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β-glucosidase enzyme screening from various parts of *Tabebuia argentea*

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Abstract

The aim of this work was to search for the novel β–glucosidase enzyme from various parts of *Tabebuia argentea*; flower, flower bud, shoot, seed shank and seed. The enzyme was extracted from the samples by using appropriated buffer. The ammonium sulfate ($(NH_4)_2SO_4$) salt precipitation in the different salt concentrations was used to initial fractionated purification steps. The monitoring of enzyme activity was carried out by using hydrolysis reaction of glycosidic bond using *p*-nitrophenyl-D-glucopyranoside (*p*NPG) as an enzyme substrate. The UV-vis spectroscopy was used to detect the corresponding *p*-nitrophenylate product under basic condition at 405 nm. The enzyme activity via *p*NPG hydrolysis of seeds extract was around 15-fold over the other plant part extracts of *Tabebuia argentea*. This was followed by selected enzyme fraction of the seeds extract subjected to optimum temperature study and temperature for the best enzyme activity was 30-40 °C. The highest enzyme activity fraction will be used for further purification and enzymatic properties test before application as a biocatalyst in biological process.

Keywords: β–glucosidase, *Tabebuia argentea*, *p*-nitrophenyl-D-glucopyranoside

1. Introduction

The glycoside hydrolase enzymes (EC 3.2) assist in hydrolysis reaction of glycosidic linkage in complex sugars (i.e., the disaccharide, oligosaccharide, polysaccharides, cellobiose, cellulose and other carbohydrates) (Andrade Pinto, De Souza, & Oliveira, 2010) with release of glucose molecules and the corresponding products. The β-glucosidase enzymes are a group of enzymes that catalyze specific bonds within naturally occurring biopolymers composed of beta-1, 4-linked glucosyl residues. Normally, β-glocosidases are found in several sources; many parts of plant, fungi, bacteria, animal, and human. They appear to differ in their specificity for β-glycosidic bond of glucosyl group and aryl-or alkyl-group. The β-glucosidases (3.2.1.21) play important roles in many of biological processes, such as growth regulation and development, lignification, phytohormone activation, cell wall degradations, defense mechanisms, and release aromatic compounds such as saponin, coumarin, quinones, stilbenoid, flavonoid etc. in plants. (Cheung, & Anderson, 1997; Dharmawardhama, Ellis, & Carson, 1995; Duroux, Delmotte, Lancelin, Keravis, and Jay-Allemand, 1998; Gírio, Fonseca, Carvalheiro, Marques and Bogel-Łukasik, 2010; Guo-Yong, Xiaonan, Binbin, Rui and Minjian, 2014)

The novel β-glucosidase enzymes from Thai plants were revealed, such as rice β-glucosidase (*Oryza sativa*), dalcochinase from Thai rosewood (*Dalbergia cochinchinensis*), cassava linamarase isolated from Cassava (*Manihot esculenta* Crantz) [7-11] and the β-glucosidase isolated from hard seed coat of Prunes (*Prunus domestica*) which is a glucose tolerance enzyme (Morant, Jorgensen, Jorgensen, & Paquette, 2008). The advantages of β-glocosidase enzymes are that they are used as catalysts in many biological processes for ethanol production via hydrolysis of lignocellulosic to sugar, followed by fermentation together with other enzymes (Nisius, 1988; Opassiri et al., 2003; Opassiri et al., 2004) The conventional method to screen is by the detection of *p*-nitrophenolate quantity released from the substrate *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) hydrolysis from β-glucosidase activity measurement under basic condition (**Figure 1**) by using UV-vis spectroscopic technique.

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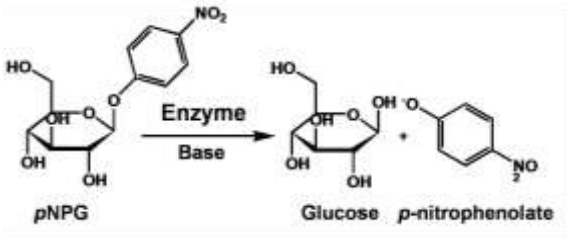

Figure 1. Enzyme activity assay by using the hydrolysis reaction of *p*NPG.

As we know, many reports of plants from *Tabebuia*

genus show that the most abundant chemical constituents are saponin, coumarin, quinones, stilbenoid, and flavonoid connected to glucopyranoside. Some of the plant extracts are used for parasite control (Hari Babu et al. 2010; Hemamalini, & Sambasiva Rao, 2014; Hommalai, Chaiyen, & Svasti, 2005; Zechel & Wihers, 2000). In this report, we screened the enzyme from various parts of *Tabebuia argentea*; flowers, flower buds, shoots, seed shanks and seeds by using *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) as an enzyme substrate. The appropriated fraction was selected for further study.

2. Materials and Methods

2.1. Materials

The chemicals used for this study were obtained from commercial suppliers and used without further purification. Double-distilled water was used in all experiments. Various parts of *Tabebuia argentea*; flowers, flower buds, shoots, seed shanks and seeds (**Figure 2**) were collected in March-April 2016 from Wangnoi district, Phra NakhonSi Ayutthaya province, Thailand. UV-vis absorption spectra were recorded on an Agilant89090A spectrophotometer.

2.2. Enzyme extraction

50 g each of *Tabebuia argentea* parts were collected and washed before the enzyme extraction, excluding seeds that had to be soaked for 24 hours before the extracts were done. For β-glucosidase enzyme, extraction was determined as follows: 50 g of each sample was blended with 400 ml of 0.1 M cold sodium acetate buffer (pH 5.5) in the presence of 0.5 µM phenylmethyl sulfonyl fluoride (PMSF) as a protease inhibitor. The homogenizing solution was kept on ice before centrifugation at 7500 rpm. Supernatant of each fraction was collected for ammonium sulfate precipitation at 4 °C before use.

2.3. Ammonium sulfate precipitation

Ammonium sulfate salt was grinded before gently adding to supernatant of each part extract using concentration 0-30 % w/w $(NH_4)_2SO_4$ salt. Precipitation was allowed to form for 45 min at 4°C with stirring, the completely precipitated solution was centrifuged at 7500 rpm at 4°C for 30 min. The supernatant was further added to the $(NH_4)_2SO_4$ salt powder slowly but steadily with thorough mixing until total concentration of 60 % w/w. The mixed solution was also stirred and left on ice for 45 min. Then solution was centrifuged at the same speed at 4°C for 30 min. The 2 desalted fractions of each sample were dissolved in 0.1 M cold sodium acetate buffer (pH 5.5) and collected at 4°C until used for activity assay and protein determination.

2.4. Enzyme activity assay

The 10 fractions were exchanged to the appropriated buffer. β-glucosidase activity assay is the reaction of the release of *p*-nitrophenolate by hydrolysis of *p*-nitrophenyl glucopyranoside (*p*-NPG) substrate under basic condition of 2 M Na_2CO_3 solution. The reaction mixture (total volume 1 ml) for activity assay containing enzyme solution (50 µl) and phosphate buffer pH 6.5 (650 µl) was pre-incubated at 37°C. The enzyme activity of each fraction occurred after adding 300 µl of 50 mM *p*-NPG as substrate, follow by further incubation for 10 min. The enzymatic reaction was stopped by adding 2 ml of 2 M Na_2CO_3 solution. The absorbance (A) of each activity assay fraction was monitored at λ = 405 nm, followed by the comparison to *p*-nitrophenolate calibration curve. The highest active fraction was selected for optimum temperature study (25- 50°C) of the enzyme by using the same condition.

2.5. Protein determination

The protein concentration determination; 200 µl biuret reagent and 800 µl protein solution were mixed, followed by incubation at room temperature for 25 min and the absorbance at 540 nm was measured against the blank reagent, which contained the same volume of distilled water instead of protein solution. The protein concentrations were determined from a calibration curve generated using 0-10 mg bovine serum

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albumin (BSA) as a protein standard.

Figure 2. Various parts of *Tabebuia argentea* a) flower buds b) flowers c) shoots d) seed shanks and e) seeds

3. Result and Discussion

The protein solution was initial purified by the steps of ammonium sulfate precipitation. The fractions of 5 parts were as follows; concentration 0-30 and 30-60 %w/w $(NH_4)_2SO_4$. The methods separated according to the ionic strength of the solution and salt concentration, the results as shown in **Table 1** and **Figure 3** The β-glucosidase activity was determined using activity assay as explained in the above. All fractions from various parts showed higher activity in the present of salt concentration 0-30 than 30-60 %w/w $(NH_4)_2SO_4$, except in the fraction of 30-60 %w/w $(NH_4)_2SO_4$ precipitation of seeds extract showed the highest activity assay and protein determination (data not show).

Table 1. The enzyme activity from various parts of *Tabebuia argentea* 30-60 % (red) w/w

Parts of Tabebuia argentea	Abs	β-glucosidase μmol/50μL
shoots	0.12	16.7±0.20
flower buds	0.02	4.3±0.10
flowers	0.01	3.0±0.10
seed shanks	0.01	3.3±0.08
seeds	0.40	51.5±0.81

The protein solution showed higher of the activity assay about 15-fold over other fractions, which displayed medium level of the protein concentration. It is indicated that there are more enzymes active to *p*NPG substrate in seed part than others like most β-glucosidase enzymes extracted from plant. The shoot part of *Tabebuia argentea* showed medium of the activity in both concentrations, it can be studied by adjusting the salt precipitation process before future purification.

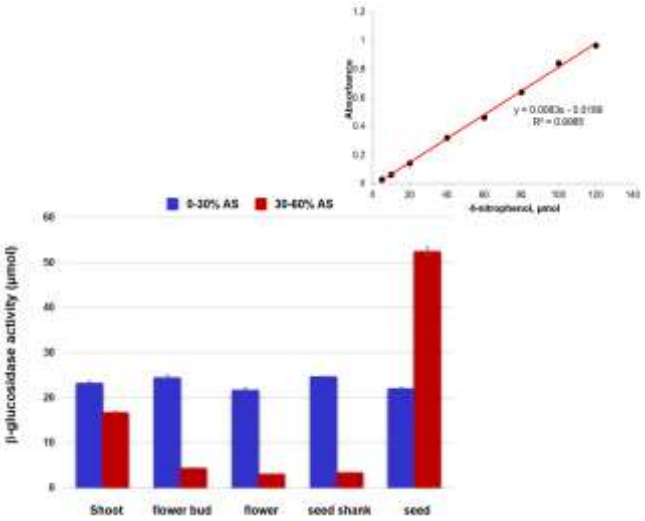

Figure 3. The enzyme activity from various parts of *Tabebuia argentea* salt 0-30 % (deep blue) and 30-60 % (red) w/w $(NH_4)_2SO_4$. Insert; change of 4-nitrophenol absorption intensity at 405 nm.

To further demonstrate its activity, the highest active fraction of 30-60% $(NH_4)_2SO_4$ salt precipitation from seeds extract was selected for optimum temperature study. The appropriated reaction of enzyme activity assay by using *p*NPG as substrate was carried out. The enzyme activity in different temperature 25, 30, 35, 40, 45, and 50°C was revealed. The results showed that at low temperature there was a weak UV-vis signal with little activity, similar to reactions at temperatures above 40 °C. The enhanced signal was observed in a temperature range of 30-35°C (**Figure 4**), the optimum temperature for this enzyme activity on *p*NPG is 30 °C. It is obvious that the appropriated condition for enzyme activity can be used in the range 30-35°C.

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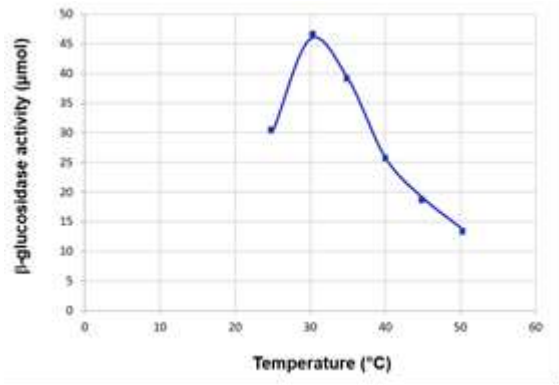

Figure 4. The optimum temperature of the enzyme from seeds extract at 25, 30, 35, 40, 45 and 50 °C.

4. Conclusions

In this study, we have demonstrated the activity assay and protein concentration diagram at different $(NH_4)_2SO_4$ salt concentrations; 0-30% and 30-60% extract from various parts of *Tabebuia argentea*. We selected the fractions from 30-60% $(NH_4)_2SO_4$ salt precipitation from seeds extract that showed the highest activity consistent to the protein concentration, with optimum temperature at which this enzyme responds to *p*-NPG is 30-35°C. This fraction was selected for future study; purification steps by using membrane cut- off, follow by column chromatographic techniques for further purification and characterization.

5. Acknowledgement

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