Green Chemistry Glucose Biosensor Development using *Etlingera elatior* Extract

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Abstract. Glucose biosensor development is one of the important strategies for early detection of diabetes mellitus disease. This study was aimed to explore the flower extract of *Etlingera elatior* for a green-analysis method of glucose biosensor. Flowers were extracted using ethanol: HCl and tested its performances as an indicator of glucose biosensor using glucose oxidase enzyme. The glucose oxidase react with glucose resulted hydrogen peroxide that would change the color of the flower extract. Furthermore, the extract was also studied including their stability to pH, oxidizing and reducing, temperature, and storage. The results showed that the *Etlingera elatior* extract had high correlation between color change and glucose concentration with regression equation of $y = -0.0005x + 0.4724$ and $R^2$ of 0.9965. The studied biosensor showed a wide linear range to detect glucose sample of 0 to 500 mM. The extract characterization showed a more stable in low pH (acid), reducing agent addition, heating treatment and storage.

1. Introduction

Diabetes mellitus (DM) is a group of carbohydrate metabolic disorder with a high blood glucose level. According to World Health Organization (WHO) (2016), there are 422 million people with diabetes worldwide, which was quadrupled since 1980 [1]. The DM complication lead to heart attack, blindness, kidney failure, lower limb amputation and stroke, which caused 1.5 million death in 2012. Indonesia is in the list of the 10 countries to have the highest number of diabetes, with diabetics of 8.4 million in 2000 and estimated to be 21.3 million diabetics in 2030 [1]. However, many people are not aware that they are living with pre-diabetes [2]. The diabetic patients are generally meet their physician in serious health complications. In this situation, it is very important to early detect diabetes.

The most popular in the diabetes early diagnostic is using a glucose biosensor, which is an analytical device in the combination of biological sensing elements and a transducer. Glucose biosensor was widely available on the market compare to other biosensor application. Improving the glucose biosensor remains interesting research topics to improve the performance [3], reduce the cost [4][5] or environment friendly method development in the analytical chemistry studies. The development of biosensors could be performed in the detection principles or the instrumentations.
On the other hand, the development of analytical technique should also consider the green chemistry for sustainable development. There are twelve principles in green analytical chemistry \[6\], which one of them would be applied in this study. The use of flower extract as biosensor colorimetric indicator was in the term from the ten list of “reagent obtained from renewable source should be preferred”. Numerous study have been reported in the green analytical chemistry development such as solventless extraction \[7\], flower extract for nanoparticle preparation \[8\], eco-friendly enzyme \[9\] and non-toxic colorant \[10\]. In this study glucose detection was based on the reactions of glucose with glucose oxidase to produce gluconolactone and hydrogen peroxide (H$_2$O$_2$). The resulted hydrogen peroxide was then reacted with the \textit{Etlingera elatior} flower extract to obtain the color change related to the glucose concentration. The performance of the \textit{Etlingera elatior} flower extract stability were also studied.

2. Materials and Methods

2.1. Materials

\textit{Etlingera elatior} was obtained from local market, which further was identified the taxonomy at the Biology Laboratorium of Universitas Jenderal Soedirman. Ethanol, hydrochloric acid, citric acid, acetic acid, sodium acetate, sodium dihydrogen phosphate, di-sodium hydrogen phosphate, hydrogen peroxide, and ascorbic acid were from E-Merck (Germany). Glucose Oxidase from Aspergillus niger (Type VII, lyophilized powder, $\geq$100,000 units/g, Sigma Aldrich)

2.2. Apparatus and measurements

Spectrophotometer (Shimadzu UV-1800) was used to measure the color change of the reactions. Temperature effect was used hotplate stirrer (Thermoline, Cimarec C), pH meter (Hanna instruments) was used to measure the pH of the solution. General laboratory instruments and glassware were also used in this research.

2.3. \textit{Etlingera elatior} flower extraction

The \textit{Etlingera elatior} flower was separated from their stalk, clean with water and grinded using blender. Solvent of mix ethanol 96% and HCl 1.5N (85:15) was then added to the flower (20 g flower / 100 mL solvent), mixed for 1 h and precipitated for 30 min. The filtrate was then separated using filter paper and keep in dark place at 40°C when it was not in use. The flower extract was measured the absorbance using spectrophotometer, scanned at 400 – 700 nm. The extract was adjusted with the solvent to get the absorbance of about 0.8.

2.4. Effect of pH

Flower extract color was studied at various pH of 3.0 to 9.0 by diluting 10 mL of extract with 100 mL of buffer solution of the corresponding pH. The dilution of the extract was based on the previous procedure (2.1). Absorbance change with the pH of the buffer solvent were then recorded at 400 – 700 nm.

2.5. Effect of oxidizing and reducing agent

The study was performed at pH of 7.0, with the addition of 0.1 M hydrogen peroxide (0.1 mL/mL extract) as oxidizing agent and ascorbic acid (5 mg/mL) as reducing agent. The color change with the oxidizing or reducing agent was studied in various incubation time, before the extract oxidized or reduced to a colorless solution.

2.6. Effect of heat treatment

Extract of 10 mL was placed in well capped bottle to avoid the solvent to evaporate during heat treatment. The extract was then kept in the water bath at 40°C and 60°C for one and two hours. The color change was measured using spectrophotometer at 400-700 nm.
2.7. Effect of storage on extract decolorization

The effect of storage was performed by storing the concentrated filtrate at 4°C in a dark bottle for a week. The color change was studied every day at 400-700 nm, by diluting the concentrated extract using pure water.

2.8. Glucose biosensor indicator study using Etlingera elatior extract

The performance of the flower extract as indicator of glucose biosensor have been tested using a standard glucose solution and glucose oxidase enzyme. Series glucose solution as samples have been prepared using phosphate buffer of pH 7.0. Five ml glucose solution was prepared in test tube, added 10 unit glucose oxidase enzyme and incubated in room temperature for 15 minutes. One ml flower extract was then added to the glucose – enzyme solution. The color change related to glucose concentration was measured using spectrophotometer at 400-700 nm.

3. Results and Discussion

3.1. Etlingera elatior flower extraction

The collected Etlingera elatior flower was confirmed that, the flower was Etlingera elatior, family of Zingiberaceae, with the local name of “kocombrang” (Fig 1).

![Figure 1. Etlingera elatior flower used as glucose biosensor color indicator](image)

Measurement of flower extract was first diluted using the solvent for ten times to rich the good absorbance (0.2 to 0.8). The spectrum showed one peak at 523 nm. The visual color of the extract was purplish red, could be a 3-glycoside anthocyanidin, which was similar to the spectral characteristic of anthocyanin showed absorbance peak at 475-560 nm [11].

3.2. Effect of pH

Anthocyanin was the major compound in the flower extract as biosensor indicator studied. In the solution, anthocyanin could be transform into several ion form, depend on the pH of the solution. In this work, the extract color was studied in the range pH of 3.0 to 9.0 would cover acidic, neutral and alkaline condition. The result (Fig. 2) showed the decreasing of absorbance from pH 3.0 to 5.0 may due to the structure change of anthocyanin with the pH which could be in a blue quinoidal base, red flavylvium cation or colorless carbinol pseudobase and calcene [12]. In the plant extract are consist of peroxidase and polyphenolase. These two enzyme were optimum at pH of 6-7 [13], catalyze the enzymatic browning which may involve in the increasing of absorbance at pH 5.0 to 6.0. The higher pH absorbance at the higher pH could also due to the structural change with the pH.
3.3. Effect of oxidizing and reducing agent

This study was to measure the color change of the extract by the addition of ascorbic acid as an example of reducing agent and hydrogen peroxide as oxidizing agent. The result show that the addition of reducing agent did not effect to the color of flower extract up to 120 minutes incubation, whereas the addition of oxidizing agent of hydrogen peroxide was decrease the flower extract color with the incubation time (Fig. 3). The reducing of extract absorbance may due to the oxidizing agent attack the red color flavylum to lose their proton, resulted colorless solution [14], and the redox reaction of anthocyanin are irreversible [15].

![Figure 2. Effect of pH on the Etlingera elatior flower absorbance](image1.png)

![Figure 3. Effect of oxidizing (hydrogen peroxide) and reducing (ascorbic acid) agent on the Etlingera elatior flower absorbance with the incubation duration](image2.png)
3.4. Effect of heat treatment

Temperature is one of important factor of anthocyanin color stability, both during their using and storing. In this work, flower extract has been treated at 40 °C and 60 °C for one and two hours, in the closed dark containers. The result (Fig. 4) showed the higher temperature and longer incubation time would decrease the extract absorbance. The anthocyanin content in the plant extract would decreased with the increasing of temperature [16] and also the absorbance decreasing showed the anthocyanin degradation which was related to the increasing of the temperature [17][18].

![Figure 4. Effect of temperature and heat treatment duration (blue = 1 hr, Red = 2 hrs) to the extract absorbance](image)

3.5. Effect of storage on extract color stability

*Etlingera elatior* extract contains of anthocyanin, pigments generally found in plants. The anthocyanin color and stability during storage are depend on the temperature and storage duration. In this work, the extract stability was studied in the room temperature and refrigerator temperature (4 °C) for one to seven days. The results showed that the extract remained their absorbance stable during storage study up to seven days (Fig. 5). The room temperature storage of seven days decrease the absorbance of the extract to 93%, whereas the refrigerator temperature storage, kept the absorbance of 100% on seven days storage duration. Thus, the extract should be kept in refrigerator (at 4 °C), in a closed dark container to prevent the color degradation. The decreasing of the color absorbance during storage may due to the intermolecular copigmentation [19]. The lower temperature (4 °C) kept the stability of the extract color compare to the room temperature, since the lower temperature would decrease the copigmentation reaction [12] and also the lower temperature would decrease the enzyme peroxidase and polyphenolase may present in the extract, thus the color was more stable.
Figure 5. Storage duration and temperature effect to the *Etlingera elatior* extract stability showed high stability for up to seven days both for room temperature (red) and 4 °C (blue).

3.6. **Glucose biosensor indicator study using *Etlingera elatior* extract**

The glucose biosensor study was based on the color change of the *Etlingera elatior* extract with the resulted hydrogen as enzymatic reaction product of glucose and glucose oxidase. The higher glucose concentration, the higher hydrogen peroxide produced, would related to the reducing of extract color. This color change has been measured at 400-700 nm using spectrophotometer (Fig. 6). Furthermore, the absorbance change of the extract was linear to glucose concentration at 0 – 100 mM, with the regression equation of $y = -0.0005x + 0.4724$ and $R^2$ of 0.9965. The coefficient of determination ($R^2$) showed a high correlation between the glucose concentration and the color (absorbance) change, thus, the *Etlingera elatior* flower extract showed a great potential as a green reagent for glucose biosensor development, specially based on colorimetric detection by the glucose oxidase enzyme.

Figure 6. Calibration curve of glucose biosensor using *Etlingera elatior* flower extract
4. Conclusion

*Etlingera elatior* flower ethanol-HCl extract could be used as an indicator of glucose detection for a green chemistry approach of early glucose detection using colorimetric methods involving the use of glucose oxidase enzyme. The proposed method showed a good linearity (0 – 100 mM, R²=0.9965) and high sensitivity (y = -0.0005x + 0.4724). Furthermore, the *Etlingera elatior* flower extract was also showed a high stability against pH, reductor, heating treatment and also stable during storage at 4 °C.

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References

[1] Wild S, Roglic G, Green A, Sicree R and King H 2004 Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030 *Diabetes Care* 27 1047–53

[2] Troughton J, Jarvis J, Skinner C, Robertson N, Khunti K and Davies M 2008 Waiting for diabetes: Perceptions of people with pre-diabetes: A qualitative study *Patient Educ. Couns.* 72 88–93

[3] Fatoni A, Numnuam A, Kanatharana P, Limbut W, Thammakhet C and Thavarungkul P 2013 A highly stable oxygen-independent glucose biosensor based on a chitosan-albumin cryogel incorporated with carbon nanotubes and ferrocene *Sensors Actuators, B Chem.* 185

[4] Fatoni A, Anggraeni M D and Dwiasi D W 2016 Simple colorimetric glucose biosensor using chitosan cryogel supporting material *AIP Conference Proceedings* vol 1746

[5] Fatoni A, Dwiasi D W and Hermawan D 2016 Alginate cryogel based glucose biosensor *IOP Conference Series: Materials Science and Engineering* vol 107

[6] Galuszka A, Migaszewski Z and Namieśnik J 2013 The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices *TrAC - Trends Anal. Chem.* 50 78–84

[7] Tobiszewski M, Mechlińska A, Zygmunt B and Namieśnik J 2009 Green analytical chemistry in sample preparation for determination of trace organic pollutants *Trends Anal. Chem.* 28 943–51

[8] Thovhogi N, Park E, Manikandan E, Maaza M and Gurib-Fakim A 2016 Physical properties of CdO nanoparticles synthesized by green chemistry via Hibiscus Sabdariffa flower extract *J. Alloys Compd.* 655 314–20

[9] Riva S 2006 Laccases: blue enzymes for green chemistry *Trends Biotechnol.* 24 219–26

[10] Sivakumar V, Anna J L, Vijayeeswarri J and Swaminathan G 2009 Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather *Ultrason. Sonochem.* 16 782–9

[11] Harborne J B 1998 *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis* vol 3

[12] Devi P S, Saravanakumar M and Mohandas S 2012 The effects of temperature and pH on stability of anthocyanins from red sorghum (Sorghum bicolor) bran *African J. Food Sci.* 6 567–73

[13] Mizobutsi G P, Finger F L, Ribeiro R A, Puschmann R, Ludmila ;, De L, Neves M, Ferreira W and Mota D 2010 Peroxidase and polyphenoloxidase activities of litchi pericarp Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp *Sci. Agric. (Piracicaba)* 67 213–7

[14] Welch C, Wu Q and Simon J 2008 Recent Advances in Anthocyanin Analysis and Characterization *Curr. Anal. Chem.* 4 75–101

[15] Janeiro P and Brett A M O 2007 Redox behavior of anthocyanins present in Vitis vinifera L. *Electroanalysis* 19 1779–86

[16] Boranhayeva T, Karadeniz F and Yilmaz E 2014 Effect of Storage on Anthocyanin Degradation in Black Mulberry Juice and Concentrates *Food Bioprocess Technol.* 7 1894–902

[17] Hou Z, Qin P, Zhang Y, Cui S and Ren G 2013 Identification of anthocyanins isolated from black
rice (Oryza sativa L.) and their degradation kinetics Food Res. Int. 50 691–7

[18] Sólyom K, Solá R, Cocero M J and Mato R B 2014 Thermal degradation of grape marc polyphenols Food Chem. 159 361–6

[19] Eiro M J and Heinonen M 2002 Anthocyanin color behavior and stability during storage: Effect of intermolecular copigmentation J. Agric. Food Chem. 50 7461–6