Journal of Applied Pharmaceutical Science Vol. 7 (04), pp. 134-137, April, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70419
ISSN 2231-3354

The Activity of Cellulase Enzymes of *Ganoderma applanatum* and *Ganoderma tropicum*

Nyi Mekar Saptarini[1*], Ginayanti Hadisoebroto[2]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jl Raya Bandung Sumedang km 21, Jatinangor, Indonesia. [2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Al Ghifari, Jl Cisaranten 140, Bandung, Indonesia.

ARTICLE INFO

Article history:
Received on: 27/09/2016
Accepted on: 20/12/2016
Available online: 30/04/2017

Key words:
Cellulolytic, colorimetric, DNS, various pH, various temperature.

ABSTRACT

Ganoderma can live as saprobes on rotting stumps and roots by decomposing lignin as well as cellulose and related polysaccharides. It is because of mushrooms cellulolytic activities.
Objective: The aim of this study was to compare the cellulolytic activity of the crude extract of *Ganoderma applanatum* and *Ganoderma tropicum* from Garut (Indonesia).
Method: Colorimetric method using dinitrosalicylic acid (DNS).
Results: The cellulolytic activity of *G. applanatum* and *G. tropicum* in the pH range of 3.5-10.0 was 0.051-0.109 and 0.063-0.131 UI/mL, respectively. The cellulolytic activity of *G. applanatum* and *G. tropicum* in the temperature range of 35-65 °C was 0.100-0.184 and 0.077-0.110 UI/mL, respectively.
Conclusion: The activity of cellulase enzymes were affected by temperature and pH. The cellulolytic activity of *G. applanatum* was the same as *G. tropicum.*

INTRODUCTION

The genus *Ganoderma* (Ganodermataceae) found in subtropical and tropical regions, because they can survive under hot and humid conditions. *Ganoderma* species contain chemical constituents such as polysaccharides, proteins, amino acids, fatty acids, terpenoids, steroids, alkaloids, and phenolic compounds, with potential nutritional and therapeutic values (Mizuno, 1995, Paterson, 2006, Singh *et al.*, 2013). *Ganoderma* species grow as a facultative parasite that can live as saprobes on rotting stumps and roots (Pilotti *et al.*, 2004) by decomposing lignin as well as cellulose and related polysaccharides (Hepting, 1971). We believe these mushrooms have cellulolytic activity which controlled and processed by cellulase system. Currently, cellulase enzyme was used in various industrial processes, such as biofuels (Vaithanomsat *et al.*, 2009), triphase biomethanation (Chakraborty *et al.*, 2000), plants and agriculture waste processing (Mswaka and Magan, 1998, Lu *et al.*, 2004). We see the opportunities of usage of *G. applanatum* and *G. tropicum* in agriculture waste processing. This study aimed to compare the cellulytic activity of the cellulase enzyme of *G. applanatum* and *G. tropicum* which grow on a dead stump of bitter bean (*Parkia speciosa*) in various pH and temperature.

* *Corresponding Author*
Nyi Mekar Saptarini, Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jl Raya Bandung Sumedang km 21, Jatinangor, Indonesia.
Email: nyi.mekar @ unpad.ac.id

MATERIALS AND METHODS

Materials

Ganoderma applanatum and *Ganoderma tropicum* were collected from a dead stump of bitter bean (*Parkia speciosa*) in the Cipangramatan village, Garut sub-district, Indonesia, in July 2015. Mushrooms were identified by School of Biological Sciences and Technology, Bandung Institute of Technology, Indonesia with No. 1996/I1.CO2.2/PL/2015. All chemical reagents are analytical grade (Merck), including glucose and dinitrosalicylic acid (Sigma Aldrich).

Preparation of Dinitrosalicylic Acid (DNS) Reagent

A total of 5 g of sodium hydroxide, 91 g of potassium sodium tartrate, 5 g of sodium sulfite, 1% phenol, and 5 g of DNS were dissolved in 500 mL distilled water. The mixture was homogenized using a magnetic stirrer. The solution was stored in amber bottles and low temperature (Benfeld, 1955).

Determination of Standard Curve

A total of 1 mL of the glucose solution (0.25, 0.50, 0.75, 1.00, and 1.25 µg/mL) in a different tube was added by 1 mL of DNS reagent. The color was then developed by boiling the mixture for 5 min. Optical densities were measured at 575 nm against a blank containing all the reagents minus glucose (Miller, 1959).

Extraction of Crude Enzyme

Mushrooms were washed and dried to a constant weight. A total of 5 g of mushrooms and 25 mL of phosphate buffer pH 7.0 were mashed for 5 min. The extract was filtered, then centrifuged for 15 min at 4000 rpm to obtain supernatant which contain the cellulase enzyme.

Determination of the Cellulolytic Activity

A total of 1 mL of the supernatant, 0.5 mL of phosphate buffer, and 1 mL of 1% CMC were mixed and incubated for 30 min at 25 °C. The reaction was terminated by adding 1 mL of DNS reagent. The color was then developed by boiling the mixture for 5 min. Optical densities were measured at 575 nm against a blank containing all the reagents minus the crude enzyme (Miller, 1959). The cellulolytic activity was determined at various pH (3.5, 5.8, 7.0, 8.0, and 10.0), and various temperature (25, 35, 45, 55, and 65 °C).

$$\text{Enzyme activity (IU/mL)} = \frac{\text{glucose level x dilution factor}}{\text{glucose MW x incubation time}}$$

Statistical Analysis

Results are presented as the mean ± standard error of the mean (SEM). Data comparisons between groups were done by two way ANOVA followed by Tukey-Kramer post hoc test. Values were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Both of ganoderma in this study were grown on a dead stump of bitter bean. The size of *G. applanatum* was 27 ± 2 cm in length, 20 ± 3 cm in width, 5 ± 1 cm in height, and brown colored. The size of *G. tropicum* was 25 ± 3 cm in length, 15 ± 2 cm in width, 5 ± 2 cm in height, and white brownish colored because of spores (Fig. 1). Both mushrooms have morphology according the literature. The growth of Ganoderma is influenced by the environmental conditions, specific host species, and the habitat nature (Turner, 1981). Different species of *Ganoderma* produced different feature and pathogenecity (Wong *et al.*, 2012).

Fig. 1: *Ganoderma applanatum* (above) and G. tropicum (below)

Determination of Standard Curve

The reaction between glucose and DNS involves the oxidation of the aldehyde functional group in the glucose and 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions, simultaneously (Miloski *et al.*, 2008). The correlation coefficient value was close to 1.00 (R^2 = 0.9961, Fig. 2) met the criteria by ICH (ICH, 2005) and showed that the instrument response was proportional to the glucose concentration.

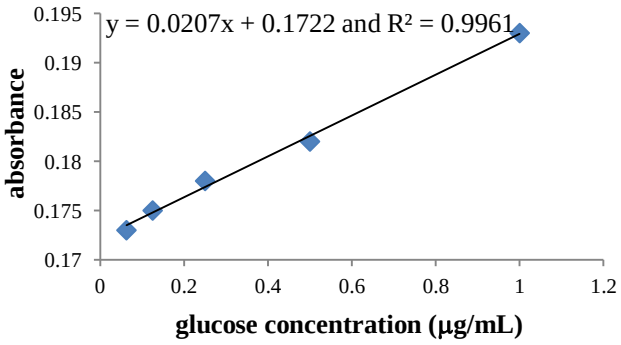

Fig. 2: Glucose calibration curve.

Extraction of Crude Enzyme

Cellulase enzymes were extracted using phosphate buffer pH 7.0 to maintain the stability and function of cellulase enzymes

in the crude extract. The extract were cloudy solution, so conducted the centrifugation to separate the supernatant from the residues. The color of clear supernatant was yellowish-white for *G. applanatum*, and brown for *G. tropicum*.

Determination of the Cellulolytic Activity

The cellulase system consists of three classes of soluble extracellular enzymes, i.e 1,4-β-endoglucanases, 1,4-β-exoglucanases, and β-glucosidases (β-Dglucoside glucohydrolases or cellobiases) (Shewale, 1982). These enzymes hydrolyze cellulose to glucose (Ryu and Mandels, 1980). Substrates for the cellulolytic activity assay was carboxy methyl celulose (CMC) which dispersed in water, the hydrophilic moieties of CMC absorb the water absorbing, then swelling, so the viscosity increased. The efficient enzymatic degradation of insoluble polysaccharides, such as cellulose, requires a tight interaction between the enzymes and substrates. In the cellulose degradation, many cellulases are known to bind to crystalline and/ or amorphous cellulose via cellulose binding domains (CBDs) which are distinct from the catalytic domains (Aderemi *et al.*, 2008).

The cellulase enzyme of *G. tropicum* is more active than *G.applanatum* at pH 3.5 - 10.0, but at pH 7.0, both enzymes have equal activity (Fig. 3). We suggested that the cellulase enzyme of *G. tropicum* is more stable to pH alteration compared to *G. applanatum*. The unfavorable pH may result in reduced enzyme activities by reducing accessibility of the substrate (Bakri *et al.*, 2008).

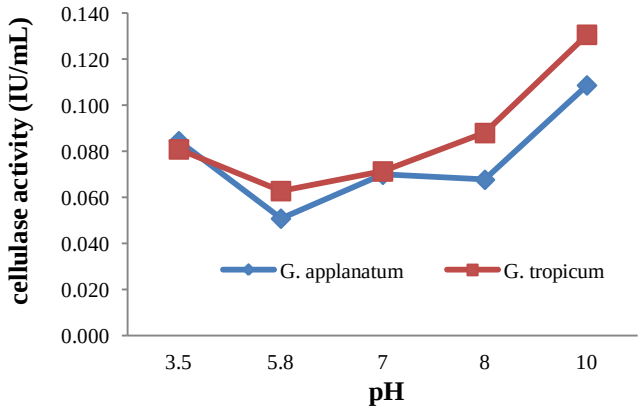

Fig. 3: Cellulolytic activity in various pH.

The cellulase enzyme of *G. applanatum* is more active than *G. tropicum* at temperature of 25-65 °C, but at 35 °C, both enzyme have equal activity (Fig. 4). We suggested that the cellulase enzymes of *G. applanatum* more stable to temperature alteration compared to *G. tropicum*. The cellulases catalytic activity is inhibited at higher temperatures. Increasing temperature was supposed to denature and unfold the proteins, thus may cause two different effects, i.e. more ionic groups of the proteins become exposed to the surface and are available to dye fixation dan more tryptophan residues formerly hidden inside the 3D-structure are revealed and thus may lead to an increase in hydrophobic interactions between protein and cellulose. Higher temperatures (above 30 °C) alter the cell membrane composition and stimulate protein catabolism, causing cell death (da Silva *et al.*, 2005).

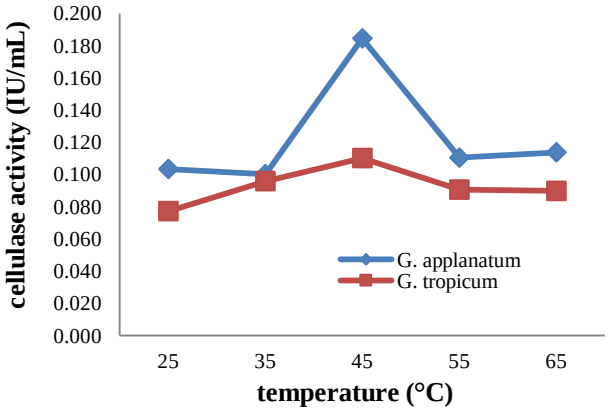

Fig. 4: Cellulolytic activity in various temperature.

Statistical Analysis

There was statistically significant on temperature (P = 0.0064) and pH (P = 0.0087), but there was no statistically significant on *Ganoderma* species for various temperature (P = 0.1576) and various pH (P = 0.1081).

CONCLUSION

The activity of cellulase enzymes were affected by temperature and pH. The cellulolytic activity of *G. applanatum* was the same as *G. tropicum*.

ACKNOWLEDGEMENTS

We are thankful to Indra Permana and Sugiarto for their technical support.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

REFERENCE

Aderemi BO, Abu E, Highina BK. The kinetics of glucose production from rice straw by *Aspergillus niger*. African J Biotech, 2008; 7:1745-52.

Bakri Y, Jawahar M, Arabi MIE. Improvement of xylanase production by *Cochliobus sativus* in submerged culture. Food Technol Biotech, 2008; 46:116-18.

Benfeld P. Amylases α and β. Methods Enzymol, 1955; 1:149-58.

Chakraborty N, Sarkar GM, Lahiri S. Cellulose degrading capabilities of cellulolytic bacteria isolated from the intestinal fluids of the silver cricket. Enviromentalist, 2000; 20:9-11.

da Silva R, Lago ES, Merheb CW, Macchione MM, Park YK, Gomes E. Production of xylanase and CMCase on solid state fermentation in different residues by *Thermoascus aurantiacus miehe*. Brazilian J Microbiol, 2005; 36:235-41.

Hepting GH. 1971. Diseases of forest and shade trees of the United States. In: Flood J, Bridge PD, Holderness M, eds. *Ganoderma* diseases of perennial crops. UK: CABI Publisher.

ICH. 2005. Validation of analytical procedures: text and methodology Q2(R1). In: Guideline ICH Harmonized Tripartite, ed. International Conference on Harmonisation of Technical Requirements for registration of pharmaceuticals for human use. Geneva.

Lu WJ, Wang HT, Nie YF. Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. J Environmental Science and Health Part B, 2004; 39:871-87.

Miller GL. Use of dinitrosalycylic acid reagent for determination of reducing sugar. Anal Chem, 1959; 31:426.

Miloski K, Wallace K, Fenger A, Bendinkas ESK. Comparison of biochemical and chemical digestion and detection methods for carbohydrates. Am J Undergrad Res, 2008; 7:7-19.

Mizuno T. Bioactive biomolecules of mushrooms: food function and medicinal effect of mushroom fungi. Food Rev Int, 1995; 11: 7-21.

Mskawa AY, Magan N. Wood degradation, and cellulase and ligninase production, by Trametes and other wood-inhabiting basidiomycetes from indigenous forests of Zimbabwe. Microbiol Study, 1998; 102:1399-404.

Paterson RRM. Ganoderna: a therapeutic fungal biofactory. Phytochem, 2006; 67:1985-2001.

Pilotti CA, Sanderson FR, Aitken AB, Armstrong W. Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. Mycopathologia, 2004; 158:251-65.

Ryu DDY, Mandels M. Cellulases: biosynthesis and applications. Enzyme and Microbial Techn, 1980; 2:91-102.

Shewale JG. Glucosidase: its role in cellulase synthesis and hydrolysis of cellulose. Int J Biochem, 1982; 14:435-43.

Singh SK, Doshi A, Pancholy A, Pathak R. Biodiversity in wood-decay macro-fungi associated with declining arid zone trees of India as revealed by nuclear rDNA analysis. Eur J Plant Pathol, 2013: 1-10.

Turner PD. 1981. Oil plam diseases and disorders. UK: Oxford University Press.

Vaithanomsat P, Chuichulcherm S, Apiwatanapiwat W. Bioethanol production from enzymatically saccharified sunflower stalks using steam explotion as pretreatment. Proceeding of World Academy of Science, Engineering and Technology, 2009; 37:140-3.

Wong CL, Bong JFC, Idris AS. Ganoderma species associated with basal stem root disaese of oil palm, Am J App Sci, 2012; 9:879-85.

How to cite this article:

Saptarini NM, Hadisoebroto G. The Activity of Cellulase Enzymes of *Ganoderma applanatum* and *Ganoderma tropicum*. J App Pharm Sci, 2017; 7 (04): 134-137.