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EDITORIAL

It is my proud privilege to welcome you all to the Researchfora International Conference at Hamburg, Germany in association with The IIER. I am happy to see the papers from all part of the world and some of the best paper published in this proceedings. This proceeding brings out the various Research papers from diverse areas of Science, Engineering, Technology and Management. This platform is intended to provide a platform for researchers, educators and professionals to present their discoveries and innovative practice and to explore future trends and applications in the field Science and Engineering. However, this conference will also provide a forum for dissemination of knowledge on both theoretical and applied research on the above said area with an ultimate aim to bridge the gap between these coherent disciplines of knowledge. Thus the forum accelerates the trend of development of technology for next generation. Our goal is to make the Conference proceedings useful and interesting to audiences involved in research in these areas, as well as to those involved in design, implementation and operation, to achieve the goal.

I once again give thanks to the Institute of Research and Journals, Researchfora, TheIIER for organizing this event in Hamburg, Germany. I am sure the contributions by the authors shall add value to the research community. I also thank all the International Advisory members and Reviewers for making this event a Successful one.

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B-GLUCOSIDASE ENZYME SCREENING FROM SHOOT OF TABEBUIA ARGENTEA

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Abstract - This work aims to purify the novel β -glucosidase enzyme from shoot of *Tabebuia argentea*. The enzyme extracted by using acetate buffer pH 5.5 from the fresh sample. The fractionation using for preliminary purification by ammonium sulfate ((NH₄)₂SO₄) salt precipitation in the different salt concentration (0-30%, 30-60%, and >60%). The enzyme activity was measured under basic condition by using hydrolysis reaction of glycosidic bond by using p-nitrophenyl-D-glucopyranoside (pNPG) as an enzyme substrate. The λ_{max} of corresponding p-nitrophenolate product under basic condition was detected at 405 nm. The enzyme activity via pNPG hydrolysis of seeds extract responds toward around 10-fold over the other part extracts of *Tabebuia argentea*, follow by selected enzyme fraction of shoot extract to subject to further chromatographic purification and enzymatic properties test before apply to be a biocatalyst in biological process.

Index Terms - β -glucosidase, shoot of *Tabebuia aurea*, p-nitrophenyl-D-glucopyranoside

I. INTRODUCTION

The group of hydrolase enzymes (EC 3.2) catalyze the hydrolysis reaction of glycosidic bond between plant carbohydrate (i.e., polysaccharides, cellulose, cellobiose and other carbohydrates) [1] or sugar and other complex molecules with release glucose molecules and the corresponding products. The β -glucosidases are the group of specific enzyme cut to the bond of a within naturally occurring biopolymer composed of beta-1, 4-linked glucosyl residues. Generally, β -glucosidases found in several sources; animal, plant, fungi, bacteria, and human. The β -glucosidases (3.2.1.21) play important roles in many of biological processes, such as growth regulation and development, cell wall degradations, phytohormone activation, lignification, defense mechanisms, and release aromatic compounds such as saponin, coumarin, quinones, stilbenoid, etc. in plants [2-6].

Many β -glucosidase enzymes from Thai plants were studied, such as rice β -glucosidase (*Oryza sativa*), cassava linamarase isolated from Cassava (*Manihot esculenta* Crantz), dalcochinase from Thai rosewood (*Dalbergia cochinchinensis*) [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 reaction of p-nitrophenyl- β -D-glucopyranoside (pNPG) as a substrate and plant extract in an appropriated buffer to yield to p-nitrophenolate released as a hydrolysis product for β -glucosidase activity measurement under basic condition (Fig. 1.) by using UV-vis spectroscopic technique.

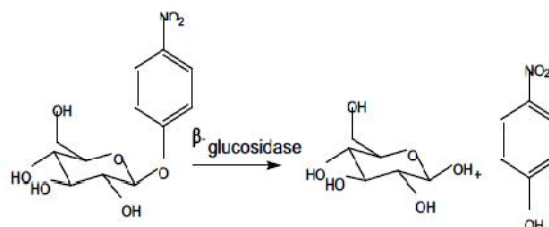


Fig. 1. The reaction of β -glucosidase screening by using pNPG as a substrate

Here in, we select the shoot part of *Tabebuia argentea* that can be found easier other parts to first study, even though the enzyme activity of seeds is high over other parts. The condition to purify β -glucosidase from *Tabebuia argentea* shoot was optimized from the previous study [16].

II. METHODS

A. materials

The chemicals used for this study obtained from commercial suppliers and used without further purification. Double-distilled water was used all experiments. Shoots of *Tabebuia argentea* (Fig. 2.) were collected on April 2016 from Wangnoi district, Phra NakhonSi Ayutthaya province, Thailand. UV-vis absorption spectra recorded on an Agilent89090A spectrophotometer.

B. Enzyme extraction

Shoot of *Tabebuia argentea* for 200 g was collected and washed before the enzyme extraction. For β -glucosidase enzyme extraction was determined as follows: water was removed from each sample then 1000 ml of 0.1 M cold sodium acetate buffer (pH 5.5) in the present of 0.5 μ M protease inhibitor phenylmethyl sulfonyl fluoride (PMSF) was mixed before blending. The appropriated buffer kept on ice

before centrifugation at 8000 rpm. Supernatant of each fraction was collected for ammonium sulfate precipitation at 4 °C before use.



Fig. 2. Shoots of *Tabebuia argentea*

C. Ammonium sulfate precipitation

Ammonium sulfate salt was grinded before gentle adding to supernatant of shoot extract using concentration 0-30 % w/w $(\text{NH}_4)_2\text{SO}_4$ salt (stirring on ice for 45 min). The precipitated solution was centrifuged at 8000 rpm at 4 °C for 30 min. The supernatant was further precipitated until total salt concentration to 60 % w/w. The mixed solution also stirred and left on ice for 45 min. The precipitated solution was centrifuged at 8000 rpm at 4 °C for 30 min. The three desalted of enzyme extractions, 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.

D. Enzyme activity assay

The three fractions were exchanged to the appropriated buffer. The activity assay of β -glucosidase enzyme is the reaction of the releasing of p-nitrophenolate by hydrolysis of p-nitrophenyl glucopyranoside (p-NPG) substrate under basic condition (2 M Na_2CO_3 solution). The reaction mixture (total volume 2 ml) for activity assay containing enzyme solution (100 μl) and phosphate buffer pH 6.5 (1300 μl) was pre-incubated. The enzyme activity of each fraction occurred after adding 600 μl of 50 mM p-NPG as substrate, follow by further incubated for 15 min. The enzymatic reaction stopped by adding 4 ml of 2 M Na_2CO_3 solution. The absorbance (A) of each activity assay fraction was monitored at $\lambda=400$ nm, followed by the comparison to p-nitrophenolate standard curve. The highest active fraction selected for optimum temperature study of the enzyme by using the same condition.

E. Protein determination

The determination of protein concentration; 200 μl biuret reagent and 800 μl 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 standard curve of various protein concentrations were

determined by using bovine serum albumin (BSA) as a protein standard (0-10 mg).

III. RESULTS AND DISCUSSIONS

The protein solution was initial purified by the steps of ammonium sulfate precipitation. The 3 fractions of shoot parts as follow; concentration 0-30% and 30-60 % and >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 I. The β -glucosidase activity was determined the amount of p-nitrophenolate released as a hydrolysis product for the activity assay as explained in the above. The fractions from shoot part of *Tabebuia argentea* showed higher activity in the present of salt concentration 0-30 than 30-60 %w/w $(\text{NH}_4)_2\text{SO}_4$. The protein solution showed higher of the activity assay about 8-fold over other fractions, which was displayed medium level of the protein concentration. The protein concentrations of 3 fractions were determined as describe in the previous, the protein concentration showed the high value in fraction 1) and 2) (data not show).

	% salt		
	1) 0-30%	2) 30-60%	3) >60%
Abs	0.02	0.20	0.01
β -glucosidase $\mu\text{mol}/50\mu\text{l}$	4.3	25.72	3.3

TABLE I: ENZYME ACTIVITY OF β -GLUCOSIDASE FROM SHOOT OF *TABEBUIA ARGENTEA*

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 reaction of 50 mM p-NPG as substrate and enzyme extract solution (100 μl) phosphate buffer pH 6.5 (1300 μl) was incubated for 10 min. The enzymatic reaction quenched by adding 4 ml of 2 M Na_2CO_3 solution. The absorbance (A) of each activity assay fraction was monitored at $\lambda=400$ nm. The results (Fig.3) showed that optimum temperature for this enzyme responds to p-NPG is 35 °C.

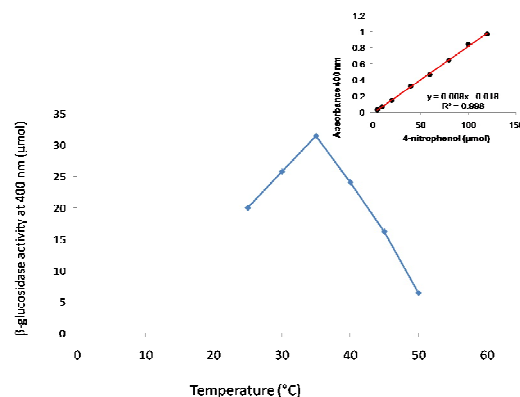


Fig. 3. The optimum temperature of the enzyme from shoot extract at 25, 30, 35, 40, 45, and 50 °C. The Insert; the calibration curve of enzyme activity at 400 nm.

CONCLUSION

In this study, we have demonstrated that the activity assay at different $(\text{NH}_4)_2\text{SO}_4$ salt concentrations; 0-30% * 30-60% and >60% extract from shoot parts of *Tabebuia argentea*. We selected the fractions from 30-60% $(\text{NH}_4)_2\text{SO}_4$ salt precipitation, which showed the highest activity consistent to the protein concentration with optimum temperature for this enzyme responds to p-NPG is 35 °C. This fraction was selected for future study; purification steps by using membrane cut-off, follow by column chromatographic techniques for further characterization.

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