ISSN: 13412051
Volume 25, Issue 06, June, 2020

ACTIVITY OF MIXED ETHANOL EXTRACT SELECTED BLACK TEA (Camelia sinensis L.) AND STEVIA (Stevia rebaudiana B.) AS AN ALTERNATIVE ANTI DIABETES HERBAL MEDICINE

Kusmiyati, Mimin[1], Trinovani, Elvi[1], Fauziyah, Roro Nur[2]

Pharmacy Departement, Health Polytechnic Ministry of Health Bandung Indonesia[1]
Nutrition Departement, Health Polytechnic Ministry of Health Bandung Indonesia[2]

ABSTRACT— Metabolic syndrome is a syndrome that has the highest risk that can cause death. People who have metabolic syndrome are more prone to Diabetes mellitus (DM) type 2. In an effort to look for alternative low-risk prevention, some plant extracts have been tested for antidiabetic activity, one of which is black tea leaf (Camellia sinensis L.) which is often consumed by people especially in Indonesia. Black tea contains several chemical compounds such as alkaloids, steroids, saponins, flavonoids, tannins, and polyphenols (Sudaryat, 2016). This research was conducted with the aim of finding out the activity of a mixture of ethanol extracts of the selected formula of black tea and stevia as an alternative antidiabetic herbal medicine. The results showed that ethanol extracts of black tea and stevia have antidepressant activity in white mice. Giving ethanol extract of black tea and ethanol extract of stevia in diabetic mice dose I containing ethanol extract of black tea 50 mg/kg BW and stevia 300 mg/kg BW, dose II containing black tea extract 100 mg/kg BW and stevia 200 mg /kg BW and dose III contain 150 mg/kg BW and stevia black tea extract 300 mg/kg BW and dose I had the highest antidiabetic activity compared to other doses with a decrease in blood glucose levels of 69.75 mg/dL.

KEYWORDS: Ethanol extract, black tea and stevia formula, antidiabetic mellitus

1. INTRODUCTION

In Indonesia, with a population of 258 million people, in 2014, more than twenty thousand people with an age range of 30 - 69 years and more than ten thousand people in the world with an age range of 70 years and over have died due to diabetes or caused due to increased levels sugar in the blood (hyperglycemia). The latest estimate is the International Diabetes Federation (IDF), there were 382 million people living with diabetes in the world in 2013. By 2035 that number is expected to increase to 592 million people. It is estimated that of the 382 million people, 175 million of them have not been diagnosed, thus threatening progressive progression into unconscious and uncomplicated complications (Ministry of Health, Republic of Indonesia, 2014). Around 37% of people in Indonesia, while for diabetes alone has caused about 6% of deaths (WHO, 2016). The therapeutic approach taken in Indonesia in general is to try to reduce hyperglycemia after meals with treatment using metformin and sulfonylureas after blood sugar levels are checked, and for those who have led to complications, dialysis and kidney transplant procedures are provided (WHO, 2016). It is known that α-glucosidase is an enzyme from the intestine that works in the digestive system which is useful for hydrolyzing starch. If this enzyme is inhibited, there will be a decrease in the retrieval of carbohydrates, so that it can reduce blood sugar levels in people with type 2 diabetes. Various studies have been conducted to find natural products from plants. In efforts to prevent diabetes, many people switch to using herbal medicine with a lower risk of side effects, one of which is consuming

black tea, green tea, white tea. Tea (Camellia sinensis) is the most consumed beverage by the public, especially in Indonesia (Dias., Et al, 2013). Black tea contains several chemical compounds such as alkaloids, terpenoids / steroids, saponins, flavonoids, tannins and polyphenols (Sudaryat, 2016). Polyphenols including EGCG (epigalokatekin galat) with HPLC / HPLC are 4.47% and can reduce the absorption of fatty acids, cholesterol, and starch in the digestive system so as to reduce the amount of nutrients that can be absorbed (Sen and Biswajit, 2016). According to in vitro research, tea extract (Camelia sinensis L) can inhibit the α-glucosidase enzyme with an IC50 value of 10.54 µg / mL (Elya B et al, 2015). Indonesian people usually drink tea using a sweetener in the form of synthetic sugar. The sweetener contains a lot of sugar and calories which are not good for diabetics. Stevia is a plant that is developed as a natural raw material of sugar (sweetener), a companion of cane sugar and a substitute for synthetic sugar with sweetness 250 - 300 times sucrose or kitchen sugar (Subroto, 2008). That by getting used to consuming tea using stevia especially for people who are at risk of developing diabetes at the age of 40 years can be used as a solution because there is a potential for the prevention and treatment of diabetes as well as to preserve the legacy of traditional ancestral medicine. Based on the description above, this time the research was conducted to test the activity of a mixture of ethanol extracts of the selected formula of black tea (Camelia sinensis L.) and stevia (Stevia rebaudiana B) as an alternative to anti-diabetic herbal medicines.

2. Methods

This study used black tea (Camelia sinensis L) and stevia leaves (Stevia rebaudiana B), the plant came from the Tea and Quinine Research Center of Mekarsari Village, Pasir Jambu District, Bandung Regency, aqua destilata, alloxan, NaCl 0.9% and ethanol. Refrigerators, rotary epavorators (Buchi), grinders, analytical scales (mettler Toledo), water baths (Memmert), thermometers, glassware, volume pipettes. The selected formula from the mixture of black tea and stevia leaves is made powder then immersed in 96% ethanol with a ratio of 1:10 using the maceration method for 6 hours while occasionally stirring and allowed to stand for 24 hours. The maserate is separated and the process is repeated 2 times with the same type and amount of solvent. The maserate obtained was then concentrated with a vacuum rotary evaporator to obtain a thick extract. Thick extract of black, green, white-stevia tea was weighed and the yield was calculated. A single dose preliminary test was performed to determine the potential of each extract as antidiabetic, the procedure carried out for the preliminary test is the same as the antidiabetic test to be performed. Doses obtained from preliminary test results which will later be used for antidiabetic testing. Mice for antidiabetic testing the selected formula of black tea and stevia extract in 6 groups each group consisted of 5 mice, as follows:

- Group I: Normal control (aquadest)
- Group II: Negative control (induction of alloxan 130 mg / kg BW)
- Group III: Aloxane 130 mg / kg body weight + glibenclamide 0.013 mg / kg body weight
- Group IV: Aloxane 130 mg / kg body weight + selected formula of black tea extract 50 mg / kg body weight and stevia 300 mg / kg body weight
- Group V: Aloxane 130 mg / kg body weight + selected formula of black tea extract 100 mg / kg body weight and stevia 200 mg / kg body weight
- Group VI: Aloxane 130 mg / kg body weight + selected formula of black tea extract 150 mg / kg body weight and stevia 300 mg / kg body weight.

Eight hours before the blood draw begins, the mice are fasted first. Before being treated, the blood glucose level of each mouse was measured as the initial blood glucose level. Mice other than the negative control group were injected with alloxan at a dose of 130 mg / kg BW in i.p on mice. Then on the 3rd day blood samples were taken both of the negative, positive, and test sample mice (Rohdiana, 2016). After mice suffering from DM (blood glucose level ≥ 1 mg / dL), given a suspension of tea and stevia extract once for

14 days orally (Holidah and Christian, 2015). Every time the blood collection of mice must be satisfied first for 8 hours. Then on the 7th day and the 14th day blood samples are taken on mice (Rohdiana, 2016). Blood collection is done through the tail veins of mice. Adequate blood is drawn and placed at the end of the test strip (One Touch Horizon) which has been installed into a glucose meter (One Touch Horizon). Blood measurements are carried out using glucose test strips (One Touch Horizon) and glucose meters (One Touch Horizon). After obtaining some blood of mice taken from the tail veins of mice. Place the blood of mice on the end of a test strip that is mounted on a glucose meter (One Touch Horizon). Within a few seconds then the results of measurements of glucose levels in the blood will be seen (Rohdiana, 2016). This study compared the reduction in blood sugar levels in mice from various doses of a mixture of ethanol extracts of black tea (Camelia sinensis L.) and stevia leaves (Steviarebaudiana B.) with a glibenclamide comparison. The results of antidiabetic activity tests were then analyzed statistically using SPSS (Statistical Package for Social Sciences). If the spread of data is normal and homogeneous, it will be tested with the Repeated anova test and continued with the Post hoc test using the LSD method to see whether the differences obtained are meaningful or not. If the data distribution is not normal and not homogeneous, then it is tested by Friedman test followed by Post hoc test to see whether the differences obtained are meaningful or not.

3. Results

Before conducting the study, the test animals were acclimatized for 7 days by being fed, drinking and weighing the mice weighed every day. The cleaning of the mouse cages was maintained by replacing the husks every 3 days. Test animals at the beginning of the acclimatization process amounted to 38 tails which were divided into 6 groups, during the research process there were 2 mice that died, so that on the last day of acclimatization the mice totaled 36 tails. The first day of acclimatization of mice's weight is 21 g to 32 g, and on the last day the acclimatization of mice's body weight is 22 g to 35 g, so that the overall mouse meets the test requirements. Mouse weight data during acclimatization can be seen in the appendix. Black tea after weighing as much as 200 g was then macerated using 96% ethanol solvent. Maserate produced from the maceration process is then concentrated using a rotary evaporator at 50 ° C until the solvent in the filtrate evaporates. The extract obtained was then transferred to a cup and then evaporated over a water bath at 50 ° C until a thick extract of 12.332 g was obtained. Black tea extract which has been thickened and then calculated the percent yield of the extract, obtained the yield of the percent extract yield of 5.78%. The extract obtained was blackish brown, thick and distinctive odor.Stevia after weighing as much as 250 g was then macerated using 96% ethanol solvent. Maserate produced from the maceration process is then concentrated using a rotary evaporator at 50 ° C until the solvent in the filtrate evaporates. The extract obtained was then transferred to a cup and then evaporated over a water bath at 50 ° C until a thick extract of 29.017 g was obtained. Stevia extract which has been thickened and then calculated the percent yield of the extract, obtained the yield of the percent extract yield of 11.6068%. The extract obtained is green, thick and characteristic odor. Preliminary Test Results Antidiabetic Activity of 96% Ethanol Extract Black Tea and 96% Stevia Ethanol Extract. The combined dose of ethanol extract of black tea and stevia used in this study were ethanol extract of black tea 100 mg / kg BW and stevia 400mg / kg BW, then each dose was converted to a dose of mice obtained 2mg / 20g BB and 8mg / kg 20g BB of mice. Then the researchers conducted a preliminary test to the test animals using this dose to determine the activity of a single dose of each extract. From each of these doses, it is obtained the result of a decrease in blood sugar levels in mice compared to normal mice and each extract has a single effect to reduce blood sugar levels in mice.

Antidiabetic Activity Test Results 96% Ethanol Extract Black Tea and 96% Stevia Ethanol Extract. The combined dose of ethanol extract of black tea and stevia used in this study were, dose I contained ethanol extract of black tea 50 mg / kg BW and stevia 300 mg / kg BW, dose II contained black tea extract 100 mg /

kg BW and stevia 200 mg / kg body weight and dose III containing 150 mg / kg body weight black tea extract and stevia 300 mg / kg body weight then each dose was converted to mice dose. Then the researchers conducted a test using these doses. From all these doses, the average result of a decrease in blood sugar levels in mice is close to the average result of a comparable decrease in blood sugar levels, which is 109.75 mg / dL.

Table 1 Average values of fasting blood glucose levels in mice.

Measurement Time	Average values of fasting blood glucose (mg/dL)					
	Control Negative	Control Positive	Comparison	Dose I	Dose II	Dose III
Day 8 (Pretest)	95.50±6.73	155.50±7.95	154.5±11.63	153.50±11.72	150.75±4.15	159.75±3.56
Day 15 (Post Test)	106.75±5.67	154.75±6.34	109.75±11.56	83.75±11.71	82.00±13.29	91.00±15.95
Decrease	-11.25	0.75	44.75	69.75	68.75	68.75

Table 1 shows the average values of fasting blood glucose in mice from the negative control group, positive control, comparison, dose I, dose II and dose III at the time of measurement day 8 and day 15. On the 8th day the mean fasting blood glucose level of mice in the negative control group was 95.5 ± 6.73 mg / dL, indicating that the fasting blood glucose level of the negative group mice was normal (<100 mg / dL). The group that was induced by alloxan, the highest average fasting blood glucose levels were the dose group III which was 159.75 ± 3.56 mg / dL and the lowest was the treatment group II which was 150.75 ± 4.15 mg / dL. On the 15th day the mean fasting blood glucose levels of mice in the negative control group were 106.75 ± 5.67 mg / dL, indicating that the fasting blood glucose levels of the negative control group of mice were above normal but still below the stated diabetes level. The average fasting blood glucose level of mice in the positive group was 154.75 ± 6.34 mg / dL, indicating that the positive control group was still diagnosed with diabetes (≥ 126 mg / dL). In the dose group I, II and III, the highest blood glucose content satisfied the highest mice was the dose group III, which was 91 ± 15.95. The first data processing is to do a data normality test. The normality test results show that the P value for all data groups is> 0.05, so it can be concluded that the distribution of all data groups is normal. After knowing that the data distribution of all data groups is nomal, we can do the variance test and see the Anova results. In the variance test, the value of P = 0.167 can be concluded that there is no difference in variance between the data groups compared to other data, meaning that the data variance is the same, because the data variance is the same, it can be continued with the Anova test. Looking at the Anova test results, P value = 0,000 was obtained, which means that there were at least significant differences in immobility time in the two groups. To find out which groups have those differences, a post-hoc analysis can be done. From the results of the post-hoc analysis, the results obtained are the control group P <0.05 which means that the control group data is significantly different from all the data groups, so the induction of alloxan in this study is said to be successful. The positive control group was significantly different from the comparison group and the dose group because the P value <0.05. The positive control group was not significantly different from the negative group because the P value> 0.05. The comparison group was significantly different from all the data groups because the P value <0.05.

The dose I group (EETH 50 mg / kg BW and EES 300 mg / kg BW) was significantly different from the negative control group, positive control group and comparison group because the P value <0.05, the dose I

group did not differ significantly from the dose group II and dosage group III because the P value> 0.05. The dose group II (EETH 100 mg / kg body weight and EES 200 mg / kg body weight) was significantly different from the negative control group, positive control group and comparison group, because the P value <0.05. The dose II group was not significantly different from the dose I group and dose III group because the P value> 0.05. The dose group III (EETH 150 mg / kg BW and EES 300 mg / kg BW) was significantly different from the negative control group, positive control group and comparison group because the P value <0.05. The dose III group did not differ significantly from the dose I group and the dose II group because the P value> 0.05.

4. Discussion

This research was conducted to determine the antidepressant activity of white tea leaf ethanol extract. The black tea (Camellia sinensis) used in the study came from the Kina Gambung Tea Research Center, Mekarsari Village, Pasir jambu District, South Bandung and Stevia (Stevia rebaudiana Bertoni) from the Manoko Experimental Garden in Cikahuripan Village, Lembang District, West Bandung District. black (Camellia sinensis) used was determined at the Gina and Stevia Quinine Tea Research Center (Stevia rebaudiana Bertoni) determined at the ITB School of Biological Science and Technology. Plant determination aims to find out the truth of plant identity and ensure that the plant is intended to be used in research. The extraction method used is the maceration method. Maceration is a process of extraction with maceration techniques carried out by shaking several times or stirring at room temperature. The advantage of this method is easy and does not need heating so it is unlikely that natural materials become damaged or decompose (Susanty, 2016) The selection of solvents based on their solubility and polarity makes it easy to separate natural materials in the sample. Work on the old maceration method and a state of rest during maceration allows many compounds to be extracted (Istiqomah, 2013). Maceration of samples was done using 96% p.a ethanol solvent because it has the ability to search with a wide polarity ranging from nonpolar compounds to polar (Saifudin et al, 2011). Compounds of black tea and stevia leaves which are suspected to have antidiabetic effects are flavonoids. According to the principle of polarization, a compound will dissolve in solvents that have the same polarity (Harborne, 1987) Flavonoid compounds are polar compounds because they have a number of bound sugars, therefore flavonoids are more likely to dissolve in polar solvents (Harborne, 1987) Filtrate obtained then concentrated using a rotary vacuum evaporator at a temperature of 50 ° C. The yield of black tea maceration extraction was 5.78% and stevia was 11.6068%. This value can be influenced by several factors, including the type of solvent, solvent concentration, particle size, solvent concentration, particle size of the simplicia, and the length of time of extraction. The success of the separation depends on differences in the solubility of the components to be separated in the solvent (Suryanto, 2012). Combination testing is done because it is expected to obtain a better glycemic control effect compared to single therapy, proving this combination can work synergistically where the effect of the combination can be greater than a single administration (Tjay, 2010). The choice of measurement with fasting blood glucose levels due to the nutritional content in the foods and drinks consumed will be absorbed into the bloodstream and can have a direct impact on blood glucose levels. Fasting for at least 8 hours will reduce the variability of this substance from the variability of other substances in the blood. This is to ensure that the results of the examination do not affect the final food consumption and can be interpreted correctly (Ministry of Health, Republic of Indonesia, 2016).

Animal selection is based on data that there is no difference in the sensitivity of mice and rats to alloxan, so that selected experimental animals with lower specifications are mice (Srinivasan, 2007). It is generally known that most female test animals show a stronger hormonal immune response compared to males (male Gupta, 1984). Based on Killic's research (2014), blood glucose levels in male mice are higher than female

mice after being induced by alloxan 150 mg / kg body weight measured every 48 hours for 8 days. This is caused by the hormones estrogen and progesterone in female mice can increase insulin secretion which will affect blood glucose levels (Nurlaela, 2016), so in this study male mice are used to avoid hormonal influences. Before testing, try animals are acclimatized, acclimatization of experimental animals is carried out for 7 days. Acclimatization aims to familiarize mice living in the environment and new treatments, as well as to limit the influence of the environment in experiments. Every day the mice are given enough food and drink and their cages are kept clean by replacing the husks for 3 days. Also carried out a daily body weight check so that none of the mice had lost weight. The day before testing the mice were fasted for 16-18 hours while still giving a drink. This aims to avoid the influence of food on the observations (Puspitasari et al, 2015). The selection of alloxan as a diabetes induction material because it has a selective mechanism of action on pancreatic β cells through GLUT2 by producing ROS (reactive oxygen species) so that pancreatic β cells do not function and cannot produce insulin (Leuzen, 2008). To get the diabetes condition, all mice were induced with alloxan monohydrate, except for the negative group mice. All mice were satisfied for 8-12 hours before being given a drink. Alloxan solution is made in a new state when it will induce dikarekan alloxan monohydrate only stable for 1.5 minutes when standing in water at 37 ° C (Lenzen, 2008). Alloxan monohydrate was given intraperitonially to mice at a dose of 130 mg / kg BW every 4 days to increase blood glucose levels (Studiawan, 2005). Measurement of blood glucose levels was carried out on days 1, 4, 8, and 15. Blood sugar levels of mice> 126 mg / dL (Malole & Pramono, 1989).

Blood sugar levels were checked using the Accu check glucometer. Measurement of blood glucose levels on day 4 and day 8 is intended to determine whether blood glucose levels have risen. Blood sampling is carried out on the tail veins of experimental animals. Provision of test preparations on experimental animals is carried out on the 9th day through the 15th day (Studiawan, 2005). To determine the effect of ethanol extracts of black tea and stevia on decreasing fasting blood glucose levels in experimental animals, the data to be compared is a decrease in glucose levels from day 8 to day 15 in each group. In this study 36 mice were used which were divided into 6 groups with 6 mice for each group. The first group was the negative control group given 0.5 ml of aquadest. The second group is a positive control group induced with alloxan without drug treatment. This group is used to ensure that the antidiabetic activity test method is correct. The next group is a comparison group of diabetic mice given glibenclamide treatment. Glibenclamide is an oral antidiabetic drug of sulfonylureas where the mechanism of action of the drug is to stimulate an increase in insulin secretion from β-pancreatic cells, so that blood glucose levels decrease (Wardani, 2016). Post Test is a measurement of fasting blood glucose levels in mice on the 15th day after treatment, the results of measurements indicate that there was a decrease in blood glucose levels in the comparison group, treatment 1,2, and 3. A decrease in the treatment group 1,2, and 3 looks significant compared to the comparison. The results of post-test measurements in the treatment group 1,2, and 3 showed a decrease in fasting blood glucose levels in mice to normal values. Hypoglycemia condition in mice and rats is a blood glucose level <40 mg / dL (Bezdawa, 2016). The combination of dose I ethanol extract of white tea 50 mg / kg BW and ethanol extract of stevia 300 mg / kg BW had a decrease in fasting blood glucose levels which is higher 69.75 mg / dL compared to dose II, dose III and comparison and when compared with administration of a single dose extract dose of 21 mg / dL and 42 mg / dL obtained from the preliminary test results. This proves that the administration of a combination of 2 types of OHO with different mechanism of action can obtain a better glycemic control effect compared with a single therapy (American Diabetes Association, 2015). Black tea (Camellia sinensis) contains flavonoid compounds which have the ability as antioxidants that work by capturing metal ions and reactive oxygen species (ROS) and triggering pancreatic β cell regeneration (Saryono, 2013). Stevia leaves have antioxidant abilities that can play an active role in reducing blood glucose levels but the mechanism as an antidiabetic is not yet known (Shukla, 2011).

Data on reducing blood glucose levels in mice after being floated and then calculating the percentage of power is data entered into the SPSS analysis. The first step taken is to test the normality and homogeneity of the data. The normality test uses the Shapiro Wilk table in the test of normality because the amount of research data is less than 50. The normality test results for each group show a p-value> 0.05, this shows that the data distribution of each group is normally distributed. Then the data homogeneity test was performed for each group, the homogeneity test results showed a P-value> 0.05, meaning that the data variance was homogeneous. Because the data variance is the same then proceed with the Anova test. In the Anova test, P = 0,000 was obtained, which means at least there was a significant difference in the decrease in blood glucose levels in the two groups. To find out which groups have significant differences, post-hoc analysis is performed. The results obtained were positive control group showed a P value <0.05 meaning that the positive control group was significantly different between pretest and posttest blood glucose levels. These results prove that test animals not given drugs and extract solutions have higher blood glucose levels. All dosage groups have significant differences with the comparison. This proves that test animals given glibenclamide suspension have lower blood glucose levels so that it can be concluded that glibenclamide has the ability as an antidiabetic. All dosage groups have significant differences. It can be concluded that all treatment groups have antidiabetic effects. From this description it can be concluded that the ethanol extract of white tea and ethanol stevia extract can reduce blood glucose levels in diabetic mice, which when compared with oral diabetes drug administration of ethanol extract of white tea and ethanol extract of stevia have greater ability to reduce blood glucose levels. However, it is important to note the possibility of the toxicity effect of ethanol extract of white tea and ethanol extract of stevia. Therefore, it is necessary to conduct further research related to the toxicity test. The most effective dosage group is the Iberisi dose group of black tea ethanol extract 50 mg / kg BW and stevia 300 mg / kg body weight with an average decrease in blood glucose levels of 69.75 mg / dL. Based on the results of this study it can be concluded that Ethanol extract of black tea and stevia have antidepressant activity in white mice.

5. Reference

[1] Anonim. (2010). Budidaya dan Pasca Panen Teh. Bogor: Badan Penelitian Dan Pengembangan Pertanian

[2] Anonim. (2010). Budidaya dan Pasca Panen Teh. Bogor: Badan Penelitian Dan Pengembangan Pertanian

[3] Anonim, (2015). “Database file for Stevia rebaudiana”, terdapat dalam Artikel Tropical Plant Database, diakses 1 November 2015 dari http://www.rain-tree.com/plants.htm

[4] Bosenberg, L. (2008). The Mechanism of Action Oral Antidiabetic Drugs: a review of recent literature. The Journal of Endrocrinology, Metabolism and Diabeticof South Africa, 80-88

[5] British Pharmacopeia Commision. (2009). British Pharmacope. (Edisi 1 & II). London: The Stationery Office

[6] Dalimartha, Setiawan dan Felix Andrian. (2012). Makanan dan Herbal UntukPenderita Diabetes Mellitus. Cetakan I. Jakarta: Penebar Swadaya

[7] Departemen Farmakologi dan Terapi FKUI. (2007). Farmakologi dan Terapi. (Edisi 5). Jakarta: Gaya Baru

[8] Departemen Kesehatan RI. (2014). Farmakope Indonesia. (Edisi V). Jakarta: Direktorat Jendral

Pengawas Obat dan Makanan Departemen Kesehatan Republik Indonesia

[9] Departemen Kesehatan RI. (1995). Material Medika Indonesia (Vol. IV). Jakarta: Departemen Kesehatan Republik Indonesia

[10] Departemen Kesehatan RI. (2000). Parameter Standar Umum Ekstrak Tumbuhan Obat.Jakarta: Departemen Kesehatan Republik Indonesia

[11] Dewi R.T, Iskandar Y.M, Hanafi M, Kardono L.B.S, Angelina M, Indah D, Banjarnahor S.D.S. (2007). Inhibitory Effect of Koji Aspergillus terreus On α-glukosidase Activity and Postprandial Hyperglycemia.Pakistan Journal OfBiological Sciene. Vol. 10 (18): 3131-3135

[12] Dias, T.R., Tomas, G., Teixeira N.F., Alves, M.G., Oliveir., dan Silva, B.M. (2013). White Tea (Camellia sinensis (L.)): Antioxidant Properties and Beneficial Health Effects. International Journal of Food Science, Nutritions Dietetics. 2(2), 19-26.

[13] Edi, Busono dan Dini Mardiani. (2015). Panduan Budidaya Stevia Sebagai PenghasilGula Rendah Kalori. Bandung: Koperasi Nukita

[14] Elya, B., Handayani, R., Sauriasari., Azizah, W., Hasyyati, U.S., Permana, I.T., dan Permatasari, Y.I. (2015). Antidiabetic Activity a Phytochemical Screening of Extra from Indonesian Plants by Inhibition of Alpha Amylase, Alpha Glucosidase and Dipeptidyl Peptidase IV. Pakistan Journal of BiologicalSciences 18(6), 279-284.

[15] Ganjdar, Ibnu Gholib dan Abdul Rahman. (2012). Analisis Obat Secara Spektroskopidan Kromatografi. (Cetakan I). Yogyakata: Pustaka Pelajar

[16] Harbone, J.B. (1987). Metode fitokimia. Bandung: ITB.

[17] Harbone, J.B. (1996). Metode fitokimia penuntun cara modern menganalisis tumbuhan.Bandung: Penerbit ITB.

[18] Hartoyo, Arif. (2007). Teh dan Khasiatnya Bagi Kesehatan. (Cetakan ke-5). Yogyakarta: Kasinus

[19] Harvey dan Ferrier. (2011). Lippincott's Ilustrated Reviews: Biochemistry (Fifth Ed). Philadelphia: Lippincott William & Wilkins, 56-62.

[20] Hilal, Y dan Engelhardt, U. (2007). Characterisation of white tea – Comparison to green and black tea. Journal of Consumer Protection and Food Safety. 2(4), 414-421.

[21] Holidah, Diana dan Christianty, Fransiska M. (2015). Uji Aktivitas Antidiabetes Ekstrak Teh Hitam Teh Oolong dan Teh Hijau Secera in Vivo. Prosiding Seminar Nasional Current Challanges in Drug Use and Development.

[22] Insanu, Muhamad, dkk. 2017. Uji Aktivitas Antibakteri Lima Belas Jenis Mutu Teh Hitam Ortodoks Rotorvane Dan Teh Putih (Camellia Sinensis Var. Assamica) Pada Staphylocoocus aureus ATCC 6538. Acta Pharmaceutica Indonesia, vol 42, no 1: 32-41.

[23] International Diabetes Federation., Nam Han Cho, David Ehiting, Leonor Guariguata, Pablo Aschner Montoya, Wolfgang Rathmann, Gojka Roglic, Jonathan Shaw, Martin Silink, D.R.R. Williams, Ping Zhang. (2013). IDF DIABETES ATLAS, 9th Edition.

[24] Istiqomah. (2013). Perbandingan metode ekstraksi maserasi dan sokletasi terhadap kadar piperin buah cabe jawa

(Piperis retrofracti fructus). (Skripsi). Jakarta: Universitas Islam Negeri Syarif Hidayatullah.

[25] Kemenkes RI. (2012). Vademekum Tanaman Obat. (Jilid 3). Jakarta: Kementerian Kesehatan RI

[26] Kim, K.Y., Nam, K.A., Kurihara, H., dan Kim, S.M. (2008). Potent α-Glucosidase Inhibitors Purified from The Red Alga (Grateloupia elliptica). Phytochemistry 69, 2820-2825.

[27] Lenzen, S., 2008. The Mechanism of Alloxan and Streptozotocin Induced Diabetes. Diabetologia 51. P. 216-226.

[28] Luqman, B. (2007). Pembuatan Gula Non Karsinogenik Non Kalori dari Daun Stevia. Tesis. Universitas Diponegoro, Semarang

[29] Martindale. (2009). Martindale: The Complete Drug Reference (Thirty-sixth Edition). London: Pharmaceutical Press.

[30] Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia. (2014). Situasidan Analisis Diabetes. Jakarta: Kementerian Kesehatan

[31] Puspitasari, Herni Pratiwi., Fitrianingsih, Sri Peni., Mulqie, Lanny. (2015). Pengaruh pemberian ekstrak jamur kuping hitam terhadap penurunan kadar kolesterol mecit swiss webster jantan. Prosiding Penelitian speSIA Unisba.

[32] Raini, Mariana dan Ani Isnawati. (2011). "Kajian: Khasiat dan Keamanan Stevia sebagai Pemanis Pengganti Gula". Dalam Artikel Elingible. 2 September.

[33] Rohdiana, Dadan., Deswati, D.A., Maryam, Z.N. (2016). Aktivitas Antidiabetes Mellitus Teh Hitam Jenis Mutu Rendah pada Mencit Putih Jantan yang Diinduksi Aloksan. Jurnal Penelitian Teh dan Kina. 19 (2): 208-214.

[34] Rukmana, Rahmat. (2003). Budi Daya Stevia. Yogyakarta: Kanisius

[35] Saifudin, Azis. (2014). Senyawa Alam Metabolik Sekunder Teori, Konsep, dan TeknikPemurnian. Edisi 1 (Cetakan ke 1). Yogyakarta: Deepublish.

[36] Sen, Gargi & Biswajit Bera. (2013). Black tea as a part of daily diet: A boon for healthy living. International Journal of Tea Sciene, vol. 9(2-3), hlm. 56-57.

[37] Setyamidjaja, Djoehana. (2008). Teh: Budi Daya Dan Pengolahan Pasca Penen. (Cetakan ke 6). Yogyakarta: Kanisius.

[38] Singh, S., Garg, V., dan Yadav, D. (2013). Antihyperglycemic and Antioxidative Ability of Stevia Rebaudiana (Bertoni) Leaves in Diabetes Induced Mice. International Journal of Pharmacy and Pharmaceutical Sciences, 5(2), 297-302.

[39] Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, Bo, Kumar, S., Placido, J., dan Zito, S.W. (2008). Alpha-Glucosidase Inhibitory Activity of Syzygium cumini (Linn.) Skeels Seed Kernel in Vitro and Goto-Kakizaki (GK) Rats. Journal Carbohydrate Research, 1278-1281.

[40] Sigma. (1996). Enzim assay of a-glukosidase. Diakses Februari 8, 2016, dari http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/General_Information/alpha_glucosidase.Par>0001.File.dat./alpha_glucosidase_sed.pdf

[41] Soraya, Noni. (2007). Sehat dan Cantik Berkat Teh Hijau. Jakarta: Penebar Plus.

[42] Studiawan, Herra. (2014). Pengembangan Dan Peningkatan Kapasitas Produksi Bahan Baku Obat Tradisional Dari Daun Kumis Kucing Dan Rimpang Temu Mangga Dalam Pilot Scale. Laporan Akhir Program Fasilitas Pengembangan Bahan Baku Obat Dan Bahan Baku Obat Tradisional. Direktorat Bina Kefarmasian Dan Alat Kesehatan Kementerian KesehatanRI. Fakultas Farmasi. Universitas Airlangga: Surabaya.

[43] Subroto, Muhammad Ahkam. (2008). Real Food True Health–Makanan SehatUntuk Hidup Lebih Sehat. Jakarta: Agro Media Pustaka

[44] Sudaryat, Yayat dan Trinovani, Elvi. (2016). Aktivitas Penghambatan Radikal Bebas dan Studi Fitokimia dari 10 Grade Teh Hitam (Camellia sinensis) yang Berbeda. Politeknik Kesehatan Bandung Jurusan Farmasi. Bandung.

[45] Sudha, P., Zinjarde, S.S., Bhargava, S.Y., dan Kumar, A.R. (2011). Potent α-Amylase Inhibitory Activity of Indian Ayurvedic Medicinal Plants. BMCComplementary and Alternative Medicine

[46] Sugiwati, Sri., Setiasih, Siswati., dan Afifah, Efy. (2009). Antihyperglycemic Activity of the Mahkota Dewa (Phaleria macrocarpa (scheff) boerl) Leaf Extracts as on Alpha-glukosidase Inhibitor. Makara, Kesehatan, Vol. 13 No. 2: 74-78

[47] Sujayanto, G. (2008). Khasiat Teh Untuk Kesehatan dan Kecantikan. Flona Serial Oktober (I)

[48] S. Shukla, a. Mehta, v.k. bajpai, s. Shukla. (2011). In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of S. rebaudiana Bert. Food Chem. Toxicol. 47, 2338-2343.

[49] Sukandar, Elin Yulinah., Andrajati, Retnosari., dan Sigit, Joseph. (2009). ISOFarmakoterapi. Cetakan kedua. Jakarta: PT ISFI Penerbitan

[50] Tjay, Tan Hoan dan Kirana Rahadja. (2013). Obat-obat Penting. Edisi ke-IV Cetakan ke-3. Jakarta: Gramedia

[51] Wardani, Novia Pegin. (2016). Uji Aktivitas Antidiabetes Ekstrak Kering Biji Mahoni Terstandar (Swietenia mahagoni Jacq) Pada Mencit yang Diinduksi Aloksan. Skripsi. Universitas Airlangga: Surabaya.

[52] Zhang, JF., Zheng, YG., dan Shen, YC. (2007). Inhibitory Effect of Valienamine on the Enzymatic Activity of Honey Bee (Apis cerana Fabr) alpha-glucosidase. Journal Pesticide Biochemistry and Physiology 83, 73-77.