Hypoglycaemic effect of *Bawang Dayak* extracts (*Eleutherine palmifolia* (L.) Merr.) on Sprague Dawley rats

Andi Early Febrinda[1*], *Nancy Dewi* Yuliana[2], *Tutik* Wresdiyati[3], *Made* Astawan[2]

[1] Department of Food Quality Assurance Supervisor, College of Vocational Studies, IPB University, Indonesia
[2] Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, Indonesia
[3] Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, IPB University, Indonesia

Abstract. The Dayak tribe in Kalimantan has long used *bawang dayak* as a traditional cure for various degenerative diseases. Previous research showed that *bawang dayak* bulb has an active compound that can inhibit the enzyme alpha-glucosidase, known as alpha-glucosidase inhibitor (AGI). Compounds that have such ability has a potential in controlling blood glucose level in diabetes mellitus. This research aims to study the effective dosage of aqueous extract and ethanolic extract of *bawang dayak* bulb as an AGI through an oral glucose tolerance test in male Sprague Dawley rats. Experimental rats were divided into 14 groups, 7 for the ethanolic extract test and 7 for the aqueous extract test. The extracts were applied in 5 dosage levels in each test, with acarbose as a drug control and distilled water as a positive control. Data were plotted to determine the area under the curve (AUC). The effective dosage was the lowest dosage that gave a significantly smaller AUC value than the AUC value of the positive control group. The effective dosage for *bawang dayak* aqueous extract and *bawang dayak* ethanolic extract was 200 mg/kg and 100 mg/kg, respectively.

1 Introduction

Diabetes mellitus (DM) is a degenerative disease that affects many of the world's population. In 2015 the number of people with diabetes globally was 422 million and was estimated to reach 642 million people in 2040. Prevalence is rising higher in low and middle-income countries. In 2015 the percentage of adults with diabetes was 8.5%, which means 1 in 11 adults live with diabetes. Diabetes became one of the largest health expenditures in the world in 2013, amounting to 612 billion dollars or 11% of total health spending. Diabetes is one of the global health emergencies in the 21st century. There are 318 million adults who have impaired glucose tolerance and are at high risk of developing diabetes [1]. Persons with impaired glucose tolerance (IGT) – 15.6% of the U.S. population

*Corresponding author: earlyfebrinda@apps.ipb.ac.id

– are at high risk for developing diabetes. In addition, IGT is an important risk factor for a number of other adverse health conditions and mortality. IGT is defined on the basis of an abnormal oral glucose tolerance test (OGTT). Persons without diabetes but with an OGTT 2-hour value of 140-199 mg/dl are considered to have IGT [2]**.** The person who develops pre-diabetes will become diabetes in several months or years, if he/she goes untreated appropriately and adequately.

One mechanism that can be used to prevent an increase in blood glucose levels is through inhibition of glucose absorption in the intestine. This mechanism involves a substance that can inhibit the action of enzymes that break down starch into glucose, known as alpha-glucosidase inhibitors (AGI). AGI works in the intestinal tract to prevent disaccharides from breaking down into glucose, thereby decreasing glucose absorption. Many studies have been carried out on various medicinal plants in vitro to determine the potential of these plants as AGI agents. *Bawang Dayak* (*Eleutherine palmifolia* (L.) Merr.) is an indigenous plant of Kalimantan Island, Indonesia. Dayak people have long used this plant as a traditional medicine for several diseases. It is known by various other names such as *bawang tiwai, bawang hantu, bawang sabrang, babawangan beureum, bawang siyem, bawang sayup, bawang kapal* and *bawang lubak*. *Bawang dayak* plants have good adaptations and can grow in various climates and soil types. In addition, this plant can also be reproduced and harvested in a short time so that it is easily developed for industrial scale [3]. *Bawang dayak* aqueous and ethanolic extracts have antioxidant and alpha-glucosidase inhibitor activities that have been proven *in vitro* [4]. The methanolic extract of *Eleutherine americana* bulb contains eleutherinoside A, a naphthalene derivative, which has strong AGI activity with IC_{50} 0.5 mM [5]. The existence of eleutherinoside A in *Eleutherine palmifolia* ethanolic extract also has been confirmed [6].

This research aims to verify the AGI activity of *bawang dayak* extracts work *in vivo* and determine the effective dosage of *bawang dayak* aqueous extract and *bawang dayak* ethanolic extract to decrease intestinal glucose uptake Oral Glucose Tolerance Test (OGTT) in male *Sprague Dawley* rats. This study will give pre-clinical proof and reveal the potential of *bawang dayak* extracts as a local resource for developing the phytopharmaceutical products in controlling and preventing hyperglycaemia in people with Impaired Glucose Tolerance (IGT).

2 Methods

2.1 Plant material and extraction process

Bawang dayak bulbs (*Eleutherine palmifolia* (L.) Merr.) were collected from Air Hitam Village, Samarinda City, East Kalimantan. The bulbs were identified and authenticated by Dr Joeni Setijo Rahajoe from Herbarium Bogoriense, Indonesian Institute of Sciences. *Bawang dayak* extraction was carried out by maceration using water and ethanol solvents according to the procedure explained by Febrinda *et al.* [4]. Fresh *bawang dayak* bulbs were added with water (or ethanol) with a ratio of 1:4 (b:v) and crushed using a blender. The solutions then were soaked for thirty minutes in an ultra-sonic bath and followed by incubation in a shaker incubator at room temperature for two hours. Then the solutions were centrifugated, and the dissolved phase was collected to be filtered using Whatman filter paper No. 1. The extract solutions obtained were frozen and dried using a freeze dryer for 48 hours, followed by pounding the extracts to become a dry powder named *bawang dayak* aqueous extract (BD aqueous extract) and *bawang dayak* ethanolic extract (BD ethanolic extract).

2.2 Experimental animal and oral glucose tolerance test

This research was conducted under the Guide for The Care and Use of Laboratory Animals [7]. 70 male Sprague Dawley rats aged around eight weeks weighing 180 to 200 g were obtained from the Food and Drug Supervisory Agency, Jakarta. The experimental animals were maintained under standard laboratory conditions (26 °C, 12 hours of light/ dark cycles) and fed and watered *ad libitum.* The animals were divided into 14 groups: 7 groups for BD aqueous extract test and the other seven groups for BD ethanolic extract test. The animals fasted for 10 hours, from 10:00 p.m. to 8:00 a.m. The following day, the fasting blood glucose levels of the experimental animals were measured immediately using a glucometer (Accu-Chek Active, Roche, Germany). The animals were grouped and given the following treatment: (1) positive control group (**PC**) treated with distilled water; (2) drug control group (**DC**) treated with 5 mg/kg BW of acarbose solution; (3) treatment group (**A1/E1**) treated with BD aqueous extract or BD ethanolic extract at a dose of 100 mg/kg BW; (4) treatment group (**A2/E2**) treated with BD aqueous extract or BD ethanolic extract at a dose of 200 mg/kg BW; (5) treatment group (**A3/E3**) treated with BD aqueous extract or BD ethanolic extract at a dose of 300 mg/kg BW; (6) treatment group (**A4/E4)** treated with BD aqueous extract or BD ethanolic extract at a dose of 400 mg/kg BW; and (7) treatment group (**A5/E5**) treated with BD aqueous extract or BD ethanolic extract at a dose of 500 mg/kg BW. Thirty minutes after the administration of treatment solution, the animals received sucrose solution with a concentration of 25% at a dose of 2 g/kg BW by oral. The solution was administered through gastric gavage. Blood glucose levels were collected and measured on the 30^{th}, 60^{th}, 90^{th}, and 120^{th} minutes after the sucrose administration. The data obtained were plotted to form a curve, and the area under the curve (AUC) was calculated for each experimental group.

2.3 Statistical analysis

The AUC values of all experimental groups were analyzed using Analysis of variance (Anova). If the Anova result showed significance, further statistical *post hoc* test would be carried out using DMRT. Data were displayed as Mean ± SD.

3 Results

3.1 OGTT result of BD aqueous extract

The curves of OGTT result on BD aqueous extract showed that the acarbose and all treatment groups had lower curves than the positive control (Fig. 1). The area measurement under the curve showed that the entire treatment group had a smaller AUC than the positive control (Fig. 2). However, the dose groups with a significantly smaller AUC than the positive control were 200, 300 and 500 mg/kg BW. These groups had AUCs, which were not significantly different from the acarbose group. Based on DMRT *post hoc* test at α 0.01, the lowest dose that gave significantly lower AUC than the positive control was 200 mg/kg BW (Fig. 2).

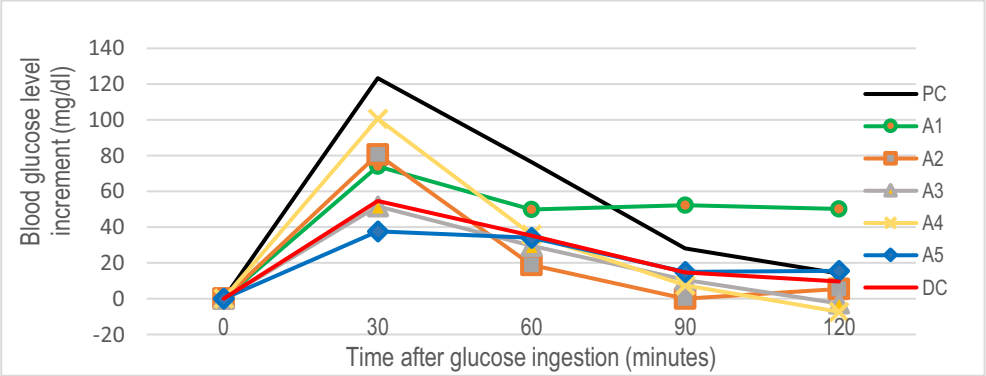

Fig. 1. OGTT curves for BD aqueous extract. PC= positive control group, treated with distilled water. A1= treated with BD aqueous extract dose 100 mg/kg BW. A2= treated with BD aqueous extract dose 200 mg/kg BW. A3= treated with BD aqueous extract dose 300 mg/kg BW. A4= treated with BD aqueous extract dose 400 mg/kg BW. A5= treated with BD aqueous extract dose 500 mg/kg BW. DC= drug control group, treated with acarbose 5 mg/kg BW.

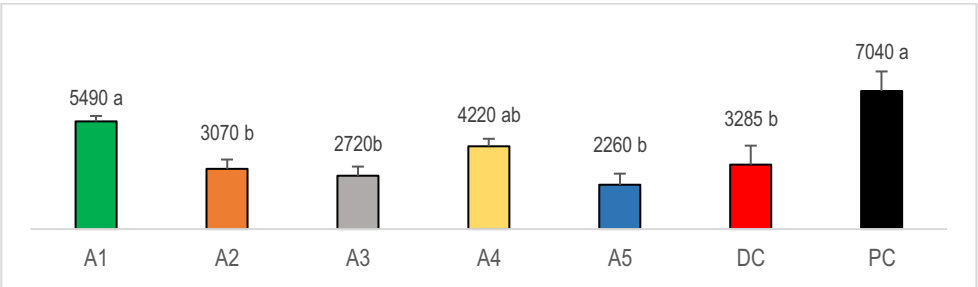

Fig. 2. The area under the curve (AUC) of BD aqueous extract. A1= treated with BD aqueous extract dose 100 mg/kg BW. A2= treated with BD aqueous extract dose 200 mg/kg BW. A3= treated with BD aqueous extract dose 300 mg/kg BW. A4= treated with BD aqueous extract dose 400 mg/kg BW. A5= treated with BD aqueous extract dose 500 mg/kg BW. DC = drug control group, treated with acarbose 5 mg/kg BW. PC= positive control group, treated with distilled water. Values not sharing common superscript differ significantly at $p<0.01$

3.2 OGTT result of BD ethanolic extract

The curves of OGTT result on BD ethanolic extract showed that the acarbose and all treatment groups had lower curves than the positive control (Fig. 3). The area measurement under the curve showed that the entire treatment group had a smaller AUC than the positive control (Fig. 4). However, the dose groups with a significantly smaller AUC than the positive control were 100, 300 and 500 mg/kg BW. These groups had AUCs, which were not significantly different from the acarbose group. Based on DMRT *post hoc* test at α 0.01, the lowest dose that gave significantly lower AUC than the positive control was 100 mg/kg BW

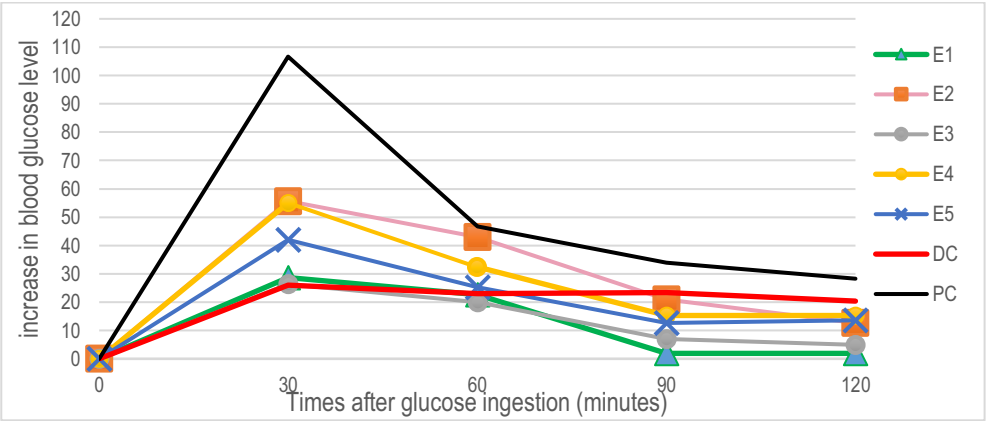

Fig. 3. OGTT curves for BD ethanolic extract. E1= treated with BD ethanolic extract dose 100 mg/kg BW. E2= treated with BD ethanolic extract dose 200 mg/kg BW. E3= treated with BD ethanolic extract dose 300 mg/kg BW. E4= treated with BD ethanolic extract dose 400 mg/kg BW. E5= treated with BD ethanolic extract dose 500 mg/kg BW. DC = drug control group, treated with acarbose 5 mg/kg BW. PC= positive control group, treated with distilled water.

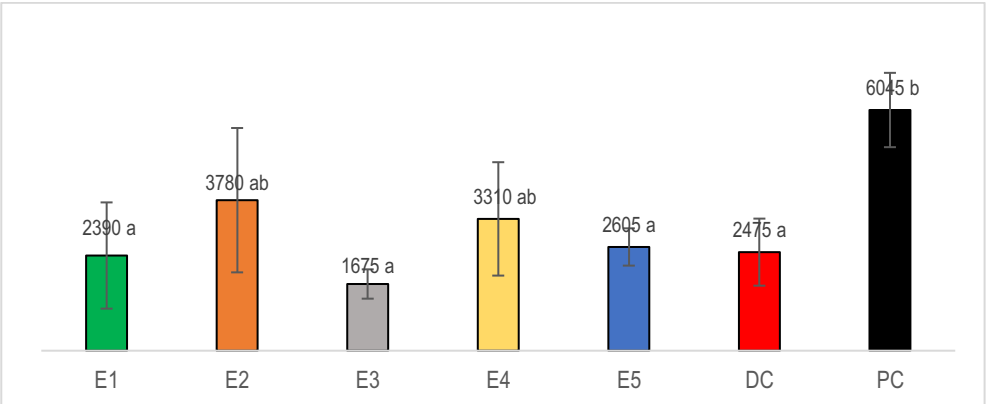

Fig. 4. Area under the curves of BD ethanolic extract. E1= treated with BD ethanolic extract dose 100 mg/kg BW. E2= treated with BD ethanolic extract dose 200 mg/kg BW. E3= treated with BD ethanolic extract dose 300 mg/kg BW. E4= treated with BD ethanolic extract dose 400 mg/kg BW. E5= treated with BD ethanolic extract dose 500 mg/kg BW. DC = drug control group, treated with acarbose 5 mg/kg BW. PC= positive control group, treated with distilled water. Values not sharing common superscript differ significantly at $p<0.01$

4 Discussion

The ability of *bawang dayak* extracts in inhibiting alpha glucosidase enzyme in the intestine was compared to acarbose as a well-known diabetes mellitus medicine. Both extracts showed that they had no significantly different ability from acarbose, although in different dose levels. BD ethanolic extract had a more remarkable ability to reduce blood glucose levels shortly after sucrose consumption than BD aqueous extract (Fig. 1 and Fig. 3). The administration of BD ethanolic extract at a dose of 100 mg/kg BW significantly gave a

lower AUC value compared to positive control, while for the aqueous extract a dose of 200 mg/kg BW was needed. This is consistent with *in vitro* research carried out previously where BD ethanolic extract had significantly lower IC_{50} of alpha glucosidase inhibition (241 ppm) than the aqueous extract (505 ppm) and not significantly different from acarbose (288 ppm) [6].

A naphthalene derivated compound named Eleutherinoside A in methanolic extract of *Eleutherine americana* bulb, could act as an alpha-glucosidase inhibitor (IC_{50} of 0.5 mM) [5]. This compound, along with two other naphthalene derivates (eleuthoside B, and eleutherol), was also found in *bawang dayak* bulb from Air Hitam Village, Samarinda (*Eleutherine palmifoli*a (L.) Merr.) that was used in this research. The presence of those compounds was shown by an NMR analysis on *Eleutherine palmifolia* ethanolic extract [4]. A result of phytochemical screening analysis on *Eleutherine palmifolia* extract also showed that phytochemical compounds presence in BD ethanolic extract was more abundant than those found in the aqueous extract. There were strong positive indications of alkaloid and triterpenoid compounds in BD aqueous extract. In contrast, there were strong positive indications for triterpenoids, flavonoids, and phenolic compounds [6]. Those compounds also had AGI activities which were stated by several research reports [8-12].

Due to the higher presence of Eleutheinoside A and phytochemical compounds in BD ethanolic extract than in the aqueous extract, the ethanolic extract had a higher ability than the aqueous extract in inhibiting alfa glucosidase enzyme activity. Those resulted in a greater decrease of glucose absorption in the intestinal tract of the experimental animal. This result was consistent with the OGTT experiment result of another Kalimantan indigenous plant, *pasak bumi* root (*E. longifolia*), which showed that the methanolic extract had a higher hypoglycaemic effect than the water infusion due to its phytochemical contents [13].

Synthetic AGI, such as acarbose, has long been used to manage type 2 diabetes mellitus. In addition to slowing down the absorption of glucose by the intestine, various diabetes drugs with the mechanism of action of AGI are reported to have side effects in the form of gastrointestinal discomfort [14,15]. Although it has a significant effect on glycemic control and insulin levels, synthetic AGI such as acarbose does not significantly affect blood lipids. On the contrary, bawang dayak extract significantly improved blood lipid profile and antioxidant status in Sprague Dawley alloxan-induced diabetic rats. Those experimental results on *bawang dayak* revealed the potential of this Kalimantan indigenous plant to be a local resource for developing phytopharmaceutical products for treating diabetes mellitus and preventing its cardiovascular complication.

5 Conclusion

The AGI activity of *bawang dayak* extracts was effective *in vivo* in reducing glucose uptake in the intestine of Sprague Dawley male rats. The effective dose of BD aqueous extract and ethanolic extract to inhibit the action of the alpha-glucosidase enzyme determined through OGTT in male Sprague Dawley rats were 200 and 100 mg/kg BW respectively. The combination of antioxidant capacity and the ability to inhibit the activity of alpha glucosidase enzyme in *bawang dayak* extracts showed that this plant had great potential as a source of the phytopharmaceutical product in controlling and preventing hyperglycaemia. Therefore, it is necessary to further assess the acute and chronic toxicity of bawang dayak extract to determine its safety for long-term usage.

References

1. International Diabetes Federation, IDF diabetes atlas 7th edition (Brussels, 2015).
2. NHANES, Oral glucose tolerance test procedure manual (CDC, Washington, 2007)
3. R.Y Galingging, JPPTP **10**, 76 (2007)
4. A.E. Febrinda, N.D Yuliana, E. Ridwan, T. Wresdiyati, M. Astawan, IFRJ, **21,** 1405 (2014)
5. T. Ieyama , M.D.P.T. Gunawan-Puteri, J. Kawabata, *Food Chem* **128**, 308 (2011)
6. A.E. Febrinda, M. Astawan, T. Wresdiyati, N.D. Yuliana, JTIP **24**, 161 (2013)
7. National Research Council, Guide for the care and use of laboratory animals 8th ed. (The National Academies Press, Washington DC, 2011)
8. S.S. Lee, H.C. Lin, C.K. Chen, Phytochem. **69**, 2347 (2008)
9. K. Tadera, Y. Minami, K. Takamatsu, T. Matsuoka, J. Nutr. Sci. Vitaminol **52**, 149 (2006)
10. M.B. Patel, S.M. Mishra, J. Funct. Foods **4,** 79 (2012)
11. Y.C. Lai, C.K. Chen, S.F. Tsai, S.S. Lee, Phytochem **74,** 206 (2012)
12. H. Wang, Y.J. Du, H.C. Song, Food Chem. **123**, 6 (2010)
13. Fransisca, G.E. Kalangi, D.C. Saptasari, P. Hendra, J. Pharm. Sci. Community **15**, 1 (2018)
14. J. Feng, X.W. Yang, R.F. Wang. Phytochem. **72,** 242 (2011)
15. F.A. Van de Laar, P.L.B.J. Lucassen, R.P. Akkermans, E.H. Van de Lisdonk, G.E.H.M. Rutten, C. Van Weel, Cochrane Database Syst. Rev. **2** (2005)