

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

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Abstract

The aim of this work is the screening for searching the novel β -glucosidase enzyme from various parts of *Tabebuia argentea*; flower, flower bud, shoot, seed shank and seed. The enzyme extracted from the samples by using appropriated buffer. The ammonium sulfate ((NH₄)₂SO₄) salt precipitation in the different salt concentration was used to initial fractionated purification steps. The monitoring of enzyme activity by using hydrolysis reaction of glycosidic bond by using *p*-nitrophenyl-D-glucopyranoside (*p*NPG) as an enzyme substrate. The UV-vis spectroscopy used to detect the corresponding *p*-nitrophenolate product under basic condition at 405 nm. The enzyme activity via *p*NPG hydrolysis of seeds extract responds toward around 15-fold over the other part extracts of *Tabebuia argentea*, follow by selected enzyme fraction of seeds extract to subject to optimum temperature study, the temperature for the best enzyme activity is 30-40 °C. The highest enzyme activity fraction will be used for further purification and enzymatic properties test before apply to be a biocatalyst in biological process.

Keywords: β -glucosidase, *Tabebuia aurea*, *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) [1] with release glucose molecules and the corresponding products. The β -glucosidase enzymes are the group of enzyme catalyze specific to the bond of a within naturally occurring biopolymer composed of beta-1, 4-linked glucosyl residues. Normally, β -glucosidases 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. [2-6]

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] The β -glucosidase isolated from hard seed coat

of Prunes (*Prunus domestica*) as glucose tolerance enzyme. [12] The advantages of β -glucosidase enzymes used as a catalyst in many biological processes for ethanol production via hydrolysis of lignocellulosic to sugar, followed by fermentation together with other enzymes. [13-15]

The conventional method to screen by the detection of *p*-nitrophenolate quantity released from the substrate *p*-nitrophenyl- β -D-glucopyranoside (*p*NPG) hydrolysis for β -glucosidase activity measurement under basic condition (Figure 1.) by using UV-vis spectroscopic technique.

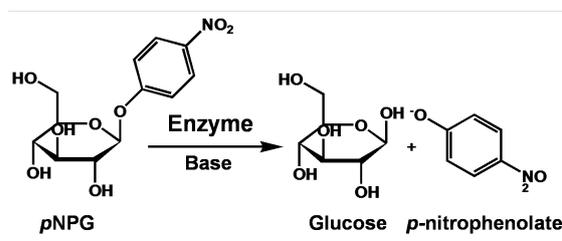


Figure 1. Enzyme activity assay by using the hydrolysis reaction of *p*NPG.

As we know that many reports for plants from *Tabebuia* genus found, the most of chemical constituents are saponin, coumarin, quinones, stilbenoid, and flavonoid connected to glucopyranoside, some of plant extract use for parasite control.^[16-20] 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 selected to further study.

2. Materials and methods

2.1. Materials

The chemicals used for this study obtained from commercial suppliers and used without further purification. Double-distilled water was used all experiments. Various parts of *Tabebuia argentea*; flowers, flower buds, shoots, seed shanks and seeds (**Figure 2**) were collected on March-April 2016 from Wangnoi district, Phra NakhonSi Ayutthaya province, Thailand. UV-vis absorption spectra recorded on an Agilent89090A spectrophotometer.

2.2. Enzyme extraction

Each *Tabebuia argentea* part for 50 g was collected and washed before the enzyme extraction. Exclude seeds that have to be soaked for 24 hours before the extracts are formed. 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 present of 0.5 μ M phenylmethyl sulfonyl fluoride (PMSF) as a protease inhibitor. The homogenizing solution 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 gentle adding to supernatant of each part extract using concentration 0-30 % w/w (NH₄)₂SO₄ salt. Allow precipitate 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 the (NH₄)₂SO₄ salt powder slowly but steadily with thorough mixing until total concentration to 60 % w/w. The mixed solution 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 using for activity assay and protein determination.



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

2.4. Enzyme activity assay

The 10 fractions were exchanged to the appropriated buffer. β -glucosidase activity assay is the reaction of the releasing of *p*-nitrophenolate by hydrolysis of *p*-nitrophenyl glucopyranoside (*p*-NPG) substrate under basic condition of 2 M Na₂CO₃ solution. The reaction mixture (total volume 1 ml) for activity assay containing enzyme solution (50 μ l) and phosphate buffer pH 6.5 (650 μ l) was pre-incubated at 37°C. The enzyme activity of each fraction occurred after adding 300 μ l of 50 mM *p*-NPG as substrate, follow by further incubated for 10 min. The enzymatic reaction stopped by adding 2 ml of 2 M Na₂CO₃ solution. The absorbance (A) of each activity assay fraction was monitored at $\lambda = 405$ nm, followed by the comparison to *p*-nitrophenolate calibration curve. The highest active fraction 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 μ l biuret reagent and 800 μ l protein solution was mixed, followed by incubated 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 albumin (BSA) as a protein standard.

3. Result and Discussion

The protein solution was initial purified by the steps of ammonium sulfate precipitation. The fractions of 5 parts as follow; concentration 0-30 and 30-60 %w/w $(\text{NH}_4)_2\text{SO}_4$. 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 $(\text{NH}_4)_2\text{SO}_4$, except in the fraction of 30-60 %w/w $(\text{NH}_4)_2\text{SO}_4$ 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 <i>Tabebuia argentea</i>	Abs	β -glucosidase $\mu\text{mol}/50\mu\text{L}$
shoots	0.12	16.7 \pm 0.20
flower buds	0.02	4.3 \pm 0.10
flowers	0.01	3.0 \pm 0.10
seed shanks	0.01	3.3 \pm 0.08
seeds	0.40	51.5 \pm 0.81

The protein solution showed higher of the activity assay about 15-fold over other fractions, which was displayed medium level of the protein concentration. It's 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.

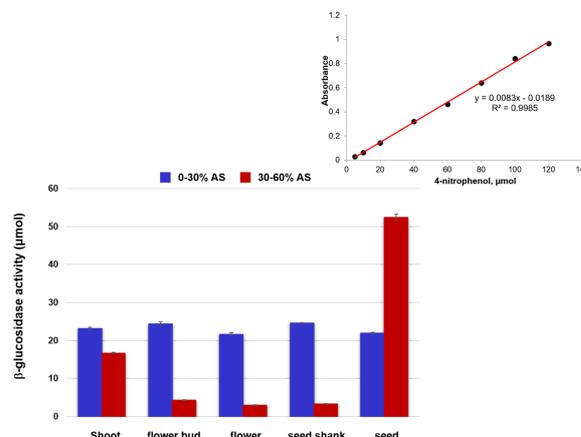


Figure 3. The enzyme activity from various parts of *Tabebuia argentea* salt 0-30 % (deep blue) and 30-60 % (red) w/w $(\text{NH}_4)_2\text{SO}_4$. Insert; change of 4-nitrophenol absorption intensity at 405 nm.

To further demonstrate its activity, the highest active fraction of 30-60% $(\text{NH}_4)_2\text{SO}_4$ 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 occurred. The enzyme activity in different temperature 25, 30, 35, 40, 45, and 50°C was revealed. The results showed that at low temperature showed a weak UV-vis signal with a small activity, also like reaction at temperatures above 40 °C. The enhance signal was observed in a temperature range of 30-35°C (**Figure 4**), the optimum temperature for this enzyme responds to *p*NPG is 30 °C. It is obvious that the appropriated condition for enzyme activity can be used in range 30-35°C.

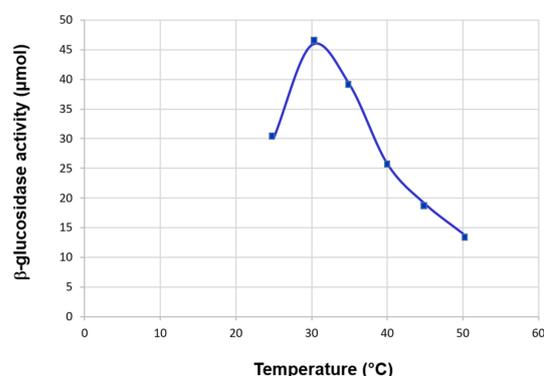


Figure 4. The optimum temperature of the enzyme from seeds extract at 25, 30, 35, 40, 45 and 50 °C.

4. Conclusions

In this study, we have demonstrated that the activity assay and protein concentration diagram at different $(\text{NH}_4)_2\text{SO}_4$ salt concentrations; 0-30% and 30-60% extract from various parts of *Tabebuia argentea*. We selected the fractions from 30-60% $(\text{NH}_4)_2\text{SO}_4$ salt precipitation from seeds extract that showed the highest activity consistent to the protein concentration with optimum temperature for 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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6. References

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