, B and H, have a carbohydrate moieties
(Dahiy a, Itzkowitz, & Byrd, 1989). Lectins are able to recognize,
to bind to the carbohydrate moiety of specific antigens, and to
agglutinate erythrocytes in a mechanism similar to antigen–anti-
body interaction (Sharon & Lis, 1972). Nachbar, Oppenheim, and
Thomas (1980) showed that about half of the known dietary lectins
are haemagglutinins that can agglutinate all blood types, whereas
the other lectins are blood-type specific in the haemagglutinating
assay. Among common experiment animals, rabbit erythrocytes
are most sensitive to lectins (Ainouz et al., 1992; Antonyuk,
Nemchenko, Tymchuk, Danileuchenko, & Stoika, 2010). Therefore,
the present study used rabbit blood and human type A, B and O
bloods in the haemagglutinating assay for lectin detection.

The results showed that HM-3A is unable to agglutinate eryth-
rocytes in rabbit blood and human blood types A, B and O (Fig. 4).
The blood type AB was not tested because it contains both antigens
A and B, and therefore a positive response from either type A or
blood type B would render it to respond positively as well. The
positive control, which was the haemagglutinating protein PHA puri-
fied from Phaseolus vulgaris, agglutinated rabbit and human
erthrocytes readily at a concentration as low as 31.25 μg/mL.
On the contrary, HM-3A failed to demonstrate any haemaggluti-
nating activity at a concentration as high as 1000 μg/mL. Based
on the above-described results of haemagglutinating assay, we
concluded that HM-3A is not a lectin.

3.3. Growth inhibitory effect of HM-3A

Fig. 5 shows the growth inhibitory effect of HM-3A against
U937 leukemia cells. The effect increased with HM-3A concentra-
tion in the range between 12.5 and 100 μg/mL and with the duration
of treatment from 24 h to 72 h, indicating the dose and time
dependency. In treating with HM-3A at the highest concentration
in the experiment, 100 μg/mL, the growth inhibition rates on
U937 cells reached 74.6%, 90.8% and 96.2% in 24, 48 and 72 h,
respectively. The growth inhibitory effect of HM-3A on otherwise
isolated MNC was also tested. HM-3A between 12.5 and 100
μg/mL, in incubating with U937 leukaemic cells. The effect increased with HM-3A concentra-

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leukaemic cell. A few mushroom glycoproteins have been reported to be inhibitory against cancer cells, including the glycoprotein fraction from *F. velutipes* that extended the lifespan of B-16 mice implanted with B-16 melanoma and Ca-755 adenocarcinoma (Ikekawa et al., 1982, 1985), a polysaccharide–peptide complex extracted from *Trametes versicolor* that inhibited MAD-MB-231 breast cancer cells (Chow, Lo, Loo, Hu, & Sham, 2003), a protein bound polysaccharide isolated from *Phellinus linteus* that inhibited SW480 human colon cancer cells (Li, Kim, Kim, & Park, 2004), and a purified glycoprotein named PCP-3A from the basidioma of *P. citrinopileatus* that inhibited U937 leukaemic cells (Chen et al., 2010). A PCP-3A molecule is composed of 10 subunits each

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**Table 1**

N-terminal amino acid sequences of HM-3A and previously reported mushroom glycoproteins.

<table>
<thead>
<tr>
<th>Species</th>
<th>N-terminal sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypsizygus marmoreus</td>
<td>ATTQWKTSAA</td>
<td>This study</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>SFDYTPNWGRGN</td>
<td>Tanaka et al., 1989</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>DFDYTPNWGRCT</td>
<td>Ko et al., 1995</td>
</tr>
<tr>
<td>Volvariella volvacea</td>
<td>NFDYTPQWQRGN</td>
<td>Hsu et al., 1997</td>
</tr>
<tr>
<td>Xerocomus spadiceus</td>
<td>CSKGGVGRGYGIG</td>
<td>Liu et al., 2004</td>
</tr>
</tbody>
</table>

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Fig. 2. The 15.0% SDS-PAGE electrophoregram of HM-3A as visualized by (A) Sypro Ruby staining (B) PAS staining. Lanes show markers, HM-3A, and PHA as the positive control, separately. The molecular mass protein markers are at 170, 130, 70, 55, 35, 25 and 15 kDa from top downward.

Fig. 3. HPAEC-PAD chromatographic profile of monosaccharides in HM-3A. The equipment was consisted of a 4 × 250 mm PA1 column and a Bioscan 817 IC System, including an electrochemical detector with gold working electrode (E1 = 0.05 V, 0.44 s; E2 = 0.80 V, 0.18 s; E3 = 0.30 V, 0.36 s). Isocratic elution was performed at 0.5 mL/min with 19 mM NaOH containing 1 mM Ba(OAc)2. Ara: arabinose; Fuc: fucose; Gal: galactose; Glc: glucose; Rha: rhamnose; Man: mannose; Xyl: xylose; GalA: galacturonic acid; GlcA: glucuronic acid.

Fig. 4. Haemagglutinating activity of HM-3A on (A) rabbit erythrocytes, and (B) human erythrocytes. PHA served as positive control. Samples treated with PBS without protein served as vehicle control.

leukaemic cell. A few mushroom glycoproteins have been reported to be inhibitory against cancer cells, including the glycoprotein fraction from *F. velutipes* that extended the lifespan of B-16 mice implanted with B-16 melanoma and Ca-755 adenocarcinoma (Ikekawa et al., 1982, 1985), a polysaccharide–peptide complex extracted from *Trametes versicolor* that inhibited MAD-MB-231 breast cancer cells (Chow, Lo, Loo, Hu, & Sham, 2003), a protein bound polysaccharide isolated from *Phellinus linteus* that inhibited SW480 human colon cancer cells (Li, Kim, Kim, & Park, 2004), and a purified glycoprotein named PCP-3A from the basidioma of *P. citrinopileatus* that inhibited U937 leukaemic cells (Chen et al., 2010). A PCP-3A molecule is composed of 10 subunits each
45 kDa in size, while an HM-3A molecules is composed of the same number of subunits each 52 kDa. These two anti-leukaemic glycoproteins differ much in size.

HM is among the popular mushrooms in the world. The supply of HM basidia for the isolation of HM-3A will be relatively easy as compared with other mushrooms. Furthermore, many efforts have been made to produce recombinant proteins from mushrooms in recent years. For example, Hsu et al. (2008) expressed the recombinant LZ-8 protein (rLZ-8) from G. lucidum using yeast Pichia pastoris protein expression system, Yeh, Yeh, Hsu, Luo & Lin (2008) expressed a functional recombinant immunomodulatory protein from G. lucidum in Bacillus subtillis and Lactococcus lactis, and Yeh, Yeh, Peng, Haung, & Peng (2009) extracellularly expressed a functional recombinant immunomodulatory protein rLZ-8 from G. lucidum in L. lactis. It will also be possible to develop the gene cloning technique for the mass production of HM-3A at a lower cost.

4. Conclusions

PAS staining, N-terminal amino acid sequence analysis and hemoagglutination assay proved HM-3A a novel non-lectin glycoprotein. The cell study proved the growth inhibitory effect of HM-3A will be among the next steps in developing HM-3A into an antitumor agent or an ingredient in health food.

References


