African Journal of Biotechnology Vol. 11(86), pp. 15241-15245, 25 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2171
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Anti-viral activity of *Hydnellum concrescens*, a medicinal mushroom

Doseung Lee[1,2#], Kyung Hwan Boo[1,2#], Jin-Man Lee[3#], Cao Dang Viet[1], Nguyen Quyen[1], Tatsuya Unno[1], Moonjae Cho[4], Key Zung Riu[1,2] and Dong-Sun Lee[1,2*]

[1]College of Applied Life Science (RISA), Jeju National University, Jeju 690-756, Republic of Korea.
[2]Subtropical Horticulture Research Institute, Jeju National University, Jeju 690-756, Republic of Korea.
[3]Department of Food & Biotechnology, Hoseo University, Asan 336-795, Republic of Korea.
[4]Biochemistry Lab, Department of Medicine, School of Medicine, Jeju National University, Jeju 690-756, Republic of Korea.

Accepted 15 August, 2012

Trafficking of viral glycoprotein to the surface of infected-cells results in syncytium formation in Newcastle disease virus (NDV)-infected baby hamster kidney (BHK) cells. *Hydnellum concrescens*, known as a medicinal mushroom, inhibited not only syncytium formation, but also trafficking of glycoprotein, hemagglutinin-neuramidase (HN) to the cell-surface. Viral glycoprotein is processed within the endoplasmic reticulum during routing into surface. Fungal extracts showed inhibitory activities (IC_{50} 15 µg/ml) against α-glucosidase. This suggested that *H. concrescens* extracts inhibited the cell-surface expression of NDV-HN glycoprotein without significantly affecting HN glycoprotein synthesis in NDV-infected BHK cells.

Key words: α-Glucosidase inhibitor, *Hydnellum concrescens*, trafficking inhibitor.

INTRODUCTION

Glycosidases play an important role in various physiological responses, including carbohydrate digestion and the processing of glycoproteins and glycolipids in mammalian cells. Furthermore, glycosidases are involved in a broad range of human degenerative metabolic disorders and other diseases such as viral infection and carcinogenesis (Dennis et al., 1987). Therefore, glycosidase inhibitors are powerful tools for studying their molecular mechanisms and could also be prospective therapeutic agents. Harmful viruses, including dengue virus, human immunodeficiency virus and hepatitis B virus are reported to be extremely sensitive to glucosidase inhibition (Courageot et al., 2000). Nojirimycin, *N*-butyldeoxynojirimycin, nectricine, and castanospermine are principally known as α-glucosidase inhibitors, and play significant roles as potent inhibitors of human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation by cell fusion *in vitro* (Dettenhofer and Yu, 2001; Johnson et al., 1989; Tsujii et al., 1996).

In general, outer membrane gp120 and transmembrane gp41 subunits, known as HIV-1 envelope glycoprotein (Env), originate from the intracellular cleavage of precursor gp160. Both glycoproteins remain non-covalently linked within oligomeric structures during the trafficking to the cell-surface (Einfeld, 1996).

After virus budding, gp120 exposed on the viral surface mediates HIV binding on CD4+ lymphocytes through interaction with cell-surface antigen CD4 (Papandréou et al., 2002). As a result, HIV infection *in vitro* induces syncytium formation by cell-to-cell fusion (Quinn, 2008). Deoxynojirimycin and analogs act as saccharide decoys that inhibit the cellular α-glucosidase activity. Accordingly, they inhibit the trimming of the Env glycan precursor (that is, the cleavage of the three Glc residues from the Glc3Man9GlcNAc2 precursor glycan) within the endoplasmic reticulum. This inhibition of glycan processing is likely to explain the decrease in the trimming activity of gp160 into gp41 and gp120 seen in HIV-infected cells treated with α-glucosidase inhibitors. This inhibition

*Corresponding author E-mail: dongsunlee@jejunu.ac.kr. Tel: 82-64-754-3343. Fax: 82-64-756-3351. #Authors contributed equally to the work.

results in a suppression of HIV replication and syncytium formation *in vitro* (Johnson et al., 1989; Fischer et al., 1995; Papandréou et al., 2002), because HIV infection induces syncytium formation by cell-to-cell fusion (Quinn, 2008). The mode of syncytium formation in Newcastle disease virus (NDV)-infected baby hamster kidney (BHK) cell is similar to those of HIV-infected cells (Gruters et al., 1987). α-Glucosidase inhibitors are expected to prevent the assembly of virus by affecting the glycoprotein processing. Recently, we found the anti-viral effect of *H. concrescens* using the screening system of α–glucosidase inhibition.

Glycoproteins are synthesized in a rough endoplasmic reticulum and secreted into cell membranes through Golgi complex. Trafficking within the cell happens from the repetitive process of budding and fusion of secretory vesicles (Muñniz and Riezman, 2000). To identify components that regulate the transport in cell, there has been a number of biochemical and genetic studies (Nakamura et al., 2003). Previously, cytosolic transport factors were identified by the application of an in vitro assay of intra-Golgi transport. Brefeldin A (BFA) effectively blocked the cell-surface expression of viral glycoprotein (Lad and Gupta, 2002). The study on the trafficking mechanisms of glycoprotein can use chemicals, and it allows discovering the primary mechanism of virus infection diseases, including cancer and other degenerative diseases. However, the transport mechanism of glycoprotein has not been well defined. Nevertheless, α-glucosidase inhibitors are expected to prevent the assembly of virus by affecting the glycoprotein processing. Recently, we found the inhibitory activity of glycoprotein trafficking in *H. concrescens* extract using the screening system of α–glucosidase inhibition.

H. concrescens is an inedible mushroom and generally known as zoned tooth fungus with brown spores (Parfitt et al., 2007). Polysaccharides extracted from mycelial culture of *H. concrescens* have an antitumor effect (Ohtsuka et al., 1973; Yang et al., 2007). Therefore, *H. concrescens*, medicinal mushroom used widely in Asia, was screened as α-glucosidase inhibitor. In this present data, the implications of the trafficking inhibition of glycoprotein and the activity of glucosidase as antiviral agents are studied.

MATERIALS AND METHODS

This mushroom was purchased from a local herbal medicine shop at Daegu, Korea. *H. concrescens* (10 g) was soaked in 100 ml methanol in a round-bottom flask at room temperature for 24 h. The extracts were filtered with Whatman No.1 filter paper. The filtrate was then evaporated *in vacuo* to remove methanol at a temperature below 40°C. The extracts were stored in airtight containers at 4°C for further testing.

H. concrescens extract inhibits various commercially available glycosidases. The assay for glycosidases was determined as described previously (Lee and Lee, 2001; Lee et al., 2010 and 2011).

RESULTS AND DISCUSSION

As shown in Figure 1, α–glucosidase was the most sensitive to *H. concrescens* extract, and the concentration required for 50% inhibition (IC_{50}) was 12 µg/ml. At higher concentrations than IC_{50}, the extracts also inhibited the activities of α-mannosidase, β-glucosidase and β-mannosidase with IC_{50} 25, 60 and 150 µg/ml, respectively. This result indicates that the activity of α-glucosidase decreased by *H. concrescens* extracts in a dose-responsive manner. The extracts inhibited α-type glycosidases more than β-type in comparison of four different types. This data also suggested that the extracts have a potent inhibitory activity on glycosylation of glycoproteins in cell.

For study on the inhibitory activity of the extracts at the cellular level, we measured its effects on levels on the synthesis and trafficking of NDV-HN glycoprotein expressed on the surface of BHK cell because the way of syncytium formation in NDV-infected BHK cell is similar to those of HIV. Inhibitory activities on syncytium formation were determined as described previously (Muroi et al., 1993; Lee et al., 2011). Syncytium formation was inhibited by the extracts at 25 µg/ml or more concentration. But, syncytium formation partly retarded at 12.5 µg/ml. The minimum inhibitory concentration (MIC) value of the extracts to suppressed syncytium formation is 12.5 µg/ml (Table 1). The decrease in syncytium formation in the presence of the extracts can be similar to the suppression mode of glycan processing in HIV-infected cells by α-glucosidase inhibition.

To determine whether the inhibition on cell-surface expression of viral glycoprotein in presence of *H. concrescens* extracts caused by the inhibition of its synthesis in cell or not, the total amount of NDV-HN synthesized in cell were measured. Synthesis of NDV-HN glycoprotein in NDV-infected cells was quantified by determining hemagglutination units (HAU) in whole lysates (Muroi et al., 1993; Lee et al., 2011). NDV-HN expressed on the cell surface was also quantified by hemadsorption (HAD) assay (Muroi et al., 1993; Lee et al., 2011).

Whole NDV-infected cultures were disrupted by brief sonication, and chicken red blood cells were added to determine hemagglutination activities in lysates. HAU was gradually reduced at concentration between 6.3 and 100.0 µg/ml crude extracts (Figure 2, white bar). On the other hand, the binding of extracellularly added chicken red blood cells to the surfaces of intact NDV-infected cells (expressed as % HAD) decreased extensively at 12.5 µg/mL (Figure 2, black) despite a slight decrease of HAU (17~14%). This data suggests that the inhibition on surface-directed trafficking of NDV-HN glycoprotein at 12.5 µg/ml extract was stronger than its synthesis in cell.

This is also in line with a fact that syncytium formation decreased at 12.5 µg/ml or more concentration (12.5 µg/ml) (Table 1, Figure 2). These results indicate that *H. concrescens* extracts inhibit the cell-surface expression of NDV-HN, but has no significant effects on its synthesis.

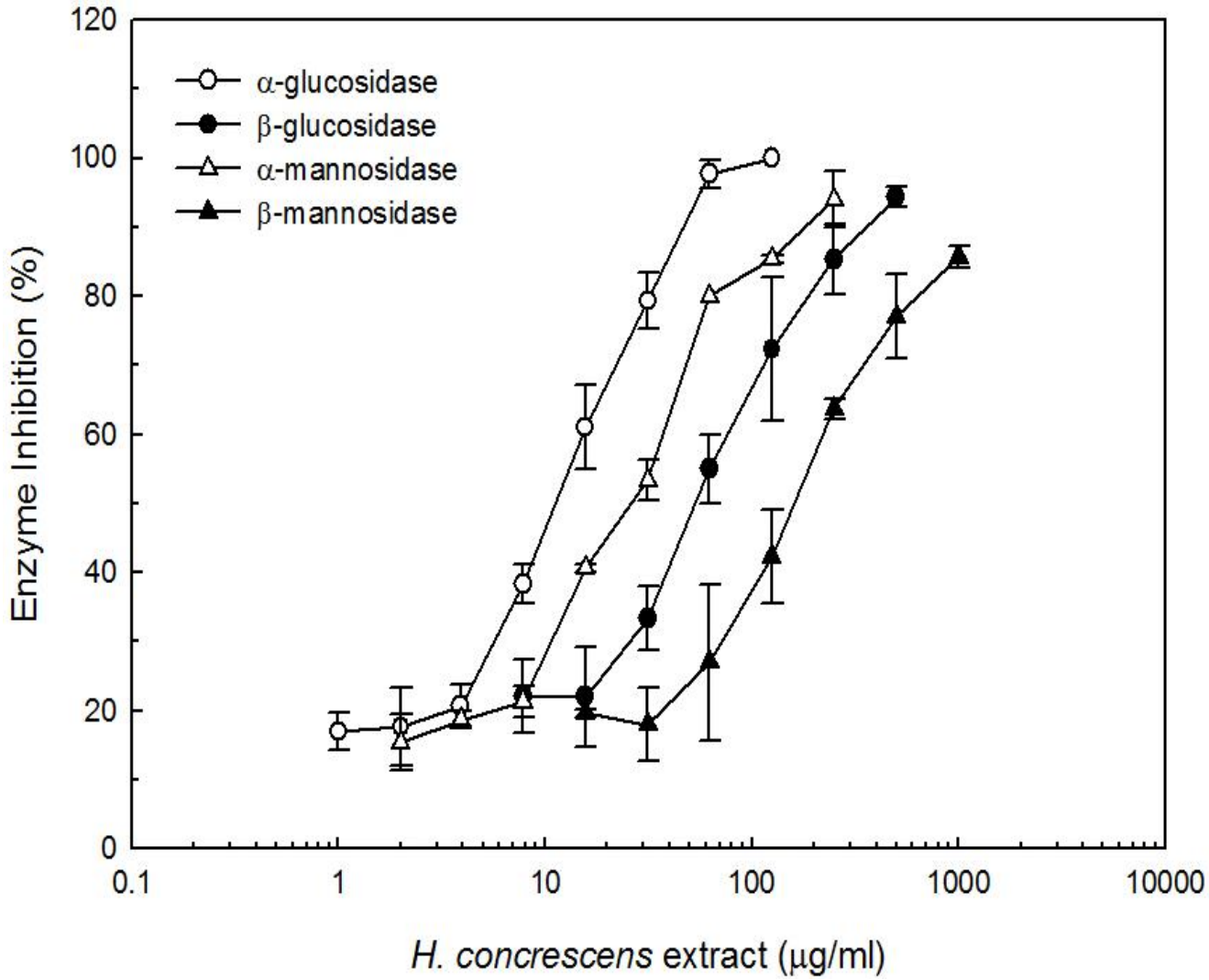

Figure 1. Inhibition by *H. concrescens* extracts against various glucosidases. Enzyme solutions were treated with designated concentrations of sample. The amounts of enzymes were as follows: 0.5 U/ml α-glucosidase (○), 0.5 U/ml β-glucosidase (●), 0.5 U/ml α-mannosidase (Δ), 0.5 U/ml β-mannosidase (▲). α-Glucosidase and the other glycosidases were assayed using 50 mM phosphate buffer at pH 6.7, and the appropriate p-nitrophenol (PNP) glycosides (at 1 mM) were used as substrates. Sample at the designated concentration was added to the enzyme solution in the buffer and incubated at 37°C for 1 h, and the substrate was then added to initiate the enzyme reaction. The enzyme reaction was carried out at 30°C for 10 min. The reaction was stopped by the addition of three volumes of 1 M Na_2CO_3, and the release of PNP was monitored at 405 nm. One unit of activity was defined as the amount of enzymes, which liberates 1.0 μmol of PNP per minute under the assay conditions. The IC_{50} values were measured graphically by a plot of percent activity versus log of the sample concentration. Data were shown as means ± SD for triplicate experiments.

Table 1. Effect of *H. concrescens* extracts on syncytium formation.

Concentration (μg/ml)	1.6	3.2	6.3	12.5	25	50	100
Syncytium formation	++	++	++	+/-	-	-	-
%HAU	100	96	97	83	71	55	43

Confluent monolayer cultures of BHK cells in 96-well titer plates were infected with NDV at a concentration of five plaque-forming units (PFU) per cell. Methanol extract was added at 1 h after infection and the cells were incubated for a further 18 h. Cells were incubated at 37˚C for 16 h in a humidified 5% CO_2-95% air incubator. The syncytium formation in NDV-infected cells was determined under an optical microscope (Muroi et al., 1993; Lee et al., 2011). The degrees of syncytium formation was expressed as follows; -, no cell fusion; +, moderate cell fusion; ++, severe cell fusion.

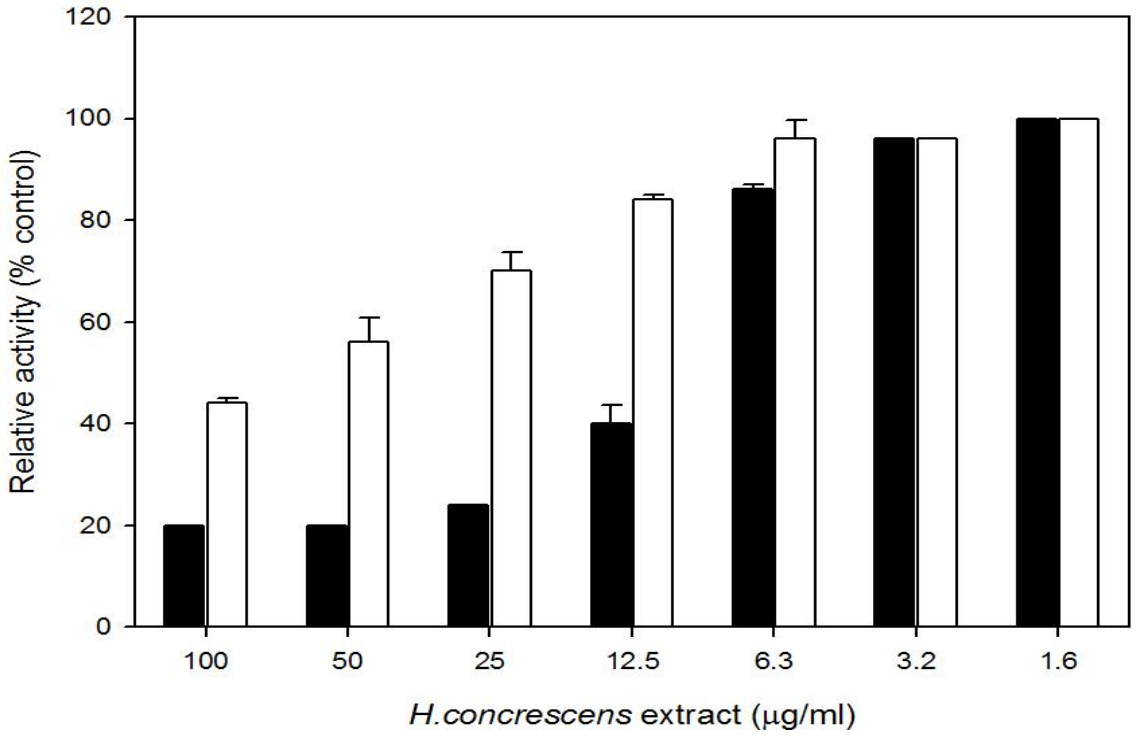

Figure 2. Blockade of the cell surface expression of NDV-HN glycoprotein by *H. concrescens* extracts with no significant effects on its synthesis. Confluent cultures of BHK cells in 6-well plates (Falcon) were infected with 2 ml of NDV at a concentration of 1 HAU/ml and incubated for 14 h at 37°C in a humidified 5% CO_2-95% air incubator. The indicated concentrations of sample were added at 1 h after infection. The medium was removed by aspiration, and then 2 ml of 1% (v/v) chicken red blood cells in cold saline was added to each well and the plate held at 4°C for 30 min with occasional gentle stirring. Unabsorbed red blood cells were removed, and the cell layers were rinsed three times with 2 ml each of cold saline. The adsorbed red blood cells were swollen in distilled water containing 1% ammonia and quantified by measuring the absorption at 550 nm. Percentages of HAU (white bar) or HAD (black bar) were determined at 14 h of infection. NDV-HN protein synthesis was quantified by determining HAU in whole lysates of infected cultures in microtiter plates, and cell surface expression quantified by measuring the amounts of chicken red blood cells adsorbed to intact infected cells in 6-well plates. Results are expressed as a percentage of the control value. Data were shown as means ± SD for triplicate experiments.

The methanol extracts of *H. concrescens* seems to be the candidate of anti-viral agent by α-glucosidase inhi-bition. To investigate further studies on anti-viral activities, activity component should be identified from *H. concrescens* extracts. The potential of trafficking inhibi-tors also requires further investigation as a candidate of anti-viral therapeutic agents.

ACKNOWLEDGMENTS

This work was supported by the grants from Priority Research Centers Program (2011-0030748), Basic Science Research Program (2012R1A1A4A01019357) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, and the Next-Generation BioGreen 21 Program (TAGC, PJ009094; CNPM, PJ009045) and the Ministry for Food, Agriculture, Forestry and Fisheries, IPET (111153-02-1-SB010), Republic of Korea.

REFERENCES

Courageot MP, Frenkiel MP, Duarte Dos Santos C, Deubel V, Despres P (2000). α-Glucosidase inhibitors reduce dengue virus production by affecting the initial steps of virion morphogenesis in the endoplasmic reticulum. J. Virol. 74:564-572.

Dennis JW, Laferté S, Waghorne C, Breitman ML, Kerbel, RS (1987). Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. Science, 236: 582-585.

Dettenhofer M, Yu XF (2001). Characterization of the biosynthesis of human immunodeficiency virus type 1 env from infected T-cells and the effects of glucose trimming of Env on virion infectivity. J. Biol. Chem. 276:5985-5991.

Einfeld D (1996). Maturation and assembly of retroviral glycoproteins. Curr. Top. Microbiol. 214:133-176.

Fischer PB, Collin M, Karlsson GB, James W, Butters TD, Davis SJ, Gordon S, Dwek RA, Platt FM (1995). The α-glucosidase inhibitor N-buthyldeoxynojirimycin inhibits juman immunodeficiency virus entry at the level of post-CD4 binding. J. Virol. 69:5791-5797.

Gruters RA, Neefjes JJ, Tersmette M, de Goede RE, Tulp A, Huisman HG, Miedema F, Ploegh HL (1987). Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. Nature 330:74-77.

Johnson VA., Walker BD, Barlow MA, Paradis TJ, Chou TC, Hirsch MS (1989). Synergistic inhibition of human immunodeficiency virus type 1

and type 2 replication *in vitro* by castanospermine and 3'-azido-3'-deoxythymidine. Antimicrob. Agents Chemother. 33:53-57.
Lad VJ, Gupta AK (2002). Inhibition of Japanese encephalitis virus maturation and transport in PS cells to cell surface by brefeldin A. Acta Virol. 46:187-190.
Lee DS, Lee SH (2001). Genistein, a soy isoflavone, is a potent α -glucosidase inhibitor. FEBS Lett. 501:84-86.
Lee DS, Kim SC, Kim DH, Kim JH, Park SP, Riu YC, Kim MY, Cho SK, Riu KZ, Lee DS (2011). Screening of *Phellinus linteus*, a medicinal mushroom, for anti-viral activity. J. Korean Soc. Appl. Biol. Chem. 54(3):475-478.
Lee JM, Kim JG, Kim TH, Lee DS, Kim JH, Cho SK, Riu KZ, Lee DS, Lee SH (2010). Nonactin hinders intracellular glycosylation in virus-infected baby hamster kidney cells. Mol. Med. Rep. 3:115-119.
Muñniz M, Riezman H (2000). Intracellular transport of GPI-anchored proteins. EMBO J. 19:10-15.
Muroi M, Takasu A, Yamasaki M, Takatsuki A (1993). Folimycin (concanamycin A), an inhibitor of V-type H(+)-ATPase, blocks cell-surface expression of virus-envelope glycoproteins. Biochem. Biophys. Res. Commun. 193:999-1005.
Nakamura M, Kono Y, Takatsuki A (2003). Mepanipyrim, a novel inhibitor of pharmacologically induced Golgi dispersion. Biosci. Biotechnol. Biochem. 67:139-150.
Ohtsuka S, Ueno S, Yoshikumi C, Hirose F, Ohmura Y, Wada T, Fujii T, Takahashi E (1973). Polysaccharides having an anticarcinogenic effect and a method of producing them from species of Basidiomycetes. UK Patent 1331513, 26 September 1973.
Papandréou MJ, Barbouche R, Guieu R, Kieny MP, Fenouillet E (2002). The alpha-glucosidase inhibitor 1-deoxynojirimycin blocks human immunodeficiency virus envelope glycoprotein-mediated membrane fusion at the CXCR4 binding step. Mol. Pharmacol. 61: 186-193.
Parfitt D, Martyn Ainsworth A, Simpson D, Rogers HJ, Boddy L (2007).
Molecular and morphological discrimination of stipitate hydnoids in the genera *Hydnellum* and *Phellodon*. Mycol. Res. 111(Pt 7): 761-777.
Quinn TC (2008). HIV epidemiology and the effects of antiviral therapy on long-term consequences. AIDS 22(Suppl 3):7-12.
Tsujii E, Muroi M, Shiragami N, Takatsuki A (1996). Nectrisine is a potent inhibitor of alpha-glucosidases, demonstrating activities similarly at enzyme and cellular levels. Biochem. Biophys. Res. Commun. 220:459-466.
Yang XL, Wang F, Shao HJ, Dong ZJ, Ding ZH, Yang WQ (2007). Study on the chemical constituents of *Hydnellum concrescens*. Natl. Prod. Res. Dev. 19:605-609.